THE UNIVERSITY OF CALGARY

REGULATION OF ARGININE VASOPRESSIN RECEPTORS

by

PAULE POULIN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF MEDICAL SCIENCE

CALGARY, ALBERTA

JUNE 1991

ISBN 0-315-71146-9

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Regulation of arginine vasopressin receptors", submitted by Paule Poulin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dr. Q.J. Pittman, Supervisor
Dept. of Medical Physiology

Dr. R. M. Cooper,
Dept. of Psychology

Dr. D. Severson,
Dept. of Pharmacology & Therapeutics

Dr. S. Weiss,
Dept. of Pathology

Dr. D.M. Dorsa, External Examiner,
University of Washington

June 27, 1991

ABSTRACT

Repeated injection of arginine vasopressin (AVP) into a lateral cerebral ventricle or into the ventral septal area (VSA) of the rat brain causes a sensitization process, whereby the first administration of AVP increases the motor responses of the animal to subsequent AVP treatment. The objective of this thesis was to investigate putative mechanism(s) underlying this sensitization process. Behavioural data showed that AVP sensitization is dependent on the dose, and on the interval of time between AVP exposures. Radioligand binding studies demonstrated the presence of AVP receptors in the VSA with properties resembling those of the V_1 receptor for AVP. In AVP sensitized animals, while VSA AVP receptor density and affinity were not altered, post-receptor response (AVP-stimulated phosphoinositide hydrolysis) was enhanced.

These studies were contrasted with a model in which AVP is chronically absent. In animals depleted of septal AVP by long-term castration, AVP-induced motor disturbances, septal AVP receptor affinity and density, and AVP-stimulated phosphoinositide hydrolysis, were not altered when compared with sham operated animals. These results suggest that the loss of the AVP content of the VSA does not result in a compensatory supersensitivity to AVP.

The recent observation that the central oxytocin (OT) receptor has high affinity for both OT and AVP raised the possibility that this "OT-AVP" receptor may be involved in AVP sensitization. The present studies, however, suggest that while pre-exposure of the rat brain to either AVP or OT induces the sensitization

process, AVP sensitization appears to be mediated via the V1 AVP receptor whereas OT sensitization appears to be mediated via the OT-AVP receptor. The ability of OT to modulate the responsiveness of the rat brain to subsequent AVP injection suggests a role for this peptide in central AVP actions.

The possibility that other physiological actions of AVP could be sensitized was also investigated. Endogenous or exogenously applied AVP within the VSA has been shown to play a role in fever suppression. The data presented here showed that pre-exposure of the rat brain to OT sensitizes the rat brain to the antipyretic action of subsequent AVP exposure. Furthermore, fever, which is known to evoke central AVP and OT release within the VSA, can mimic the effect of centrally administered OT or AVP in sensitizing the rat brain to the motor disturbances induced by exogenously administered AVP. These data indicate that AVP may play a role in the genesis of febrile convulsion.

In conclusion, these data document the presence of a V_1-like vasopressin receptor in the VSA of the rat brain. The data indicate that the temporal characteristics of repeated agonist exposure can affect the responses to the agonist. The results also indicate that receptor binding, while critical to hormone action, is not the sole factor for determining responsiveness; rather postreceptor mechanism(s) appear most important in determining AVP responsiveness.

ACKNOWLEDGEMENTS

My sincere thanks to Dr. Q.J. Pittman for his invaluable support, guidance and knowledgeable supervision of my graduate work. I would also like to thank Dr. Q.J. Pittman, Dr. K.E. Cooper and Dr. W.L. Veale for providing an excellent environment in which to carry out this investigation. Thanks are extended to Dr. D. McMaster for his special encouragement and many helpful discussions. Thanks to L. Bauce, D. Ko, L. Franklin, Y. Takahashi and to other members of our laboratory and faculty who were there when advice, technical expertise and encouragement were needed. Thanks are also extended to Dr. D. Severson, and Dr. S. Weiss for serving on my Supervisory Committee and to Dr. R.M. Cooper and D.M. Dorsa for agreeing to serve on my Examination Committee. Finally, I acknowledge financial support from the Alberta Heritage Foundation for Medical Research.

DEDICATION

To the Rocky Mountains

TABLE OF CONTENTS

LIST OF FIGURES

ABBREVIATIONS

ANOVA	Analysis of variance
AVP	Arginine vasopressin
^{3}H-AVP	Tritiated-arginine-vasopressin
AVP 4-9	Arginine-vasopressin 4-9
AVT	Arginine-vasotocin
B_{max}	Binding sites concentration
BSA	Bovine serum albumin
BST	Bed Nucleus of the Stria Terminalis
d(CH_2)$_5$ Tyr(Me)AVP	1-(ß-Mercapto-ß ,ß-cyclopentamethylenepropionic acid),2-(O-methyltyrosine) arginine-vasopressin
d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH_2^9]OVT	[1-(ß-Mercapto-ß,ß-cyclopentamethylenepropionic acid), 2-O-methyltyrosine,4-threonine,8-ornithine, 9-tyrosylamide]vasotocin; compound 6
dAVP	Desglycinamide arginine-vasopressin
DDAVP	1-desamino-8-D-arginine vasopressin,
desGly^9-dAVP	Desglycinamide deamino-arginine vasopressin
GPI	Glycero-phosphoinositol
HPC	Hippocampus
HPLC	High performance liquid chromatography
i.c.v.	Intracerebroventricular

i.p.	Intraperitoneal
IL-1	Interleukin-1α
IP_1	Inositol-1-phosphate
^3H-IP_1	Tritiated-Inositol-1-phosphate
K_d	Binding site dissociation constant
KRB	Krebs-ringer bicarbonate buffer
LH-RH	Luteinizing hormone releasing hormone
LS	Lateral septum
LVP	Lysine-vasopressin
MIF	Melanocyte-stimulating hormone-release inhibitor factor
Myo-[2-^{3}H(N)]-inositol	Tritiated inositol
NMDA	N-methyl-D-aspartate
OT	Oxytocin
^3H-OT	Tritiated oxytocin
PI	Phosphatidylinositol
^3H-PI	Tritiated phosphatidylinositol
SPM	Synaptic plasma membrane
[Thr^4,Gly^7]OT	Threonine-4, glycine-7-oxytocin
TRH	Thyrotropin-releasing hormone
V1	Vasopressor receptor
V2	Antidiuretic receptor
VSA	Ventral septal area

I. INTRODUCTION

A. Overview

In recent years, it has become apparent that the acute effects of neurotransmitters or hormones may differ considerably from their chronic influences. In particular, following chronic exposure, the phenomena of tolerance, addiction, withdrawal and sensitization have been observed. Because the vast majority of neurotransmitters and hormones elicit their actions via an interaction with specific cell surface receptors, it has been suggested that these phenomena may result, at least in part, from changes in the affinity, number or responsiveness of these receptors (for review, see Creese and Sibley, 1980; Klein et al.1989; Wonnacott, 1990).

Recently, direct investigation of receptors, using radioligand binding techniques, has confirmed that the majority of receptor systems possess the flexibility of up or down "regulation" depending on stimulus characteristics. For example, exposure to agonist, or conditions that increase the concentration of the natural ligand, typically result in a desensitization and or downregulation of the target receptor system (for reviews, see Catt et al.1979; Creese and Sibley, 1980; Sibley and Lefkowitz, 1985; Klein et al.1989). Conversely, chronic exposure to antagonist, or conditions that decrease the concentration of the natural ligand, have been reported to result in a sensitization and/or upregulation of the target receptor system (Creese and Sibley, 1980; Zukin and Tempel, 1986). Agonist-induced desensitization, downregulation and tolerance phenomena or conversely

antagonist-induced sensitization and upregulation phenomena have been postulated to represent mechanisms for the maintenance of homeostatic regulation of receptor function in response to varying levels of physiological activity in vivo (Raff, 1976; Creese and Sibley, 1980; Mahan, 1987; Klein et al.1989).

Paradoxically, however, several reports have described enhanced response to the repeated administration of agonists (Post, 1980; Post and Weiss, 1989; Robinson and Becker, 1986; Kita and Nakashima, 1989; Wonnacott, 1990 for reviews). Agonist-induced sensitization or upregulation phenomena have been associated with a special kind of plasticity for positive reinforcement mechanisms and strengthening of synapses with use (Klein et al.1989). From these observations, it appears, therefore, that an agonist may have the "choice" to activate mechanisms involved in desensitization and downregulation or sensitization and upregulation in a given system in part depending on stimulus characteristics.

Elucidation of the putative mechanisms underlying agonist-induced sensitization, involving the neurohypophysial peptide arginine vasopressin (AVP), is the main subject of this thesis. Because receptors are at a pivotal point of signal transduction, the focus of our analysis will mainly be on possible alteration in receptor function that may underlie AVP sensitization. Although it is important to realize that changes in physiological responsiveness to agonist exposure may result from processes either proximal or distal to the receptor, such issues will not be dealt with in detail.

In this manuscript, desensitization and tolerance, regardless of the mechanisms, will refer to the process whereby exposure to a ligand results in decrease in responses, while the phenomenon of receptor "downregulation" will refer to the specific loss of cell surface receptors. Conversely, sensitization, regardless of the mechanisms, will refer to the process whereby exposure to a ligand results in an increase in responses, while the phenomena of receptor "upregulation" will refer to the specific increased number of cell surface receptors.

B. Agonist-induced sensitization

1. General background

AVP is not the only ligand that has been observed to evoke a sensitization phenomenon in response to repeated exposure. In fact, the phenomenon of agonist-induced sensitization was first observed in the late 1960s in experiments in which repeated amphetamine (a drug that cause endogenous catecholamine release and blocks its reuptake) treatments were given (Ellinwood, 1967; Ellinwood, 1968; 1971; Ellinwood et al.1973). In particular, it was observed that while repeated administration of amphetamine induced the expected development of tolerance to the autonomic effects of amphetamine, including those on body temperature, blood pressure, heart rate and respiration (Costa and Garattini, 1970; Kosman and Unna, 1968 for reviews), many behaviours including stereotypy (e.g. Klawans et al.1975), rotational behaviour (Robinson, 1984), drinking behaviour (Rowland et al.1981), acoustic startle behaviour (Kokkinidis, 1984), and intracranial self-stimulation (Predy and Kokkindis, 1984) were shown to be enhanced. This

agonist-induced enhancement of behaviour, over and above that produced by a single injection, became referred to as agonist-induced "behavioural sensitization".

Many studies have since been conducted on the phenomenon of agonist-induced sensitization and it has been observed with several additional agonists, including opiates (Broekkamp et al.1979; Joyce and Iversen, 1979; Kalivas et al.1983; Kalivas, 1985; Kalivas et al.1985; Kalivas and Duffy, 1987; Vezina et al.1987), nicotine (Kita and Nakashima, 1989; Wonnacott, 1990 for reviews), gamma amino butyric acid (GABA) receptor inverse agonists (Hirano et al.1989), and luteinizing hormone-releasing hormone (LH-RH) (Aiyer et al.1974; Fink et al.1976; Curtis et al.1985).

Reviewing these studies, several authors have concluded that some general principles of agonist-induced sensitization may exist. In general, it was observed that while chronic, continuous stimulation of receptors by agonist is often associated with the development of desensitization, downregulation or tolerance phenomena, chronic intermittent stimulation with the same agonist, under some circumstances, is associated with sensitization or upregulation phenomena (Post, 1980; Post and Weiss, 1989; Robinson and Becker, 1986; Jenner and Marsden, 1987; Mattingly et al.1988; Di Paolo et al.1989). It was also observed that only a single injection of agonist can be sufficient to enhance the effect of subsequent agonist injection given weeks later (e.g. Magos, 1969). It was therefore concluded that intermittent versus continuous exposure to agonist was of primary importance for agonist-induced sensitization.

It was further observed that frequent treatments with high dose of agonist may preferentially evoke tolerance, whereas treatments with low dose of agonist may preferentially evoke sensitization (Post, 1980, 1981; Antelman and Chiodo, 1981). Thus, it was also concluded that the dose of agonist used may, in some case, be an additional key element in agonist-induced sensitization.

C. Agonist-induced sensitization and receptor regulation

1. General background

The idea that agonists may regulate their actions via alteration in receptors came from the observations that agonists had been shown to regulate the concentration and/or properties of their own receptors. The finding that hormones and neurotransmitter ligands could alter the number of their cell surface receptors was first described when some hormones such as insulin (de Meyts et al., 1973; Kahn et al.1973), growth hormone (Lesniak et al.1977) and thyrotropin-releasing hormone (TRH) (Hinkle and Tashjian, 1975) or neurotransmitters such as α-adrenergic agonists (Mukherjee et al.1975) were observed to bind to their membrane receptors and induce the loss of their own surface receptor (for a review, see Raff, 1976). It thus became apparent that receptors are at a pivotal point for regulating cellular responses to agonist exposure.

As will be reviewed next, agonist-induced sensitization has been associated with a variety of receptor regulatory phenomena.

2. Agonist sensitization and receptor upregulation

In the simplest model proposed, agonist-induced sensitization has been shown to be related to post-synaptic receptor upregulation and/or supersensitivity. A number of experiments have shown that chronic noradrenergic agonist treatments induce an increase in adrenergic receptors. For example, (Banerjee et al.1979) found that chronic amphetamine treatment (which acts as an indirect agonist by increasing the synaptic levels of catecholamine including norepinephrine) led to an 80% increase in ^{3}H-dihydroalprenolol (a ß-adrenergic antagonist) binding. Because chronic treatment with amphetamine and cocaine (see above) causes sensitization to stereotyped behaviour and locomotor activity, and because norepinephrine is involved in the control of locomotor activity, it has been suggested that ß-adrenergic receptor upregulation may be related to these alterations in behaviour (Bailey and Jackson, 1978; Segal et al.1980).

Similarly, a number of experiments have shown that chronic dopamine agonist treatments (Martres et al.1977; Schwartz et al.1978) or amphetamine (Borison et al.1979; Klawans et al.1979; Robertson, 1983) induce an increase in central dopamine receptors. Because dopamine agonist or amphetamine treatment has also been shown to induce behavioural sensitization, dopamine receptor upregulation and/or supersensitivity has been suggested to be related to receptor upregulation (Creese and Sibley, 1980; Robinson and Becker, 1986 for reviews). These conclusions, however, are controversial because other studies on dopamine receptor binding do not support the postsynaptic dopamine receptor

supersensitivity hypothesis in dopamine sensitized rats (Robinson and Becker, 1986).

Here again, there appear to be a number of factors that must be taken into consideration when investigating a receptor hypothesis to explain behavioural phenomena including the injection paradigm, the receptor subtype, and the region of the brain investigated. For example, when elevated brain concentrations of dopamine agonists or amphetamine are maintained for a few days (either by the continuous administration or by frequent repeated high dose injections), a decline in the number of central dopamine receptors has been observed (Mishra et al.1978; Costall and Naylor, 1979; Howlett and Nahorski, 1979; Muller and Seeman, 1979; Ellison and Eison, 1983). Under these conditions, it has been proposed that it is more likely that tolerance will develop and less likely that sensitization will occur (Scatton and Worms, 1978; Schwartz et al.1978; Post, 1980). The direction of the regulation (sensitization versus tolerance and upregulation versus downregulation), therefore, may depend upon the magnitude, duration and frequency of agonist treatment.

Studies with other agonists such as nicotine, have provided similar results in that following chronic nicotine exposure, an increase in receptor numbers (Marks et al.1985; Schwartz and Kellar, 1985; Marks and Collins, 1985; Lapchak et al.1989; Nordberg et al.1989; Wonnacott, 1990) and an increase in behavioural responses (Ksir et al.1987; Clarke et al.1988) has been reported. These studies, however, reveal that the increase in nicotinic receptor is sometimes correlated with

the development of tolerance phenomenon (Marks et al.1985). In summary, there is some evidence to suggest that agonist-induced sensitization may in some, but not all, cases be related to the receptor upregulation phenomenon.

3. Agonist sensitization and receptor downregulation

Paradoxically, however, there are several studies indicating that agonist-induced receptor downregulation may be related to the behavioural sensitization phenomenon. For example, the effects of dopaminergic agonist exposure on dopamine receptor regulation has been shown to be even more complex due to the fact that both pre-synaptic (also called autoreceptor) and post-synaptic receptors have been identified (Roth, 1979). Dopamine autoreceptors have been associated with negative feedback regulatory mechanisms and it has been suggested that, in some instances, the effect of agonist administration is to desensitize and/or downregulate presynaptic dopamine autoreceptors (Beaudry et al.1977; Matres et al.1977; Muller and Seeman, 1979; Antelman and Chiodo, 1981). In keeping with this idea, several studies have shown that chronic dopaminergic agonist treatment, while causing behavioural sensitization, results in a decreased number of central dopamine binding sites (Howlett and Nahorski, 1979; Mishra et al.1978; Muller and Seeman, 1979). Thus, in principle, if the receptor is pre-synaptic in nature, and mediates a negative feedback loop regulatory function, then agonist-induced sensitization is expected to be correlated with receptor downregulation.

Similarly, behavioural sensitization has also been correlated with receptor downregulation when the receptor is inhibitory in nature. The central inhibitory neurotransmitter gamma amino butyric acid receptor (GABA receptor) is inhibitory, and it has been suggested that removal of such an inhibitory component, by GABA receptor downregulation, is correlated with the enhanced seizure activity of repeated GABA inverse agonist administration. For example, repeated administration of a GABA inverse-agonist results in a downregulation of the GABA receptor (Corda et al.1987; Hirano et al.1989) while producing sensitization to its effects in that seizure develops (Little et al.1986; Loscher and Stephens, 1988; Corda et al.1988).

Thus, these results also points to the possibility that behavioural sensitization may be a phenomenon associated with receptor downregulation (and/or desensitization) if the receptor mediates inhibitory activity.

4. Agonist sensitization and post-receptor alteration

A variety of events following the occupancy of receptor by agonists have been suggested to take part in the regulation of receptor function (for reviews, see Raff, 1976; Catt et al.1979; Hosey et al.1990; Nathanson, 1989; Klein et al.1989; Hollenberg, 1990; Lewis et al.1990; Spiegel, 1990; Leidenheimer, 1991). Another possibility, therefore, is that agonist-induced sensitization is not correlated with alteration of the receptor per se; it may, however, be correlated with alteration of post-receptor intracellular mechanisms. For example, the increased responsiveness to repeated LH-RH exposure has been shown to be correlated with an increase

in protein systhesis (Curtis et al.1985; Mobbs et al.1989; Mobbs et al.1990a, 1990b) but not with an alteration of LH-RH receptor number or affinity (Mitchell et al.1988). Thus, in some cases, alteration in receptor responsiveness may be revealed by analyzing the activation of second messenger systems but not be detected by ligand binding studies.

Finally, it is also possible that altered behavioural responsiveness to repeated agonist exposure may result from processes distal to the receptor, requiring intact circuits for the complete expression of the response. For example, it has been suggested that some of the motor stimulating effects of opiates are due to opiate-dopamine interactions. In support of this, Kalivas (1985) has shown that daily injections of enkephalins into the ventral tegmental area (dopamine containing cells projecting to the nucleus accumbens) of rats, while producing no change in opioid receptors in the ventral tegmental area, produces enhanced dopamine turnover in the nucleus accumbens in response to a subsequent similar challenge of enkephalin when compared to non-sensitized animals. Repeated agonist administration, therefore, may produce some of its effects on behaviour by altering distant receptor systems.

It is concluded, therefore, that agonist-induced sensitization may be correlated with several aspects of receptor regulation; upregulation, downregulation, regulation of post-receptor mechanisms as well as regulation of distant receptor systems.

Because knowledge of the mechanisms involved in receptor regulation may help us to understand the phenomenon of agonist-induced sensitization, the next section will briefly review some of the best known mechanisms of agonist-induced receptor regulation which potentially could be involved in the agonist-induced sensitization phenomenon. Because the field of receptor regulation is very complex, and a large number of agonist-induced receptor regulatory processes have been described (see Creese and Sibley, 1980; Bouvier et al.1989; Chuang, 1989a, 1989b; Klein et al.1989; Nathanson, 1989; Hausdorff et al.1990; Hollenberg, 1990; Hosey et al.1990; Lefkowitz et al.1990; Homcy et al.1991 for reviews), only one example of a mechanism that best represents each of the agonist-induced receptor regulatory phenomena (upregulation, downregulation, and regulation of post-receptor mechanisms) that may underlie the sensitization phenomenon will be presented.

D. Agonist-induced sensitization and receptor regulation mechanisms.

1. General background

With increased understanding of signal transduction mechanisms, it has now become clear that the amplification reactions triggered by agonists can lead to cellular responses as well as to feedback control of the receptor function. Molecular analysis of several receptor molecules has revealed that, no matter which of the superfamilies a receptor molecule belongs to [e.g. ligand-gated ion channel; ligand-regulated enzymes; transmembrane proteins which interact in a ligand-regulated manner with other membrane-associated proteins such as the

guanine nucleotide binding proteins (G proteins)], all receptor molecules studied so far have been shown to contain similar "regulatory sites" in their amino acid sequence upon which feedback regulators act (Hollenberg, 1990). Regulation of a receptor molecule through activation of these sites, by feedback regulators, has been shown to include the control of activity per receptor molecule (desensitization/sensitization) and the control of the number of receptors per cell (down-regulation/up-regulation) (for a review, see Klein et al.1989).

Despite considerable evidence that cellular response to external signals is modulated not merely by quantitative changes in the extracellular "first messenger" ligands, but also by qualitative and quantitative changes at the level of the receptor and the feedback regulators, the underlying mechanisms of receptor regulation have only recently begun to be elucidated.

2. Agonist-induced receptor downregulation mechanism

There are many examples of agonist-induced receptor downregulation in the literature (Chuang, 1989a; Hausdorff et al.1990; Lefkowitz et al.1990; Spiegel, 1990 for reviews). Of particular interest, however, was the recent observation that one of the factors that may govern the interaction of receptor molecules with feedback regulators, ligand concentration, appears to be similar to a factor that governs agonist-induced sensitization. A particularly well studied example of differential regulation of a receptor regulation by ligand concentration is that of the ß-adrenergic receptor coupled to the adenylate cyclase system (Hausdorff et al.1990; Lefkowitz et al.1990 for reviews).

In this system, at least two major pathways of ß-adrenergic receptor regulation have been described. Firstly, agonist activation of the ß-adrenergic receptor has been shown to lead to the production of an intracellular messenger (cyclic AMP) which activates, among others, an intracellular enzyme (kinase A). Kinase A then has been shown to phosphorylate membrane receptor proteins including the ß-adrenergic receptor itself. ß-adrenergic receptor phosphorylation by kinase A has been shown to result in the desensitization of the ß-adrenergic receptor. Because kinase A can be activated by a multitude of agonists that also activate the production of cAMP, ß-adrenergic receptor phosphorylation by kinase A could account for some form of heterologous receptor desensitization (heterologous receptor regulation is refered to if the presence of a given agonist leads to altered response to multiple agonists that bind to distinct receptors) (Hausdorff et al.1990; Lefkowitz et al.1990).

Secondly, recent evidence (O'Dowd et al.1989; Hausdorff et al.1990; Lefkowitz et al.1990) indicates that when an agonist binds the ß-adrenergic receptor, it is phosphorylated by a specific enzyme (termed ß-adrenergic receptor kinase (BARK) (although it will modify many receptor types) that specifically phosphorylates the agonist-bound form of the ß-adrenergic receptor. This accounts for some forms of homologous receptor desensitization (homologous receptor regulation is referred to if a given agonist leads to altered response specific only to the cell surface receptor being activated).

The most interesting observation regarding this dual ß-adrenergic phosphorylation, however, is that the regulation of the ß-adrenergic receptor phosphorylation by protein kinase A preferentially occurs at "low" levels of agonist exposure, and occurs in the absence of receptor downregulation, whereas ß-adrenergic receptor phosphorylation through BARK kinase is preferentially activated by "higher" concentrations of agonist, and involves receptor sequestration and, depending on the conditions, receptor downregulation (O'Dowd et al.1989; Hausdorff et al.1990; Lefkowitz et al.1990). These results represent an example of how, under certain conditions, an agonist can activate different molecular mechanisms leading to differences in receptor regulation. This is interesting because it demonstrates some possibilities as to how changes in agonist exposure can result in changes in the way the receptor responds to that agonist.

3. Agonist-induced receptor upregulation mechanism

In the above example, it was demonstrated that alteration in agonist exposure can activate different pathways of receptor regulation, leading to differences in the magnitude of the desensitization and/or downregulation of that receptor. It is interesting to note, here, that the magnitude of receptor desensitization has recently been proposed to be important in initiating receptor upregulatory mechanisms. The best studied example of this may be the upregulation of the acetylcholine nicotinic receptor following repeated agonist exposure.

There is a growing body of evidence that chronic administration of nicotinic agonists upregulates nicotinic receptors in the central nervous system, while downregulating nicotinic receptors at the periphery (Wonnacott, 1990 for a review). It has been suggested that the reason central nicotinic receptors are upregulated after chronic agonist exposure while the peripheral ones are downregulated is a function of the magnitude of the desensitization of the receptor. Because central nicotinic receptors undergoe a profound desensitization after chronic agonist exposure, this desensitization may actually result in "functional blockade" of the receptor and therefore underlie the mechanism of agonist-induced nicotinic receptor upregulation (Ashizawa et al.1982; Robinson and McGee, 1985; Schwartz and Kellar, 1985; Berg et al.1989; Wonnacott, 1990). As suggested by Wonnacott (1990), because it was observed that a single injection of nicotine is without effect on receptor numbers whereas repeated daily injections induce measurable upregulation of the receptor, under low drug concentrations (single injection) resensitization must occur to provide additional activatable receptors, while under high agonist concentrations (repeated injections), desensitization may persist causing a "functional blockade" which results in receptor upregulation.

How exactly "functional blockade" of receptors can initiate receptor upregulation or sensitization is not known, but it may be similar to the mechanisms involved in antagonist-induced receptor upregulation. In other systems, antagonist-induced homologous receptor upregulation has been shown to be mediated by increased transcription of gene(s) expression responsible for receptor production.

An example of this is the regulation of dopamine D2 receptor levels after chronic reserpine (a drug that depletes dopamine stores) treatment. Norman et al.(1987) have shown that dopamine receptor upregulation in response to chronic reserpine treatment is an active process dependent on an increase in receptor production rate rather than a decrease in the degradation rate. Similarly, because the half-maximal increase in nicotinic receptor number occurs about three days after the start of nicotine treatment, agonist-induced nicotinic receptor upregulation is compatible with altered protein turnover which could result from increased receptor synthesis or assembly (e.g. Marks et al.1985). Agonist initiation of processes that regulate receptor number may be via control of receptor mRNA levels (Hadcock and Malbon, 1988). How control of the receptor message is linked to receptor activity is unknown. Transcriptional regulation plays an important role in specifying the regional and cellular expression of receptors (Schofield et al.1990), but although probes for neuronal receptor mRNA and specific antibodies to receptors have become available, there is little information as yet regarding regulation of receptor gene expression in the brain. Depending on the stimulus characteristics, however, specific expression of a receptor gene can theoretically be induced, maintained or repressed.

4. Agonist-induced alteration in receptor affinity

Alteration of receptor affinity can also indicate altered "sensitization" states of receptor (Bevan et al.1989; Oriowo et al.1989; Bevan and Shreeve, 1989). In

fact it has been observed that the sensitivity of the biological response of a tissue to an agonist may be related not only to the number of specific receptors involved but also to the affinity of the receptor for the agonist. For example, Oriowo et al.(1989) demonstrated that there is a linear relationship between the contractile response to norepinephrine for vascular α-adrenergic receptors and norepinephrine affinity. Therefore, in some cases, one can determine whether the ability of the receptor to transduce intracellular signal has been altered in a "pathophysiologic" state by quantitating the percentage of receptors in the high and low affinity states. Alteration in receptor affinity in response to agonist has been described for other agonists (e.g. Changeux et al.1990).

5. Agonist-induced alteration in post-receptor mechanisms

In addition to regulatory mechanisms acting at the receptor itself, regulation of receptor function has also been shown to depend on the presence of intracellular factors. The best example of an association of agonist-induced sensitization and alteration of intracellular factors is probably that of LH-RH induced sensitization.

In 1976, Pickering and Fink suggested that the priming effect of LH-RH (the capacity of this neuropeptide to increase pituitary responsiveness to itself upon repeated exposure) may be dependent upon protein synthesis as the priming effect of LH-RH could be blocked by administration of inhibitors of protein synthesis (Pickering and Fink, 1976, 1979). In 1985, Curtis et al. demonstrated that the priming effect of LH-RH involves the synthesis of a new protein as well as post-

translational changes (possibly phosphorylation) in two other proteins. Subsequently, (Mobbs et al.1989, 1990a) reported the identity of the LH-RH induced protein to be HIP-70, and recently, HIP-70 has been found to by identical to an isoform of phosphoinositol-specific phospholipase C-α (PLC-α) (Mobbs et al.1990b). PLC-α is one of a family of enzymes that generate the phosphoinositide-derived messenger molecules, including inositol phosphates (which mobilizes intracellular calcium), and diacylglycerol (which activate protein kinase C) (for reviews see, Berridge, 1987; Chuang, 1989a; Farago and Nishizuka, 1990). In keeping with this, Mitchell et al.(1988) previously observed that although the priming effect of LH-RH was not correlated with changes in LH-RH receptor numbers or affinity, it involved enhanced LH-RH-induced production of the second messenger inositol 1,4,5-trisphosphate and calcium mobilization. Taken together, these results provides strong evidence for a functional relationship between LH-RH hormonal induction of HIP-70 (PLC-α) and LH-RH sensitization of gonadotropin secretion following repeated exposure.

This work is of particular interest with respect to AVP-induced sensitization, because there is evidence suggesting that the phosphoinositol pathway is activated by stimulation of V1 AVP receptors both at the periphery and centrally (for reviews, see Jard et al.1983a, 1983b, 1987).

The phosphoinositide cascade, which results in the generation of inositol phosphates and diacylglycerol, has been shown to be triggered by activation of phospholipase C-coupled receptors (Berridge, 1987; Chuang, 1989a for reviews).

Therefore, as in the case of LH-RH induced sensitization, it is possible that AVP-induced sensitization may also be mediated by the induction of an enzyme of the family that generate the phosphoinositide-derived second messenger molecules.

Furthermore, if such mechanisms underlie AVP-induced sensitization, one may predict that the sensitization phenomenon may be heterologous in nature because several ligand receptors have been shown to be phospholipase C-coupled receptors (Chuang, 1989a). In keeping with this, with respect to LH-RH actions, it is interesting to note that the sensitization of lordosis behaviour induced by LH-RH infused into midbrain central gray in female rats (Sakuma and Pfaff, 1979) has also been induced with the administration of phorbol esters (which mimic the action of diacylglycerol in stimulating protein kinase C) in that same brain region (Mobbs et al.1989). It would, therefore, be interesting to test whether the sensitization of the rat brain to AVP-induced motor disturbances could be achieved by agents such as phorbol esters.

In summary, in this section evidence was provided to demonstrate that alteration in stimulus characteristics can lead to alteration in receptor regulatory mechanisms. In particular, evidence was provided to support the hypothesis that repeated agonist exposure may lead to alteration in receptor number, affinity or alteration in post-receptor mechanisms. In some cases, evidence was also provided to suggest that agonist-induced alteration in receptor regulatory mechanisms may underlie, at least in part, the phenomenon of agonist-induced sensitization. Although many more possible mechanisms could underlie the

phenomenon of agonist-induced sensitization, it is also possible that one of these mechanism may be involved in AVP-induced sensitization.

Because this thesis is primarily concerned with the phenomenon of AVP sensitization, the following discussion will review the data presently available with respect to AVP sensitization.

E. Central actions of vasopressin and receptor regulation

1. AVP as a neurotransmitter

The neurohypophyseal hormone arginine vasopressin meets most of the criteria which are usually applied to be assigned a role as a neurotransmitter or a neuromodulator (Buijs, 1983; Riphagen and Pittman, 1986 for reviews). Briefly, AVP has been shown to be synthesized at multiple sites in the brain in neurons that have widespread extrahypothalamic projections in the central nervous system (Buijs et al.1983; De Vries and Buijs, 1983; Sofroniew, 1983). A calcium-dependent release of AVP can be evoked by potassium or veratridine in various areas of the rat brain (Buijs and Van Heerikhuize, 1982). Central AVP release has also been shown by push-pull perfusion in several brain areas in response to electrical stimulation (Pittman et al.1984; Naylor et al.1988; Neumann et al.1988; Landgraf et al.1990), or in response to physiological stimuli such as fever (Landgraf et al.1990), haemorrhage and hypertonic saline (Demotes Mainard et al.1986; Landgraf et al.1988). Autoradiographic and radioligand binding studies have demonstrated the presence of putative receptors for AVP in many areas of the brain (for a review, see Jard et al.1987). Both direct application of exogenously

applied AVP and AVP endogenously released following afferent stimulation similarly affects the electrical activity of several neuronal groups in the brain (Dreifuss et al.1982; Joëls and Urban, 1982; Lawrence and Pittman, 1985; Disturnal et al.1987). In addition, numerous reports have suggested the involvement of central AVP in processes such as memory consolidation (Koob et al.1985; van Wimersma Greidanus et al.1986; Dantzer et al.1987; Le Moal et al.1987; Yehuda, 1987; Dantzer et al.1988 for reviews), tolerance (Hoffman and Tabakoff, 1984; Ritzmann et al.1985; Mannix et al.1986; Speisky and Kalant, 1986; Szabo et al.1988), temperature (Kovacs and De Wied, 1983; Ruwe et al.1983; Cooper et al.1987; Naylor et al.1987; Pittman et al.1988; Kasting, 1989) and blood pressure (Martin et al.1985; Pittman and Franklin, 1985; Brooks, 1989; Laycock and Lightman, 1989; Perez et al.1989; Riphagen and Pittman, 1989; Ryan et al.1989) regulation, and several behaviours including flank marking behaviour (Ferris et al.1988, 1990), and motor behaviour (Kruse et al.1977; Kasting et al.1980; Burnard et al.1983, 1985, 1986, 1987; Boakes et al.1985; Naylor et al.1985a; Wurpel et al.1986a, 1986b, 1988; Balaban et al.1988).

This latter effect of AVP is of particular interest because it involves a "sensitization" process (see below). This thesis is primarily concerned with the phenomenon of AVP sensitization, thus the following section will review the data presently available with respect to AVP sensitization.

2. AVP-induced motor disturbances and sensitization

a. General background. Studies of the motor effects of AVP (Kruse et al.1977; Kasting et al.1980; Burnard et al.1983, 1985, 1986, 1987; Yamada and Furukawa, 1984; Boakes et al.1985; Naylor et al.1985a; Wurpel et al.1985, 1986a, 1986b, 1988; Balaban et al.1988; Thurston et al.1988), and lysine-vasopressin (Kruse et al.1977; Abood et al.1980) given into either the cerebral ventricles or central neuronal structures have reported acute transient disequilibrium and convulsive like activity and long-term changes in the central nervous system function. The acute specific motor dysfunction after a central AVP administration in rats includes transient bouts of head and body sway, ataxia, nystagmus, and listing of the body in the direction of the head sway. This progresses in many animals to a characteristic behaviour termed barrel rotation (Cohn and Cohn, 1975), where the animal repeatedly rolls about its longitudinal axis. Repetitive bouts of barrel rotation may appear intermittently for a period ranging from less than 5 min to 30 min. After bouts of symptoms of disequilibrium, some animals display symptoms of dyspnea, apnea and clonic and/or tonic convulsive-like behaviour. These convulsive syndromes can lead to significant mortality, which is frequently associated with pulmonary edema. Barrel rotation, ataxia, nystagmus and tonic/clonic convulsive-like behaviour, in quadruped mammals, have been reported to be similar to head torsion, spontaneous nystagmus and loss of balance in bipeds (primates and humans) (for a review, see Balaban et al.1989).

In addition to acute disequilibrium symptoms, central administration of AVP has been shown to have long-term effects, in that a pre-exposure to the peptide enhances (sensitizes) the likelihood of more severe motor disturbances following subsequent injections of AVP.

AVP-induced sensitization was first described by Kasting et al. (1980) when the authors observed that an initial intracerebroventricular injection of AVP into conscious rats evoked minor motor disturbances including pauses and prostration, whereas a similar AVP injection repeated two days later caused enhanced motor disturbances including swaying movements of the head and ataxia, barrel rotation, clonic and tonic movements of the limbs and sometimes death associated with lung edema. Sensitization to the effects of i.c.v. AVP has been observed reproducibly by many investigators (Burnard et al.1983, 1986, 1987, 1985; Boakes et al.1985; Naylor et al.1985a; Wurpel et al.1986a, 1986b, 1988; Balaban et al.1988).

b. Dose response of AVP-induced motor disturbances and sensitization. A review of the literature shows that on a first exposure to AVP, the severe motor disturbances (mainly barrel rotation) induced by i.c.v. AVP have been described as an all-or-none response with a relatively flat dose-response function (Wurpel et al.1986a). In fact there are no reports of a dose of AVP that produces barrel rotation in 100% of naive rats tested. For example, Kruse et al.(1977) reported barrel rotation in 4 of 12 rats at an 8ng bolus dose, 4 of 12 rats at a 40ng dose, 6 of 12 rats at a 200ng dose, 5 of 10 rats at a 1 μg dose, and 9 of 10 rats at a 5

μg dose. This indicates a fairly flat relationship over a wide dose range, since the proportion of responsive rats did not differ significantly for doses between 8ng and 1 μg.

The minimum dose of i.c.v. AVP that reportedly elicits barrel rotation ranges from 8 to 250ng in studies employing bolus injection protocols (Kruse et al.1977; Abood et al.1980; Boakes et al.1985; Wurpel et al.1986a); a minimum dose of 3.5ng was reported in continuous infusion experiments (Wurpel et al.1986a). A dose of 1ng in the fourth ventricle was reported to produce barrel rotation in one study (Boakes et al.1985). These doses were lower that the 100 pmol ($\approx$ 100 ng) dose reported to produce barrel rotation after bilateral infusion into either ventral septal area or anterior hypothalamic area in either naive or sensitized rats (Naylor et al.1985), but were in the same range as the doses that produced barrel rotation after intracerebellar injection (Maiti et al.1986). Whatever the threshold in naive animals, it appears that sensitization not only enhances the incidence of severe motor behaviour but also decreases the threshold dose required (Kasting et al.1980; Wurpel et al.1986a). In sensitized animals however, no dose response relationship has been established.

Lysine vasopressin, oxytocin, vasotocin, and oxypressin are four peptides related to AVP that have also been reported to produce barrel rotation when administered i.c.v. to rats (Kruse et al.1977; Abood et al.1980; Kordower and Bodnar, 1984). The doses, however, are much higher that those reported for AVP. Severe motor disturbances such as barrel rotation have been reported for lysine

vasopressin over an i.c.v. dose range of 1.5 to 200 ng (Kruse et al.1977; Abood et al.1980), for vasotocin over a 200ng to 5 μg dose (Kruse et al.1977), for oxytocin over a 500ng to 5 μg dose range (Kruse et al.1977; Kordower et al.1984), and for oxypressin at a 5 μg dose (Kruse et al.1977). Thus oxypressin and oxytocin seemed to be the least potent, and neither ring nor tail fragments of AVP were effective in eliciting barrel rotation (Kruse et al.1977). The effect of these compounds on the sensitization process has not yet been tested.

c. Interactions with other transmitter systems.

Changes in physiological responsiveness to agonist exposure may result from processes distal to the receptor. For example, there exist some anatomical (Freund-Mercier et al.1988) and biochemical (Yamada and Furukawa, 1984) evidence to indicate that the motor response resulting from administration of AVP may involve dopaminergic, GABAergic or cholinergic pathways. Furthermore, there is evidence of effects of systemic drugs on AVP-induced motor disturbances and sensitization.

i. Cholinergic agents. Atropine produced only a 50% depression in the incidence of AVP-induced barrel rotation in naive rats (Wurpel et al.1986b), and did not affect lysine-vasopressin induced barrel rotation (Kruse et al.1977). Although a dose response relationship was not determined, a cholinergic contribution does not appear to be necessary for AVP motor disturbances.

ii. Catecholaminergic agents. The evidence regarding catecholaminergic involvement in AVP-induced barrel rotation is suggestive but not conclusive. Kruse

et al.(1977) reported that pretreatment with haloperidol, propranolol, phentolamine or methysergide did not affect barrel rotation incidence in response to lysine vasopressin, but that the incidence was depressed by chlorpromazine. By contrast, Yamada and Furukawa (1984) reported enhanced barrel rotation responses to a subthreshold dose of a vasopressin analog (10 μg of i.c.v. aminosuberyl^{1},6-arginine^{8}-vasopressin) after either systemic pretreatment with haloperidol, fluphenazine, α-methyl-p-tyrosine or i.c.v. pretreatment with 6-hydroxydopamine, suggesting that dopaminergic mechanisms inhibit the generation of barrel rotation by this analog. Paradoxically, the incidence of AVP barrel rotation is attenuated by bilateral destruction of substantia nigra with the neurotoxin 6-hydroxydopamine (Wurpel et al.1985). A similar depression in incidence was obtained after bilateral kainic acid lesions of the basal ganglia, and neither nigral nor caudate-putamen lesions were found to affect sensitization to a subsequent dose of AVP (Wurpel et al.1985). Given the lack of characterization of the properties of aminosuberyl^{1},6-arginine^{8}-vasopressin at AVP receptors, and its low potency for eliciting barrel rotation (30 μg threshold), it is not possible to reconcile the discrepancy in effects. Although these data suggest the possibility of a nigrostriatal dopaminergic components for the trigger for AVP barrel rotation, more rigorous and complete experimental investigations are required to characterize the role of dopaminergic mechanisms in AVP barrel rotation and sensitization.

iii. GABAergic agents. Diazepam has been reported to inhibit significantly the incidence of AVP barrel rotation (Wurpel et al.1986b). The decreased incidence in

sensitized rats was accompanied by a significantly longer latency for barrel rotation onset (Wurpel et al.1986b). A potential role of GABAergic circuits is consistent with reports that barrel rotation appears after i.c.v. picrotoxin (Zainos et al.1984; Wurpel, 1986), or after multiple injections of bicuculline methiodide in substantia nigra (Kelly et al.1977). Finally, an interaction between GABAergic and AVP mechanisms in sensitization to AVP barrel rotation is indicated by the finding that prior exposure to i.c.v. picrotoxin (Wurpel, 1986) sensitized rats to effects of a subsequent dose of AVP (Wurpel, 1986). Thus picrotoxin pretreatment mimicked the effects of prior AVP exposure. These observations support the view that sensitization may be activated by many mechanisms.

iv. Antiseizure drugs. Reports of the efficacy of antiseizure medications in preventing neuropeptide-induced barrel rotation are limited. Phenytoin (100-200 mg/kg i.p.) attenuated AVP barrel rotation in either naive or sensitized rats (Abood et al.1980; Wurpel et al.1986b). Phenobarbital (50 mg/kg dose) reduced the incidence of AVP barrel rotation (Wurpel et al.1986b). Valproic acid (125 or 250 mg/kg i.p.) also reduced the incidence of AVP barrel rotation in sensitized rats (Wurpel et al.1986b). Unfortunately, it is difficult to infer mechanisms or sites of actions from these studies.

3. Locus of AVP-induced motor disturbances and sensitization.

Several distinct sites have been identified for AVP-induced motor disturbances and sensitization.

a. Substantia nigra and basal ganglia. Examination of the literature clearly indicates that asymmetric pharmacological manipulations of substantia nigra can result in horizontal circling that progresses to barrel rotation (Iwamoto and Way, 1977; Kelly et al.1977). Injection of 100 pmol (≈100 ng) AVP, however, failed to produce motor disturbances in six rats tested (Naylor et al.1985a). This latter finding suggests, then, that the AVP-like immunoreactive fiber plexus (Sofroniew, 1983), and AVP binding (Tribollet et al.1988b) in substantia nigra do not reflect a primary site of action of AVP in triggering barrel rotation and/or sensitization. Other lines of evidence, however, suggest a role of nigrostriatal circuits in generation of the response to i.c.v. AVP. Chemical ablation of substantia nigra and the neighbouring ventral tegmental area with 6-hydroxydopamine depressed the incidence of barrel rotation in response to AVP, but sensitization to a second dose of the peptide was unaffected (Wurpel et al.1985). These data indicate that an intact nigrostriatal system and/or ventral tegmental area is not necessary for either triggering AVP barrel rotation in naive animals or for the sensitization process. They suggest, however, that these circuits are involved in complete expression of the response.

b. Vestibular nuclear complex and cerebellar nucleus. Another possible site for AVP-induced motor disturbances and/or sensitization appears located in the vicinity of the medulla or cerebellum. Boakes et al.(1985) reported that the fourth ventricle was a highly sensitive site for AVP-induced barrel rotation, eliciting a 50% incidence after the injection of 1 ng dose. Of particular interest was the fact that

several lines of evidence implicate some nuclear groups adjacent to the fourth ventricle, including the vestibular nuclear complex in barrel rotation (for review, see Balaban et al.1989). Wurpel et al.(1986b) found that microinjection of AVP directly into the vestibular nuclear complex elicited barrel rotation.

Other nuclear groups adjacent to the fourth ventricle have been implicated in AVP sensitization and barrel rotation. Maiti et al.(1986) presented evidence that AVP injection in the fourth ventricle, and 1 μl infusions of 20 to 200 pmol ($\approx$20 to 200 ng) into the region of the cerebellar nodulus in rats induce barrel rotation. Prior exposure to AVP at this site sensitized rats to subsequent application of the peptide, and these effects were blocked by kainic acid lesions of the cerebellar site (Maiti et al., 1986). In keeping with this, Wurpel et al.(1986b) demonstrated that perturbation of cerebellar afferents influences the incidence and sensitization of rats to AVP barrel rotation. These afferents arise in the inferior olive and after destruction of the inferior olive by 3-acetylpyridine intoxication, the initial incidence of barrel rotation was depressed when AVP was administered under normal ambient illumination (Wurpel et al.1986b). Wurpel et al.(1986b), however, observed that a different response pattern was obtained when 3-acetylpyridine-treated rats were given AVP in darkness: the initial incidence of barrel rotation was identical to control rats given the peptide in either light or darkness and sensitization did not occur. Since the incidence of AVP barrel rotation is reduced and the sensitization phenomenon abolished after chemical ablation of the inferior olive, Wurpel et al.(1986b) suggested that an extraolivary visual compensatory mechanism inhibits

the incidence of barrel rotation and that both visual input via extraolivary pathways and intact climbing fibres projections to the cerebellum are necessary for sensitization to AVP barrel rotation. Although, these data suggest a role of the cerebellum in the mediation of AVP barrel rotation, it is not the sole trigger site, because cerebellectomy depressed but did not eliminate barrel rotation incidence after i.c.v. AVP (Wood et al.1979).

c. Amygdaloid complex. A third site is found in the amygdaloid complex. Intra-amygdaloid injection of 100 pmol (≈100 ng)/ 0.5 μl AVP has recently been shown to elicite a low incidence of motor disturbances after an initial exposure (Wilcox, 1991). Administration of the same dose after 1 day revealed a potent sensitization to the effects of the peptide as severe motor disturbances were observed. These AVP-induced motor disturbances and the sensitization process appear to be mediated by the V1 AVP receptor because pre-infusion of the V1 antagonist d(CH_2)$_5$Tyr(Me)AVP) into the amygdaloid complex could block both of these events. Interestingly, however, the sensitization process appears more complex as it could also be initiated by the V2 receptor agonist DDAVP (Wilcox, 1991). The distribution of immunoreactive AVP like peptide in the amygdaloid complex (Urban et al.1990) and high affinity binding sites (Dorsa et al.1984) are consistent with a role in AVP-induced motor disturbances and sensitization.

d. Ventral septal area. A fourth site for AVP-induced motor disturbances and sensitization is found in the basal forebrain or rostral hypothalamus. Direct administration of 100 pmol (≈100 ng)/ 0.5 μl AVP into the ventral septal area (an

area extending from the area of the diagonal bands of Broca to the anterior hypothalamus; VSA) of the rat basal forebrain elicited a low incidence of barrel rotation after an initial exposure (Naylor et al.1985a). Administration of the same dose after 1 or 2 days revealed a potent sensitization to the effects of the peptide as severe motor disturbances were observed. Inspection of their illustration revealed that, in the rat basal forebrain, repeated administration of AVP outside the VSA into the nucleus accumbens, the dorsal lateral septum, the dorsomedial hypothalamus or the substantia nigra did not elicit barrel rotation. Therefore, the VSA appears to be a specific site for AVP barrel rotation and sensitization. The distribution of endogenous AVP in the VSA is consistent with a role in barrel rotation and sensitization. Immunocytochemical and transport studies have demonstrated that the VSA contains fibres immunoreactive for AVP (De Vries and Buijs, 1983). These fibres have their origins in the AVP cell bodies in the bed nucleus of the stria terminalis (BST) (Buijs et al.1983; De Vries and Buijs, 1983; Van Leeuwen et al.1985).

Although these results suggest that AVP-induced motor disturbances and sensitization reflects specific actions of the peptides at multiple sites, because the action of AVP in the VSA has been best characterized, our particular interest has focused on the VSA site.

4. AVP-induced motor disturbances and sensitization and the VSA

a. General background. Structure-activity studies with a variety of agonists and antagonists of the AVP molecule provide the best pharmacological evidence indicating that AVP sensitization and motor disturbances in the VSA are mediated via specific AVP receptors. AVP sensitization appears to be mediated by the V1 receptor because pretreatment with a V1 antagonist [1-(ß,ß-Mercapto-ß,ß-cyclopentamethylenepropionic acid),2-(O-methyl)tyrosine]AVP [d(CH_2)$_5$ Tyr(Me)AVP], blocks AVP sensitization (Burnard et al.1986). Similarly, use of the same V1 AVP antagonist d(CH_2)$_5$ Tyr(Me)AVP, and the AVP antidiuretic V2 receptor agonist, 1-desamino-8-D-arginine vasopressin (DDAVP), strongly suggests that the receptors involved in AVP-induced motor disturbances also resemble the V1 receptor (Burnard et al.1986). Thus, although these studies indicate that AVP appears to act in the VSA on a receptor resembling the peripheral V1 receptor, characterization of AVP receptors specificically in the VSA has not yet been done. The next section, therefore, will describe the known characteristics of central AVP receptor subtypes.

b. Central AVP-receptor subtypes. Based on differing ligand selectivities and the effector mechanism involved, it has long been recognized that, in the periphery, at least two types of AVP receptors exist. One type is referred to as the V2 (antidiuretic) receptor and is coupled to cAMP dependent mechanisms, the other type of peripheral AVP receptor is referred to as the V1 (pressor) receptor and is coupled to phosphatidylinositol mechanism (for reviews see, Jard, 1983b, 1987; Gubitz et al.1987).

Direct evidence from autoradiographic and radioligand binding studies also support the existence of vasopressin receptors in the brain. Recent autoradiographic studies have revealed AVP binding sites in many areas of the rat brain (Baskin et al.1983; Dorsa et al.1983, 1984, 1988; Van Leeuwen and Wolters, 1983; Yamamura et al.1983; Biegon et al.1984; Brinton et al.1984; de Kloet et al.1985; Junig et al.1985; Brinton et al.1986; Kiraly et al.1986; Petracca et al.1986; Raggenbass et al.1987, 1989; Tribollet et al.1988b, 1990; Voorhuis et al.1988; Dreifuss et al.1989; Fahrenholz and Gerstberger, 1989; Ko et al.1989; Snijdewint et al.1989; Gerstberger and Fahrenholz, 1989).

In studies using membrane fractions derived from whole brain or from anatomically defined areas, several subtypes of central ^{3}H-AVP binding sites have been pharmacologically characterized as receptors. (1) A central AVP receptor which resemble the antidiuretic V2 type present in the kidney, has been found in the supraoptic nucleus (Van Leeuwen, 1987) of the rat brain. (2) A central AVP receptor which resemble in many respects the vascular V1 type present in blood vessels and in the liver, has been found in numerous brain areas (e.g. Barberis, 1983; Dorsa et al.1984, 1988; Cornett and Dorsa, 1985; Shewey and Dorsa, 1986, 1988; Raggenbass et al.1987; Van Leeuwen et al.1987; Fahrenholz and Gerstberger, 1989; Raggenbass et al.1989; Shewey et al.1989). (3) A third type of AVP receptor, that is neither V1 nor V2, has been characterized in the rat adenohypophysis (e.g. Antoni, 1984; Antoni et al.1985; Baertschi and Friedli, 1985; Jard et al.1986). Like the V1 receptor it is coupled to a Ca^{2+} dependent,

phosphatidylinositol mechanism but it possesses neither a vasopressor nor an antidiuretic ligand selectivity. This receptor has been classified as a V1b receptor while the original V1 receptor is now often referred to as V1a or simply V1 receptor. (4) A fourth putative subtype of central AVP receptor has been uncovered in the presence of Ni^{2+}. This Ni^{2+}-dependent AVP binding site was detectable in numerous brain areas (Pearlmutter et al.1983; Costantini and Pearlmutter, 1984). (5) A putative fifth subtype of central "AVP" receptor has recently been described that binds both OT and AVP with high affinity in the limbic system (Ferrier et al.1983) and in the hippocampus (Audigier and Barberis, 1985; Elands et al.1988a, 1988b) of the rat brain, which will be referred to as the OT-AVP receptor. And finally, (6) there is the possibility that more subtypes of "AVP" receptors exist in the brain since aminopeptidases located in several brain areas have been shown to generate several biologically active fragments of AVP (Burbach et al.1983; Burbach and Lebouille, 1983; De Wied et al.1984, 1987; Kovacs et al.1986, 1989; Wang et al.1986). Receptors specific for these fragments have been characterized in the rat brain and based on their differential distribution are apparently different from other central AVP receptors (de Kloet et al.1985; Brinton et al.1986) .

Thus, because these studies present evidence of the possibility that the involvement of various neurohypophyseal neuropeptide receptor types might be more complex that originally suggested it is particularly important that the VSA AVP receptor be characterized.

c. Central AVP receptors and second messengers. Another way to study receptors is by investigating their second messenger coupled mechanisms. Although the second messengers coupled to most of the central receptors have not been investigated, by analogy with the data obtained on peripheral vasopressin receptors, the possibility that central V2 receptor might be coupled to adenylate cyclase and that central V1 receptor might be coupled to phospholipase C and inositol phosphate hydrolysis has been investigated by several authors.

i. Adenylate cyclase. The many documented behavioural actions of intracerebral DDAVP have provided indirect evidence for the presence of central V2-like receptors (Beckwith et al.1984; Davis, 1984). All attempts, however, to demonstrate a vasopressin sensitive adenylate cyclase in membranes from the nervous system were unsuccessful (Barberis, 1983; Courtney and Raskind, 1983; Audigier and Barberis, 1985). Vasopressin, however, was found to alter dopamine and norepinephrine -induced adenylate cyclase (Courtney and Raskind, 1983; Hamburger Bar and Newman, 1985; Petit et al.1988; Brinton and McEwen, 1989). As suggested by Brinton et al.(1989) the reported modulation by vasopressin of the effects of several agents active on cAMP production might reflect a vasopressin-induced liberation of these agents. The modulation might also be analogous to the well documented potentiation by vasopressin of the CRF-induced cAMP production by corticotropic cells from the adenohypophysis (Giguere and Labrie, 1982). Together, these results suggest that central V2-like AVP receptors

are not directly coupled to adenylate cyclase in a similar fashion as peripheral V2 receptors. Therefore it is possible that other coupling mechanisms exist.

ii. Phosphoinositide hydrolysis. Conversely, studies of phosphatidyl inositol hydrolysis suggest that in many areas of the brain, the central V1 receptor may be similar to peripheral V1 receptors. Vasopressin was shown to enhance the metabolism of phosphatidyl inositol in the rat brain (Jolles et al.1979), and to stimulate the accumulation of inositol phosphates in rat lateral septal slices (Shewey and Dorsa, 1988; Shewey et al.1989; Lebrun et al.1990), hippocampal slices (Stephens and Logan, 1986), in isolated rat cervical sympathetic ganglia (Bone et al.1984; Horwitz et al.1986; Kiraly et al.1986; Horn and Lightman, 1988) and in rat adenohypophysial cells (Guillon et al.1987).

Studies on the lateral septal area indicate that the AVP effect on labelled phosphate accumulation is mediated by AVP receptors analogous to the peripheral V1 AVP receptors in that the effect was blocked in the presence of the V1 antagonist but not stimulated by OT or the V2 agonist (Shewey and Dorsa, 1988). In addition, in synaptic plasma membrane from the hippocampus, AVP was shown to alter protein F1 (also called B50) phosphorylation (Hinko et al.1986; Hinko and Pearlmutter, 1987). Because B50/F1 has been suggested to be a substrate for protein kinase C, it has been suggested that AVP may act by altering protein kinase C activity (Hinko and Pearlmutter, 1987). Therefore, evidence indicates that in many areas of the brain, the AVP receptor may resemble the peripheral V1 receptor.

d. Central AVP receptor regulation

i. Brattleboro rat. As mentioned in the beginning, the absence or presence of a ligand at a receptor site provides a useful model to study receptor regulation. The homozygous Brattleboro rat, which has a genetic deficit in the ability to synthesize AVP (Sokol and Valtin, 1982), provides a useful model for the study of receptor regulation. Membrane binding studies, conducted to examine the effect of AVP deficiency on the pharmacological characteristics of the septal AVP receptors, revealed an increased number of AVP receptors (Shewey and Dorsa, 1986), correlated with an increased AVP-induced stimulation of inositol phosphates accumulation in septal slices (Shewey et al.1989) in homozygous Brattleboro rat. In contrast, Burnard et al.(1985), found that the motor responses to a first injection of AVP do not differ between the homozygous Brattleboro rats and their genetic parent, the Long-Evans strain. Differences in sensitivity, however, were observed after a second central AVP injection (Burnard et al.1985). The apparent need for a priming dose of AVP in order to reveal increased sensitivity to AVP barrel rotation is not presently understood (Burnard et al.1985). Because Brattleboro rats have several endocrinological, morphological and metabolic abnormalities in addition to their AVP deficiency (Sokol and Valtin, 1982), interpretation of the increased severity of motor disturbances to a second i.c.v. injection of AVP in these animals as a consequence of septal AVP receptor upregulation may thus be too simple.

ii. Long-term castration. One of the most interesting features of the BST-VSA AVP system is that the BST vasopressinergic projection to the VSA can

be eliminated by long-term castration. Following long-term castration, both immunoreactive AVP cell bodies in the BST, as well as immunoreactive AVP fibres projecting to the septal area, were virtually eliminated (De Vries and Buijs, 1983; De Vries et al.1983; De Vries et al.1984, 1986; Van Leeuwen et al.1985; De Vries and al Shamma, 1990). In keeping with these findings, both immunoassayed AVP content of the septal area (Pittman et al.1988), and perikaryal AVP mRNA content in the BST (Miller et al.1989), were shown to be reduced in long-term castrated rats. The reduction of the AVP content in the VSA appears to be functionally significant in that, in the absence of AVP in the VSA, the typical antipyretic action of endogenous AVP appears reduced in long-term castrated rats since they display enhanced fever when challenged with a pyrogen (Pittman et al.1988). Thus, the long-term castrated rats gives us a unique opportunity to study the effect of the absence of AVP on the regulation of AVP receptor and the sensitization system.

e. Vasopressin and endogenous antipyresis in the VSA. The VSA has an additional interesting feature that makes it an ideal site to study, in that the VSA also appears to be a major site at which AVP is capable of causing antipyresis (for review, see Pittman et al.1988). For example, it has been shown that perfusion of AVP through the VSA suppresses the rise in core temperature evoked by peripheral (Kasting et al.1979; Naylor et al.1985b), and central (Naylor et al.1985b; Ruwe et al.1985) administration of a pyrogen.

Furthermore, evidence to support the hypothesis that AVP function within the VSA as an antipyretic under physiological conditions has been obtained from

release studies and from experiments in which the effects of endogenously released AVP have been blocked either with the V1 AVP antagonist [d(CH_2)$_5$Tyr(Me)AVP] or specific AVP antibodies. Therefore, during fever, AVP has been shown to be released centrally (Cooper et al.1979; Malkinson et al.1987; Landgraf et al.1990) and injection of the AVP V1 antagonist (Cooper et al.1987), or perfusion of specific AVP antisera (Malkinson et al.1987) within the VSA, was shown to enhance the febrile response to a pyrogen challenge. Similarly, injection of the V1 antagonist prevented the antipyretic action of exogenously administered AVP (Kasting and Wilkinson, 1986; Naylor et al.1987), whereas a V2 agonist did not alter fever when injected into the brain (Naylor et al.1987). Furthermore, recently, it has been shown that electrical stimulation of the BST suppresses fever (Naylor et al.1988); this fever suppression appears to be brought about through release of AVP into the VSA, because prior administration of a V1 antagonist into the VSA will completely abolish the effects of the BST stimulation (Naylor et al.1988).

The observations that exogenously administered or endogenously released AVP acts in the VSA to induce sensitization, motor disturbances and antipyresis, raise the possibility that these phenomena may be related. Of particular interest is the hypothesis, put forward by Kasting et al.(1980), that AVP may play a role in the initiation or the development of febrile convulsions (Kasting et al.1980; for a review, see Pittman et al.1988). This is a particularly interesting hypothesis because a feature common to febrile convulsions [that an infant which has previously

convulsed will be more likely to convulse subsequently (Lennox-Buchthal, 1973)] is similar to the feature seen in the sensitization paradigm (Kasting et al.1980). These observations, therefore raise the possibility that AVP-induced antipyresis may also involve a sensitization process and that AVP released during fever may alter subsequent AVP-induced motor disturbances. Evidence for such a phenomenon would provide a strong suggestion that endogenous release of central AVP may act as a "sensitizing stimulus" and therefore play a role in febrile convulsions. Such "endogenous-sensitization" has been previously reported in that potent stimuli causing the release of central AVP, e.g. haemorrhage and hypertonic saline (Demotes Mainard et al.1986; Landgraf et al.1988) have also been shown to mimic the effect of a central injection of AVP in sensitizing the rat brain to the motor action of centrally injected AVP (Burnard et al.1983).

In summary, evidence was provided to suggest that the VSA may contain V1 AVP receptors which, upon activation, may mediate sensitization, motor disturbances and antipyresis.

F. Rationale for research

This research was undertaken in an attempt to understand the basic mechanism(s) underlying the sensitization phenomenon evoked by repeated central administration of AVP. The rat was used as the experimental animal because 1) this laboratory had previously demonstrated that rats respond to repeated central AVP administration with severe motor disturbances involving a

sensitization process, 2) the central vasopressinergic system of the rat is well documented.

To determine possible neuronal mechanisms responsible for this sensitization process, I:

1. characterized the dose and temporal profile of the sensitization process.
2. characterized AVP receptors in the VSA of the rat brain and investigated the hypothesis that AVP sensitization may be mediated by alteration in the number and/or affinity of these receptors.
3. investigated the hypothesis that AVP sensitization may be mediated by alteration in the signal transduction mechanism of VSA AVP receptors (phosphatidylinositol hydrolysis).

To further study the general aspect of AVP receptor regulation, I utilized an animal model (long-term castrated rats) in which septal AVP is known to be depleted. In these experiments I tested the hypothesis that:

1. septal AVP depletion would a) alter the sensitization to exogenous AVP; b) alter septal AVP receptor properties (number and affinity); c) alter septal post-receptor mechanism (phosphatidylinositol hydrolysis).

To study the specificity of AVP sensitization to the V1 AVP receptor, I investigated the possibility that the recently described central OT-AVP receptor may be involved

in the sensitization phenomenon by using specific agonists and antagonists to the central OT-AVP receptor.

Finally, because AVP acts in the VSA to cause both motor disturbances and antipyresis, I investigated whether the central antipyretic actions of AVP could also be sensitized.

II. SUBCELLULAR LOCALIZATION AND CHARACTERIZATION OF VASOPRESSIN BINDING SITES IN THE VENTRAL SEPTAL AREA, LATERAL SEPTUM, AND HIPPOCAMPUS OF THE RAT BRAIN

A. Introduction

Studies with AVP analogues suggest that AVP-induced motor disturbances result from an interaction of AVP with V_1-like AVP receptors in the VSA (Naylor et al.1985a; Burnard et al.1986). In the present study, we have used synaptic plasma membrane (SPM) preparations for radioligand binding studies to characterize AVP receptors in VSA tissue and compare them with those characterized in the lateral septum (LS) and the hippocampus (HPC). These data provide the basis for future investigations examining possible changes in receptor properties in the VSA following pretreatment with AVP.

B. Methods

For each experiment, male Sprague-Dawley rats (weighing 200-250 g) were decapitated, and the brain was rapidly removed. Three areas were dissected: (a) the LS, delineated by the coordinates 1.2 mm anterior to and -0.3 mm posterior to the bregma (Praxinos and Watson, 1982), laterally at this level by the lateral ventricles and ventrally by a cut just above the anterior commissure; (b) the VSA, defined as the area ventral to the LS sample, between the anterior commissure and the ventral surface of the brain and within 2.0 mm of the midline; and (c) complete HPC. All tissue samples were immersed in ice-cold 0.32 M sucrose containing the proteolysis-inhibiting peptide bacitracin (1.0 mg/ml). Brain tissues

were homogenized in a Teflon-glass homogenizer and centrifuged at 1,000 g for 10 min at 4° C. The resulting pellet was discarded, and the supernatant (S1) was centrifuged at 12,000 g for 20 min at 4° C to prepare a crude mitochondrial-synaptosomal pellet (P2) and a supernatant (S2) containing the "microsomal" components (Whittaker et al.1964). The mitochondrial-synaptosomal pellet (P2) was subjected to an osmotic shock in distilled water for 15 min at 0° C followed by centrifugation at 12,000 g for 20 min at 4° C. The resulting pellet (P2'), containing mitochondria, myelin, and synaptic junctions, was resuspended in 50 mM Tris-HCl buffer (pH 7.4) containing 3.0 mM $MgCl_2$, stored at -70° C, and used later in binding experiments. The resulting supernatant (S2')(containing SPMs and vesicles) and the S2 (microsomal) supernatant were both centrifuged at 100,000 g for 80 min at 4° C. The resulting SPM and microsomal (M) pellet fractions were resuspended in 50 mM Tris-HCl (pH 7.4) containing 3.0 mM $MgCl_2$ and were used immediately for binding assays or stored at -70° C for up to 2 months. Protein content was determined by the method of Lowry et al.(1951) using bovine serum albumin as the standard.

^{3}H-AVP binding assay

Membranes (50-200 μg of protein/assay) from the P2', M, and SPM fractions were incubated in a final volume of 300 μl of a medium composed of 50 mM Tris-HCl (pH 7.4) containing 3 mM $MgCl_2$, bovine serum albumin (1 mg/ml), bacitracin (1 mg/ml), and various amounts of ^{3}H-AVP (specific activity = 64 - 70 Ci/mmol), AVP, related and unrelated peptides, or other substances. The assays,

in triplicate, were initiated by addition of membranes and were terminated by addition of 5 ml of rinsing buffer [50 mM Tris-HCl (pH 7.4) containing 3 mM $MgCl_2$] at 4° C. Unless otherwise stated, incubations were done at 22 ° C for 60 min. At the end of the incubation period, the contents of the assay tubes were filtered immediately through Whatman GF/B glass filters that had been presoaked in incubation medium (as this reduced by 50% the nonspecific binding of ^{3}H-AVP to filters). Filters were washed rapidly three times with 5 ml of rinsing buffer, dried by high vacuum, and placed in scintillation vials. Radioactivity retained on the filters was measured by liquid scintillation spectrometry using a LKB beta counter with a counting efficiency of 32%. Nonspecific binding was defined as the binding occurring in the presence of 1.0 μM AVP. Binding of ^{3}H-AVP to filters in the absence of membrane was 0.4% of total radioactivity added and was not displaceable by 1.0 μM AVP (filter Blank). Under these conditions, depending on the amount of radioligand used, 35-69% of the binding measured was specific binding.

Subcellular distribution of ^{3}H-AVP binding

Various membrane fractions (P2', M, SPM) were incubated with ^{3}H-AVP (1 nM) at 22° C for 60 min with in the presence or absence of 1 μM AVP. One hundred micrograms of protein was used in the VSA and LS membrane fractions, 150 μg of protein was used in the HPC membrane fractions. Nonspecific binding was defined as the amount of ^{3}H-AVP binding in the presence of 1.0 μM AVP.

Heat lability of ^{3}H-AVP binding

Membrane fractions (SPM and M) were incubated as described above at either 22 or 50° C for 60 min.

Time course studies and reversibility of ^{3}H-AVP binding

Time course experiments were done using the standard conditions described above. Synaptic plasma membranes (100 μg of protein) were incubated at 22° C with 1.0 nM ^{3}H-AVP in the presence or absence of 1.0 μM AVP for the time indicated up to one hour. The reversibility of the binding was studied by diluting (tenfold) the assay mixture with rinsing buffer [50 mM Tris-HCl (pH 7.4) containing 3 mM $MgCl_2$], and by adding excess (final concentration = 0.1 μM) unlabeled AVP.

Reversibility of the specifically bound ^{3}H-AVP under mild acidic conditions.

Synaptic plasma membranes (100 μg of protein) were incubated with 1.0 nM ^{3}H-AVP at 22° C in the presence or absence of 1 μM AVP. After 40 min, the assay mixtures were acidified to pH 4.0 by adding 30 μl of 0.01% acetic acid. After different intervals under acid conditions, ^{3}H-AVP specific binding was measured and reported as a percentage of control (time 0) binding. To measure the possible degradation of receptors that mild acidification may cause, an additional set of assay tubes were tested. After incubation for 20 min at pH 4.0, membranes were centrifuged at 15,000 g for 5 min. The supernatant was discarded, and the pellets were washed twice with rinsing buffer and resuspended in standard incubation buffer (pH 7.4) containing 1.0 nM ^{3}H-AVP in the presence or absence of AVP.

After an additional 40 min of incubation at pH 7.4, ^{3}H-AVP specific binding was measured.

Ligand degradation

Degradation of ^{3}H-AVP during the binding assays was assessed by HPLC using the method of Gunther et al.(1980) and by rebinding studies. Synaptic plasma membranes from the VSA (150 μg) were incubated in a final volume of 300 μl with 1.3 nM ^{3}H-AVP (19,500 cpm) under standard conditions. After 60 min at 22° C, the samples were centrifuged at 15,000 g for 5 min. The supernatant (300 μl, containing free ligand) was removed and saved on ice; the pellets were washed twice in cold assay buffer, acidified by resuspension in 200 μl of 10% trifluoroacetic acid (TFA), and incubated on ice for 30 min. Following recentrifugation for 5 min, 150 μl of the acid eluate (membrane-bound radioactivity), along with 50 μl of the original supernatant (free radioactivity), and standard ^{3}H-AVP (5 μl) were analyzed by HPLC for the distribution of radioactivity. Fifty microliters of the supernatant containing the free radioactivity and 25 μl of the acid eluate containing the bound radioactivity were counted directly to estimate the recovery from the HPLC assay and to calculate the percentage of bound radioligand. The standard ^{3}H-AVP was analyzed by HPLC after the samples to avoid contamination of the samples by carry-over of radioactivity. A Waters HPLC system with a C-18 Bondapak column (Waters; pore size - 10 μm, 30 X 0.39 cm inner diameter), a Bondapak C-18/Corasil guard column, and a 214-nm ultraviolet detector were used. Separation was achieved using a linear gradient of 20-40% solvent B over a 30-min period

(flow rate = 1 ml/min). Solvent A was 0.1% aqueous TFA; solvent B was 60:40 CH_3CN/H_2O (containing 0.09% TFA). Fractions (0.5 min) were collected and counted by liquid scintillation to determine the radioactivity distribution. In rebinding studies, after 40 min at 22° C, the samples were centrifuged at 15,000 g for 5 min, and the supernatant was removed. One hundred micrograms of membranes was then incubated, for an additional 40 min, either with the supernatant or with new incubation medium, and specific binding was measured as described above.

Linearity of tissue binding

Tritiated AVP (1 nM) was incubated at 22° C for 60 min with increasing amounts (50-200 μg of protein) of fresh or frozen SPM fractions from the VSA, LS, and HPC in the presence or absence of 1 μM AVP.

Equilibrium binding studies

Synaptic plasma membranes (100 μg of protein for VSA and LS and 200 μg of protein for HPC) were incubated at 22° C for 60 min with increasing concentrations of ^{3}H-AVP (0.1-10 nM) in the presence or absence of 1.0 μM AVP.

Specificity of ^{3}H-AVP binding

Synaptic plasma membranes (100 μg of protein) were incubated in the presence of a constant amount of ^{3}H-AVP (0.5 nM) and increasing amounts of unlabeled peptides at 22 ° C for 60 min. The amount of specific binding measured at equilibrium in the absence of competitor was taken as 100%. Substances tested includes AVP, oxytocin (OT), Lys-vasopressin (LVP), Arg-vasotocin (AVT), [pGlu^4,

Cyt^{6}]-AVP 4-9 (AVP 4-9), desglycinamide AVP ([desGly^{9}]-AVP), and desglyciniamide deamino-AVP ([desGly^{9}]-dAVP), [deamino^{1}]-D-AVP (DDAVP), [1-(ß-mercapto-ß,ß-cyclopentamethylene propionic acid), 2-(O-methyl)tyrosine]-AVP [d(CH$_2$)$_5$Tyr(Me)AVP], presinoic acid, melanocyte-stimulating hormone-release inhibitor factor (MIF), thyrotropin-releasing hormone (TRH), somatostatin, angiotensin II (human), substance P, arginine, lysine, prostaglandin-E$_1$ (PGE$_1$), salicylate, glutamate, and N-methyl-D-aspartate (NMDA).

Materials

^{3}H-AVP (specific activity = 64 - 70.0 Ci/mmol) was obtained from New England Nuclear Corp. AVP, OT, LVP, AVT, presinoic acid, MIF, TRH, somatostatin, angiotensin II (human), and substance P were obtained from Bachem U.S.A. The vasopressin metabolite neuropeptide AVP 4-9, desGly^{9}]-AVP, and [desGly^{9}]-dAVP were obtained from Peninsula Laboratories (Palo Alto, CA, U.S.A.). Arginine, lysine, and salicylate were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.), NMDA from Fisher Chemical Co., and PGE$_1$ from Upjohn (U.S.A.). DDAVP was a gift from Ferring Pharmaceuticals (Malmö, Sweden), and the vasopressor antagonist [d(CH$_2$)$_5$Tyr(Me)AVP] was a generous gift from Dr. M. Manning (Medical College of Ohio).

C. Results

Subcellular distribution of ^{3}H-AVP binding

Specific ^{3}H-AVP binding was compared among the three subcellular particulate fractions P2', M, and SPM. Specific binding was low in the P2'

particulate fraction of the VSA (22%) and LS (16%), whereas 40% of apparent specific binding was found in the P2' fraction of the HPC. Specific binding was high in both the M (51% in the VSA, 61% in the LS, and 53% in the HPC) and the SPM (59% in the VSA, 63% in the LS, and 49% in the HPC) fractions (Fig. 1, top panel). Within the VSA, 49% of the total specific binding measured was found in the SPM fraction, 39% in the M fraction, and 12% in the P2' fraction (Fig. 1, lower panel). A similar distribution was seen in the LS, with 46% of the total specific binding measured found in the SPM fraction, 47% in the M fraction, and 6% in the P2' fraction. In the HPC, however, the specific binding was more widely distributed, with 27% of the specific binding found in the P2' fraction, 37% in the M fraction, and 36% in the SPM fraction (Fig. 1, lower panel).

Heat Lability

Previous experiments had indicated that AVP binding was temperature dependent. In this study, the heat lability of the apparent specific binding in M and SPM fractions was tested. At 50°C, the amount of apparent specific binding present in both the M and the SPM fractions was reduced to 2 and 18% in the VSA, 18 and 22% in the LS, and 30 and 21% in the HPC, respectively (Fig. 2). Because the specific binding in the P2' fraction of the HPC appeared to be moderately high (40% specific binding), we tested its heat lability. At 50° C, the amount of specific binding increased by 93% (data not shown), thereby suggesting that a chemical reaction with the radioligand, rather than a receptor binding interaction, may have occurred. Although ^{3}H-AVP binding tended to be rather

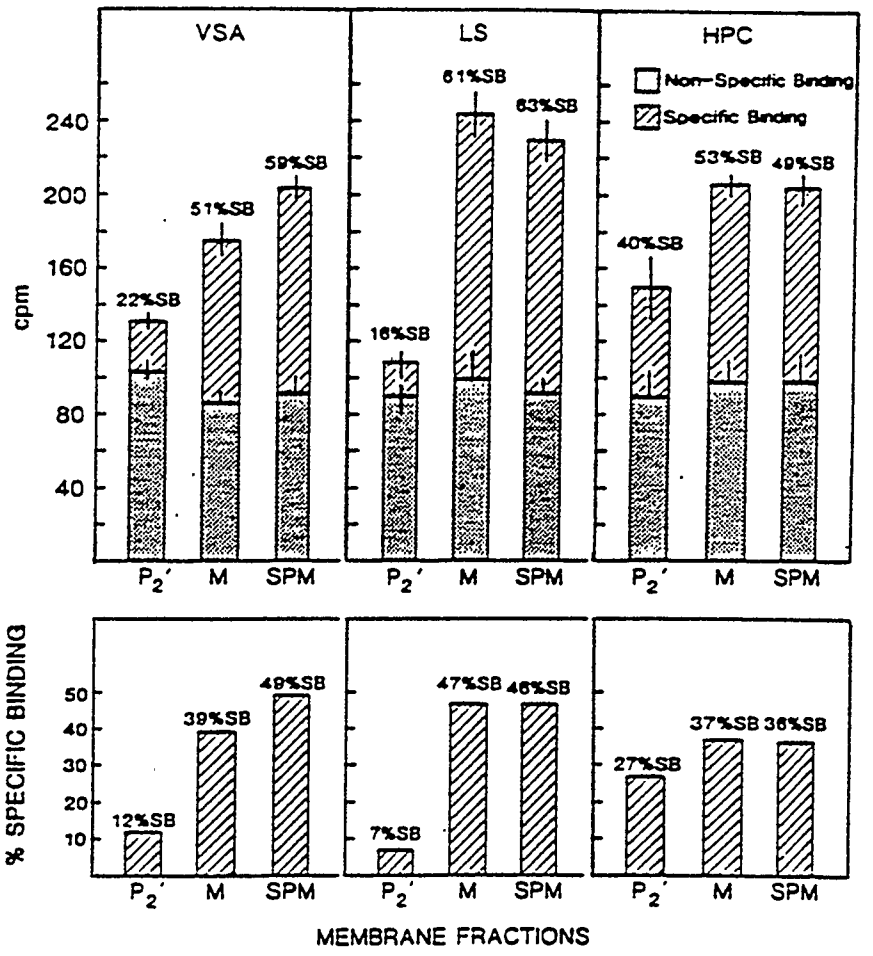

Figure 1. Upper panel: subcellular distribution of ^{3}H-AVP binding. Each bar diagram (mean ± S.E.M. from two determinations, each done in triplicate) represents the amount of total binding; the shaded area, the amount of nonspecific binding; and the hatched area, the calculated amount of specific binding. Numbers represent, in percentages, the amount of specific binding (SB) contained in each membrane fraction. Lower panel: Distribution of ^{3}H-AVP specific binding was calculated for each region of the rat brain. Numbers represent the amount, in percentage, of specific binding found within each fraction.

widely distributed among the three membrane fractions (Fig. 2), the SPM fraction was chosen for further investigations because it has a higher degree of homogeneity than the M fraction; in the M fractions, glial and other nonsynaptic components may be present (Burt, 1985).

Time course studies and reversibility of ^{3}H-AVP binding

The time course studies of ^{3}H-AVP binding indicated (a) that the binding of ^{3}H-AVP to SPMs reached equilibrium within 40 min at 22° C (Fig. 3) and (b) that the binding was stable for an additional 80 min (data not shown). Association and dissociation constants were calculated from the first-order equation $\ln [B_{eq}/(B_{eq} - B_t)] = k_{+1} \times (L)T \times (R)T/B_{eq}$, as described by Weiland and Molinoff (1981), where B_{eq} is the concentration of specifically bound ^{3}H-AVP at equilibrium, B_t is the concentration of specifically bound ^{3}H-AVP at time t, (L)T is the total concentration of ligand, and (R)T is the total concentration of binding sites. Figure 3, inset, represents the pseudo-first-order plot of $1n[B_{eq}/(B_{eq} - B_t)]$ vs. time, where the slope (k_{obs}) is equal to $k_{+1} \times (L)T \times (R)T/B_{eq}$. k_{+1} is equal to the association rate constant. The observed association rate constant (k_{obs}) was calculated to be 0.087 min^{-1} for the VSA, 0.090 min^{-1} for the LS, and 0.070 min^{-1} for the HPC. The kinetic constants for association are 5.38×10^7 for the VSA, 3.98×10^7 for the LS, and 3.57×10^7 for the HPC. No significant dissociation of ^{3}H-AVP binding to VSA and LS SPMs was observed after either a tenfold dilution of the incubation medium with rinsing buffer [50 mM Tris-HCl (pH 7.4) containing 3 mM $MgCl_2$] over a period

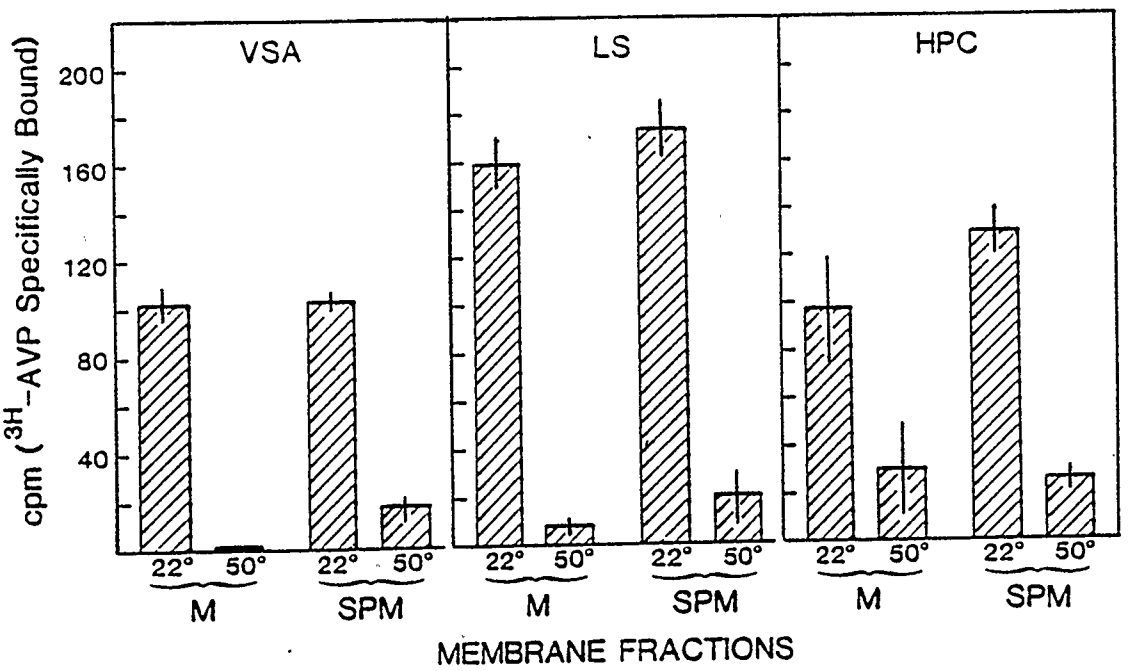

Figure 2. Heat lability of ^{3}H-AVP specific binding. The results are expressed as the amount of specific binding at 22 and 50° C for each membrane fraction. Data are mean ± S.D. (bars) values from triplicate determinations.

of 60 min (VSA) or 20 min (LS) or after addition of excess (final concentration = 0.1 μM) of unlabeled AVP. In the HPC SPM, however, 50-60% of ^{3}H-AVP specific binding was consistently reversible on tenfold dilution of the assay mixture. No additional dissociation was observed on further addition of an excess (final concentration = 0.1 μM) of unlabeled AVP. The calculated dissociation constant k_{-1} was 0.109 min^{-1}. In the HPC, using the rate constants k_{+1} and k_{-1}, we calculated a dissociation constant (K_d) of 3.05 nM, which is in good agreement with the dissociation constant determined from equilibrium experiments.

Failure to demonstrate dissociation of ^{3}H-AVP in the VSA and LS SPM or to demonstrate complete dissociation of ^{3}H-AVP in the HPC SPM suggested the possibility of side reactions occurring during the course of the binding measurements. Possible candidates include (a) the internalization or uptake of the ligand, (b) the secondary formation of a high-affinity covalent complex, and (c) the degradation of the peptide ligand during the incubation period. To evaluate the first two possibilities, we demonstrated that the specific binding was completely reversible under mild acidic conditions (pH 4.0) and, furthermore, that this effect was reversed on return to pH 7.4 (Fig. 4). These data thus indicate that the ligand is not internalized and that the ligand does not form a covalent complex with the binding site, because a pH of 4.0 would not be likely to disturb such a complex. To evaluate the third possibility, we demonstrated using HPLC analysis that the membrane-bound or free radioactivity present in the incubation mixture was not metabolized or degraded (Fig. 4).

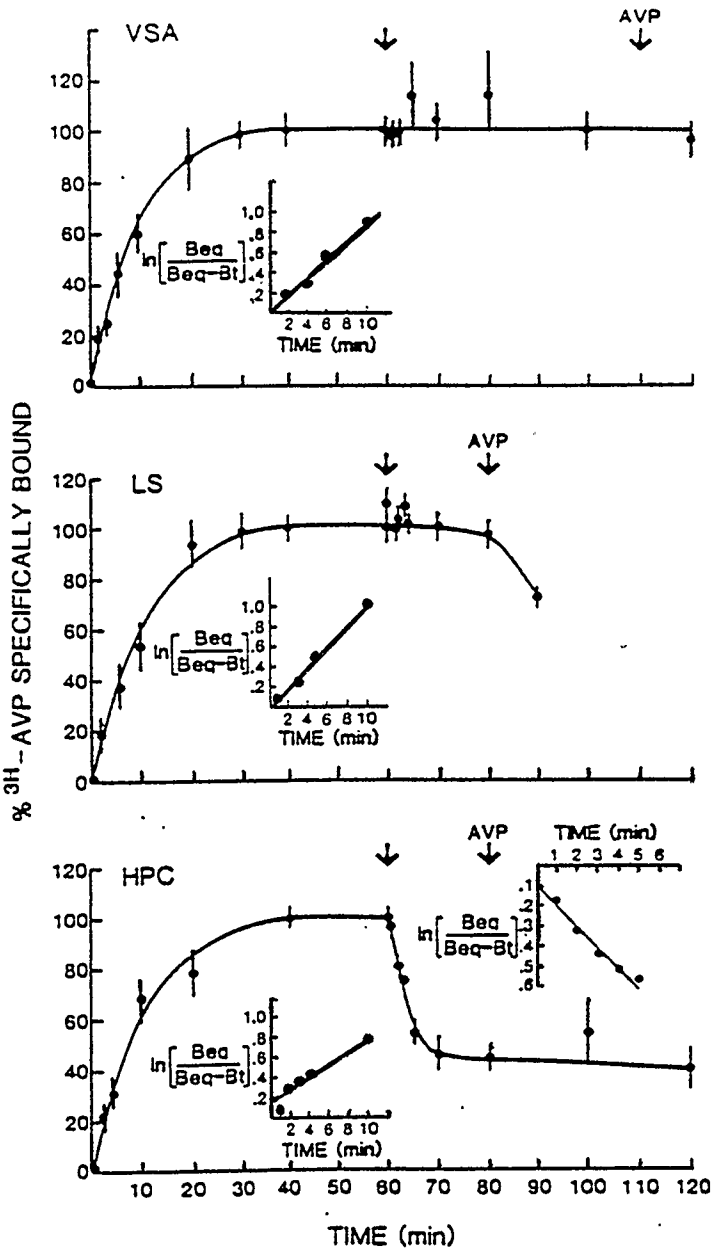

Figure 3. Time course study of ^{3}H-AVP specific binding in SPM fractions of the VSA, LS, and HPC. Data are mean ± S.D. (bars) values from triplicate determinations of a representative experiment. Binding reversibility: first arrow indicates a tenfold dilution with rinsing buffer and the second arrow indicates the addition of unlabeled AVP (final concentration = 0.1 μM). Binding data are expressed as percentages of ^{3}H-AVP specific binding. Data are mean ± S.D. (bars) values from triplicate determinations in a representative experiment. Inset: The same data plotted according to the pseudo-first-order rate equation as described in method.

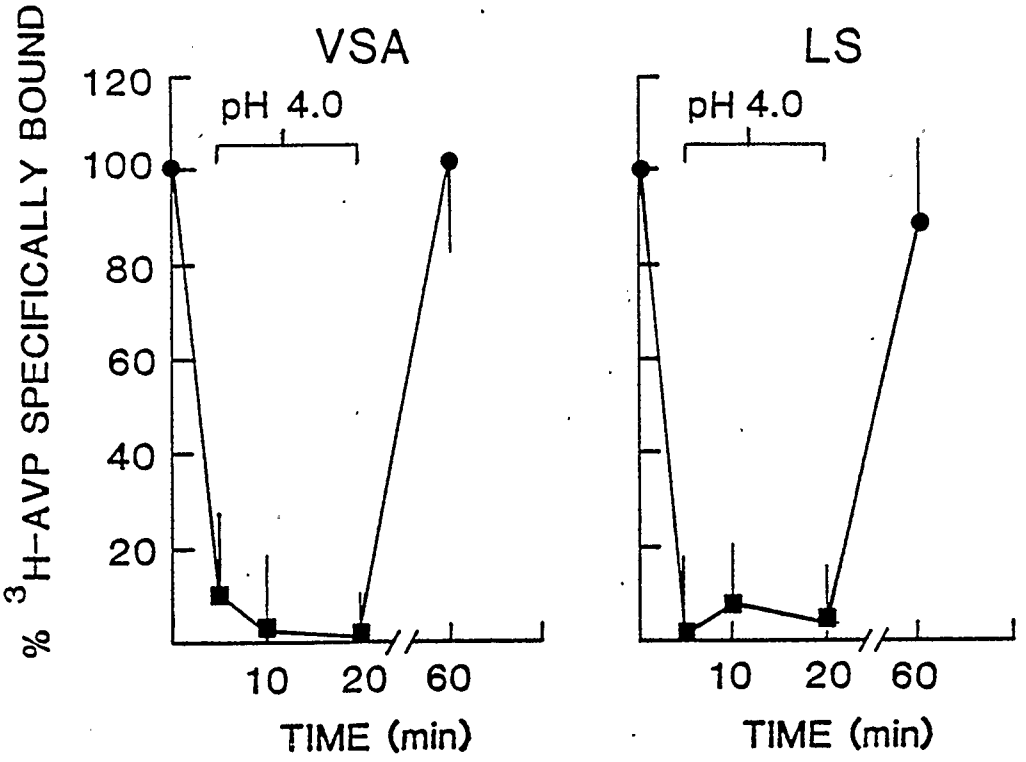

Figure 4. Reversibility of the specifically bound ^{3}H-AVP under mild acidic conditions. Solid circles represent possible degradation of receptors that mild acidification may cause measured as described in method. Data are mean ± S.D. (bars) values from triplicate determinations.

Ligand integrity and effect of membrane storage

Figure 5 compares the HPLC migration patterns of the standard labeled ligand ^{3}H-AVP not exposed to membranes, the free ^{3}H-AVP that had been exposed to membranes, and the ^{3}H-AVP bound to the membranes, showing that all forms of the ligand migrated in a similar pattern. The control assay showed that <10% (3,810 cpm/379,000 cpm) of the standard ^{3}H-AVP radioactivity was found in eluted fractions other than those associated with AVP. In the supernatant containing the free AVP and in the supernatant containing the bound AVP, also <10% of the radioactivity was found in fractions not associated with AVP. Thus, no significant degradation of radioactive ligand (free or bound) could be detected. From this experiment, it was calculated that 1.6% of the total radioactivity added to the incubation mixture was bound to the membranes (305 cpm bound/19,500 cpm added). Although no apparent degradation of ^{3}H-AVP was measured using HPLC analysis, it is not known whether or not the ^{3}H-AVP exposed to the membranes had retained its binding ability. Thus, rebinding studies using ^{3}H-AVP preexposed to membranes were done. These studies showed that ^{3}H-AVP preexposed to membranes retains its binding ability, because 95 ± 16% of the specific binding was detected using preexposed ^{3}H-AVP as compared with standard ^{3}H-AVP (data not shown).

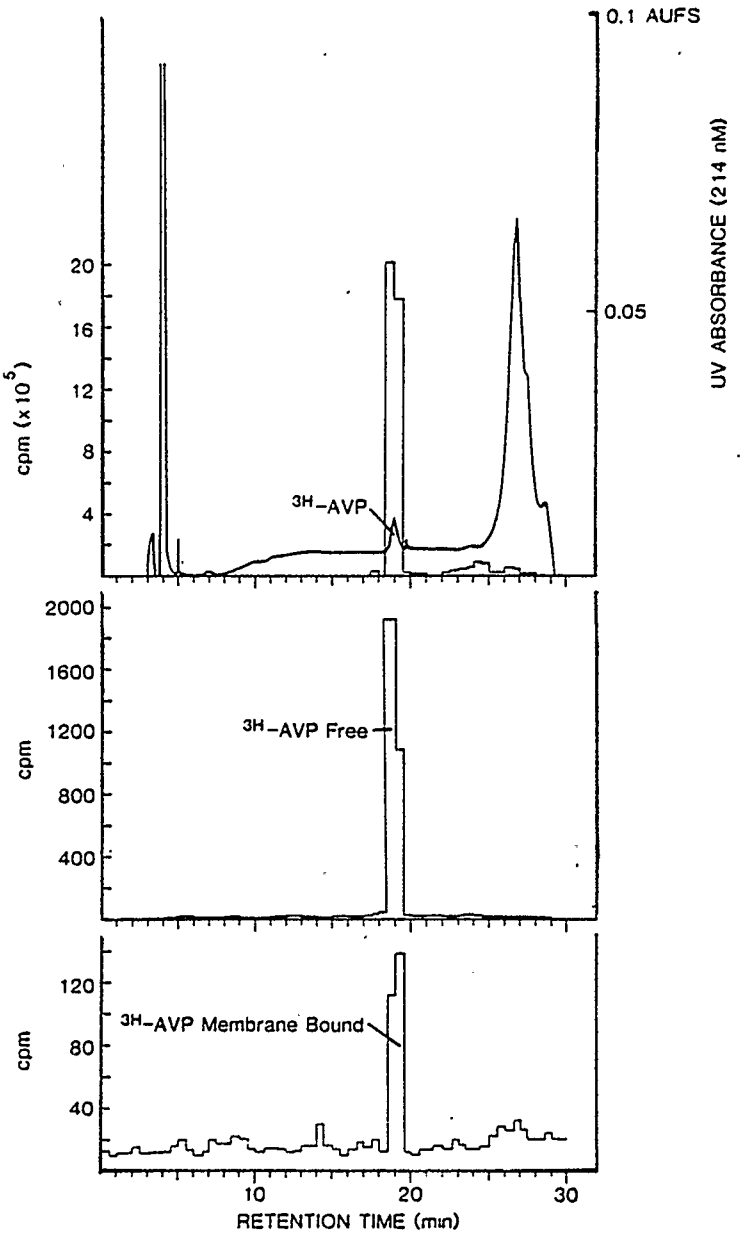

Figure 5. Identity of the bound ligand shown by detection of potential ^{3}H-AVP metabolism using HPLC analysis of both the membrane-bound and the free ^{3}H-AVP. HPLC migration pattern of 10 μl of the standard ^{3}H-AVP not exposed to the membrane (upper panel), 50 μl of the free ^{3}H-AVP that had been exposed to the membrane (middle panel), and 150 μl of the ^{3}H-AVP bound to the membrane (lower panel). AUFS, absorbance full scale.

Linearity of binding

Linearity of binding, with different membranes concentrations, is also important in binding studies to demonstrate the absence of receptor or ligand degradation during the incubation period. Incubating increasing amounts (50 - 200 μg) of SPMs from the VSA, LS, or HPC resulted in a linear increase in ^{3}H-AVP specific binding, thus further supporting the stability of the bound ligand and validating the conditions of the binding assay. For study of the effect of storing membranes frozen on the specific binding of ^{3}H-AVP in SPMs, increasing amounts (50 - 200 μg) of both fresh and frozen SPMs from the VSA, LS, and HPC were incubated with a constant amount of ^{3}H-AVP (1 nM). Figure 6 shows that both the ^{3}H-AVP specific binding and the tissue linearity were preserved in frozen membranes, thereby indicating that frozen SPMs could be used in further experiments.

Equilibrium binding studies

When SPMs were incubated with increasing concentrations (0.1-10 nM) of ^{3}H-AVP, specific binding was shown to be saturable in the VSA, LS, and HPC (Fig. 7, left panel). Scatchard analysis of ^{3}H-AVP binding to VSA, LS, and HPC SPMs yielded linear plots (Fig. 7, right panel). The data were consistent with a single binding site model. Each linear plot could be used for estimating apparent dissociation constants and maximal binding capacities. The calculated values for dissociation constants (K_d) and maximal binding capacities

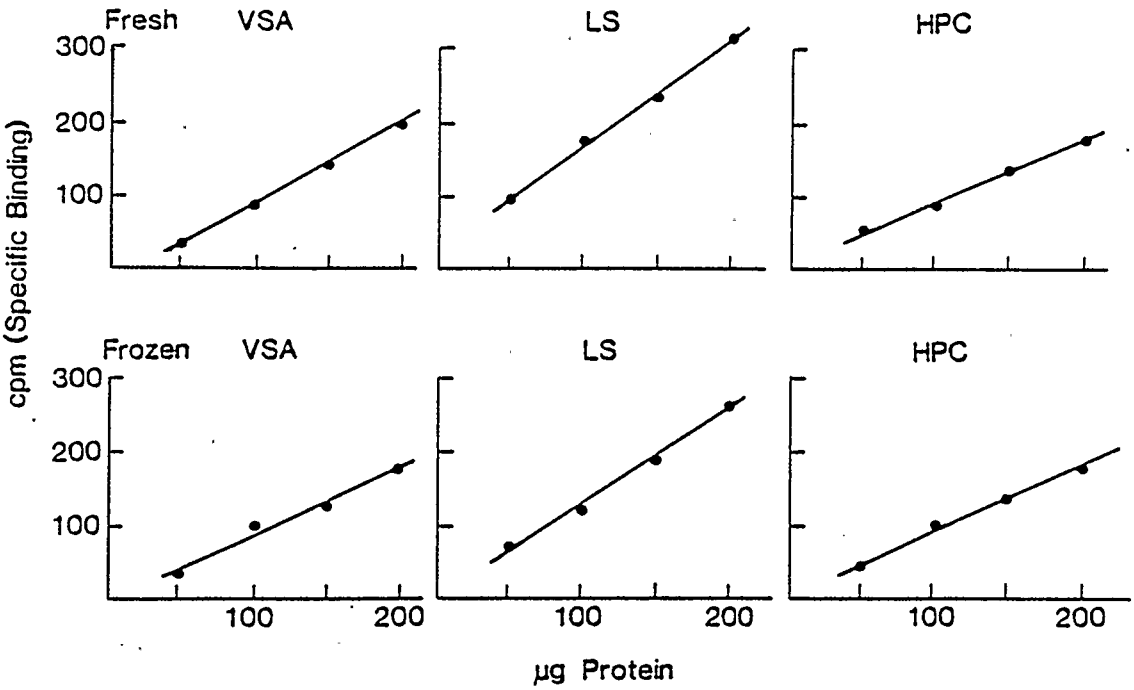

Figure 6. Linearity of ^{3}H-AVP binding to tissue. Binding of ^{3}H-AVP (1 nM) to increasing amount (50-200 μg of protein) of fresh (top panel) or frozen (lower panel) SPM fractions from the VSA, LS, and HPC in the presence or absence of 1 μM AVP. The results represent the amount of specific binding observed at each membrane protein concentration. Each point represents the mean of triplicate determinations.

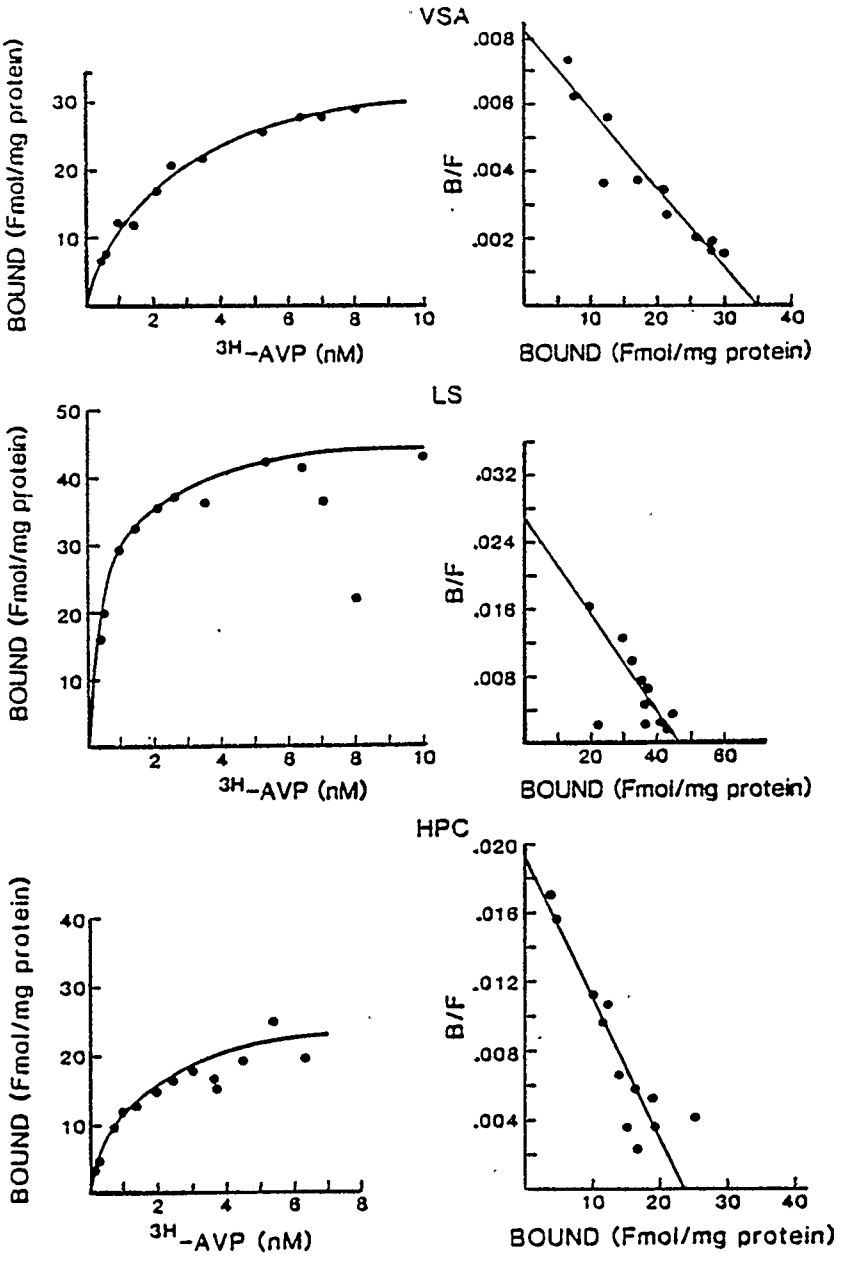

Figure 7. Saturation curves (left panel) and Scatchard analysis (right panel) of ^{3}H-AVP specific binding in VSA, LS, and HPC SPM fractions. Each point is the mean of triplicate determinations from a representative experiment.

(B_{max}), respectively, were 1.06 ± 0.39 nM and 24.0 ± 7.01 fmol/mg of protein (mean ± SEM; n=3) for the VSA, 0.92 ± 0.13 nM and 47.0 ± 4.96 fmol/mg of protein (n=3) for the LS, and 0.91 ± 0.13 nM and 25 ± 5.0 fmol/mg of protein (n=3) for the HPC.

Specificity of ^{3}H-AVP binding

The specificity of ^{3}H-AVP binding was determined by measuring the ability of various fragments and analogues of AVP to compete for specific high-affinity ^{3}H-AVP binding to VSA, LS, and HPC SPMs. Figure 8 reveals that the binding site discriminated very poorly among AVP, AVT, and LVP(AVP ≥ AVT ≥ LVP), whereas these high-affinity binding sites discriminated very effectively between AVP and analogues exhibiting enhanced peripheral oxytocic or antidiuretic selectivity. Thus, OT and dDAVP inhibited ^{3}H-AVP binding with a low potency (AVP > OT > dDAVP). The analogue ([desGly^9]-dAVP), which is devoid of peripheral activity but which retains the AVP-like potency in preventing extinction of a conditioned avoidance response (VanRee et al.1978), was found to be a poor competitor for ^{3}H-AVP. With the vasopressor antagonist d(CH_2)$_5$Tyr(Me)AVP, the displacement curve demonstrated an apparent two-component displacement. At lower concentrations, the capacity of the antagonist to displace ^{3}H-AVP was similar to that of AVP, whereas at higher concentrations, there was a tendency for the antagonist to be less potent than AVP in displacing ^{3}H-AVP binding. In contrast, AVP-related peptides, pressinoic acid, AVP (4-9), MIF, nonrelated peptides such as somatostatin, substance P, TRH, and other compounds such as PGE_1, salicylate,

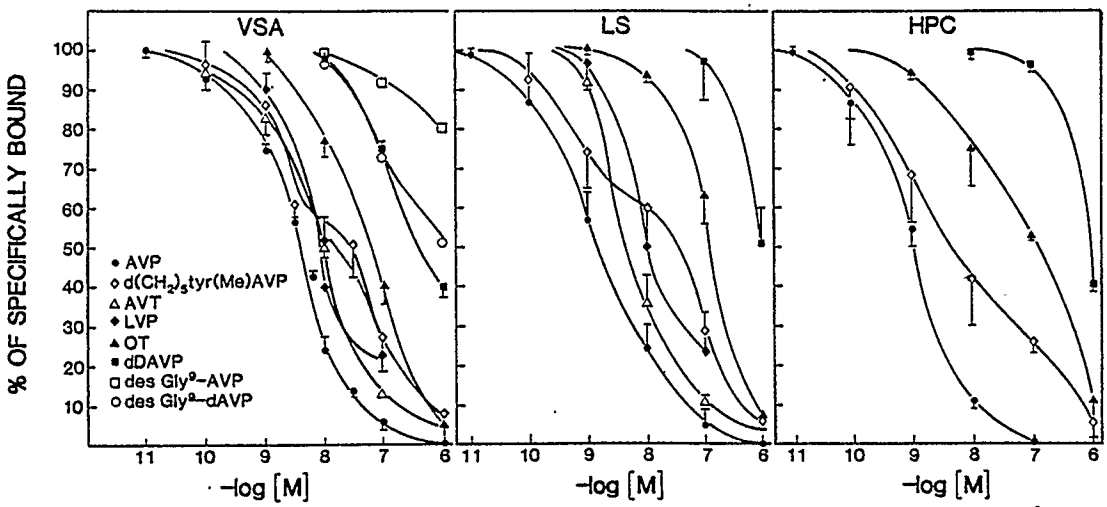

Figure 8. Specificity of ^{3}H-AVP binding. Dose-dependent inhibition of ^{3}H-AVP binding to VSA, LS, and HPC SPM fractions was produced by unlabeled peptides. The amount of specific binding measured at equilibrium in the absence of competitor was taken as 100%. Values of specific binding measured in the presence of unlabeled peptides were expressed as percentages of the specific binding measured in the absence of competitors. Data are mean ± S.E.M. (bars) values from one to seven separate experiments, each performed in triplicate.

glutamate, NMDA, arginine, and lysine failed to displace ^{3}H-AVP even at high concentrations (1 X 10^{-6} M).

D. Discussion

This study demonstrates the presence of specific high-affinity binding sites for AVP in SPMs of the VSA of the rat brain as well as of the LS and HPC. These sites show binding characteristics that are consistent with those of high-affinity ^{3}H-AVP binding sites reported in different areas of the rat brain (e.g. Barbaris, 1983; Dorsa et al.1983, 1984; Audigier and Barberis, 1985; Cornett and Dorsa, 1985; Junig et al.1985). It appears that at least three different subtypes of AVP receptors which bind the V1 antagonist, d$(CH_2)_5$Tyr(Me)AVP, may exist in the rat brain, namely, (a) a high-affinity site (K_d = 0.44-1.5 nM) that discriminates very efficiently between AVP and analogues exhibiting enhanced oxytocic or antidiuretic selectivity on peripheral organs (e.g. Barberis, 1983; Dorsa et al.1984; Cornett and Dorsa, 1985; Audigier and Barberis, 1985; Shewey and Dorsa, 1988), (b) a high-affinity OT-AVP binding site that discriminates very poorly between OT and AVP (K_d = 3.0 nM) (Audigier and Barberis, 1985; Di Scala-Guenot et al.1990), and (c) a low-affinity ^{3}H-AVP binding site (K_d = 11-13 nM) (Pearlmutter et al.1983; Costantini and Pearlmutter, 1984; Junig et al.1985) that also discriminates very poorly between OT and AVP but, in contrast to the two high-affinity sites, discriminates very poorly between the behaviourally active analogues ([desGly^{9}]-AVP) (VanRee et al.1978) and AVP (4-9) (de Kloet et al.1985) and AVP (Costantini and Pearlmutter, 1984).

The high-affinity ^{3}H-AVP binding site we detected in our study discriminated well between AVP and OT. This selectivity is consistent with that found for the receptor responsible for AVP-induced motor disturbances (see below).

The specific ^{3}H-AVP binding in the "microsomal" membrane fraction has been suggested to reflect receptors that may be in the process of synthesis or degradation (Burt, 1985) as well as receptors localized on plasma membranes (Pearlmutter et al.1983). It is also possible that the M membrane fraction may contain receptors in vesicular compartments derived from receptor-mediated endocytosis and recycling. When the subcellular localization of the specific binding was compared among the different brain regions, an equivalent amount of specific binding was observed in the M and SPM fractions. This result contrasts with the distribution of the low-affinity ^{3}H-AVP binding site, which, in the LS and HPC, has been shown to be present in greater proportions in the SPM fraction than in the M fraction (Pearlmutter et al.1983). Thus, the low- and high-affinity ^{3}H-AVP binding sites may be distributed differently in the different membrane fractions. The different distribution of high- and low-affinity sites, however, may also reflect a different rate of synthesis and degradation of these receptors and/or relative rates of membrane recycling.

Ligand degradation was not evident under our study conditions as tested by HPLC analysis of free and membrane-bound ligand: (a) The free radioactivity or that bound to the membranes comigrated with intact ^{3}H-AVP, (b) the binding of standard ^{3}H-AVP and that of membrane pre-exposed ^{3}H-AVP to SPMs were

similar, and (c) the binding of ^{3}H-AVP to SPMs was linear with respect to increasing protein concentrations. These data suggest that both free and membrane-bound ligand was intact ^{3}H-AVP.

The ^{3}H-AVP binding reached an equilibrium after 40 min at 22° C, and the binding was stable for at least an additional 80 min (Fig. 3). The observed rate constants (k_{obs}) of 0.087 min^{-1} for the VSA, 0.090 min^{-1} for the LS, and 0.074 min^{-1} for the HPC are comparable to those calculated for the high-affinity ^{3}H-AVP site in the amygdala observed by Dorsa et al.(1984). Although dissociation has generally not been studied, in agreement with the data obtained in the amygdala by Dorsa et al.(1984), ^{3}H-AVP dissociation from the high-affinity binding sites was only partial in HPC SPMs. Dissociation of ^{3}H-AVP binding from VSA and LS SPMs was not observed after either a tenfold dilution of the incubation medium with rinsing buffer or after addition of excess unlabeled AVP but was complete after mild acidification of the incubation mixture. Failure to observe dissociation except under mild acidic conditions does not exclude receptor identification but does mean that a further explanation must be sought. One possible explanation is that there is a formation, over time, of a high-affinity complex. If so, in reversal experiments, taking measurements at earlier time points could reveal some partial reversibility.

In all three regions of the brain studied, ^{3}H-AVP binding was saturable. Scatchard analyses yielded linear plots and similar dissociation constant values for each site (Fig. 7). Scatchard analyses yielding linear plots have been observed previously in hippocampal synaptosomes (Barberis, 1983; Audigier and Barberis,

1985), septal crude membranes (Shewey and Dorsa, 1986), and amygdala and dorsal hindbrain crude membranes (Dorsa et al.1983, 1984). Even under conditions that reveal both high- and low-affinity AVP binding sites (Junig et al.1985), the high-affinity site was shown to have a dissociation constant consistent with linear Scatchard K_d estimates of the high-affinity site reported here, and by others (Barberis, 1983; Dorsa et al.1984; Audigier and Barberis, 1985; Cornett and Dorsa, 1985; Shewey and Dorsa, 1986).

The binding of related and non-related AVP peptides indicated that the high-affinity AVP binding site has recognition properties similar to those described in other regions of the brain (Barberis, 1983; Dorsa et al.1984; Audigier and Barberis, 1985; Cornett and Dorsa, 1985; Junig et al.1985). All indications are that it resembles a V_1 site as described by Michell et al.(1979). In keeping with this observation, the V_1 antagonist $d(CH_2)_5$Tyr(Me)AVP competed effectively with AVP for the binding site. Of particular interest, however, was the shape of the competition curve obtained with $d(CH_2)_5$Tyr(Me)AVP. As shown in Fig. 8, the displacement curve obtained by this V_1 antagonist appears biphasic which suggests that ^{3}H-AVP may bind to two sites. In fact, one of these populations might be the so-called high-affinity OT-AVP binding site, which was shown to have a high affinity (K_d = 3.7 nM) for AVP (Audigier and Barberis, 1985). A similar biphasic (two-components) competition curve was observed by Audigier and Barberis (1985) using an oxytocic receptor agonist (OH-Thy^4-Gly^7-OT) in displacing ^{3}H-AVP binding from hippocampal membranes; this finding was also interpreted

as suggesting the possibility that ^{3}H-AVP labeled two populations of sites with similar affinities for AVP. On the basis of ligand specificities from this study and others, it appears that the specific high-affinity AVP binding site has similarities with the V_1 peripheral receptor but is distinct from the V_2 peripheral receptor, from the low-affinity CNS AVP receptor, and from the OT-AVP subtype of CNS receptor. A more detailed analysis of the structural requirements for AVP binding to central and peripheral AVP and OT receptors may be needed to define subclasses of AVP-like or OT-like receptors.

Pharmacological and physiological studies support the possibility that the high-affinity AVP binding site localized to the SPM fraction studied here is a functional AVP receptor localized in the VSA of the rat brain. Examination of the rank order potency for AVP, dDAVP, and OT in inducing motor disturbances (Abood et al.1980; Kasting et al.1980; Naylor et al.1985a; Burnard et al.1986), antipyresis (Naylor et al.1987), and inhibition of glutamate-induced excitation of VSA cells (Disturnal et al.1987) and in competitive binding studies reported here reveals a close correlation among the four models investigated. Furthermore, the V_1 antagonist d(CH_2)$_5$Tyr(Me)AVP was shown to block both the behavioural and the physiological consequences of AVP applications to the VSA as well as the specific binding.

In summary, we characterized an AVP binding site located in the VSA that probably reflects the receptor involved in mediating AVP's actions, including those on motor control, in this region. Because the latter phenomenon displays a

sensitization process, it will be of interest to investigate whether this sensitization process is mediated by changes in AVP receptor number and properties in the VSA.

III ENHANCED BEHAVIOURAL AND CELLULAR ACTIONS OF CENTRAL AVP

A. Introduction

The neural mechanism(s) underlying agonist-induced sensitization are not well understood. In particular, little is known with respect to AVP sensitization. Thus, using a standard AVP sensitization protocol (e.g. Kasting et al.1980; Burnard et al.1983; Wurpel et al.1986a; Balaban et al.1988) we tested the hypothesis that AVP sensitization may result in an increase in the number of septal V1 receptors. The unexpected results obtained in these binding experiments, however, (i.e. no apparent changes in septal ^{3}H-AVP binding site density or affinity, see below) led us to further investigate the phenomenon of AVP sensitization. In particular, because it has been observed that agonist-induced sensitization is typically responsive to the paradigm of administration used [i.e. dose and time allowed between agonist administration (for reviews, see Post, 1980; Robinson and Becker, 1986)] we first investigated the possibility that the development of AVP sensitization may be altered by the dose and the temporal pattern of AVP administration. Secondly, because several components of agonist receptor-effector interactions have been shown to be susceptible to modification as a result of prior agonist exposure (Mitchell et al.1988), we have also tested the hypothesis that in sensitized rats, the signal transduction mechanism of septal AVP V1 receptor [phosphatidylinositol (PI) hydrolysis (Shewey and Dorsa, 1988)] may be enhanced.

B. Methods

Adult male Sprague Dawleys rats (200-250 gm) were anaesthetized with sodium pentobarbital (50 mg/kg) and a stainless-steel 23-gauge guide cannula was implanted stereotaxically under aseptic conditions to allow access to a lateral cerebral ventricle. Rats were then allowed 5-7 d to recover. The rats were housed in groups of four per cage on a 12-hr light cycle and allowed food and water ad libitum. Using an appropriate concentration of synthetic AVP (Bachem, USA) in sterile, pyrogen-free, physiological saline, intracerebroventricular injections of AVP solution were administered by gravitational flow through a 27-gauge injection cannula into awake, freely moving animals. For behavioural observations, after an injection the animals were placed in a large plexiglass chamber and observed for the development of motor disturbances for a 10-min period. Motor disturbances were defined and scored at 1-min intervals on a scale modified from that developed by Kasting et al.(1980) as follows: 0, no effect; 1, pauses as defined by periods (10 sec or longer) of absence of activity; 2, prostration; 3, large head swaying and locomotor difficulties; 4, barrel rotation as indicated by the animal's rotation along the long axis of the body; 5, myotonus or myoclonus; 6, death. Experiments were all performed between 10:00 and 18:00 hours. Behavioural results are presented as the highest score each animal received during the 10-min observation period.

Radioligand binding studies:

In these experiments, we addressed the question of whether pretreatment with AVP would alter the number and/or affinity of central ^{3}H-AVP binding sites. Our attempt to identify possible AVP receptor changes in sensitized rats has focused on the VSA AVP system because AVP-induced sensitization has been observed after AVP injections in to the VSA of the rat basal forebrain (Naylor et al.1985a). We also investigated, however, possible alterations in AVP receptors in other brain areas known to contain AVP receptors such as the LS and the HPC (Audigier and Barberis, 1985; Jard et al.1987; Raggenbass et al.1987 and see chapter II) because i.c.v. administration of AVP is expected also to reach these brain regions. Thus, in these experiments, cannulated rats received either an i.c.v. injection of AVP (1 nmol, n=30, sensitized) or saline alone (n=30, control) and their behaviour observed as described above. Two days later, to confirm the enhanced motor actions of a second AVP administration, 5 animals from each group was given a second injection of AVP (100 pmol) and their behaviour observed as described above, while the remaining animals (n=25 controls, and n=25 sensitized) were used for ^{3}H-AVP saturation binding studies using ligand binding techniques previously described (chapter II).

Data from saturation experiments were analyzed using a computerized, interactive curve fitting procedure to generate representative Scatchard plots. Each linear plot could be used for estimating apparent dissociation constants (K_d) and binding sites concentration (B_{max}). Statistical analysis comparing Scatchard plot

data from sensitized and control rats was performed using regression analysis (Kleinbaum et al.1988), to consider two basic questions: 1) Are the two slopes ($1/K_d$) the same or different? 2) Are the two x intercepts (B_{max}) the same or different?

Behavioural observations:

Dose response relationship. The first set of experiments investigated the dose response relationship of AVP-induced sensitization. In these experiments, nine groups of animals (n=4-8) were subjected to a first injection of AVP (1, 10 or 100 pmol), or as control physiological saline alone. Two days later, all animals received physiological saline followed 5-min later by an injection of AVP (1, 10 or 50 pmol). After each injection, the behaviour of the animals was scored as described above. Results were statistically analyzed by the non-parametric Wilcoxon matched-pairs signed-ranks test. The i.c.v. saline injection did not elicit any behavioural score greater that one (data not shown). Because the above experiment indicated that repeated doses of 10 pmol of AVP was sufficient to induce severe motor disturbances in more than 90% of the sensitized animals, this dose was used in subsequent experiments.

Time course study. In a second set of behavioural experiments, we studied the time course of the development and the duration of AVP sensitization after a first exposure to AVP. In these experiments ten groups of animals were used. Each group was given a first exposure to AVP (10 pmol) at time zero, and the behaviour of the animals scored as described above. Then, each group of animals was given

a second exposure to AVP (10 pmol) at a specified time following the initial AVP exposure [1 (n=4), 3 (n=6), 6 (n=6), 9 (n=6), 16 (n=6), 24 (n=6) hours; 2 (n=8), 4 (n=5), 6 (n=4), and 8 (n=4) days] and the behaviour was scored as described above. Results were statistically analyzed by the non-parametric Wilcoxon matched-pairs signed-ranks test.

Time interval between injections. In a third set of experiments, we investigated the importance of allowing time between treatments. Three groups of animals were given repeated injections of AVP (10 pmol) either once daily (n=5) for up to 6 days, once hourly (n=4) for up to 16 hours, or once weekly (n=5) for up to 4 weeks. After each injection, the behaviour of each animal was observed as described above. Results were statistically analyzed by the non-parametric Friedman two-way analysis of variance by ranks.

Assay of [^{3}H]inositol-1-phosphate (IP_1)

In these experiments, we tested whether pretreatment with AVP would enhance septal PI hydrolysis in response to a second challenge with AVP. Cannulated rats received either an i.c.v. injection of AVP (10 pmol, n=24, sensitized) or saline alone (n=24, control). One day later, AVP-induced ^{3}H-IP_1 accumulation in septal slices was performed essentially using the method developed by Shewey and Dorsa (1988) with slight modifications as described below. Briefly, (250 x 250 μm) slices (Sorval tissue chopper) were prepared from sensitized or control rats septal tissue (n=5 animals per group in each experiments) and were incubated for 30 min in a modified Krebs-Ringer

bicarbonate (KRB) buffer containing (mM): 118 NaCl, 4.7 KCl. 1.3 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 5.0 $MgCl_2$, 25.0 $NaHCO_3$, 11.7 glucose, pH = 7.4, at 37° C under 95% O_2/5% CO_2 in a shaking water bath. Dispersed slices were labelled with 0.3 μM ^{3}H-inositol (Myo-[2-^{3}H(N)]-inositol, 20.0 Ci/mmol, New England Nuclear Corp.) in KRB buffer under 95% O_2/5% CO_2 for 1h at 37° C and then washed 3 times with fresh KRB buffer to remove extracellular label. Gravity packed, labelled slices (25 μl, approximately 400-600 μg of protein/well) were then added to tissue culture wells (to maximize gas exchange) containing 250 μl KRB buffer containing 10 mM LiCl under 95% O_2/5% CO_2 at 37° C. After 10 min, agonists were added in the appropriate concentrations and the incubation continued for an additional 60 min. In some experiments, an antagonist was added 10 min prior to the agonist. The reaction was stopped by the addition of ice cold chloroform:methanol (1:2). The mixture was then transferred to polypropylene tubes, vortexed and separated into two phases by centrifugation. Labelled inositol phosphates, in the water-soluble phase, were analyzed using modifications of the method described by Berridge et al.(1983) employing anion-exchange resin columns. Briefly, a 1 ml aliquot of the water-rich phase of each tube was diluted to 3.2 ml with distilled water and loaded onto a 1 ml Dowex formate column (Dowex AG1-X8, Bio-Rad Laboratories). The columns were washed with an additional 1 ml of distilled water. Three ml of 5 mM sodium tetraborate in 60 mM sodium formate, followed by 1 ml distilled water, were used to elute the glycero-phosphoinositol (GPI) fraction. Inositol monophosphate (IP_1) was then eluted from the columns by the addition of

4 ml of 0.2 M ammonium formate in 0.1 M formic acid. The remainder of the inositol phosphates were eluted with 4 ml of 1 M ammonium formate in 0.1 M formic acid. The radioactivity contained in aliquots of the GPI, IP_1 and inositol phosphates fractions, and in the chloroform-rich phase containing the phosphoinositides (PI), was measured by scintillation counting after the addition of 6 ml of Ready Gel (Beckman; Missisauga, Ontario). In order to compensate for the variability associated with pipetting slice suspensions, the radioactivity accumulating in the ^{3}H-IP_1 fraction was expressed as a ratio of the label incorporated in the ^{3}H-PI fraction. The remaining ^{3}H-inositol phosphates and GPI accounted for less than 1% of the total radioactivity. Results were statistically analyzed using two-way analysis of variance (two-way ANOVA) for all dose-response curves, and where further post hoc testing was required, the Scheffé multiple-range test was used. Values of $p < 0.05$ were considered significant.

C. Results

Binding studies. In order to confirm the sensitization state of the AVP pre-treated animals we first conducted a behavioural experiment on 5 animals from each group (saline or AVP pre-treated). In these experiments, we observed that after saline injection animals displayed no sign of motor responses and after the first exposure to AVP (1 nmol), all rats displayed minor behavioural responses consisting of pauses and prostration (behavioural score $\leq$ 2; data not shown). After a second AVP exposure (100 pmol), saline pre-treated animals displayed minor motor responses including pauses whereas all AVP pre-treated animals showed

significantly increased ($p < 0.05$, Wilcoxon matched pairs signed-ranks test) motor responses including barrel rotation, myotonus and myoclonus and death (data not shown). These results confirm that AVP pre-treated animals are highly sensitized whereas saline pre-treated animals are not. Using the rest of the animals from each group, saturation radioligand binding experiments were performed using ^{3}H-AVP binding to synaptic plasma membranes (SPMs) prepared from the VSA, LS and HPC. Binding of ^{3}H-AVP to SPMs was saturable over ligand concentrations from 0.1 to 10 nM and specific binding of up to 67% was observed (data not shown). Scatchard analysis of ^{3}H-AVP binding to VSA, LS, and HPC SPMs yielded linear plots (Fig 9), which is consistent with a single binding site model. The calculated K_d for ^{3}H-AVP binding from controls were not significantly different from the K_d for ^{3}H-AVP binding from sensitized animals: 0.7 versus 0.9 nM, $p > 0.37$, VSA; 0.9 versus 0.8 nM, $p > 0.85$, LS; and 0.45 versus 0.5 nM, $p > 0.13$, HPC, for controls and sensitized animals respectively. Figure 9 also shows that the B_{max} for ^{3}H-AVP from controls were not significantly different from the B_{max} for ^{3}H-AVP from sensitized rats: 17 versus 23 fmol/mg protein, $p > 0.36$, VSA; 36 versus 38 fmol/mg protein, $p > 23$, LS; and 19 versus 21 fmol/mg protein, $p > 0.65$, HPC, for controls and sensitized rats respectively. These results indicate that AVP sensitization does not appear to alter ^{3}H-AVP binding, based on values for dissociation constants and density of binding sites, in any of the 3 regions of the rat brain studied.

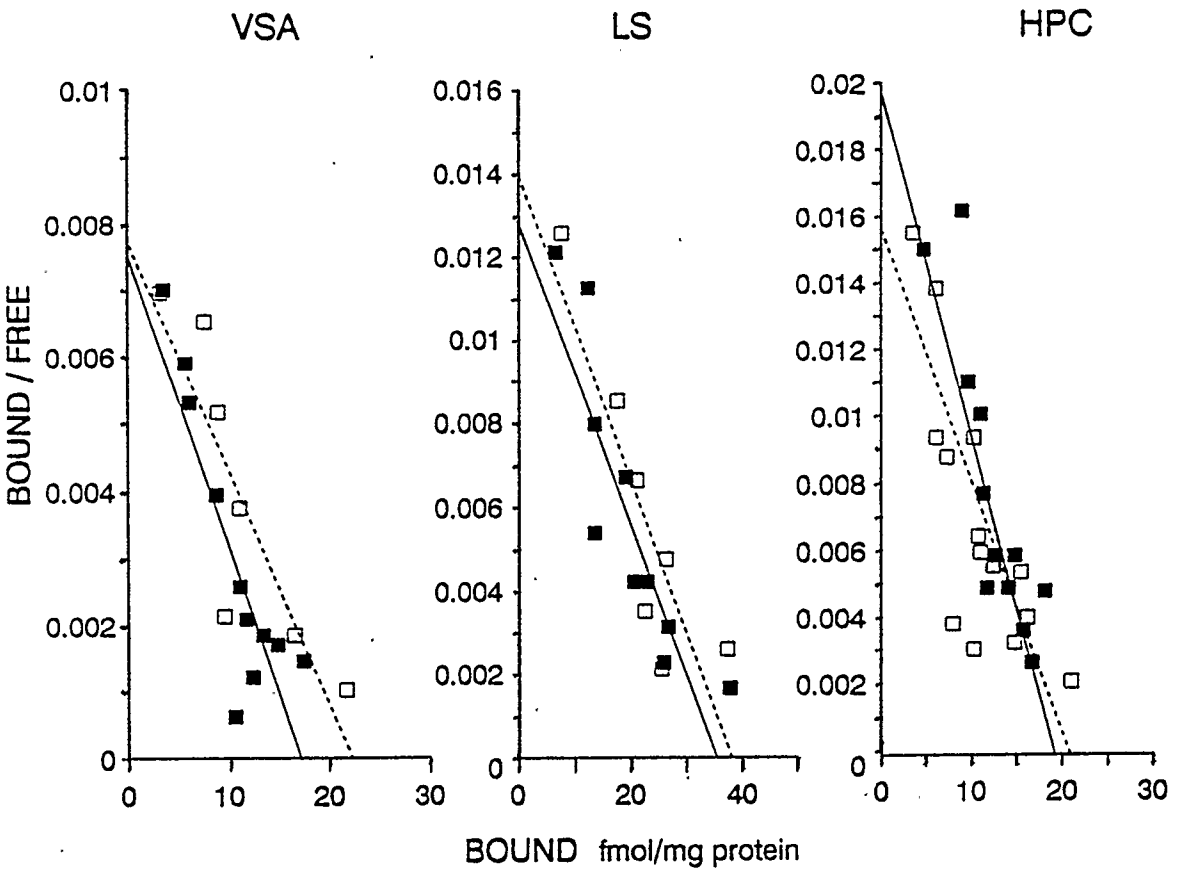

Figure 9. Scatchard plot analysis of ^{3}H-AVP specific binding in SPM prepared from the VSA, LS and the HPC of non sensitized controls (saline pretreated;■–■) or sensitized (1 nmol AVP pretreated; □–□) rats. Each point is the mean of triplicate determinations.

Behavioural observations

Dose response relationship. Figure 10 shows the results of experiments designed to investigate whether AVP sensitization is a dose dependent phenomenon. The behavioural scores of rats receiving a first (1st exposure) and a subsequent (2nd exposure, two days later) i.c.v. injection of AVP at several doses are shown in Figure 10. After the first exposure to AVP (1, 10 or 100 pmol), all rats displayed minor behavioural responses consisting of pauses and prostration with no obvious dose-response relationship (see Fig 10, 1st exposure). When the animals were retested two days later (2nd exposure), however, it can be seen that the motor responses of the animals were significantly increased ($p < 0.04$, Wilcoxon matched pairs signed-ranks test, in all paired groups) from those of the first exposure in a dose dependent manner.

The data obtained in Figure 10 are replotted in Figure 11 to illustrate the dose-response relationship for the proportions of rats (expressed as percent) exhibiting severe motor disturbances (including barrel rotation, myotonic/myoclonic convulsions and death; score ≥ 4) after an AVP injection according to the sensitized state of the animals. This figure illustrates that the potency of AVP to induce motor disturbances depends upon the sensitization state of the animals. The greater the sensitizing dose of AVP the animals received on the first AVP exposure, the greater the number of animals displaying severe motor disturbances on the second AVP exposure.

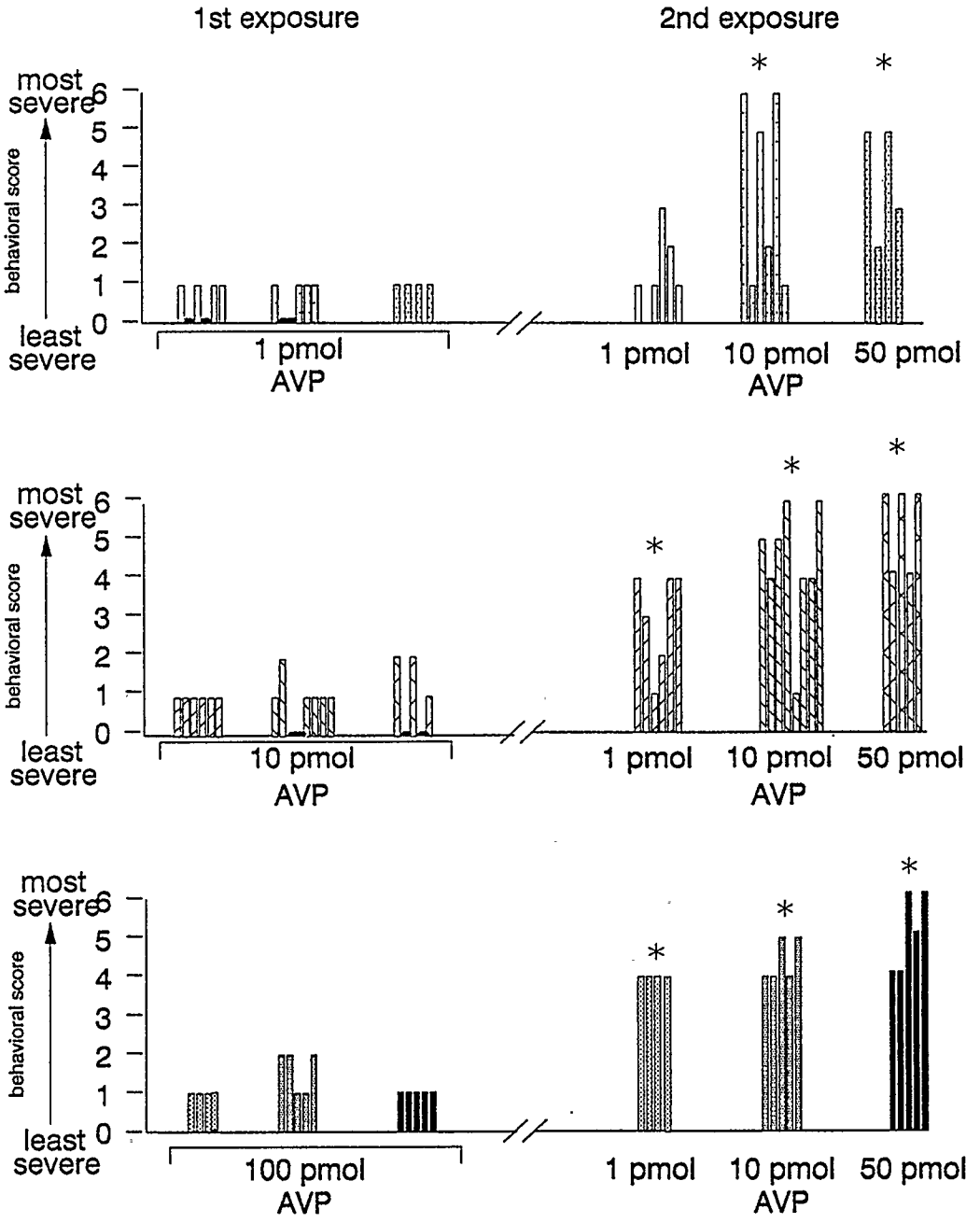

Figure 10. AVP-induced motor disturbances in 9 groups of animals (n=4-8) in response to an initial (1st exposure) and a subsequent (2nd exposure) i.c.v. injections of AVP, 2 days later, at varying doses. Each bar represents the most severe behavioral score an individual animal received during the 10-min observation period following the AVP injection. Each group is represented by a unique bar pattern on the first and second AVP exposure, and asterisks indicate significant ($p < 0.05$, Wilcoxon matched pairs signed-ranks test) increase in the severity of the motor disturbances between the first and the second AVP exposure.

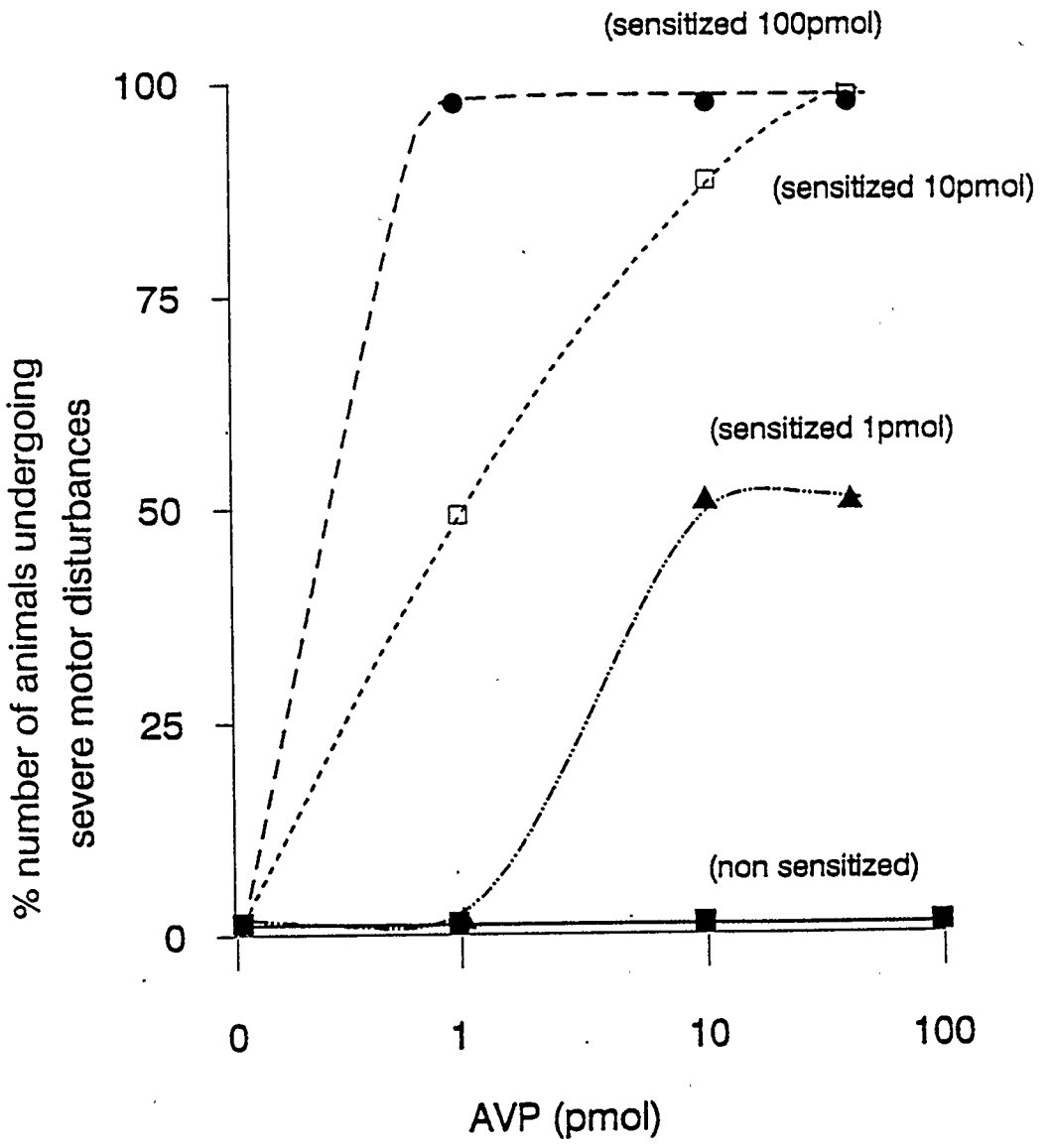

Figure 11. Data from figure 10 are replotted to show the proportions of rats (expressed as percent) exhibiting severe motor disturbances (including barrel rotation, myotonus, myoclonus and death, score ≥ 4) in response to various doses of AVP on the second AVP exposure as a function of the sensitization state (dose of AVP received as a first exposure) of the animals.

Time course study. Figure 12 shows the results of the experiments designed to elucidate the time course of the development and duration of the sensitization process. After the first exposure to AVP (10 pmol), all animals displayed minor behavioural responses (scores $\leq$ 2) consisting of pauses and prostration (data not shown). After the second exposure to AVP (10 pmol) the behavioural scores of animals given the second AVP injection 1 or 3 hours later were not significantly different ($p > 0.6$, Wilcoxon matched pairs signed-ranks test) from the initial response. When the second AVP injection was administered at least 6 hours and up to 6 days following the initial AVP injection, a significant increase in the behavioural score was observed ($p < 0.03$, Wilcoxon matched pairs signed-ranks test). This increase in the motor responses to a second AVP exposure was no longer observed ($p > 0.46$, Wilcoxon matched pairs signed-ranks test) if the second exposure to AVP was given 8 days after the initial AVP exposure.

Time interval between AVP administrations. Figure 13 shows the results of the experiments designed to elucidate whether allowing time between treatments is important for the development of sensitization. Figure 13 (top panel) shows that if AVP (10 pmol) was repeatedly administered at a time interval for which sensitization was seen (i.e. daily) for up to 6 consecutive days, the sensitization of the rat brain could be induced and maintained for at least 5 days ($p < 0.05$, Friedman two-way ANOVA). If, however, repeated AVP injections were given hourly, (i.e. at a time interval shorter than that seen for the development of the sensitization process to occur) or weekly (i.e. at a time interval longer than the

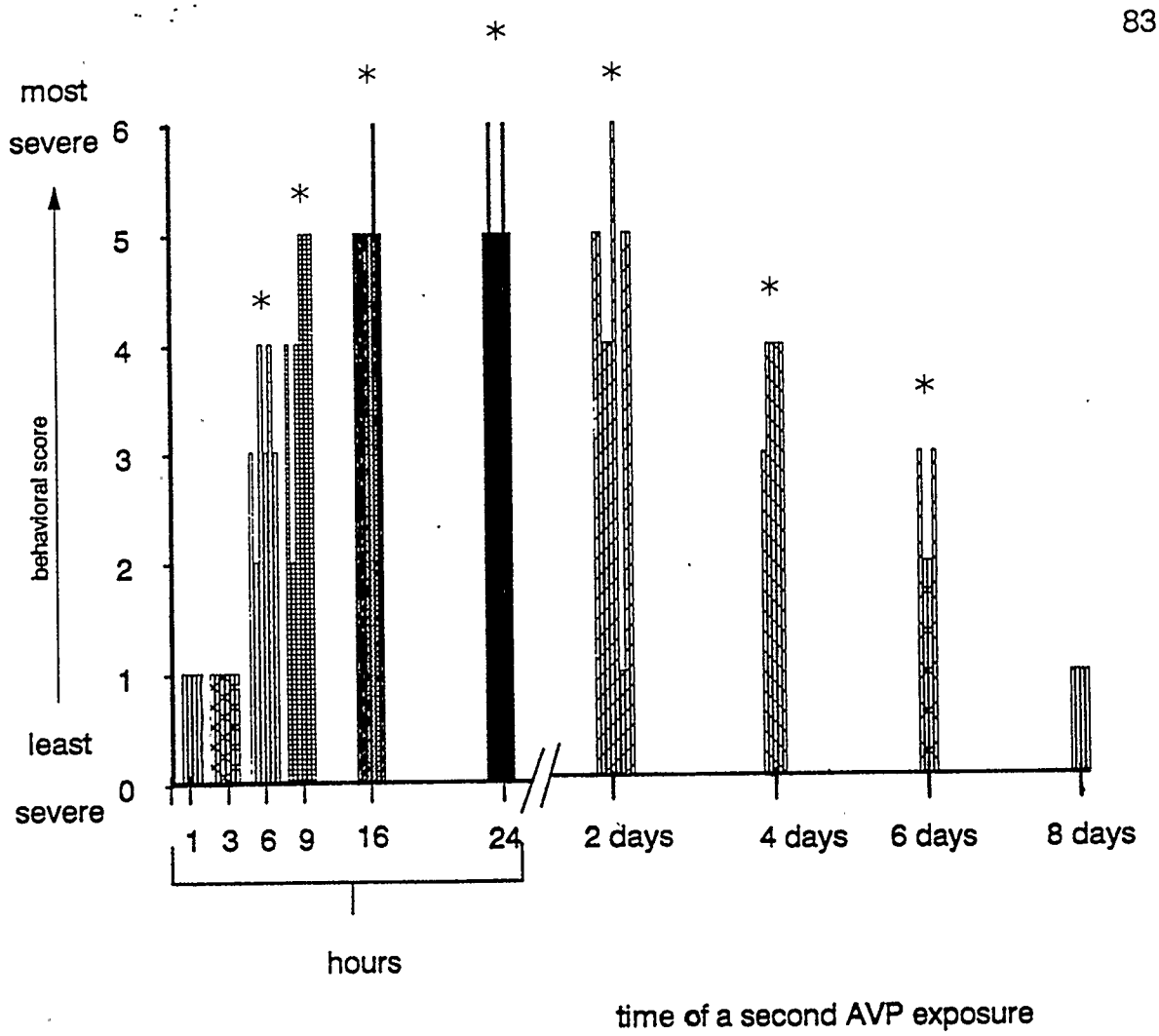

Figure 12. Time course study of AVP-induced sensitization. Ten groups of rats (n=4-8) were first given an i.c.v. dose of AVP (10 pmol) at time zero and their motor behavior scored (data not shown; all scores ≤ 2). At different times (1, 3, 6, 9, 18, 24 hours and 2, 4, 6, and 8 days) following the initial injection, each group was given a second i.c.v. injection of 10 pmol AVP and their motor behavior scored. Each bar represent the maximal score each animal received following the second AVP injection. Asterisks indicate significant ($p < 0.05$, Wilcoxon matched pairs signed-ranks test) increase in the severity of the motor disturbances between a first (data not shown) and a second AVP exposure.

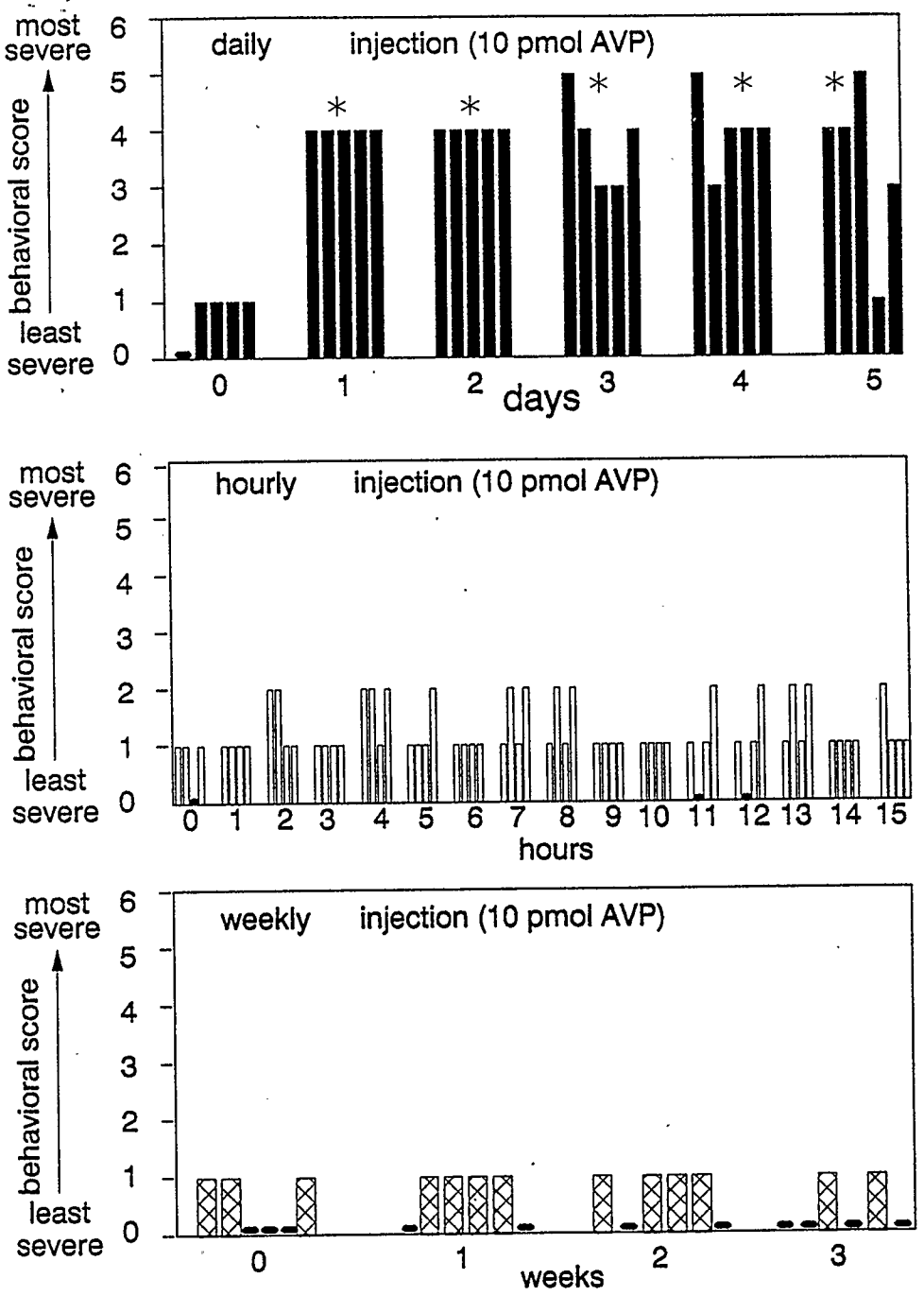

Figure 13. Time interval between AVP administrations. Each bar represent the maximal score each animal received following an AVP injection. Three groups of rats were given repeated daily (top panel), hourly (middle panel) or weekly (bottom panel) i.c.v. AVP (10 pmol) injections and their motor behavior scored. Asterisks indicate significant ($p < 0.05$, Friedman two-way analysis of variance by ranks) increase in the severity of the motor disturbances between the first and the subsequent AVP exposure.

duration of the sensitization), AVP sensitization was not revealed ($p > 0.05$, Friedman two-way ANOVA; figure 13, middle and lower panel). These results suggest that the sensitization of the rat brain to AVP appears to be dependent on the time interval between AVP exposures.

Assay of ^{3}H-inositol-1-phosphate (^{3}H-IP$_1$)

In order to determine the effects of central AVP pre-treatment on AVP stimulated cellular events, we measured AVP-induced ^{3}H-IP$_1$ accumulation in septal slice preparations of saline (control) or AVP-pretreated (sensitized; 10 pmol, 24 hrs earlier) animals. Central pretreatment with AVP did not affect the unstimulated phosphoinositide breakdown in the absence of AVP (18.7 ± 4.4 % controls versus 15.1 ± 3.2 % sensitized (n=3); ^{3}H-IP$_1$ accumulation was expressed as a ratio of the total label incorporated in the ^{3}H-PI fraction). Figure 14 shows that ^{3}H-IP$_1$ accumulation over basal levels in septal slices of AVP sensitized rats was significantly higher (35.4 ± 2.1% over basal; n=3, $p < 0.003$; two-way ANOVA, Scheffé multiple range test) than in septal slices from saline treated animals (6.1 ± 4.2 % over basal, n=3) at an AVP concentration of 0.5 μM. Carbachol (1 mM), used as a control for the assay procedure, resulted in a similar stimulation of ^{3}H-IP$_1$ accumulation over basal of 85 ± 12% in controls and 89 ± 18% in sensitized rats ($p > 0.87$; two-way ANOVA, Scheffé multiple range test, n=3; data not shown). In order to examine the specificity of the enhanced AVP stimulated ^{3}H-IP$_1$ accumulation in AVP sensitized rats, we tested the ability of oxytocin and the V2 receptor agonist DDAVP to stimulate ^{3}H-IP$_1$ accumulation. Figure 14, inset, shows

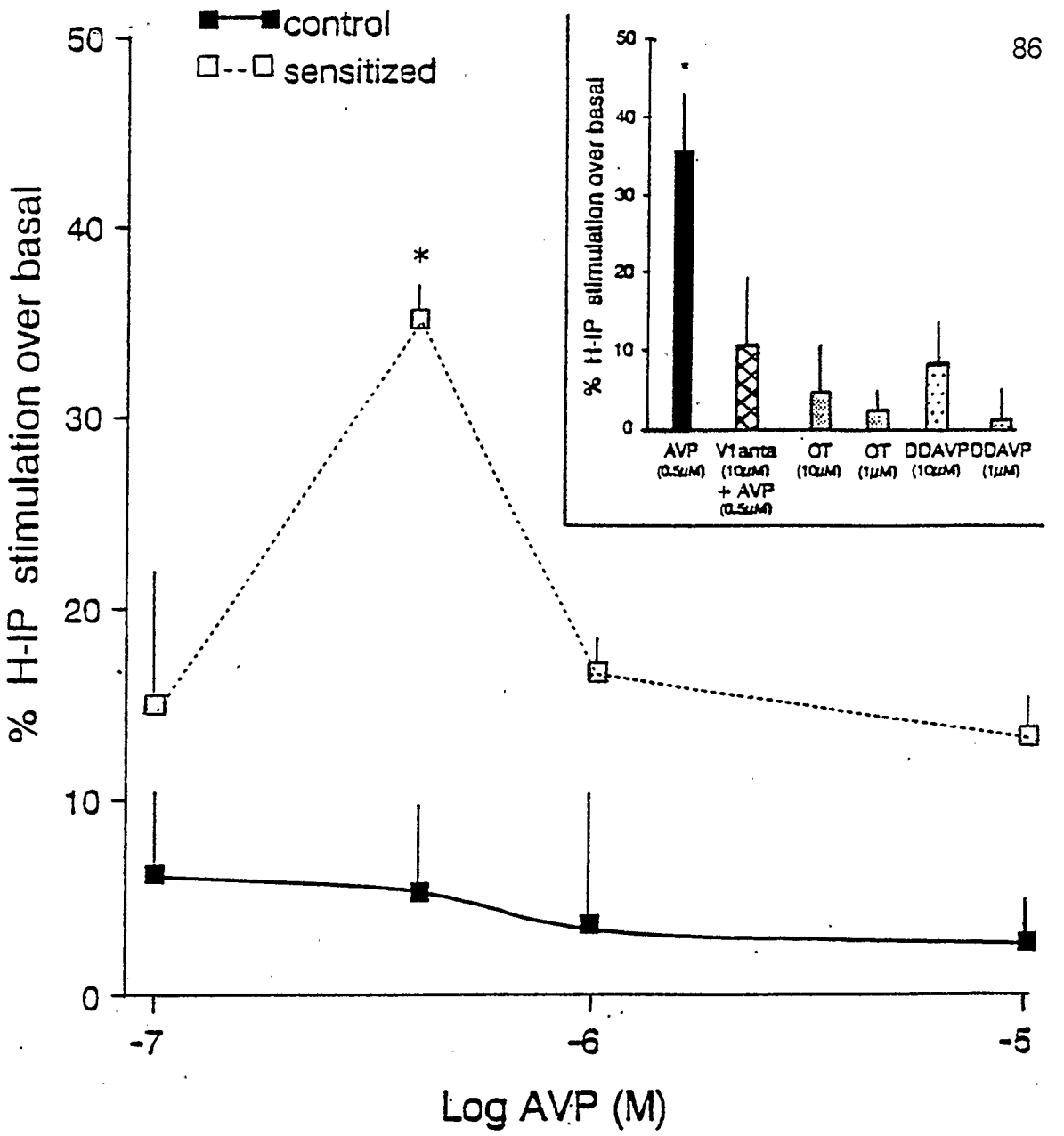

Figure 14. Dose-response relationship for AVP-stimulated ^{3}H-IP$_1$ accumulation in septal slices from non-sensitized saline pretreated controls (■–■) and from sensitized rats (□–□) pretreated, 24 hr earlier, with an i.c.v. AVP (10 pmol) injection. Inset: Pharmacological profile of AVP stimulation of ^{3}H-IP$_1$ accumulation in septal slices of sensitized animals following the addition of AVP (0.5 μM); 1 or 10 μM oxytocin (OT) alone; or 1 or 10 μM DDAVP alone. Values are means ± S.E.M. of 2 or 3 separate experiments each performed in triplicate. Data were analyzed using two-way ANOVA in conjunction with Scheffé multiple-range test. Asterisks indicate significant ($p < 0.05$) increase in ^{3}H-IP$_1$ accumulation over basal.

that both oxytocin and DDAVP at concentrations of 1 and 10 μM showed virtually no effects. To confirm that the enhanced AVP-induced ^{3}H-IP$_1$ accumulation in septal slices of sensitized animals is mediated by the V1 receptor, we tested the ability of the V1 antagonist d(CH$_2$)$_5$Tyr(Me)AVP to inhibit the enhanced AVP response. Figure 14, inset, shows that the addition of the V1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP (10 μM) caused a significant reduction of ^{3}H-IP$_1$ accumulation in response to AVP (0.5 μ^{M}) in sensitized rat septal slices (from 36.0 $\pm$ 7.8% to 11.0 $\pm$ 8.1%) ($p < 0.04$, Scheffé multiple range test). These results indicate that a V1 receptor may be involved in mediating the enhanced AVP stimulation of ^{3}H-IP$_1$ accumulation.

D. Discussion

In this study, we have shown that a single i.c.v. injection of AVP enhances (sensitizes) the motor response produced by a subsequent injection of AVP 6 hours to 6 days later. AVP sensitization is a dose dependent phenomenon, in that the greater the sensitizing dose of AVP the animals receive on the first AVP exposure, the greater the number of animals displaying severe motor disturbances on a second AVP exposure. AVP sensitization is also seen after multiple intermittent (daily) injections of AVP, but the importance of allowing time between treatments has been denoted by the observations that sensitization is not observed in rats that received hourly or weekly AVP administration. Although the enhanced motor actions of AVP in sensitized animals have been shown to be mediated via VSA V1 receptor (Burnard et al.1986), the phenomenon of AVP sensitization is not

readily explained by alterations in the number or affinity of septal ^{3}H-AVP binding sites. Rather, enhanced intracellular signal transduction (PI hydrolysis) may, in part, contribute to the enhanced motor response to AVP observed in sensitized rats.

Several studies have shown that in agonist-induced sensitization, the paradigm used for agonist administration is important in that increased responsiveness (agonist-induced sensitization) is observed following intermittent, low dose treatment regimens, whereas diminished responsiveness (agonist-induced tolerance) is observed after frequently repeated (or continuous administration), high dose treatments (for reviews, see Post, 1980; Robinson and Becker, 1986; Martin Iverson et al.1988). In agreement with these observations, our results and those of others show that AVP sensitization was observed in animals given intermittent (i.e. daily) administration of relatively low doses of AVP, whereas no AVP sensitization has been reported in animals implanted intraventricularly with an AVP-containing Accurel mini device which slowly and continuously released AVP centrally for 5 days (Shewey et al.1989). In addition, it has also been shown that a single agonist administration is sufficient to alter subsequent agonist responses for a long period of time. For example, a single injection of amphetamine enhanced the stereotypy (Ellison and Morris, 1981), and rotational behaviour (Robinson et al.1982a) produced by a subsequent injection given weeks later. Similarly, our behavioural data revealed that a single injection of AVP produces a long-lasting (for up to 6 days) enhancement in the motor response to a subsequent challenge with AVP. Thus, our results and those of others support

the observations that both the temporal pattern and the amount of agonist to which a receptor is exposed determine which regulatory mechanisms (sensitization or tolerance) are activated.

A number of hypotheses have been entertained to explain the phenomenon of agonist-induced sensitization (Kalivas et al.1985; Robinson and Becker, 1986; Vezina et al.1987; Martin Iverson et al.1988; Mitchell et al.1988; Di Paolo et al.1989; Karler et al.1989; Kita and Nakashima, 1989; Hurd et al.1990; Mobbs et al.1990a, 1990b; Wonnacott, 1990). In our study, we first tested the possibility that the enhanced responsiveness to AVP may be related to an enhanced number of septal AVP receptors. Our results indicate, however, that neither the number nor the affinity of ^{3}H-AVP binding to septal AVP receptors were altered in AVP sensitized animals. Because i.c.v. administration of AVP is expected to reach several brain regions also known to contain central V1 receptors including the LS and HPC (Jard et al.1987) and see chapter II), we investigated the possibility that these AVP receptors may also be upregulated by prior AVP exposure. Our results indicate that the LS and HPC AVP receptor appear to be similar to those of the VSA AVP receptor in that neither the number nor the affinity of ^{3}H-AVP binding to hippocampal or lateral septal AVP receptors were altered in response to prior AVP exposure [although the electrophysiological response of hippocampal cells to AVP was previously observed to be enhanced in sensitized animals (Burnard et al.1987)].

This apparent lack of septal AVP receptor "upregulation" concomitant with an increased responsiveness to a second AVP exposure may be explained, however, by a potentiation of intracellular signal(s) transduction mechanisms at some step beyond receptor occupation. Therefore, because previous AVP sensitization studies are indicative of an action of AVP at septal (Naylor et al.1985a) V1-like AVP receptors (Burnard et al.1986) and because septal V1 AVP receptors have been shown to be coupled to PI hydrolysis (Shewey and Dorsa, 1988), we tested the hypothesis that AVP sensitization could be related to enhanced ^{3}H-IP$_1$ accumulation in response to AVP stimulation in septal slices of sensitized animals. In these experiments, we found that (1) AVP failed to stimulate ^{3}H-IP$_1$ production in septal slices of rats that were not pretreated with AVP (2), that basal ^{3}H-IP$_1$ production was not altered by AVP pre-treatment and (3) that, in septal slices of AVP sensitized rats, the stimulation of ^{3}H-IP$_1$ in response to AVP was enhanced. Interestingly, this enhanced ^{3}H-IP$_1$ production in response to AVP was observed at an AVP concentration (0.5 μM AVP) at which Shewey and Dorsa (1988) previously found maximal ^{3}H-IP$_1$ stimulation in response to AVP in septal slices of Long Evans rats. In addition, using similarly sensitized male Wistar rats, Lebrun et al.(1990) found similar enhanced ^{3}H-IP$_1$ stimulation in response to AVP in septal slices. Taken together, our results and those of others suggest that the phenomenon of AVP sensitization is not readily explained by alterations in the number or affinity of V1 receptors. Rather, alterations in other components and

interactions of the AVP receptor effector complex seem to relate more closely to the sensitization phenomenon.

While the mechanism(s) by which the enhanced intracellular signal in response to AVP is brought about is yet unknown, if one considers the time required to attain sensitization (approximately 6 hours), it is tempting to suggest that AVP may cause an alteration in synthesis of a protein whose availability may play an important role in regulating the receptor function. For example, pre-exposure to LH-RH has been shown to cause a characteristic potentiation of subsequent secretory responses to LH-RH (Pickering and Fink, 1976, 1979) and a potentiation of inositol phosphate accumulation (Mitchell et al.1988). This enhanced response has been associated with protein synthesis which has recently been identified as HIP-70; an isoform of phosphoinositol-specific phospholipase C-α (Mobbs et al.1990a, 1990b). In this respect, it would be interesting to investigate whether AVP sensitization may utilize similar regulatory mechanisms. It would be interesting to test if there is a correlation between the time taken for the sensitization of the motor disturbances and the enhancement of the intracellular signal to develop.

Furthermore, it now becomes apparent that agonist-induced sensitization may be important for endocrine regulation in general in that repeated intermittent exposure to small doses of agonists may be used naturally or experimentally to induce a progressive enhancement in many agonist-induced responses (while avoiding the development of tolerance). Thus it would be interesting to investigate

whether the peripheral AVP actions may be similarly responsive to prior AVP exposure. Alteration in the availability of "factors" has already been suggested to play a role in the regulation of peripheral V1 receptor coupling to G-proteins in peripheral V1 receptor function (Bielinski et al.1988).

Although at first glance agonist-induced sensitization appears to be a phenomenon somewhat at variance with the generally accepted view that the presence of an agonist at the receptor site induces down-regulation and/or desensitization and/or tolerance phenomena, other agonist-induced sensitization-like phenomena have been observed before. For example, kindling has been described as a long-term enhancement of a response after repeated intermittent stimulus (either chemical or electrical) exposure (for review, see McNamara, 1988). Interestingly, similarities between the phenomenon of sensitization and the phenomenon of kindling have been described before (Post and Weiss, 1989). Similarly, because several lines of evidence suggest a role for AVP in kindling (Gillis and Cain, 1986; Greenwood et al.1989), it is tempting to suggest that the underlying mechanism(s) mediating the role of AVP in sensitization and kindling may be similar.

The physiological role of AVP-induced sensitization is not known, but the fact that the AVP system undergoes a sensitization process is of particular interest for at least two reasons: (1) Sensitization-like phenomena would be ideal for strengthening of synaptic connections and could be involved in the well known actions of AVP on learning and memory (Koob et al.1985, 1989). (2) Sensitization-

like phenomena may also play a role in other physiological actions of AVP (see chapter VI).

In summary, this study has shown that repeated intermittent AVP administration can induce motor disturbances ranging from mild pauses and prostration to severe barrel rotations and myotonus and myoclonus, in part depending on the number, duration and dose of AVP administrations. This progressive enhancement in the motor responses to AVP is not readily explained by alterations in the number or affinity of central AVP receptors. Rather, alterations in receptor effector coupling process(es) seem to relate more closely to the sensitization phenomenon.

IV. SEPTAL AVP RECEPTOR REGULATION IN RATS DEPLETED OF SEPTAL AVP FOLLOWING LONG-TERM CASTRATION

A. Introduction

Because long-term castration drastically reduces the AVP content of the VSA (De Vries et al.1986), in the present study we tested the possibility (1) that the brain of long-term castrated rats could not be sensitized to AVP by stimulus (hypertonic saline)-induced release of endogenous AVP, and (2) that the brain of long-term castrated rats would, however, have up-regulated VSA AVP receptors which, (a) following a central injection of AVP would result in a supersensitive response to the motor actions of AVP; (b) would show an increase in receptor numbers and/or affinity as measured by ^{3}H-AVP binding studies; (c) would show an increase in AVP-stimulated phosphoinositide hydrolysis measured in septal slices. We also measured AVP receptor properties in the lateral septal area (LS) and the hippocampus (HPC) because immunocytochemical studies of long-term castrated rats have demonstrated that immunoreactive AVP fibres are also depleted in these regions (De Vries et al.1986). Furthermore, as plasma testosterone level has been shown to alter AVP gene expression (Miller et al.1989) in the cells of the BST within 24 hours of treatment, and as steroid hormones have been shown to significantly alter central neurohypophysial receptor expression within 24 hours of steroid treatment (Johnson et al.1989) we also measured the characteristics of AVP receptors in short-term (24 h castrated) testosterone depleted rats.

B. Methods

Adult male Wistar rats (200-250 gm) were anaesthetized with sodium pentobarbital (50 mg/kg) and, under aseptic conditions, half of the rats were castrated. The remaining rats underwent sham surgery, in which the scrotum was opened and then sutured closed. The rats were housed in groups of four per cage on a 12 h light cycle and allowed food and water ad libitum.

AVP-induced motor disturbances.

Twenty weeks following castration, the animals were re-anaesthetized with sodium pentobarbital, and a stainless steel 23 gauge guide cannula was implanted stereotaxically to allow access to a lateral cerebral ventricle. Rats were then allowed 5 to 7 days to recover. Prior to experimentation, synthetic AVP (Bachem, Torrance, California) was made up into the appropriate concentrations using sterile, pyrogen-free physiological saline. Behavioural experiments were conducted 2 days apart in order to examine the effect long-term castration may have not only on the initial motor responses to a central AVP injection (1st exposure), but also to reveal possible effects of castration on the "sensitization" phenomenon of AVP-induced motor disturbances (2nd exposure) previously described (see chapter III). On each experimental day following each injection, the animals were placed in a large plexiglass chamber and observed for the development of motor disturbances for a 10 min period as described in chapter III. Experiments were all performed between 10:00 and 18:00 hours. Results are presented as the highest score each animal received throughout a 10 min observation period. Results were

statistically analyzed by the non-parametric Mann-Whitney U-Test and the Wilcoxon matched-pairs signed-ranks test.

Using the method described above two series of behavioural experiments were conducted. In the first set of experiments we tested the possibility that the brain of long-term castrated rats would not become sensitized by a stimulus known to release endogenous AVP. On the first experimental day (1st exposure), castrated rats (n=6) or sham controls (n=7) were given an intraperitoneal (i.p.) injection of 1.0 ml of 1.5 M hypertonic saline to stimulate endogenous release of AVP into the brain (Demotes Mainard et al.1986; Landgraf et al.1988), or as control, 1.0 ml of physiological saline i.p. (n=4 for each group). Two days later (2nd exposure), all animals received an i.c.v. (i.c.v.) injection of 100 pmol of AVP in 5 μl saline or physiological saline alone and their motor responses recorded as described above.

In the second set of experiments we tested the possibility that the brain of long-term castrated rats would have up-regulated VSA AVP receptors which, following a central injection of AVP, would result in a supersensitive motor response to AVP. On the first experimental day (1st exposure) castrated rats (n=18) or sham controls (n=14) were given an i.c.v. injection of AVP (10 pmol in 5 μl saline). Two days later (2nd exposure), all animals received an i.c.v. injection of AVP [10 pmol (n=8, controls; n=9 castrated) or 1 pmol (n=6, controls; n=8, castrated)] in 5 μl saline and the behaviour of the animals was scored as described above. As additional controls, on each experimental day in addition to

receiving AVP i.c.v., each animal was given a control injection of the vehicle alone (physiological saline, 5μl i.c.v.) and the behaviour of the animals scored as described above.

Radioligand binding studies.

Twenty weeks or 24 h following castration or sham surgery (n = 30 per group), rats were decapitated, and the brains rapidly removed and partially purified SPM from the VSA, LS and HPC of rat brains were prepared. In addition, because receptor upregulation may also be observed as an increase in the rate of receptor synthesis or as a decrease in receptor degradation and because the presence of receptors in the "microsomal" membrane fraction has been suggested to reflect receptors that may be in the process of synthesis or degradation (Burt, 1985), we also prepared microsomal membrane fractions of the VSA and LS. Binding studies were conducted as previously described in chapter II.

Data from saturation experiments were analyzed using a computerized, interactive curve fitting procedure to generate representative Scatchard plots. Statistical analysis comparing Scatchard plot data from castrated rats and sham controls was performed using regression analysis (Kleinbaum et al.1988), to consider two basic questions: 1) Are the two slopes ($1/K_d$) the same or different? 2) Are the two x intercepts (B_{max}) the same or different?.

Assay of [^{3}H]Inositol-1-phosphate (IP_1)

Brain septal slices (250 x 250 μm) from long-term castrated rats or sham controls (n = 5 animals per group, in each experiments) were prepared and the

accumulation of $^{3}H\text{-}IP_1$ measured as described in chapter III. Results were statistically analyzed using two-way analysis of variance (two-way ANOVA) for all dose-response curves, and where further post hoc testing was required, the Scheffés' multiple-range test was used. Values of $p < 0.05$ were considered significant.

C. Results

AVP-induced motor disturbances.

A first experiment was designed to establish whether long-term castrated rats were functionally deficient (in terms of AVP-induced motor disturbances), in AVP content of the VSA. In this first experiment, the method used was essentially that of Burnard et al.(1983) in which a hypertonic stimulus is used to stimulate the central release of AVP from endogenous stores to sensitize the rat brain to the motor effects of subsequent injections of AVP. Figure 15 illustrates the behavioral scores of long-term (20 weeks) castrated rats and of sham controls in response to an injection of 100 pmol AVP i.c.v. 2 days after i.p. injection of either 1.0 ml of hypertonic (1.5 M) saline or 1.0 ml of physiological saline as control. As seen in figure 15, AVP-induced motor disturbances in sham control rats that received the hypertonic saline pre-treatment are significantly higher ($p < 0.008$, Mann Whitney U-test) than the AVP-induced motor disturbances observed in sham controls that received the control physiological saline pre-treatment. The behavioral scores show that sham control rats which received the hypertonic saline pre-treatment displayed

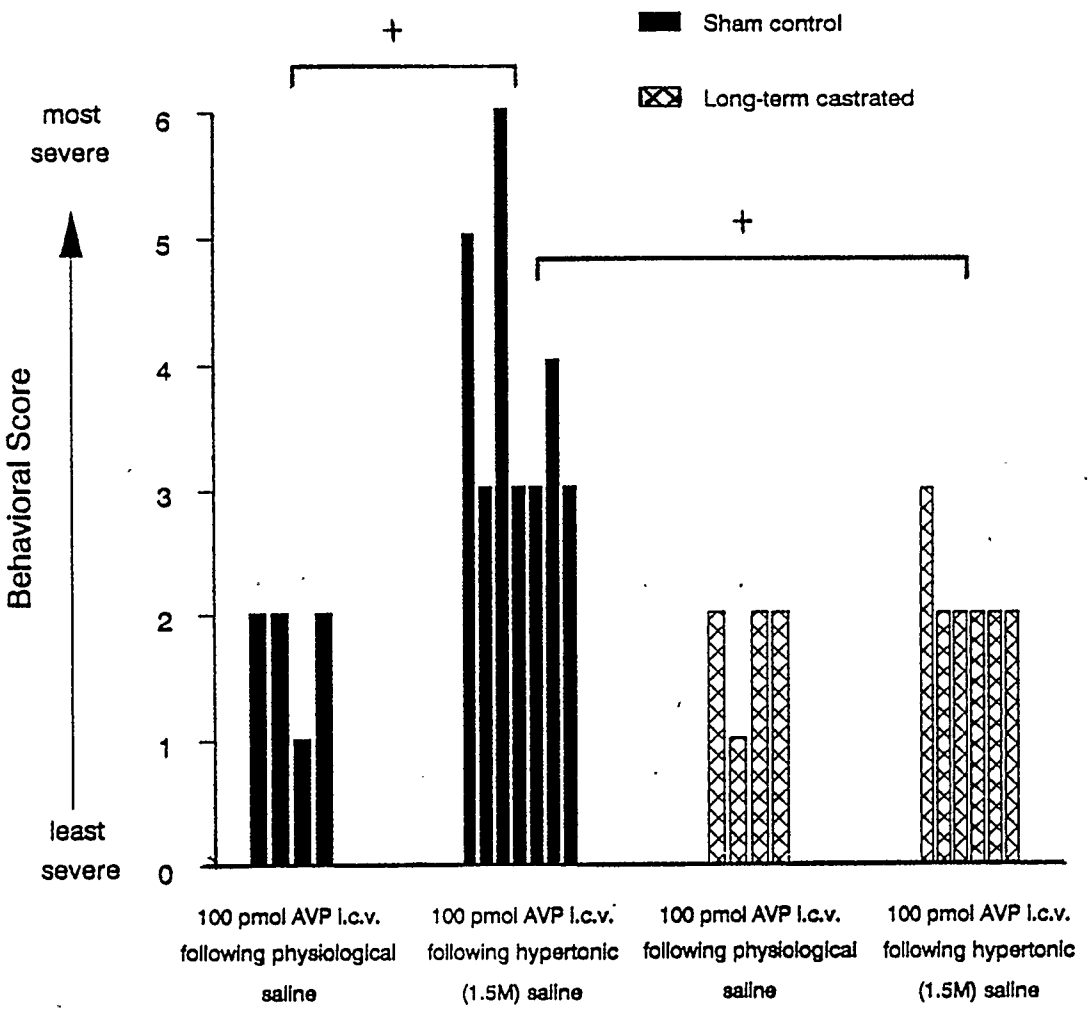

Figure 15. Endogenous release of AVP. Each bar represents the most severe behavioral score of a sham control (filled bars) or a long-term castrated male Wistar rat (hatched bars) in response to 100 pmol AVP i.c.v. two days after receiving, intraperitoneally, either 1 ml of physiological saline (n=4, each group) or 1 ml of 1.5 M hypertonic saline (n=6, controls; n=7, castrated). Each bar represents the behavioral score of an individual animal. (+) indicate significant difference ($p < 0.05$, Mann-Whitney U-test).

locomotor difficulties and head swaying (n = 4), barrel rotation (n = 1), myotonus and myoclonus (n = 1) and even death (n = 1). Motor disturbances of this magnitude are not normally seen following a first injection of 100 pmol AVP i.c.v. (chapter III). This suggests that, in sham controls, hypertonic saline pre-treatment on day 1 mimics the previously described effect (Burnard et al.1983) of an i.c.v. injection of AVP on day 1, in causing sensitization and hence increased behavioural responses to the second i.c.v. injection of AVP.

In contrast, the motor responses of long-term castrated rats to the injection of 100 pmol of AVP i.c.v., following either the physiological saline or the hypertonic saline pre-treatments, are not significantly different. All rats exhibited minor motor disturbances rating scores of 1 (ataxia) and 2 (prostration) and one rat exhibited some locomotor difficulties. These behaviours are also seen following a first injection of 100 pmol AVP i.c.v. (chapter III) indicating that in castrated rats, no sensitization occurred following the hypertonic saline pre-treatment. It can therefore be seen in Fig. 15 that sham control rats displayed increased sensitivity to 100 pmol of AVP i.c.v. when compared to castrated rats ($p < 0.004$, Mann Whitney U-test) following hypertonic saline i.p. pre-treatment.

A second series of behavioural experiments was conducted in order to examine whether the prolonged lack of AVP in the VSA has rendered the AVP receptor supersensitive to exogenous AVP administration. Figure 16 illustrates the behavioural scores of long-term (20 weeks) castrated rats and sham controls following a first (1st exposure) and a subsequent (2nd exposure) i.c.v. injection of

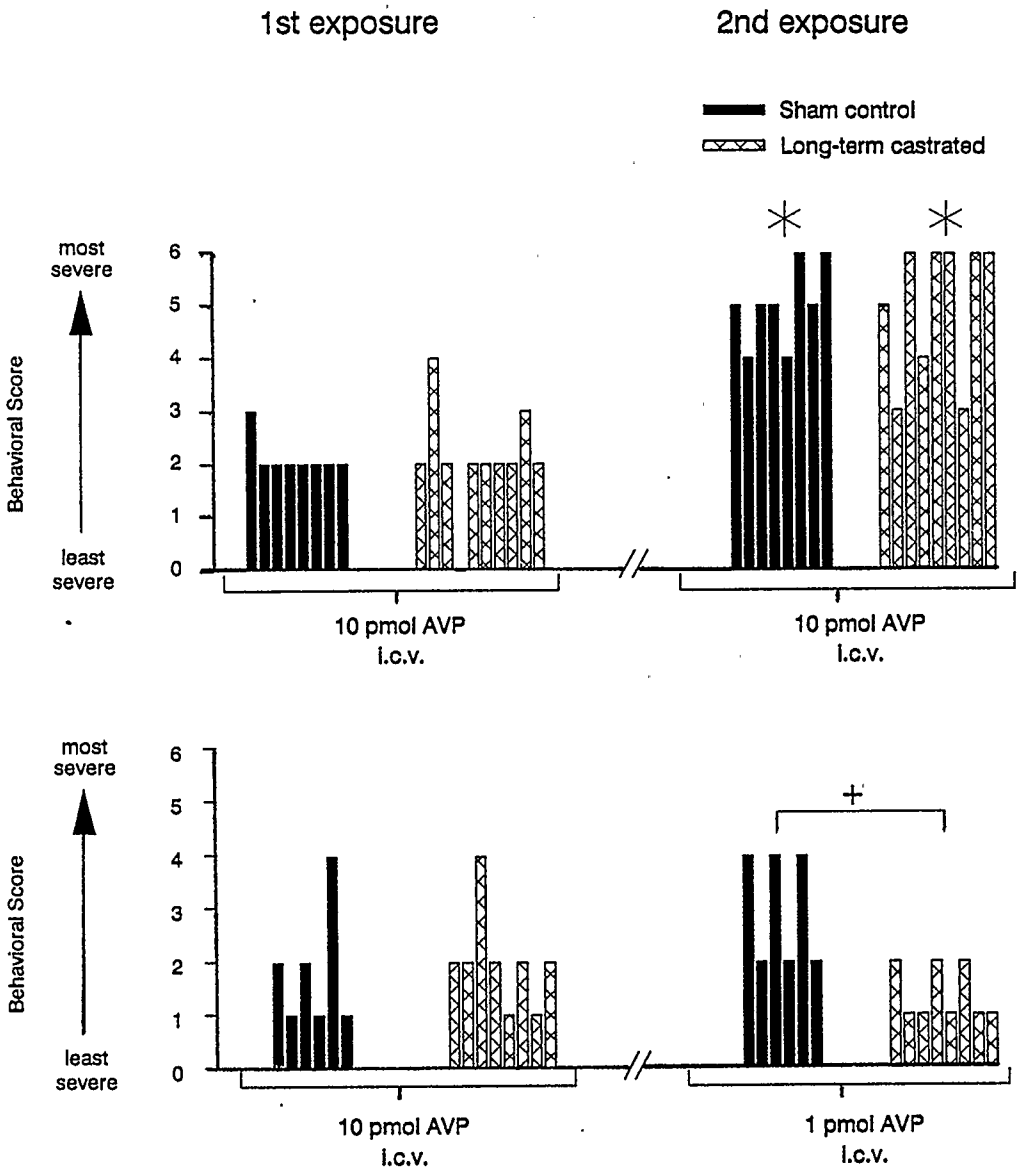

Figure 16. Lack of behavioral supersensitivity following castration. Each bar represents the most severe behavioral score of a sham control (filled bars) or a long-term castrated (hatched bars) male Wistar rat in response to AVP i.c.v.. In the top panel, animals received 10 pmol AVP i.c.v. on the 1st and the 2nd exposure. In the bottom panel, animals received 10 pmol AVP i.c.v. on the 1st exposure and 1 pmol AVP i.c.v. on the 2nd exposure. Asterix (*) indicate significant ($p < 0.05$; Wilcoxon matched-pairs signed ranks test), and (+) indicate significant differences ($p < 0.05$ Mann-Whitney U-test).

AVP. As indicated in figure 16, on the first day of exposure to AVP i.c.v. (10 pmol), both sham controls and castrated rats displayed minor behavioural responses consisting of ataxia, prostration and some locomotor difficulties. No significant differences were observed between sham controls and castrated rats ($p > 0.5$ Mann-Whitney U-test). When the animals were retested two days later (2nd exposure), however, it can be seen that a second injection of AVP i.c.v. induced enhanced motor responses in a dose dependent manner, in that; (1) at the highest doses of AVP (10 pmol AVP i.c.v. on the 1st exposure and 2nd exposure, figure 16, top panel), the motor responses of the animals on the 2nd exposure were significantly increased ($p < 0.001$, Wilcoxon matched pairs signed-ranks test) from those of the 1st exposure, with both groups of animals displaying severe motor disturbances including barrel rotation, convulsions and death. In the top panel, it can be seen that no significant differences in the AVP-induced motor disturbances either on the 1st exposure or on the 2nd exposure were observed between castrated and sham control rats ($p > 1.0$ top panel; Mann-Whitney U-test). Thus although it appears that there are no significant differences in the motor actions of centrally injected AVP either on the 1st exposure or on the 2nd exposure, it could be that on the 2nd exposure too high of a dose was used since near maximal motor disturbances was observed even in the control group. Therefore a sub-maximal dose of AVP was used on the 2nd exposure in another group of rats. As can be seen in Fig. 16 bottom panel, when lower doses of AVP (10 pmol AVP i.c.v. on the 1st exposure and 1 pmol AVP i.c.v. on the 2nd exposure; bottom panel)

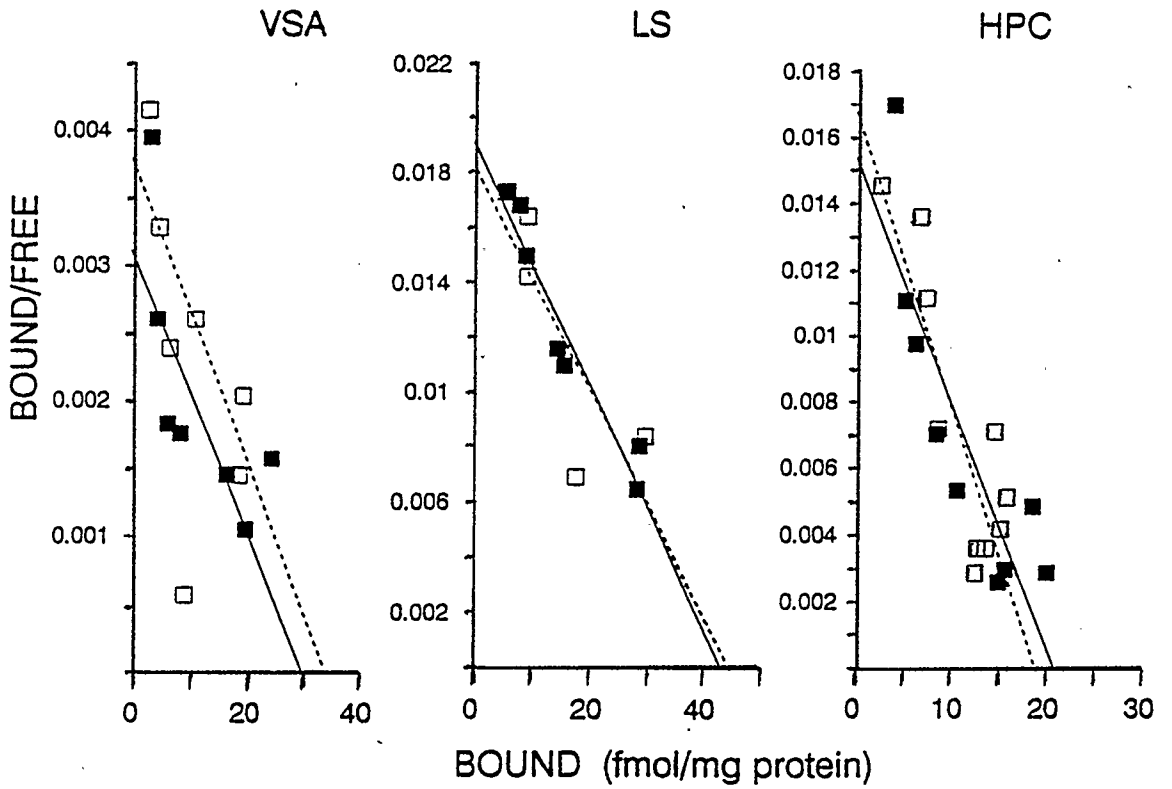

Figure 17. Scatchard plot analysis of ^{3}H-AVP specific binding in SPM prepared from the VSA, LS and the HPC of sham controls (■–■) or long-term (20 weeks) castrated (□) male Wistar rats. Each point is the mean of triplicate determinations.

were used on the 2nd exposure castrated rats were shown to be somewhat less sensitive to the motor effects of 1 pmol AVP than sham controls ($p < .02$; Mann-Whitney U-test). In all cases, i.c.v. saline injections did not elicit any motor disturbances rating greater than a score of 1 (data not shown). An additional control experiment was conducted in which one group of animals was given an i.c.v. saline injection on the 1st exposure followed by 10 pmol of i.c.v. AVP on the 2nd exposure two days later. In this experiment, the behavioural scores of animals on the 2nd exposure to 10 pmol AVP i.c.v. did not significantly differ from behavioural scores of animals given 10 pmol i.c.v. AVP on the 1st exposure, indicating that an injection of saline i.c.v. on the 1st exposure is insufficient to sensitize the rat brain to AVP-induced motor disturbances (data not shown). It can therefore be concluded that, when compared to sham controls, long-term castrated rats did not develop a supersensitive response to the motor effects of centrally injected AVP. Long-term castrated rats may even be somewhat less sensitive to the motor actions of centrally injected AVP.

Binding studies.

The effect long-term castration may have on AVP receptor number and/or affinity was measured. The binding of ^{3}H-AVP to SPMs prepared from the VSA, LS and HPC of long-term castrated rats or sham controls was measured using saturation experiments. In all cases, binding of ^{3}H-AVP to SPMs was saturable over ligand concentrations from 0.1 to 10 nM and specific binding of up to 70% was observed. Scatchard analysis of ^{3}H-AVP binding to VSA, LS, and HPC SPMs

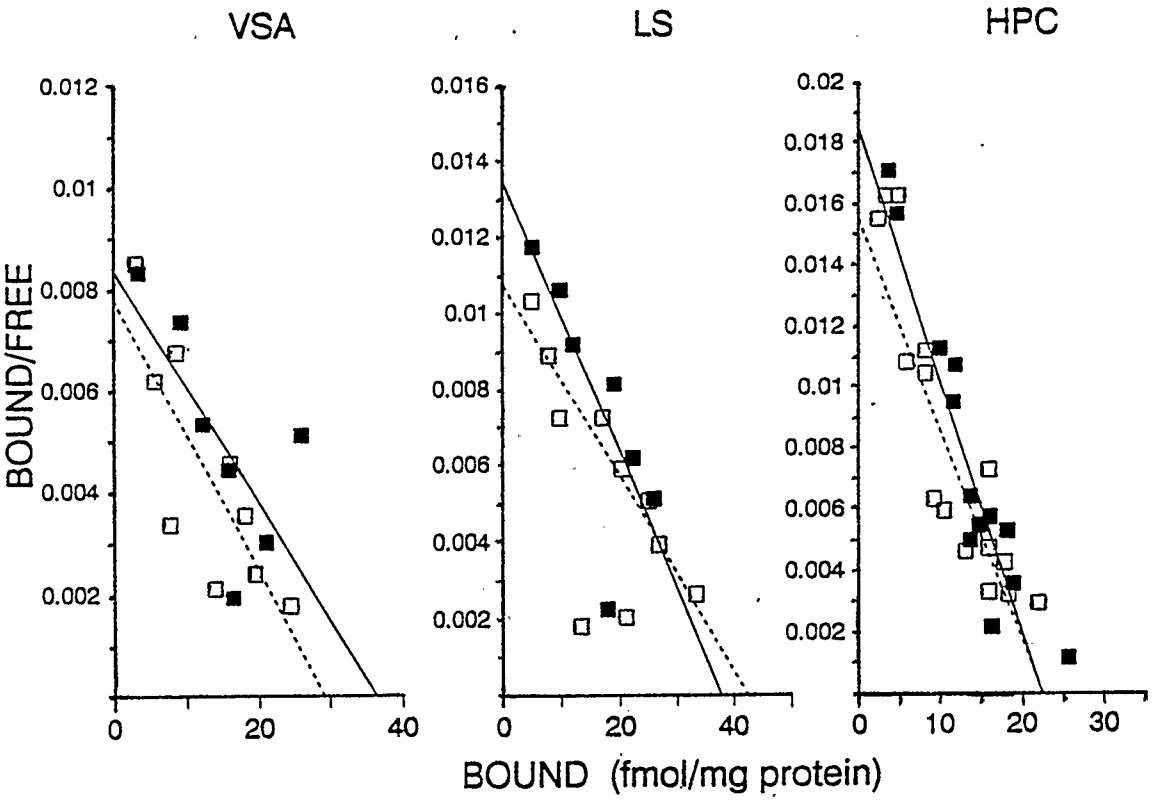

Figure 18. Scatchard plot analysis of ^{3}H-AVP specific binding in SPM prepared from the VSA, LS and the HPC of sham controls (■–■) or short-term (24 h) castrated (□) male Wistar rats. Each point is the mean of triplicate determinations.

yielded linear plots (Figure 17 and 18) which is consistent with a single binding site model. Each linear plot could be used for estimating apparent dissociation constants (K_d; 1/slope) and binding sites concentration (B_{max}; x intercept). Figure 17 shows that the K_d values of the binding site for ^{3}H-AVP in brain tissues of sham controls were not significantly different from the K_d values obtained from brain tissues of long-term castrated rats. The K_d values of the AVP binding sites from sham controls and long-term castrated rats were calculated to be 3.2 vs 3.0 nM, $p > 0.94$, VSA; 0.76 vs 0.82 nM, $p > 0.89$, LS; and 0.47 vs 0.57 nM, $p > 0.49$, HPC, respectively. Figure 17 also shows that the binding sites concentration (B_{max}) for ^{3}H-AVP from sham controls was not significantly different from the B_{max} for ^{3}H-AVP from long-term castrated rats. The B_{max} values were calculated to be 30 vs 33 fmol/mg protein, $p > 0.48$, VSA; 44 vs 45 fmol/mg protein, $p > 0.84$, LS; and 22 vs 20 fmol/mg protein, $p > 0.89$, HPC, respectively. These results suggest that long-term castration does not alter ^{3}H-AVP binding site apparent dissociation constant (K_d) or ^{3}H-AVP binding sites concentration (B_{max}) in SPMs of any regions of the rat brain studied.

Since the presence of receptors in the "microsomal" membrane fraction has been suggested to reflect receptors that may be in the process of synthesis or degradation (Burt, 1985) we also investigated AVP binding sites concentration in the microsomal membrane fraction in the VSA and in the LS of sham-controls and long-term castrated rats. ^{3}H-AVP binding sites concentration of the microsomal membrane fraction from sham controls and from long-term castrated rats were

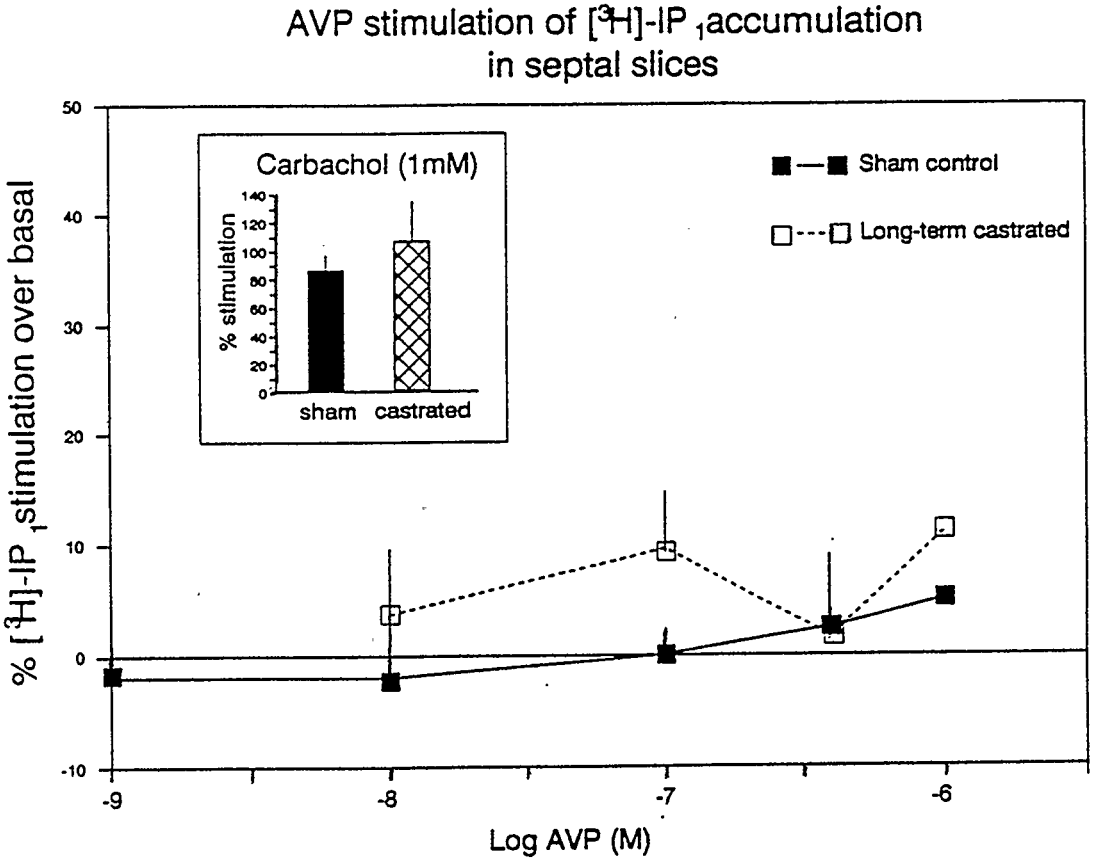

Figure 19. Dose-response curves for AVP-stimulated ^{3}H-IP$_1$ accumulation in septal slices from long-term castrated and sham control male Wistar rats. Values are means ± S.E.M. of 3 separate experiments performed in triplicates. (no S.E.M. is shown at 0.001 and 1 μM AVP because the experiment was done only once). Inset: Carbachol-induced ^{3}H-IP$_1$ accumulation in septal slices from long-term castrated and sham control male Wistar rats.

calculated to be 40 and 38 fmol/mg protein, VSA; 42 and 50 fmol/mg protein, LS respectively (data not shown).

AVP receptor regulation was also measured in short-term testosterone depleted (24 h castration) rats. As can be seen in figure 18, the K_d for ^{3}H-AVP binding sites measured in membranes prepared from sham controls VSA SPMs is not significantly different from the K_d of ^{3}H-AVP binding sites from short-term castrated rats VSA SPMs (1.5 vs 1.2 nM; $p > 0.64$). Similar results were obtained when binding studies were performed in membranes prepared from the LS (0.95 vs 1.4 nM; $p > 0.44$) or the HPC (0.49 vs 0.48 nM; $p > 0.41$). Taken together, the K_d values of the binding site for ^{3}H-AVP in brain tissues of sham controls were not significantly different from those obtained from brain tissues of short-term castrated rats. Figure 18 also shows that the B_{max} for ^{3}H-AVP in VSA membranes prepared from sham controls was not significantly different from the B_{max} for ^{3}H-AVP in VSA membranes prepared from short-term castrated rats (38 vs 30 fmol/mg protein; $p > 0.2$). Similar results were obtained when binding studies were performed in membranes prepared from LS (38 vs 41 fmol/mg protein; $p > 0.17$) or HPC (23 vs 23 fmol/mg protein; $p > 0.85$). These results indicate that short-term hormone depletion (castration) does not appear to alter the AVP binding site dissociation constant or concentration in any of the regions of the rat brain studied.

Assay of [^{3}H]Inositol-1-phosphate (IP_1)

To determine the effect septal AVP depletion, caused by long-term castration of rats, has on septal AVP post receptor mechanisms, we measured

AVP-induced ^{3}H-IP$_1$ accumulation in septal slice preparations. We have previously shown (chapter III) that although PI hydrolysis induced by AVP is not detectable in control rats, our assay procedure is sensitive enough to detect the enhanced PI hydrolysis in sensitized rats. In keeping with these observations, figure 19 shows that AVP induced no significant ($p > 0.9$; two-way ANOVA) ^{3}H-IP$_1$ accumulation in septal slices either from long-term castrated rats or from sham controls over AVP concentrations of 10^{-9} to 10^{-6} M. Although carbachol (1 mM) resulted in an increase in the stimulation of ^{3}H-IP$_1$ accumulation over basal (85% ± 10% in sham controls and 105% ± 28% in castrated rats), no significant differences were observed between the two groups ($p > 0.89$; Scheffé's Multiple range test).

D. Discussion

The VSA has been shown to be a site in which AVP acts to cause motor disturbances (Naylor et al.1985a). We and others (Kasting et al.1980; Burnard et al.1983, 1985, 1986; Wurpel et al.1986a; Balaban et al.1988) have previously demonstrated that the motor disturbances produced by central injections of AVP involve a "sensitization" phenomenon in that a first injection of AVP increases the severity of the motor disturbances caused by subsequent central injections of AVP. This phenomenon was also shown following central release of endogenous AVP. For example, Burnard et al.(1983) demonstrated that in rats, an i.p. hypertonic saline pre-treatment can mimic the effect of a central injection of AVP in sensitizing the brain to the motor effects of subsequent injections of AVP. These results were

interpreted to suggest that hypertonic saline pre-treatment caused release of central AVP such that the rat brain became "sensitized". In agreement with this, additional evidence from several laboratories has also shown that i.p. hypertonic saline treatment causes release of central AVP (Demotes Mainard et al.1986; Landgraf et al.1988). We report here that more than five months after castration, in the absence of AVP in the VSA, no sensitization of the rat brain was obtained using stimulus-induced release of endogenous AVP. We interpret our results to suggest that i.p. hypertonic saline pretreatment in long-term castrated animals does not sensitize the rat brain to AVP-induced motor disturbances possibly because no AVP was released in the VSA. The lack of stimulus-induced sensitization of the rat brain to AVP further supports previous findings suggesting that the reduction of the AVP content in the VSA following long-term castration appears to be functionally significant (Pittman et al.1988). The present study was also designed to investigate the properties of the brain AVP receptor following removal of its ligand, as removal or absence of a receptor ligand has been shown to typically result in an up-regulation of receptors and in the development of behavioural supersensitivity (Catt et al.1979; Klein et al.1989). Thus in view of the fact that long-term castration of adult male rats causes a dramatic reduction in the AVP content of the VSA where sensitive sites for the motor effects of centrally injected AVP have been identified (Naylor et al.1985a), we hypothesized that long-term castration of adult male rats could result in the animals becoming "supersensitive" to the motor effects of centrally injected AVP and in the

"up-regulation" of VSA AVP receptors. We report here that no "supersensitivity" in VSA AVP receptors was found in that, (1) the motor effects of centrally injected AVP were no greater in long-term castrated rats than in sham controls (2), neither the number of AVP receptors nor the apparent affinity of the receptor sites, as determined by ^{3}H-AVP binding, was altered by long-term castration and (3), AVP stimulation of phosphoinositide hydrolysis in septal slices was not increased in long-term castrated rats. In addition, since receptor up-regulation may also be observed as an increase in the rate of receptor synthesis or as a decrease in receptor degradation and since the presence of receptors in the "microsomal" membrane fraction has been suggested to reflect receptors that may be in the process of synthesis or degradation (receptor in vesicular compartments derived from receptor-mediated endocytosis and recycling; Burt, 1985), we also investigated the AVP binding site concentration in the microsomal membrane fraction in the VSA and in the LS of sham-controls and long-term castrated rats. When ^{3}H-AVP specific binding concentration is compared between the SPM and the microsomal membrane fraction, equivalent concentrations of ^{3}H-AVP binding sites are observed in these membrane fractions. These results are similar to what has previously been shown (chapter III) and further suggests that the rate of synthesis and degradation of ^{3}H-AVP binding sites does not appear to differ between sham controls and long-term castrated rats.

The VSA of long-term castrated rats contains some residual AVP (Pittman et al.1988), although a markedly reduced amount compared to that of the control

animals, thus it is possible that it may be sufficient to maintain AVP receptors under normal regulation. It does not seem, however, that we can explain our failure to find an "up-regulation" of VSA AVP receptors by a failure of the long-term castration to functionally reduce the AVP content of the VSA (see above discussion).

Our study, therefore, suggests that the septal AVP receptor system displays potentially unique properties in that the reduction in the concentration of its ligand, during adulthood, fails to "up-regulate" the receptor and fails to result in a behavioural "supersensitivity" to AVP. In this respect, it resembles the V1 receptor in the periphery which also appears to be relatively independent of the level of circulating AVP (Butlen et al.1984). To explain the lack of dynamic regulation of the V1 receptor, one might consider that receptor supersensitivity has been proposed to be a compensatory homeostatic response to the loss of stimulation by ligand. Thus supersensitivity to a ligand would be expected to occur in tonically active systems. In contrast, in systems not tonically active, the removal of the ligand may not be expected to produce a supersensitivity response because no great loss in basal ligand stimulation would occur. As the loss of vasopressinergic innervation of the VSA did not result in a compensatory supersensitivity response in VSA AVP receptors, our results would therefore suggest that the central VSA AVP system may not be tonically active. This may well be true as several months after castration are required before immunoreactive vasopressin is eliminated from

the BST cells (De Vries et al.1986), despite an almost immediate arrest in AVP synthesis (Miller et al.1989).

Receptor "supersensitivity" has been described mainly under conditions [including denervation, chronic treatment with antidepressants and neuroleptics (for example, see (Jenner and Marsden, 1987)] that decrease the synaptic concentration of transmitter in a very short period of time (days). It is therefore possible that because the depletion of central AVP level is occurring very slowly after castration (De Vries et al.1986), the receptor system may have the time to "adjust" to this slowly changing agonist concentration which may explain why no alteration in receptor is observed five months following castration despite an almost complete depletion of VSA AVP stores.

It is also possible that castration may cause a transient alteration in central AVP receptors. Our results, however, demonstrated that AVP receptor regulation is not affected 24 hours after castration. This is an important observation because neurohypophysial hormone receptors expression have been shown to be directly influenced by steroid hormones within 24 hours of steroid treatment (Johnson et al.1989). Direct steroid influences on neurohypophysial hormone receptors have also been observed in other systems. For example, Voorhuis et al.(1988) have shown that testosterone increases the density of ^{3}H-AVP binding sites in certain brain nuclei of birds. In the rat, de Kloet et al.(1986) have shown that oestradiol treatment increases the density of the central oxytocin-vasopressin receptors in discrete brain regions. It is interesting to note that these investigators have

suggested that the putative V1-type of AVP receptor, located in the septal area of the rat brain, does not appear to be responsive to this steroid treatment (de Kloet et al.1986). A recent report by Tribollet et al.(1990) also indicates that whereas the oxytocin receptor in the brain is regulated by steroids, the V1 receptor concentration is unaltered by manipulating gonadal hormone levels.

While in our study the elimination of androgen hormones following castration did not alter the affinity and/or number of central AVP receptors, nor did it affect the post-receptor response of VSA AVP receptors, this does not argue against the possibility that AVP receptor regulation may, in itself, be dependent upon steroid hormones derived from the testes. It may be that the absence of such circulating steroids (eg. androgens) would prevent the up-regulation of the AVP receptors which might normally result from reduced ligand exposure. In fact, Antoni et al.(1985) demonstrated that rat anterior pituitary AVP receptors appear to be regulated through a mechanism that requires the presence of the adrenal glucocorticoid hormone, corticosterone. Although some central actions of AVP have been shown to be altered by gonadal steroids (Albers et al.1988), the present results do indicate that the induction of the "sensitization" phenomenon following central AVP administration does not appear to be dependent upon circulating steroids, because a second exposure to AVP causes enhanced behavioural responses in both sham controls and long-term castrated rats.

Using Homozygous Brattleboro rats, which are also deficient (genetically) in AVP, Burnard et al.(1985) have shown that Brattleboro rats are "supersensitive"

to the motor effects elicited from central AVP injections when compared to Long-Evans. In keeping with these behavioural observations, Shewey and Dorsa (1986) have shown that Brattleboro rats have an apparent increase in the number of septal AVP receptors (as well as a lower affinity), when compared to their heterozygous littermates. In addition, Brattleboro rats were also shown to display increased AVP-stimulated phosphoinositol hydrolysis (Shewey et al.1989) in septal slices. While these results suggest that the absence of AVP may result in septal AVP-receptor up-regulation, it is possible that the absence of AVP during a "critical" neonatal period in Brattleboro rats, but not on long-term castrated rats, results in differences in receptor regulation. In fact, neonatal AVP exposure has been shown to decrease the number of vasopressin binding sites in adult animals (Handelman and Sayson, 1984).

Besides self-regulation, the regulation of central AVP receptors may depend on other factors. For example, Antoni et al.(1985) showed that oxytocin was equipotent with AVP in regulating anterior pituitary AVP receptors. Interestingly, long-term castration has been shown to be without effect on the oxytocin content of the VSA, LS or HPC (De Vries et al.1986; Pittman et al.1988). It is therefore possible that this ligand is capable of maintaining normal regulation of central AVP receptors in long-term castrated animals. In this respect, we have obtained preliminary evidence that oxytocin may indeed be important in the regulation of central AVP receptor function in the rat brain (see chapter V).

It is also possible that age may alter AVP receptor regulatory mechanisms most particularly since it has been shown that age affects AVP levels in a number of areas of the rat brain (Fliers et al.1985). Thus an additional consideration is that both castrated and sham control rats used in our study were over 8 months of age. Our results indicate that VSA AVP receptors in older animals, (both long-term castrated or sham controls), appear to have a somewhat lower affinity for AVP (3.2 and 3.0 nM, respectively) as compared to VSA AVP receptors affinity of younger (both short-term castrated and sham controls; 1.5 and 1.2 nM, respectively) animals. Although it is possible that as animals age receptor regulation become less responsive, such animals were, in our study, able to display the expected "sensitization" to AVP seen in younger animals.

In conclusion, our study shows that the septal AVP receptor displays potentially unique properties. Firstly, as has been previously shown, repeated treatments with AVP results in the "sensitization" of the rat brain to AVP. Secondly, our study further suggests that the septal AVP receptor may not behave in a typical fashion since it is not subject to the development of the up-regulation typically observed after removal of a ligand (even though the removal of the ligand appears functionally significant).

V. OXYTOCIN PRE-TREATMENT ENHANCES AVP-INDUCED MOTOR DISTURBANCES AND AVP-INDUCED PHOSPHOINOSITOL HYDROLYSIS IN RAT SEPTUM

A. Introduction

Recently a ^{3}H-oxytocin (^{3}H-OT) binding site which has been found to bind both OT and AVP with high affinity has been characterized and described as an OT-AVP receptor in the limbic system (Ferrier et al.1983) and the hippocampus (Audigier and Barberis, 1985; Elands et al.1988a, 1988b) of the rat brain. Studies using the V1 antagonist, d$(CH_2)_5$ Tyr(Me)AVP, indicated that the V1 receptor may be involved in AVP-induced sensitization (Burnard et al.1986). This V1 antagonist d$(CH_2)_5$ Tyr(Me)AVP, however, has also recently been shown to lack selectivity in that, although it discriminates between the V1 and the V1b or the V2 binding sites (for review, see Jard et al.1987) it displays considerable antioxytocic activity (Kruszynski et al.1980) and has been found to bind with high affinity the OT-AVP receptor (Di Scala-Guenot et al.1990). The role of this OT-AVP binding site is not known at the present time but the unexpected high affinity of the OT-AVP receptor for both OT and AVP raises the possibility that this OT-AVP receptor may be involved in some of the central actions of AVP.

This study was therefore undertaken to test the possibility that the OT-AVP receptor may be involved in the sensitizing actions of centrally injected AVP. Thus, we compared the ability of the specific OT-AVP receptor antagonist, compound 6, and the V1 AVP receptor antagonist, d$(CH_2)_5$ Tyr(Me)AVP, to block AVP

sensitization. We also tested if OT and the OT-AVP receptor agonist, [Thr^4,Gly^7]OT were able to induce sensitization and to induce the enhanced motor responses.

B. Methods

Adult male Sprague Dawley rats (200-250 gm) were anaesthetized with sodium pentobarbital (50 mg/kg) and a stainless steel 23-gauge guide cannula was implanted stereotaxically under aseptic conditions to allow access to a lateral cerebral ventricle. During the 5-7 days recovery, the rats were housed in groups of four per cage on a 12 h light/dark cycle and allowed food and water ad libitum.

In this study a standard AVP sensitization protocol (see chapter III) was used in which a first exposure to AVP or to a peptide solution was given to sensitize the animals to a subsequent (24 hrs later) AVP or peptide administration. On each experimental day (between 10:00 and 18:00 hours), i.c.v. injections of peptide solution in sterile, pyrogen free, physiological saline, were administered by gravitational flow through a 27-gauge injection cannula into awake, freely moving animals. After each injection, the animals were placed in a large plexiglass chamber and observed for the development of motor disturbances for a 10 min period. Motor disturbances were defined and scored at 1 minute intervals on a scale modified from that developed by Kasting et al.(1980) as described in chapter III. Results were statistically analyzed by the non-parametric Mann-Whitney U-Test, the Wilcoxon matched-pairs signed-ranks test.

Behavioural observations

In the first set of experiments, we tested the ability of the V1 antagonist and of compound 6 to block AVP-induced sensitization. In these experiments, on the first peptide exposure, four groups of animals received either AVP (10 pmol, n=8) alone, or the V1 antagonist (100 pmol) 2 min prior the injection of AVP (10 pmol, n=8); AVP (10 pmol, n=6) alone, or compound 6 (100 pmol) 2 min prior the injection of AVP (10 pmol, n=8). One day later, all animals received an injection of AVP (10 pmol). After each injection, the behaviour of the animals was observed as described above.

In the next set of experiments, we tested the ability of OT to sensitize the rat brain to the motor effects of a subsequent AVP exposure. On the first peptide exposure, two groups of animals received either AVP (10 pmol, n=5) alone, or OT (10 pmol, n=5) alone. One day later, all animals received AVP (10 pmol). After each injection, the behaviour of the animals was observed as described above.

The finding that OT was capable of sensitizing the rat brain to AVP-induced motor disturbances led us to test the dose-response of OT-sensitization and compare it with our previously established dose-response of AVP-sensitization. In these experiments, six groups of animals were used. Each group was given a first exposure to OT [either 1000 (n=5), 100 (n=5), 10 (n=8), 1 (n=4), 0.1 (n=5), or 0.01 (n=6) pmol]. One day later, all animals were given AVP (10 pmol). After each injection, the behaviour of the animals was observed as described above. Because this experiment indicated that a first exposure to 1 pmol OT or 10 pmol AVP is

sufficient to sensitize the animals such that a subsequent AVP (10 pmol) injection, 24 hrs later, induces severe motor disturbances in more than 90% of the animals, these doses were used in subsequent experiments.

In the next set of experiments, we tested the ability of the V1 antagonist and of compound 6 to block OT-induced sensitization. In these experiments, on the first peptide exposure, four groups of animals received either OT (10 pmol, n=5) alone, or the V1 antagonist (100 pmol) 2 min prior the injection of AVP (10 pmol, n=7); OT (10 pmol, n=6) alone, or compound 6 (100 pmol) 2 min prior the injection of OT (10 pmol, n=5). One day later, all animals received an injection of AVP (10 pmol). After each injection, the behaviour of the animals was observed as described above.

To further evaluate the role played by the OT-AVP receptor in sensitization, a more selective OT-AVP receptor agonist (Audigier and Barberis, 1985; Elands et al.1988a) was used. In particular we tested the possibility that the specific OT receptor agonist [Thr^4,Gly^7]OT may induce sensitization and the ability of compound 6 to block [Thr^4,Gly^7]OT-induced sensitization. In these experiments, on the first peptide exposure, two groups of animals received either [Thr^4,Gly^7]OT (10 pmol, n=8) alone, or compound 6 (100 pmol) 2 min prior the injection of [Thr^4,Gly^7]OT (10 pmol, n=8). One day later, all animals received an injection of AVP (10 pmol). After each injection, the behaviour of the animals was observed as described above.

Because testing for sensitization involves measuring the motor response of an animal to a challenge dose of AVP, it is possible that OT might directly influence the motor response to AVP, rather than modulate sensitization per se. Therefore, we tested the possibility that OT or [Thr^4,Gly^7]OT may either induce motor disturbances in their own right or may interfere with AVP-induced motor disturbances. In the next set of experiments, four groups of animals were used. The first group of animals was given a first exposure to [Thr^4,Gly^7]OT (10 pmol, n=8) and the rest of the animals (group 2,3) were given a first exposure to OT (10 pmol). One day later, the [Thr^4,Gly^7]OT pretreated animals received a second injection of [Thr^4,Gly^7]OT (10 pmol), one group of OT sensitized animals received a second injection of 100 pmol OT (n=5) and the other received a second injection of 1000 pmol OT (n=7). After each injection, the behaviour of the animals was observed as described above.

In the next set of experiments, three groups of animals were given a first exposure to AVP (10 pmol). One day later, each group of animals received either AVP (10 pmol, n=8) alone, OT (100 pmol) 2 min prior the AVP (10 pmol, n=8) injection, or OT (100 pmol, n=5) alone. After each injection, the behaviour of the animals was observed as described above.

Assay of [^{3}H]inositol-1-phosphate (IP_1)

It has previously been shown that AVP sensitization enhances the magnitude of ^{3}H-IP_1 accumulation in response to subsequent AVP exposure in septal slices (see chapter III). Thus, we tested the possibility that the magnitude

of ^{3}H-IP$_1$ accumulation in response to subsequent AVP exposure in septal slices of OT sensitized animals would be similarly enhanced. Cannulated rats received either an injection of OT (10 pmol, sensitized) or saline (5 μl saline, control) alone. One day later, ^{3}H-IP$_1$ accumulation in septal slices in response to AVP was performed as previously described in chapter III. Results were statistically analyzed using two-way analysis of variance (two-way ANOVA), and where further post hoc testing was required, the Scheffés'multiple-range test was used. Values of $p < 0.05$ were considered significant.

C. Results

Behavioural observations

Figure 20 compares the ability of the V1 antagonist and compound 6, to prevent AVP-induced sensitization. As can be seen in Fig. 20, after a first peptide exposure to either AVP or AVP in combination with antagonists, all rats displayed minor motor disturbances (scores $\leq$ 2). One day later, when AVP was administered, the motor responses of animals that were pretreated with AVP alone or AVP in combination with compound 6 were significantly enhanced ($p < 0.05$, for all paired groups) when compared to the first peptide exposure. In contrast, rats that were pretreated with AVP plus the V1 antagonist exhibited significantly reduced behavioural score ($p < 0.002$, Mann-Whitney U-Test) following a second exposure to AVP when compared to AVP alone or to AVP and compound 6.

The next series of experiments were conducted to determine whether oxytocin would induce sensitization. Results from these experiments are shown in

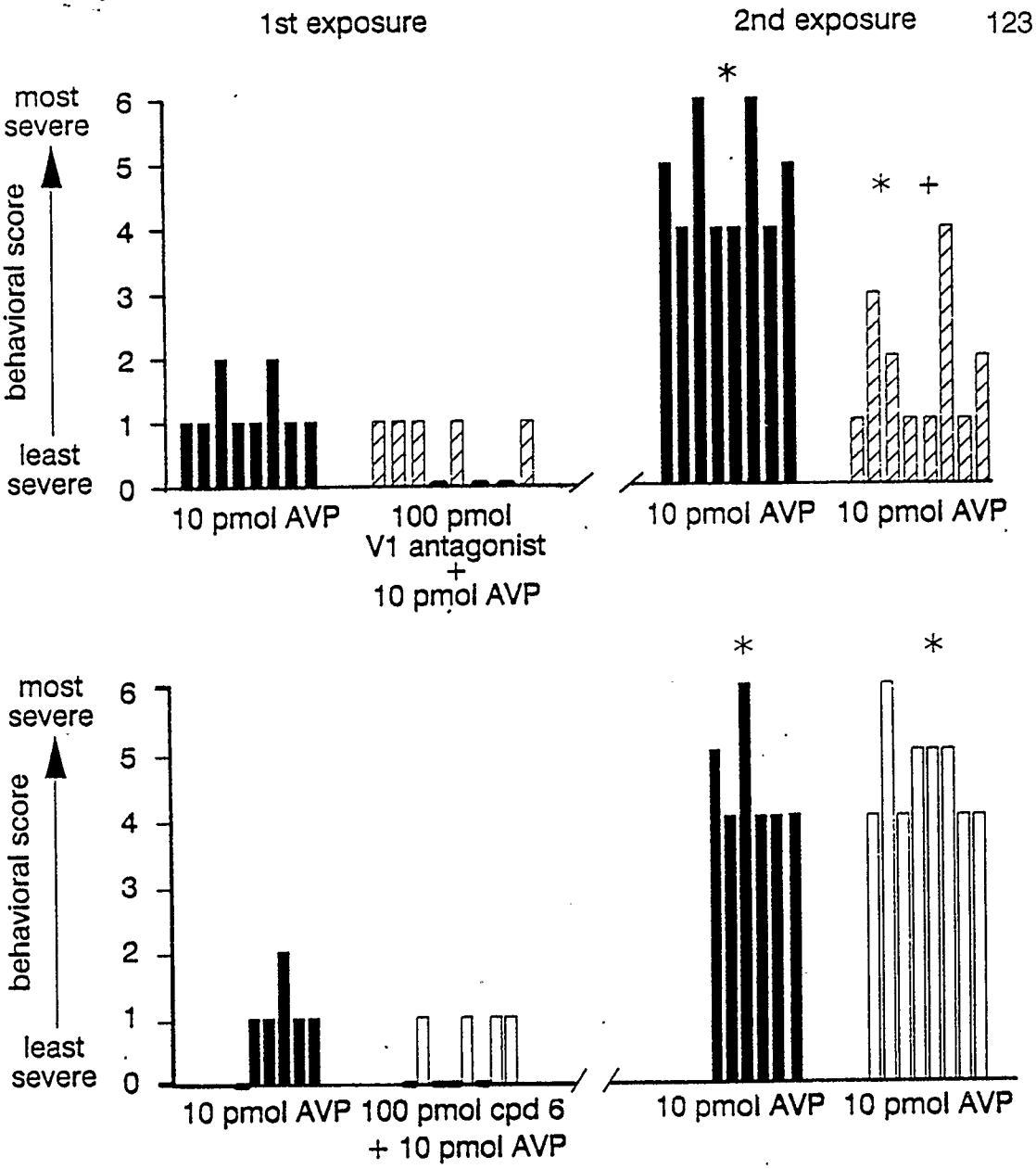

Figure 20. Motor disturbances induced in 4 groups of animals in response to an initial (1st exposure) i.c.v. injection of AVP alone, or in combination with the V1 antagonist or compound 6, and to a subsequent (2nd exposure) i.c.v. injection of AVP. Each bar represents the most severe behavioral score an individual animal received during the 10 min observation period following each injection. Asterisks indicate significant ($p < 0.05$, Wilcoxon matched pairs signed-ranks test) increase in the severity of the motor disturbances between the first and the second peptide exposure. + indicate significant ($p < 0.05$, Mann-Whitney U-test) difference between groups on the same day of exposure.

Fig. 21. After the first injection of AVP or OT, rats typically displayed periods of immobility, as indicated by the low behavioural scores. One day later, when these rats received AVP (10 pmol), however, the motor response of the animals were significantly enhanced ($p \leq 0.02$, Wilcoxon matched pairs signed-ranks test). These consisted of barrel rotations, myoclonic and myotonic convulsive movements and sometimes death, with no differences between the AVP or OT sensitized animals ($p \geq 0.4$, Mann-Whitney U-test). Thus, OT, as well as AVP, is a very powerful sensitizing agent.

Figure 22 shows the dose-response relationships of OT sensitization. After the first exposure to OT (0.01, 0.1, 1, 10, 100 or 1000 pmol), all rats displayed minor behavioural responses consisting of pauses with no obvious dose-response relationship (see Fig. 22, 1st exposure). When the animals were retested 24 hrs later in response to an AVP injection (10 pmol), however, it can be seen that the motor responses of the animals were significantly increased ($p < 0.05$, Wilcoxon matched pairs signed-ranks test, in all paired groups).

Dose-response data previously obtained for AVP sensitization (Chapter II) and data obtained in Figure 22 for OT sensitization are replotted together in Figure 23 to compare and illustrate the dose-response relationship for the proportions of rats (expressed as percent) exhibiting severe motor disturbances (including barrel rotation, myotonic/myoclonic convulsions and death; score ≥ 4) following an AVP (10 pmol) injection according to the sensitized state of the animals (either sensitized with a first exposure to AVP (0.1, 1, 10 or 100 pmol) or to OT (0.01, 0.1,

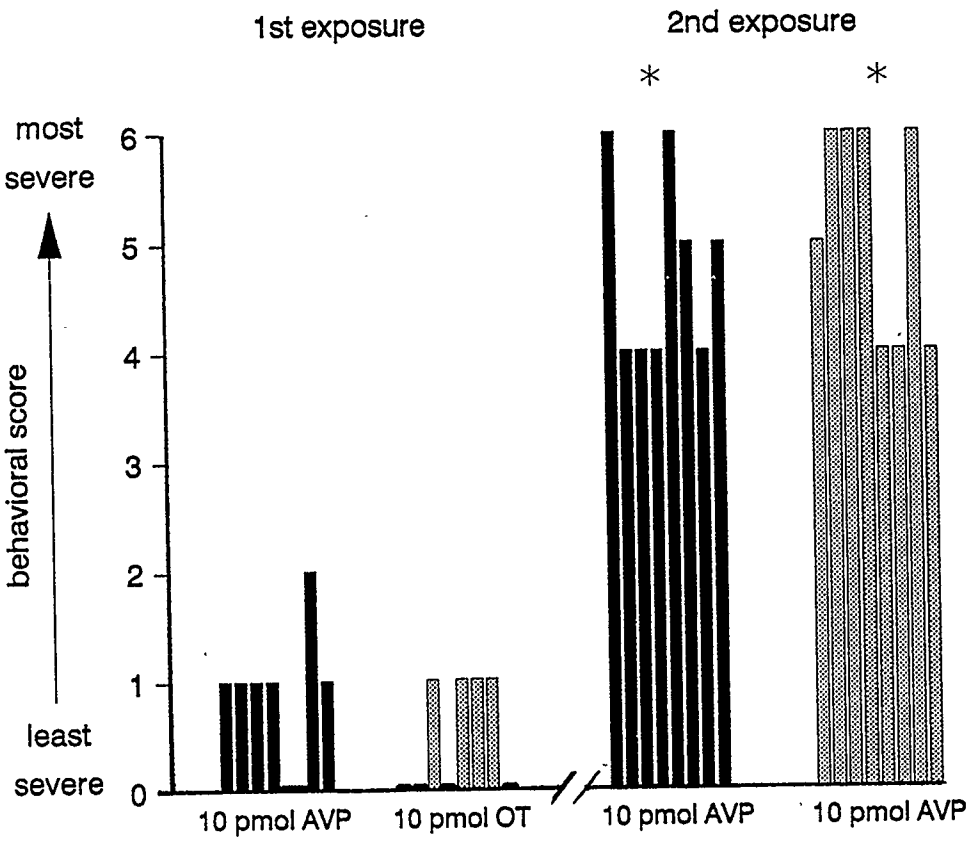

Figure 21. Motor disturbances in 2 groups of animals in response to an initial (1st exposure) i.c.v. injection of AVP or OT and a subsequent (2nd exposure) i.c.v. injections of AVP. Each bar represents the most severe behavioral score an individual animal received during the 10 min observation period following each injection. Asterisks indicate significant ($p < 0.05$, Wilcoxon matched pairs signed-ranks test) difference in the severity of the motor disturbances between the first and the second peptide exposure.

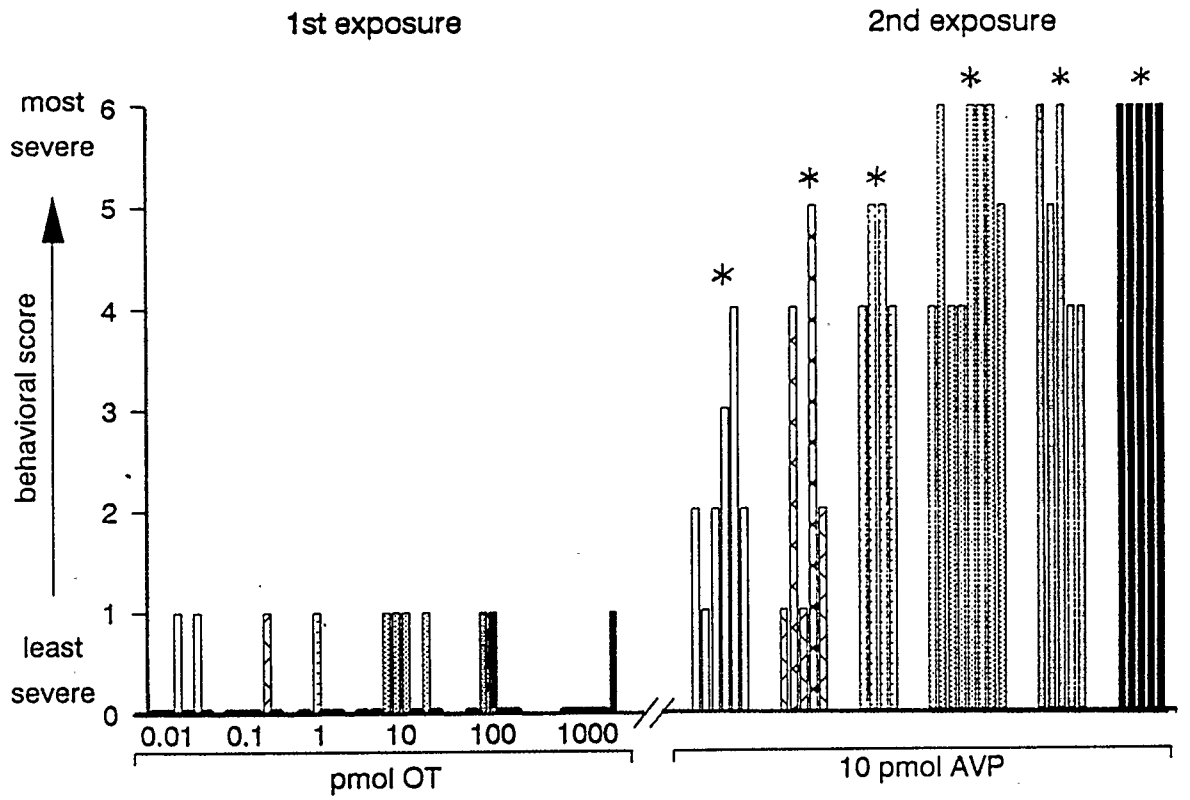

Figure 22. Motor disturbances induced in 6 groups of animals in response to an initial (1st exposure) i.c.v. injection of OT and to a subsequent (2nd exposure) i.c.v. injection of AVP. Each bar represents the most severe behavioral score an individual animal received during the 10 min observation period following each injection. Each group is represented by a unique bar pattern on 1st and the 2nd peptide exposure. Asterisks indicate significant ($p < 0.05$, Wilcoxon matched pairs signed-ranks test) difference in the severity of the motor disturbances between the first and the second peptide exposure.

1, 10 or 100 pmol). As can be seen in Figure 23, a comparison of the dose response curves of AVP-induced motor disturbances in OT-sensitized and in AVP-sensitized animals, shows that OT sensitization shifts the dose-response of the motor effects of subsequent AVP to the left.

Figure 24 compares the ability of the V1 antagonist and compound 6, to prevent OT-induced sensitization. As can be seen in Figure 24, after a first peptide exposure to OT alone or OT in combination with antagonists, all rats displayed minor motor disturbances (scores $\leq$ 1). One day later, when the animals were administered AVP, the motor responses of animals that were pretreated with OT alone were significantly enhanced ($p < 0.05$, Wilcoxon matched pairs signed-ranks test, in all paired groups) when compared to the first peptide exposure. Rats that were pretreated with OT plus the V1 antagonist exhibited enhanced behavioural scores following a second exposure to AVP ($p < 0.02$, Wilcoxon matched pairs signed-ranks test), and the behavioural scores of the V1 antagonist pretreated groups were not significantly different ($p > 0.06$, Mann-Whitney U-Test) from the agonist alone pretreated rats. Although the behavioural scores of the compound 6 pretreated group was slightly but not significantly ($p > 0.05$, Wilcoxon matched pairs signed-ranks test) enhanced, they were significantly less that the OT pretreated group alone ($p < 0.05$, Mann-Whitney U-test). Furthermore, the compound 6 pre-treated rats had behavioural scores significantly less ($p < 0.05$, Mann-Whitney U-test) that the V1 antagonist pre-treated rats.

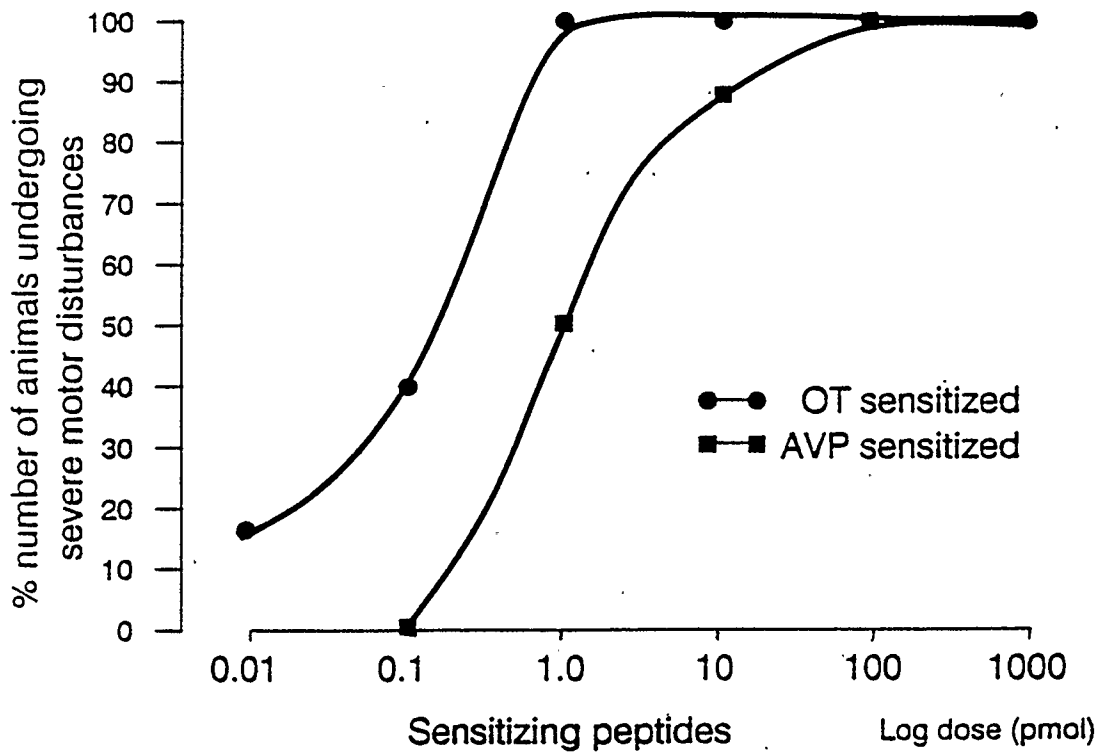

Figure 23. The proportions of rats (expressed as percent) exhibiting severe motor disturbances (including barrel rotation, myotonus, myoclonus and death, score ≥ 4) in response to an i.c.v. injection of AVP (10 pmol) are re-plotted together as a function of OT (dose response, figure 3) or AVP (dose response data of AVP sensitization obtained in chapter III) sensitization.

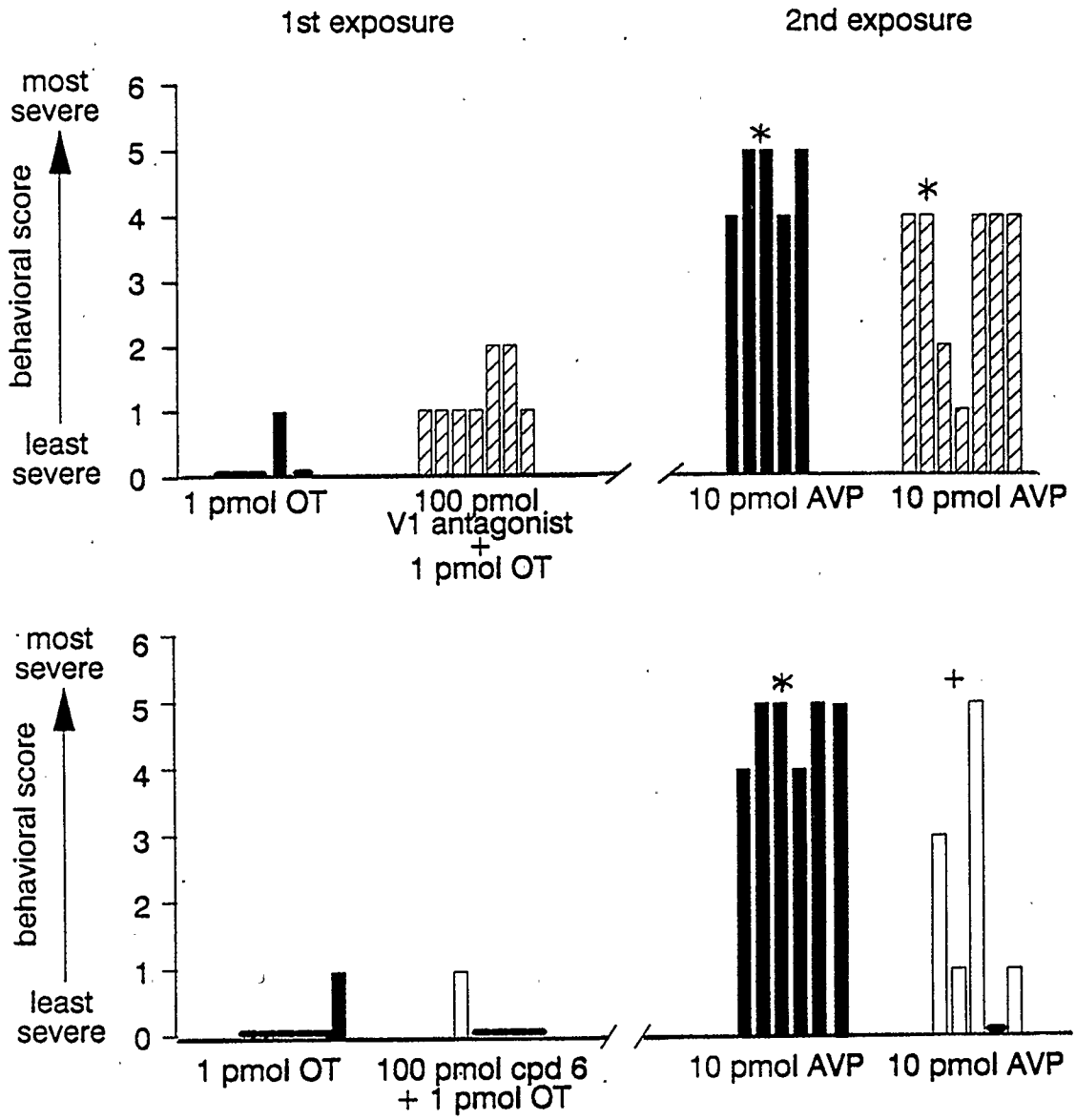

Figure 24. Motor disturbances induced in 4 groups of animals in response to an initial (1st exposure) i.c.v. injection of OT alone, or in combination with the V1 antagonist or compound 6, and to a subsequent (2nd exposure) i.c.v. injection of AVP. Each bar represents the most severe behavioral score an individual animal received during the 10 min observation period following each injection. Asterisks indicate significant ($p < 0.05$, Wilcoxon matched pairs signed-ranks test) difference in the severity of the motor disturbances between the first and the second peptide exposure. + indicate significant ($p < 0.05$, Mann-Whitney U-Test) difference between groups on the same day of exposure.

The ability of the specific OT receptor antagonist, compound 6, to prevent [Thr^4,Gly^7]OT sensitization was also tested. As can be seen in Figure 25, after a first peptide exposure, all rats displayed minor motor disturbances (scores ≤ 1). One day later, when the animals were administered AVP, the motor responses of animals that were pretreated with agonists [Thr^4,Gly^7]OT alone were significantly enhanced ($P \leq 0.02$, Wilcoxon matched pairs signed-ranks test) when compared to the first peptide exposure. Similarly, rats that were pretreated with [Thr^4,Gly^7]OT plus compound 6 exhibited an enhanced behavioural score following a second exposure to AVP ($p \leq 0.04$, Wilcoxon matched pairs signed-ranks test), there were a significant differences between the [Thr^4,Gly^7]OT and the [Thr^4,Gly^7]OT plus compound 6 pretreated animals ($P < 0.005$, Mann-Whitney U-Test).

Figure 26 and 27 shows the results of experiments conducted to test whether activation of the OT-AVP receptor could directly affect motor disturbances. Figure 26 shows that OT does not induce severe motor disturbnces unless high doses are used. As can be seen in Figure 26, after a first peptide exposure, all rats displayed minor motor disturbances (scores ≤ 1). One day later, when the animals were administered OT, only the motor responses of animals treated with very high dose of OT (1 nmol) displayed significantly enhanced motor responses ($p < 0.04$, Wilcoxon matched pairs signed-ranks test)

Figure 27 shows that OT does not interfere with AVP-induced motor disturbances. After the first injection of 10 pmol AVP, all rats typically displayed periods of immobility, as indicated by the low behavioural scores. One day later,

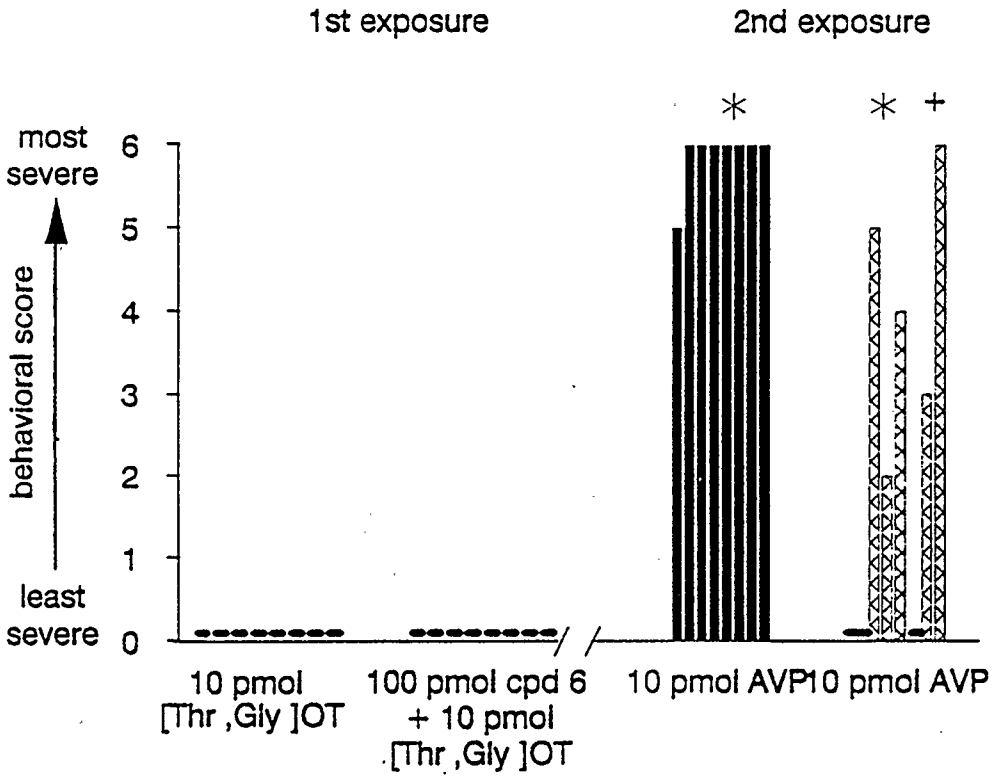

Figure 25. Motor disturbances induced in 2 groups of animals in response to an initial (1st exposure) i.c.v. injection of [Thr^4,Gly^7]OT or [Thr^4,Gly^7]OT in combination with compound 6, and to a subsequent (2nd exposure) i.c.v. injection of AVP. Each bar represents the most severe behavioral score an individual animal received during the 10 min observation period following each injection. Asterisks indicate significant ($p < 0.05$, Wilcoxon matched pairs signed-ranks test) difference in the severity of the motor disturbances between the first and the second peptide exposure. + indicate significant ($p < 0.05$, Mann-Whitney U-test) difference between groups on the same day of exposure.

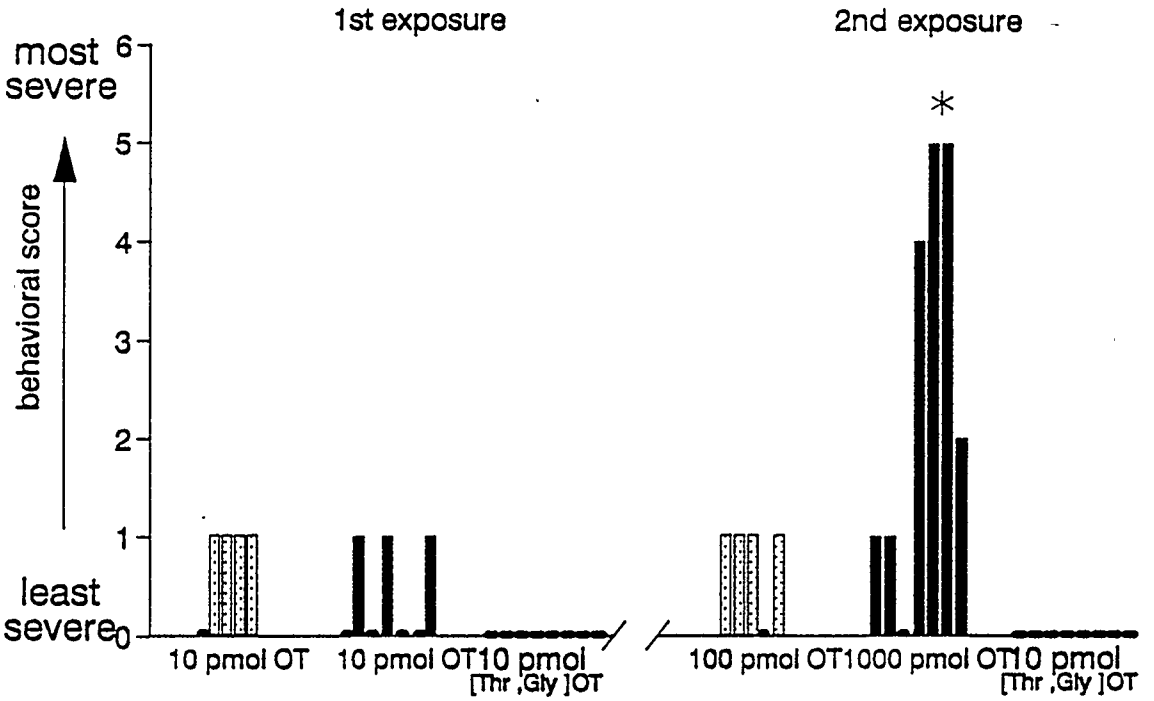

Figure 26. Motor disturbances induced in 3 groups of animals in response to an initial (1st exposure) i.c.v. injection of OT or [Thr^4,Gly^7]OT, and to a subsequent (2nd exposure) i.c.v. injection of OT or [Thr^4,Gly^7]OT. Each bar represents the most severe behavioral score an individual animal received during the 10 min observation period following each injection. Asterisks indicate significant ($p < 0.05$, Wilcoxon matched pairs signed-ranks test) difference in the severity of the motor disturbances between the first and the second peptide exposure.

when the animals received either 10 pmol AVP alone or 100 pmol OT 2 min prior to receiving 10 pmol AVP, the motor responses of the animals were significantly enhanced ($p \leq 0.05$, Wilcoxon matched pairs signed-ranks test) from the first exposure. Animals given 100 pmol OT alone, however, exhibited only minor behavioural anomalies. Furthermore, on the second peptide exposure, when the behavioural scores of AVP alone treated rats were compared to those of the AVP and OT treated rats, no significant differences were observed ($p > 0.5$, Mann-Whitney U-test). These results incidate that OT, at this dose, does not induce severe motor disturbances even in AVP sensitized animals and does not block AVP induced motor disturbances.

In order to determine the effects of central OT pretreatment on AVP stimulated cellular events, we measured AVP-induced ^{3}H-IP_1 accumulation in septal slice preparations of saline (control) or OT-pretreated (sensitized; 10 pmol, 24 hrs earlier) animals. Central pretreatment with OT did not affect the unstimulated phosphoinositide breakdown in the absence of AVP (14.5 ± 3.9, controls versus 17.1 ± 3.1, sensitized (n=3) % ^{3}H-IP_1 accumulation expressed as a ratio of the total label incorporated in the ^{3}H-PI fraction; data not shown). Figure 28 shows that ^{3}H-IP_1 accumulation over basal levels in septal slices of OT-sensitized rats was significantly higher (35.4% ± 2.1% over basal; n=3, $p < 0.003$; two-way ANOVA, Scheffé multiple range test) than in septal slices from saline pretreated animals, 6.1% ± 4.2% over basal (n=3) at an AVP concentration of 10.0 μM. Carbachol (1 mM), used as a control for the assay procedure, resulted in a similar stimulation

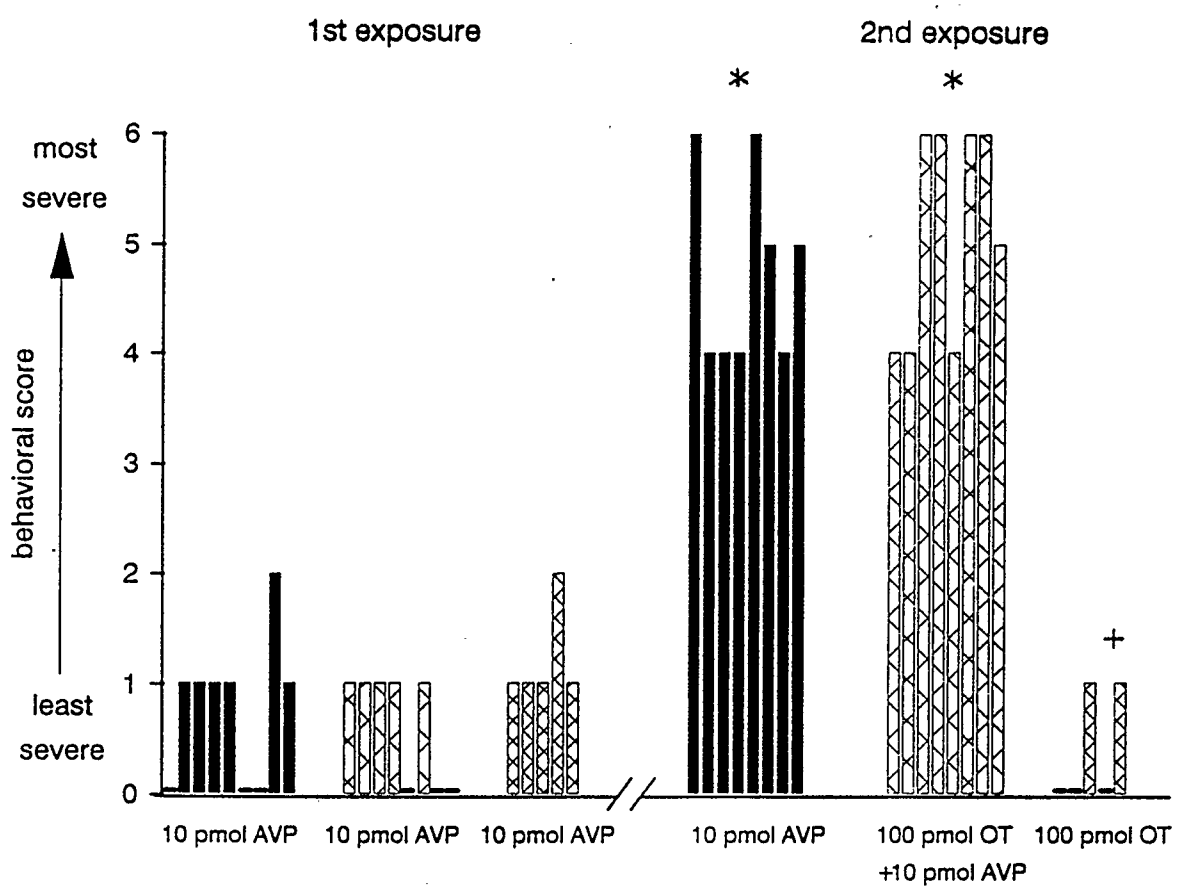

Figure 27. Motor disturbances induced in 3 groups of animals in response to an initial (1st exposure) i.c.v. injection of AVP, and to a subsequent (2nd exposure) i.c.v. injection of either AVP and OT alone, or in combination. Each bar represents the most severe behavioral score an individual animal received during the 10 min observation period following each injection. Asterisks indicate significant ($p < 0.05$, Wilcoxon matched pairs signed-ranks tes) difference in the severity of the motor disturbances between the first and the second peptide exposure. + indicate significant ($p < 0.05$, Mann-Whitney U-Test) difference compared to the AVP alone treated group on the same day.

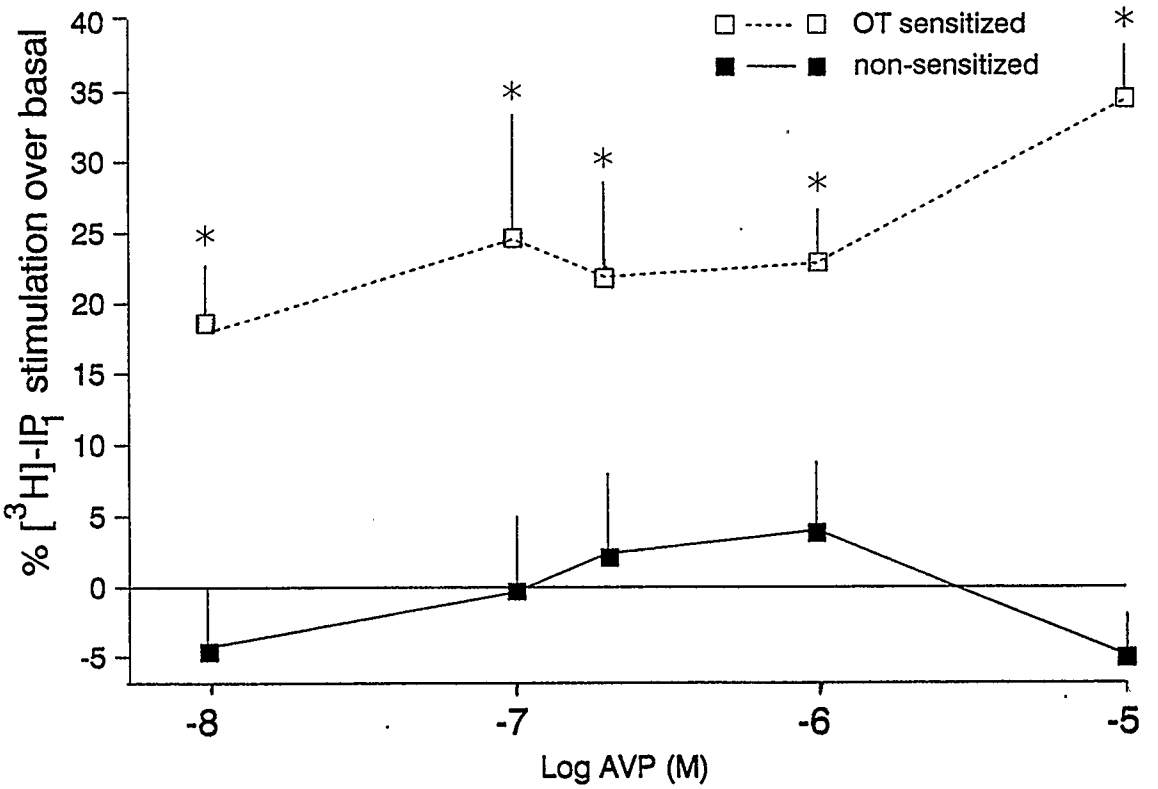

Figure 28. Dose-response relationship for AVP-stimulated ^{3}H-IP$_1$ accumulation in septal slices from non-sensitized controls and OT-sensitized rats. Values are means ± S.E.M. of 3 separate experiments each performed in triplicate. Data were analyzed using two-way ANOVA in conjunction with Scheffé's multiple-range test. Asterisks indicate significant difference in ^{3}H-IP$_1$ accumulation between control and sensitized animals. * $p < 0.05$..

of ^{3}H-IP$_1$ accumulation over basal of 85% ± 12% in controls and 89% ± 18% in sensitized rats ($p > 0.87$;two-way ANOVA; Scheffé multiple range test, n=3; data not shown).

D. Discussion

The recent discovery that central oxytocin binding sites have high affinity for both OT and AVP (Audigier and Barberis, 1985) raises the possibility that some of the central actions of AVP may be mediated via this receptor. Because pre-exposure of the rat brain to AVP has been shown to enhance (sensitize) the central actions of subsequent AVP administration (Kasting et al.1980; Burnard et al.1983, 1985, 1986, 1987; Boakes et al.1985; Naylor et al.1985a; Wurpel et al.1986a, 1986b, 1988; Balaban et al.1988), in this study we investigated the possibility that AVP-induced sensitization could be mediated via the OT-AVP receptor. Our present results confirm previous findings that AVP-induced sensitization appears to be mediated via the V1 receptor (Burnard et al.1986). In addition, a surprising finding from this study was the observation that OT is itself a potent sensitizing agent. OT sensitization appears to be mediated via the OT-AVP receptor and to enhance both AVP-induced motor disturbances and ^{3}H-IP$_1$ accumulation in septal slices in response to subsequent AVP administration.

Several studies have shown that the V1 antagonist, d(CH$_2$)$_5$ Tyr(Me)AVP, binds both the OT-AVP (Di Scala-Guenot et al.1990) and the V1 receptor (Dorsa et al.1984; Cornett and Dorsa, 1985 and see chapter II), whereas compound 6 selectively binds the OT-AVP receptor (Elands et al.1988b; Di Scala-Guenot et

al.1990). Our observation that AVP-induced sensitization could be blocked by $d(CH_2)_5$ Tyr(Me)AVP, but not by compound 6, strongly suggest that AVP sensitization is not mediated via the OT-AVP receptor.

As mentioned above, a surprising observation in this study was the finding that OT is a potent sensitizing agent, even more potent than AVP (see Figure 23). Furthermore, our observation that OT-induced sensitization could be blocked by the specific OT-AVP receptor antagonist, compound 6, but not by $d(CH_2)_5$ Tyr(Me)AVP strongly suggests that OT sensitization is mediated via the OT-AVP receptor. The findings that the specific OT-AVP receptor agonist, [Thr^4,Gly^7]OT, could also act as a sensitizing agent, and that compound 6 could block [Thr^4,Gly^7]OT-induced sensitization confirms that activation of the OT-AVP receptor, by OT-like agonist, can sensitize the rat brain to AVP-induced motor disturbances.

Even though compound 6 blocked OT sensitization, which suggests a specific effect of OT at OT receptors, the possibility remains that OT could induce sensitization by causing the endogenous release of AVP. The observation, however, that the V1 antagonist $d(CH_2)_5$ Tyr(Me)AVP although effective in blocking AVP sensitization did not block OT sensitization does not favor this possibility.

Because this $d(CH_2)_5$ Tyr(Me)AVP is known to bind both the OT-AVP receptor (Di Scala-Guenot et al.1990) and the V1 receptor (Dorsa et al.1984; Cornett and Dorsa, 1985); and see chapter II), it would be expected to have blocked OT sensitization. However, there are at least two possible explanations why $d(CH_2)_5$ Tyr(Me)AVP did not block OT sensitization under the present

experimental conditions. The first is that, although the OT-AVP receptor binds both OT and AVP, it binds OT with a higher affinity than it binds AVP or V1 antagonist (Audigier and Barberis, 1985; Di Scala-Guenot et al.1990). The second is that OT is at least ten times more potent than AVP as a sensitizing agent (see Figure 23). Both of these observations suggest that much higher dose of the V1 antagonist would be required to block OT sensitization.

Despite the fact that OT can sensitize the rat brain to AVP-induced motor disturbances, OT given 5 min prior to an acute AVP injection, does not block the motor effects of AVP, unless very high doses are used (see Abood et al.1980). Similarly, OT injected alone, even in sensitized animals, does not in itself induce motor responses unless very high doses are used (see Figure 26) (a dose at which it would be expected to act as a partial agonist). This finding underlines the fact that the motor disturbances themselves do not result from action at an OT receptor site.

Previous studies indicate that the magnitude of ^{3}H-IP$_1$ accumulation in septal slices in response to AVP is enhanced in AVP-sensitized animals (see chapter III; (Lebrun et al.1990). In this study we have found similar results in that (1) ^{3}H-IP$_1$ accumulation in septal slices of saline pretreated rats was not stimulated by AVP; (2) basal ^{3}H-IP$_1$ accumulation was not altered by OT pre-treatment; (3) ^{3}H-IP$_1$ accumulation in septal slices of OT pretreated rats in response to AVP was enhanced. Taken together, our results and those of others (Lebrun et al.1990) suggest that AVP or OT sensitization results in enhanced AVP-induced ^{3}H-IP$_1$

accumulation in septal slices. The observation that OT sensitization results in an enhanced intracellular response to subsequent AVP exposure is in line with our previous conclusion that the phenomenon of sensitization is more closely related to alterations in post-receptor mechanisms rather than in alteration in the V1 receptor itself.

During the last decade, several attempts have been made to characterize central AVP receptors on membrane fractions derived from whole brain or from anatomically defined areas (see intro and chapter II). When ^{3}H-AVP binding is determined in the presence of 10 mM Ni, high capacity ^{3}H-AVP binding sites also bind both AVP and OT with similar affinities (Pearlmutter et al.1983; Costantini and Pearlmutter, 1984). Thus another possibility is that AVP- and OT-induced sensitization may be mediated via action at this receptor. Further characterization of this receptor will be required to investigate this possibility.

Whether the sensitization obtained by AVP and OT pre-exposure occurs via similar mechanisms, however, remains unknown. There is some electrophysiological evidence that AVP and OT exert a similar excitatory effect on a small proportion of neurons located in the septal area (joëls and Urban, 1982).

Whether AVP and OT have a similar site of action in the rat brain to induce sensitization is also not known. It is interesting to note, however, that autoradiographic studies of the rat basal forebrain, an area including the VSA, was recently shown to contain both ^{3}H-AVP and ^{3}H-OT binding sites (e.g. Biegon et al.1984; de Kloet et al.1985; Raggenbass et al.1987; Freund-Mercier et al.1988;

Tribollet et al.1988a). This observation raises the posibility that both AVP and OT may act in the ventral septal area via their respective receptors to induce sensitization.

Finally, it is interesting to note that several studies have previously indicated the importance of endogenous neurohypophyseal peptides in processes related to tolerance and sensitization phenomena. Although some controversies exist as to whether AVP and/or OT facilitate (e.g. Van Ree and De Wied, 1976; Post et al.1982) or inhibit (Kovács et al.1985, 1987; Kovács and Telegdy, 1987) the development of tolerance, evidence indicates that AVP and related peptides influence the activity of neuronal pathways that subserve central adaptive processes involved in tolerance and/or sensitization phenomena. It is possible, therefore, that tolerance and sensitization may be highly related phenomena, in that, both are altered responsivities based on previous exposure. It would be of interest to determine if the present observations of altered responsiveness to these neurohypophyseal peptides had relevance to the studies on tolerance.

VI. OT SENSITIZATION OF AVP-INDUCED ANTIPYRESIS

A. Introduction

There is now considerable evidence that AVP can act as an antipyretic agent in the brain. Central AVP administration to a variety of species causes a dose-dependent, receptor-mediated reduction in the febrile response but not in normal body temperature (for a review, see Pittman et al.1988). The VSA appears to be a major site at which AVP is capable of causing antipyresis (Naylor et al.1988) and several lines of evidence suggest a role for endogenous AVP in fever suppression within the VSA (for reviews, see Naylor et al.1987; Cooper et al.1987).

Although both AVP and OT are released into the VSA during fever (Landgraf et al.1990), structure activity studies suggest that the central receptor mediating the antipyretic action of AVP in the brain resembles the V1 rather than the V2 or OT-AVP receptor, in that; (1), an antagonist of the V1 receptor d$(CH_2)_5$ Tyr(Me)AVP prevents the antipyretic action of AVP in the brain (Naylor et al.1987); and (2) the V2 agonist DDAVP and oxytocin were shown to be inactive as antipyretic agents (Kovacs and De Wied, 1983; Cooper et al.1987; Naylor et al.1987). Thus, a role for the OT released during fever is not yet known.

As discussed in previous chapters, pre-exposure of the rat brain to AVP (Kasting et al.1980; Burnard et al.1983, 1985, 1986, 1987; Boakes et al.1985; Naylor et al.1985a; Wurpel et al.1986a, 1986b, 1988; Balaban et al.1988 and previous chapters) or OT (chapter V) cause a sensitization to the motor actions

of subsequent AVP exposure. Therefore, in this section we tested the hypothesis (1) that pre-exposure of the rat brain to OT may similarly sensitize the rat brain to the antipyretic action of subsequent AVP exposure, and (2) that AVP or OT released during fever, could sensitize the brain to the subsequent motor actions of central AVP.

B. Methods

Male sprague Dawleys rats initially weighing 250g were anesthetized with sodium pentobarbital (50 mg/Kg) and a stainless-steel 23-gauge guide cannula was implanted stereotaxically under aseptic conditions to allow access to a lateral cerebral ventricle. In addition, pre calibrated, paraffin-covered temperature-sensitive radio-transmitters (Mini-Mitter) were inserted in the intraperitoneal cavity for the future remote recording of body temperature. Animals were then housed in groups of four per cage on a 12-hr light cycle, allowed food and water ad libitum and allowed 5-7 days to recover from surgery.

Because interleukin-1 (IL-1) has been shown to be effective in causing fever following i.c.v. injection (Duff and Durum, 1983), we have utilized IL-1 to induce fever. Stock solutions of IL-1, AVP and OT were diluted to the appropriate concentrations with sterile saline immediately prior to i.c.v. injections. Injections were performed, in a volume of 5 μl, by gravitational flow through a 27-gauge injection cannula into awake, freely moving animals.

OT sensitization, fever and antipyresis

In a first set of experiments, we addressed the questions of whether pretreatment with OT would (1) alter the fever response to IL-1, (2) alter the antipyretic response to an exogenous i.c.v. AVP administration. Cannulated rats were given either an i.c.v. injection of saline as control (n=6), or were sensitized with an i.c.v. injection of 0.1 pmol OT (n=5), 1.0 pmol OT (n=5), or 10 pmol OT (n=5). One day later, each animal was placed individually in a plexiglass cage located on an antennae designed to receive the signals emitted by the intraperitoneally implanted transmitters. These signals were converted to body temperature measurements with a Heath Computer System, and monitored at 5 min intervals for at least 60 min (baseline body temperature recording). Once a stable baseline body temperature was established, each animal was given an i.c.v. injection of 20 Units of IL-1 and body temperature monitored for an additional 120 min. To investigate the antipyretic potency of exogenously administered AVP, at 120 min post IL-1 injection, 1 pmol of AVP in 5 μl saline was administered i.c.v. and body temperature monitored for an additional 120 min.

OT sensitization and OT antipyresis

To test whether the antipyretic response induced by the i.c.v. AVP injection was specific to AVP, an additional group of animals (sensitized one day earlier with 10 pmol OT i.c.v.) were given an i.c.v. injection of 10 pmol of OT at 120 min post IL-1 fever and the body temperature was measured as described above.

Endogenous sensitization and AVP-induced motor responses

In a second series of experiments, we tested the possibility that fever (a known stimulus for the release of central AVP and OT) may sensitize the rat brain to the motor actions of subsequently administered AVP. On the first experimental day, two groups of animals were given either a fever, induced by the i.c.v. injection of 20 Units of IL-1 in 5 μl physiological saline (n=8) or as control, 5 μl physiological saline alone (n=8). One day later all animals received an i.c.v. injection of 100 pmol AVP (a dose previously utilized in endogenously sensitized animals) in 5 μl physiological saline. The animals were then placed in a large plexiglass chamber and observed for the development of motor distubances for a 10-min period as described in chapter III. Results were statistically analyzed by the non-parametric Mann-Whitney U-Test.

Body temperature measurements are presented as changes in body temperature, obtained by subtracting the mean baseline core temperature from each temperature measurement obtained during the various experimental manipulations. The changes in body temperature were then subjected to an analysis of variance (ANOVA) and Scheffé post-hoc test.

C. Results

OT sensitization, fever and antipyresis

Figure 29, illustrates the febrile response of non-sensitized and OT sensitized animals. The average baseline body temperature (data not shown) of non-sensitized control animals (38.4 ± 0.3 ° C) was not significantly different from

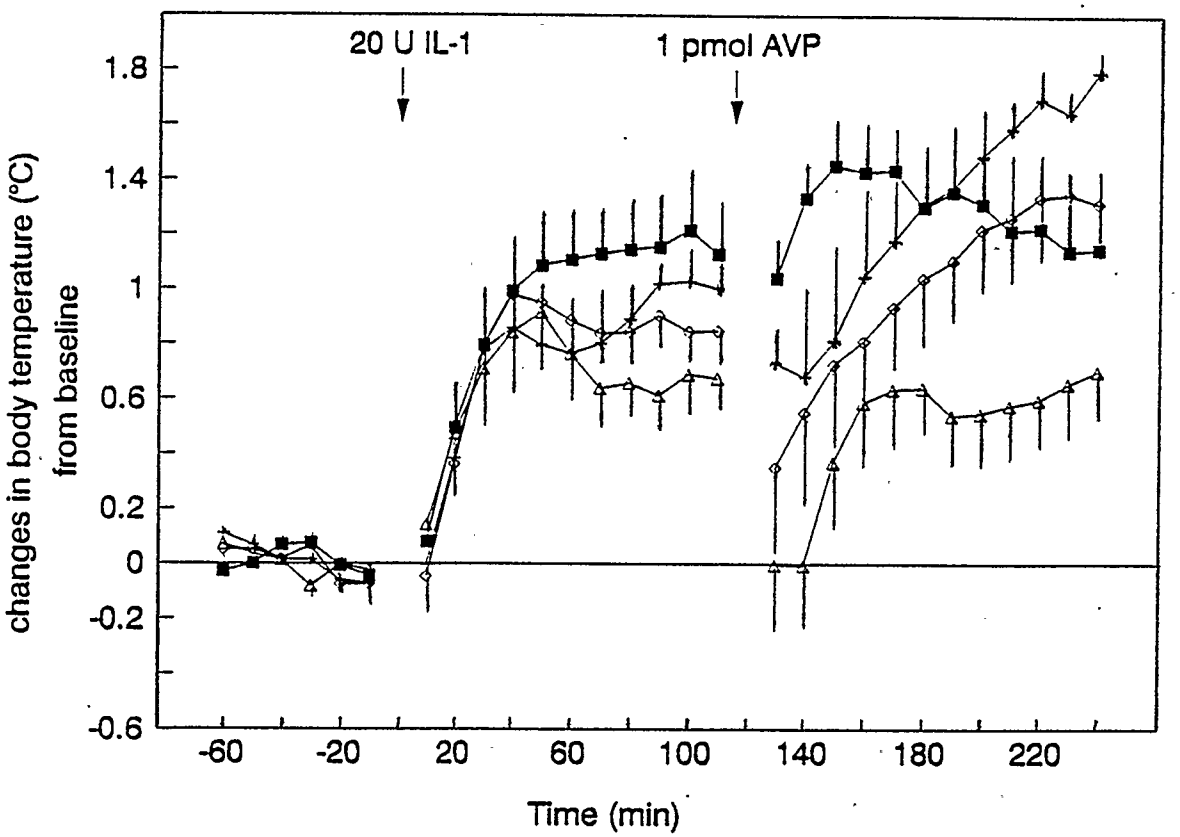

Figure 29. Mean (± S.E.M.) change in body temperature of animals pre-treated on day 1 with i.c.v. injections of saline (n=6, ■–■); 0.1 pmol OT (n=5, + - +); 1.0 pmol OT (n=5, ◇–◇); 10.0 pmol OT (n=5, △–△). At time 0, 20 units of IL-1 was administered i.c.v.. Two hours later, animals received an i.c.v. injection of 1 pmol AVP.

the baseline body temperature of animals sensitized either with 0.1 pmol OT (38.0 ± 0.3 ° C), 1.0 pmol OT (38.1 ± 0.3 ° C), or 10 pmol OT (37.5 ± 0.3 ° C) ($p > 0.1$). Similarly, the febrile responses to IL-1 (Fig 29) were not significantly different ($p > 0.1$) between control and sensitized animals. Inspection of the mean temperature records (Fig. 29) reveals, however, that whereas the initial rise in body temperature was almost identical in control and sensitized animals, sensitized animals displayed evident tendencies toward greater defervescence during the later phase of the fever response (i.e., lower body temperature between 70 and 120 min post IL-1 injection). When the average body temperature is calculated during this later, stabilized phase of fever (Fig. 30), it becomes apparent that associated with a larger sensitizing dose of OT, is a greater tendency for lower body temperature.

In addition, Figure 29 also illustrates the effect of exogenously administered AVP on febrile body temperature. As illustrated in Figure 29, the febrile body temperature was attenuated by AVP to a significantly ($p < 0.1$) greater extent in sensitized (10 pmol OT) animals than in non-sensitized controls. This becomes more apparent on calculation of the maximal decrease in body temperature induced by AVP as a function of the dose of OT used to sensitize the animals (Fig. 31).

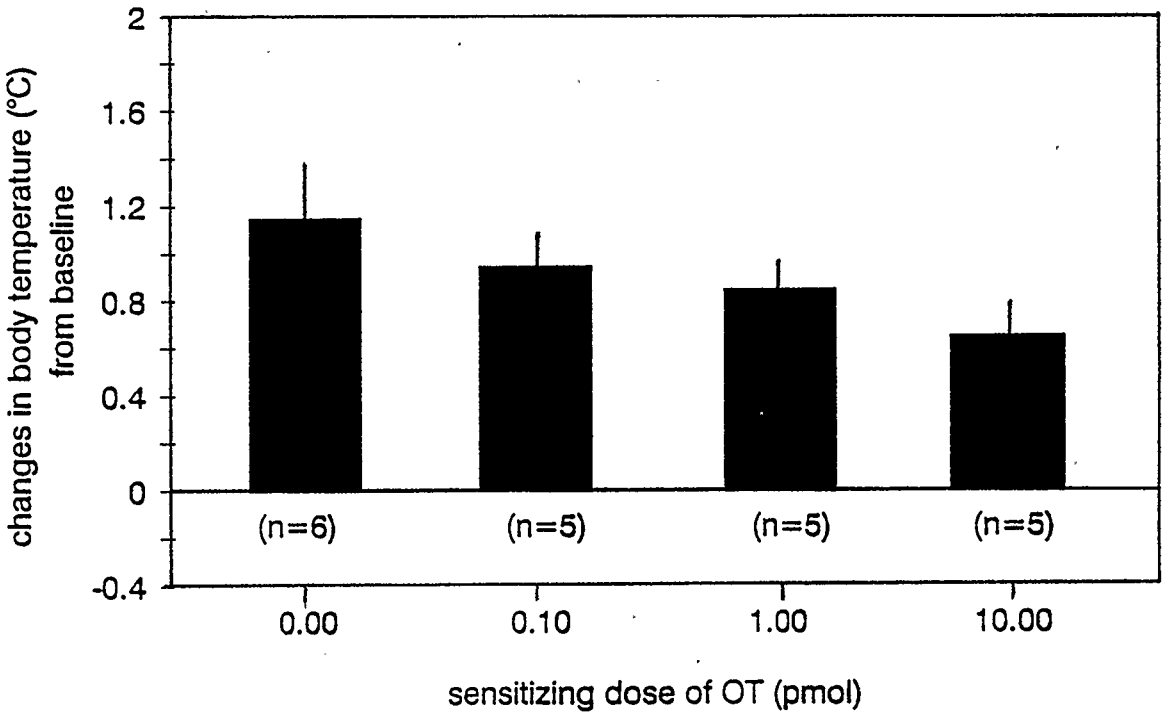

Figure 30. Body temperature (Mean ± S.E.M.) changes from baseline during the defervescence phase of fever (70 - 120 min post IL-1) as a function of OT-induced sensitization.

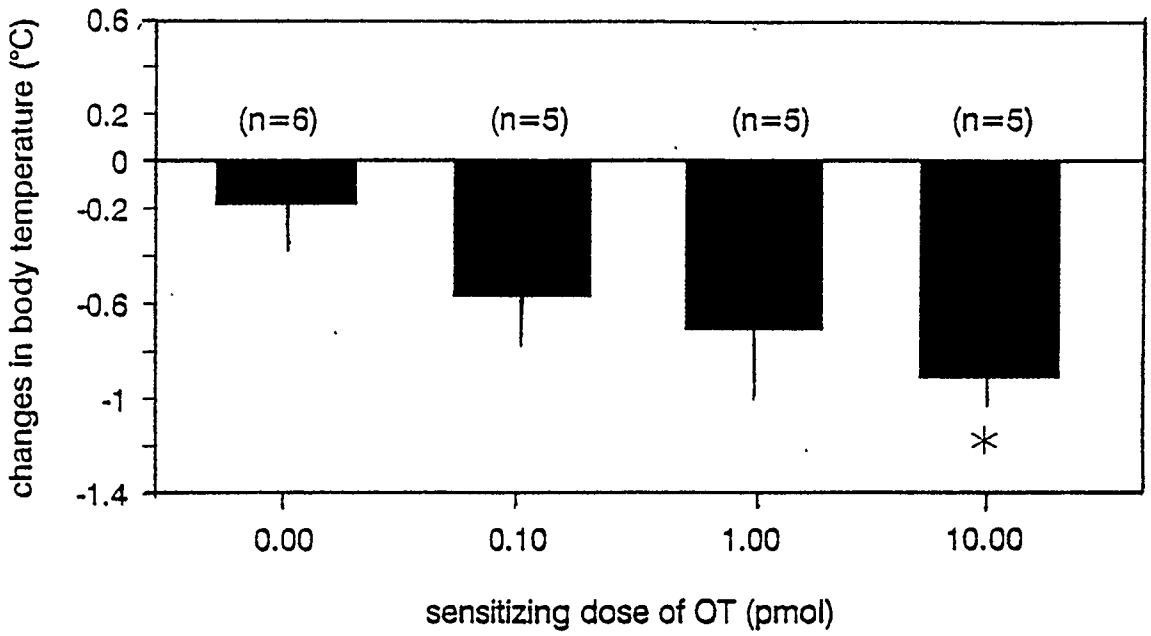

Figure 31. Maximal body temperature (Mean ± S.E.M.) changes from pre AVP injection baseline (average at time 100 - 120 min) after an i.c.v injection of 1 pmol AVP. Asterisks indicate a significant difference in body temperature between the saline pre-treated and sensitized animals. *$p < 0.1$.

OT sensitization and OT antipyresis

When febrile sensitized (10 pmol OT) rats were given 10 pmol OT i.c.v. 120 min post IL-1 administration, no decrease in body temperature was observed (Fig. 32).

Endogenous sensitization and AVP-induced motor responses

Following an IL-1 fever (20 Units, i.c.v.), animals exhibited maximal rise in body temperature of 2.15 ± 0.25 ° C above baseline whereas saline treated rats exhibited maximal rise in body temperature of 0.28 ± 0.05 ° C (figure 33, top panel). When rats received 100 pmol AVP i.c.v. one day later, the behavioural effects were striking. Whereas 100 pmol AVP in saline pre-treated rats evoked minor motor responses including pauses and prostration, it evoked severe motor disturbances including barrel rotation myoclonic/muotonic convulsion and even death in fever-pretreated animals (Fig. 33, lower panel). Thus the response of saline pre-treated rats to an i.c.v. injection of AVP is significantly different ($p < 0.05$, Mann-Whitney U-test) from the response of rats pre-treated with fever (figure 33, lower panel).

D. Discussion

The results of the experiments reported here indicate that pre-exposure (sensitization) of the rat brain to a single injection of OT enhances the antipyretic response to injection of AVP into a lateral ventricle in febrile animals. Although basal body temperature and the initial rate of rise in body temperature to IL-1 were not altered in sensitized rats, there was a tendency toward lower body

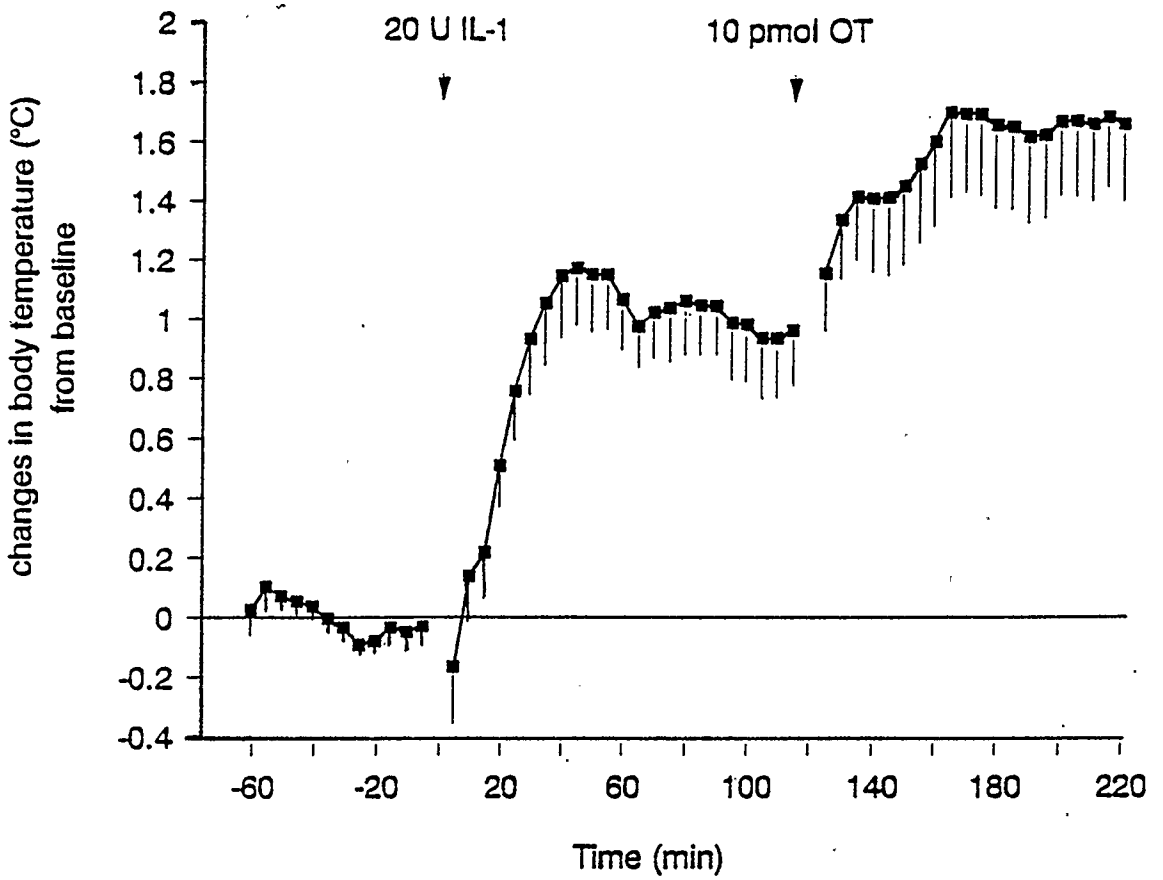

Figure 32. Mean (± S.E.M.) change in body temperature of animals pre-treated on day 1 with an injections of 10.0 pmol OT i.c.v. (n=6, ▪–▪). One day later, at time 0, 20 units of IL-1 i.c.v. was administered. Two hours later, animals received an i.c.v. injection of 10 pmol OT.

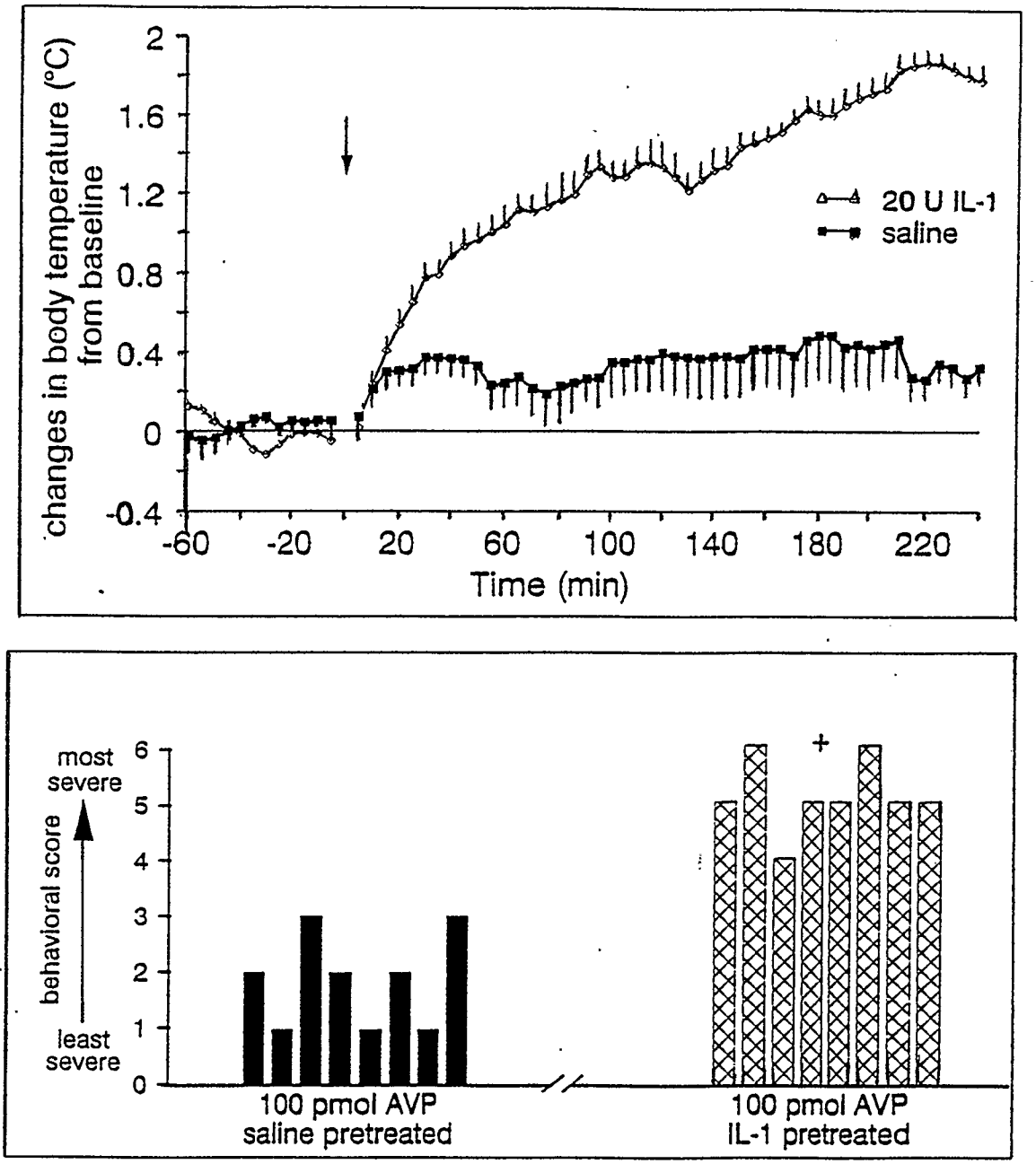

Figure 33. Top panel: Mean (± S.E.M.) change in body temperature of animals given, at time 0, an i.c.v. injection of saline (n=8) or 20 U IL-1 (n=8). Lower panel: Motor disturbances induced in saline or IL-1 pre-treated animals, in response to an i.c.v. injection of 100 pnol AVP. Each bar represents the most severe behavioral score an individual animal received during the 10 min observation period. Asterisks indicate significant increased in the severity of the motor disturbances between the saline and the IL-1 pretreated animals (Mann-Whitney U-test). + $p < 0.05$.

temperature during the latter phase of the fever response. This is particularly interesting because AVP has been shown to play a role as an endogenous antipyretic agent during the latter phase of fever. For example, rats made febrile and given AVP antagonists develop fevers in which the initial rise in body temperature was not altered but in which defervescence was delayed (Cooper et al.1987). Furthermore, Brattleboro rats which are genetically deficient in AVP (Cooper et al.1987) and long-term castrated rats which are also deficient in septal AVP (Pittman et al.1988) develop fevers in which defervescence is delayed. The fact that we found that exogenously applied AVP has an enhanced antipyretic action in sensitized animals, and the fact that several reports provide evidence to support the hypothesis that endogenous AVP may affect fever duration by facilitating defervescence (Cooper et al.1987; Pittman et al.1988) suggest that the tendency toward enhanced endogenous antipyresis in sensitized animals made febrile, may be due to the action of endogenous AVP being released during fever.

Previously, we have demonstrated that the enhanced action of AVP in sensitized animals appears to be due to enhanced post receptor activation (see chapter III). Our observation that exogenously applied AVP is more effective in the OT sensitized rats supports the hypothesis that the enhanced antipyresis is mediated at the receptor level and is not due to increased endogenous release. However, further studies utilizing push-pull perfusion of the VSA may be required to determined if release of AVP is also altered in the sensitized rats.

The observation that OT in itself does not decrease the body temperature of febrile sensitized animals is in line with previous observations concerning the ineffectiveness of this peptide in causing antipyresis (Kovacs and De Wied, 1983). These data further support the hypothesis that the enhanced action of AVP in sensitized animals is not mediated via the OT-AVP receptor (see chapter V). Nontheless, the observation that centrally administered OT can sensitize the antipyretic actions of AVP raises the possibility that endogenous OT, which is known to be released along with AVP into the VSA during fever (Landgraf et al.1990), may play a role in modulating fever responses.

Recent studies suggest that the development of tolerance to a pyrogen-induced fever by repeated injections of endotoxin may involve AVP mediated endogenous antipyresis. For example, Cooper et al.(1988) observed that the development of fever tolerance in guinea pigs was associated with changes in central immunoreactivity for AVP. Recently, Wilkinson and Kasting (1990a) have observed that animals made fever tolerant by successive daily injections of endotoxin developed enhanced fevers following application of an AVP antagonist into the VSA and interpreted their results to suggest a role for centrally released AVP during pyrogenic tolerance in the rat. The present observations raise the possibility that sensitization of endogenous antipyresis mechanisms may also contribute to the pyrogenic tolerance.

Because fever can mimic the effect of a central injection of OT in sensitizing the rat brain to the motor effects of AVP we interpret these results to

suggest that fever stimuli caused the release of central OT (or possibly AVP) so as to sensitize the brain to the motor anomalies induced by exogenously administered AVP. However, it is also possible that some other aspect of the responst to IL-1 (i.e. elevated body temperature or release of cytokinines etc.), could also have participated in the sensitization process.

Because the VSA of the rat basal forebrain appears to be a major site at which AVP is capable of causing both antipyresis (Kasting et al.1982) and motor disturbances (Naylor et al.1985a), it has been suggested previously (for a review, Pittman et al.1988) that AVP, released during fever, could be involved in the genesis of febrile convulsions. The present observations are the first to demonstrate a causal link between the occurence of a pyrogen induced fever and a subsequent enhanced susceptibility to the convulsive and motor actions of AVP.

VII. GENERAL DISCUSSION AND CONCLUSIONS

There have been numerous reports in the literature of the effects of acute central administration of a variety of neurotransmitters. For many of these substances, however, relatively little is known about the effects produced by their chronic or repeated administration. As discussed in the introduction, it is now apparent, at least for some substances, that dramatic changes in their effects are seen upon repeated administration.

The aim of the work reported in this thesis was to investigate putative mechanism(s) underlying the phenomenon that repeated central administration of AVP causes a sensitization process, in that a first exposure to AVP increases the responsiveness of the animals to subsequent AVP exposure.

The behavioural data indicate that sensitization to repeated administration of AVP is dependent on dose, and on the interval of time between exposures to AVP. While many other variables can affect the development of sensitization, it is suggested that both the amplitude (dose) and the temporal (intermittent exposure) characteristics of agonist exposure are important in determining subsequent responsiveness. This has been observed in a number of other systems (reviewed in the introduction), all of which appear to possess the flexibility of up or down regulation depending on the stimulus characteristics. The possibility exists that many other neurotransmitters or hormones may similarly evoke enhanced responsiveness upon repeated exposure. Experiments such as

those described in chapter III could profitably be carried out upon other agonists and hormones.

The fact that the temporal characteristics of a stimulus may be important in regulating the magnitude of the response has functional significance for the following reasons; first, enhanced responsiveness of a tissue to an agonist would provide a mechanism whereby an optimal (enhanced) effect could be obtained without requiring massive amounts of ligand to be synthesized, released and degraded. Secondly, because optimal responsiveness could be achieved without requiring large concentrations of the ligand, this may prevent the downregulation of receptors which occurs in some systems upon continuous exposure to high concentrations of agonist. Thirdly, if the system has a different responsiveness to repeated agonist exposure as compared with a single agonist exposure, this suggests that the system could use frequency as well as amplitude coding. This could increase the information handling capacity of tissues and could contribute to neural plasticity, in that response characteristics could change as a function of previous experience.

To determine possible neuronal mechanisms responsible for this sensitization process, AVP receptors in the VSA of the rat brain were first characterized. The theoretical basis of the method used to characterize receptors was essentially that discussed by Burt (1985), in which binding assays require the preparation of a receptor-containing fraction of the tissue of interest, by a process of homogenization and differential centrifugation. A radioactive form of the agonist

of interest is incubated with the tissue fraction under appropriate conditions for a set period of time. Unbound ligand is then separated from bound ligand, for example by filtration, and bound radioactivity is quantified. Because this binding represents binding to both receptor and other non-specific sites, it is necessary to include samples of binding in the presence of an excess of unlabelled ligand (e.g. 100 times the K_d concentration). This is assumed to displace all the specific (receptor) bound radioligand, but none of the non-specific binding, since the latter has an almost infinite capacity. The relationship between specific binding and concentration of ligand(s) is then compared with a mathematical model to yield values for the number of receptors and for the affinity of the receptor. Before the binding sites can be assigned the term "receptor", however, there are certain criteria which must be satisfied. These have been discussed in detail elsewhere (Burt, 1985) and include factors such as saturability, reversibility, high affinity, sensitivity to temperature and tissue linearity. Furthermore, certain precautions must be taken before results of binding assays can be deemed reliable. For example, the stability of the labelled ligand during the incubation should be tested.

In this study, the results from radioligand binding studies indicate that many of the criteria have been fulfilled for establishing as receptors the AVP binding sites characterized in the VSA of the rat brain. Additional radioligand binding studies have further demonstrated that this VSA AVP receptor has properties resembling those of the V_1 receptor. This is in keeping with the

pharmacological properties of AVP-induced motor-disturbances (Burnard et al.1986), antipyresis (Naylor et al.1987), and also of the electrophysiological actions of AVP on VSA cells (Disturnal et al.1987).

Having identified and described a receptor which fulfilled the criteria for a V1 receptor, the hypothesis that AVP sensitization may be associated with an alteration in VSA AVP receptor number or affinity was investigated. Experiments carried out in chapter III indicated that in AVP sensitized animals, VSA AVP receptor density and affinity are not altered. This suggests that the increased sensitivity of the animals to AVP's motor effects is not mediated via an increase in receptor number or affinity.

From studies of post receptor mechanisms, results presented in chapter III revealed that repeated AVP exposure alters the sensitivity of the VSA to AVP at the cellular level. AVP-stimulated phosphoinositide hydrolysis was found to be enhanced in septal slices of AVP sensitized animals. This is the first time that evidence has been presented to show that repeated AVP exposure can alter AVP receptor effector mechanisms. Subsequent to completion of this work, it has recently been demonstrated that repeated agonist exposure for another hormone (LH-RH; Mitchell et al.1988; Mobbs et al.1990a, 1990b) also leads to enhanced receptor efficacy without apparent alteration in receptor number and affinity. This further supports the contention that such agonist-induced regulation at the post-receptor level may be an important form of regulatory mechanism in general. In addition, these authors have demonstrated that the enhanced LH-RH receptor

responsiveness due to repeated LH-RH administration is caused by an increase in the synthesis of an enzyme (PLC-α) involved in the generation of intracellular messengers. Thus the possibility that AVP may similarly evoke enhanced responsiveness by altering the synthesis of such a protein should be investigated. The problem with this type of experiment in differentiated neuronal tissue, however, is that the number of AVP receptors is very small. Work on peripheral AVP receptors may help to reveal the mechanisms by which central AVP receptors operate. In particular, because the VSA AVP V1 receptor resembles the peripheral V1 type vasopressor receptor (Jard et al.1987), it would be of interest to determine whether similar sensitization occurs in peripheral systems. If so, further research using tissues such as liver cells (which contain high numbers of V1 AVP receptors) may avoid some of the technical difficulties of working with brain tissue in which the level of receptors is very low.

Pretreatment of rats with OT also sensitizes the rat brain to AVP-induced motor disturbances (chapter V). Use of AVP and OT antagonists and analogues suggests that whilst pre-exposure of the rat brain to either AVP or OT induces the sensitization process, AVP sensitization appears to be mediated via the V1 AVP receptor whereas OT sensitization appears to be mediated via the OT-AVP receptor. It is of interest that many physiological stimuli that activate central AVP release also activate the release of OT in the same brain areas (Landgraf et al.1990; Landgraf et al.1988). Therefore, the ability of OT to modulate the

responsiveness of the rat brain to subsequent AVP injection suggests a role for this peptide in central AVP actions.

Cross-sensitization of AVP-induced motor disturbances with a synthetic convulsant (pentylenetetrazol), or a peptide (somatostatin), has previously been shown (Burnard, 1985), suggesting that sensitization of the rat brain to AVP is heterologous in nature. This is in keeping with the observation that the mechanism of AVP-induced sensitization may occur at a site away from the receptor and supports the possibility that other ligands may also sensitize AVP responses via alteration of post receptor mechanisms.

In fact, the mechanisms underlying agonist-induced sensitization may be similar to those known for the "priming" effect of one hormone upon another. and for which there are many examples in the literature. For example, steroid hormones such as estradiol are well known to increase the pituitary responsiveness to LH-RH. Interestingly, (Mobbs et al.1989, 1990a) have recently shown that the mechanism underlying the priming of the pituitary tissue by estradiol is the same as the mechanism for the self-priming of the pituitary tissue by LH-RH. Therefore, it may be a general principle that the mechanism(s) known for the priming effects of many ligands may also be the underlie agonist induced sensitization.

The studies on receptor regulation in sensitized animals (chapter III) were contrasted with studies of receptor regulation using an animal model in which AVP is chronically absent (chapter IV). In animals depleted of septal AVP by long-

term castration, AVP-induced motor disturbances, septal AVP receptor affinity and density, and ^{A}VP-stimulated phosphoinositide hydrolysis were not altered when compared with sham operated animals. These results suggest that the loss of the AVP content of the VSA does not result in a compensatory supersensitivity to AVP and does not alter the ability of repeated AVP exposure to activate the sensitization process. It is interesting to compare these results with those obtained from Brattleboro rats which also have no AVP. In contrast to the results on castrated rats presented in this thesis, the Brattleboro rat was shown to have an enhanced number of receptors in the septal area (Shewey and Dorsa, 1986). However, it must be recalled that Brattleboro rats have many other physiological and biochemical abnormalities in addition to the absence of AVP; similarly, castrated rats exhibit hormonal and pharmacological alterations to systems other than the BST-VSA AVP pathway. Which of the two AVP deficient models represents the best model of chronic AVP absence is not known.

The question was also asked, whether other physiological actions of AVP could be sensitized. For example, endogenous or exogenously applied AVP within the VSA has been shown to play a role in fever suppression (Naylor et al.1987; Pittman et al.1988). The results presented here showed that pre-exposure of the rat brain to OT can sensitize the antipyretic action of subsequent AVP exposure (chapter VI).

It has been known for some years that certain animals may undergo a time during which they are unable to develop fever to a normal pyrogenic stimulus (for

review, see Pittman and Wilkinson, 1991). Subsequently, it has been established that endogenous fever suppression may be due to activity in endogenous AVP systems (Kasting et al.1979; Kasting et al.1981; Cooper et al.1987; Merker et al.1980). The fact that the response to AVP may be altered due to a sensitization process raises the possibility that enhanced responsiveness to 'normal' levels of AVP could account for some states of enhanced endogenous antipyresis. This could be investigated by examining AVP-induced phosphatidylinositol hydrolysis in animals exhibiting enhanced endogenous antipyresis.

In 1969, Goddard showed that the appearance of seizures in response to repetitive subconvulsive stimulation (kindling) of specific brain regions could be induced not only by repeated electrical stimulations but also by repeated microinjections of agonists. The phenomenon of chemical kindling has provided strong evidence that convulsions can be the pathological expression of physiological adaptation in synaptic and cellular excitability. In chapter VI, results were presented indicating that fever can induce sensitization of the brain to the motor effects of AVP; presumably this occurs due to the release of endogenous AVP or OT in the VSA known to occur during fever (Landgraf et al.1990). This is the best evidence to date that there may be a link between the AVP or OT released during fever and the subsequent development of febrile convulsions. It is curious however, that rats do not generally convulse during repeated experimental fevers; this could be accounted for by the powerful inhibiting mechanisms present in the mature brain to prevent aberrant operation. Perhaps,

an animal model in which some of the inhibitory synapses are blocked would increase the likelihood of rats to develop convulsions and motor abnormalities during fever. If this model could be developed it would be possible to test whether AVP is the critical agent precipitating the phenomenon of febrile convulsions.

In conclusion, I have used the phenomenon of AVP-induced sensitization and motor disturbances as a tool to arrive at a new understanding of principles underlying receptor regulation. Physiological studies reported herein demonstrated that the phenomenon of sensitization may be of considerable relevance to animal physiology and/or pathophysiology.

VIII REFERENCES

Abood, L.G., R. Knapp, T. Michell, H. Booth, and L. Schwab (1980) Chemical requirements of vasopressin for barrel rotation convulsions and reversal by oxytocin. J. Neurosci. Res. 5: 191-199.

Aiyer, M.S., S.A. Chippa, and G. Fink (1974) A priming effect of luteinizing hormone releasing factor on the anterior pituitary gland in the female rat. J. Endocrinol. 62: 573-588.

Albers, H.E., S.Y. Liou, and C.F. Ferris (1988) Testosterone alters the behavioral response of the medial preoptic-anterior hypothalamus to microinjection of arginine vasopressin in the hamster. Brain. Res. 456: 382-386.

Antelman, S.M. and L.A. Chiodo (1981) Dopamine autoreceptor subsensitivity: A mechanism common to the treatment of depression and the induction of amphetamine psychosis? Biol. Psych. 16: 717-727.

Antoni, F.A. (1984) Novel ligand specificity of pituitary vasopressin receptors in the rat. Neuroendocrinology 39: 186-188.

Antoni, F.A., M.C. Holmes, and J.Z. Kiss (1985) Pituitary binding of vasopressin is altered by experimental manipulations of the hypothalamo-pituitary-adrenocortical axis in normal as well as homozygous (di/di) Brattleboro rats. Endocrinology. 117: 1293-1299.

Ashizawa, T., B.E. Stanton, and S.H. Appel (1982) Interaction of myasthenic immunoglobulins and cholinergic agonists on acetylcholine receptors of rat myotubes. Ann. Neurol. 11: 22-27.

Audigier, S. and C. Barberis (1985) Pharmacological characterization of two specific binding sites for neurohypophyseal hormones in hippocampal synaptic plasma membranes of the rat. EMBO. J. 4: 1407-1412.

Baertschi, A.J. and M. Friedli (1985) A novel type of vasopressin receptor on anterior pituitary corticotrophs. Endocrinology 116: 499-502.

Bailey, R.C. and D.M. Jackson (1978) A pharmacological study of changes in central nervous system receptor responsiveness after long-term dexamphetamine and apomorphine administration. Psychopharmacology 56: 317-326.

Balaban, C.D., D.A. Fredericks, J.N. Wurpel, and W.B. Severs (1988) Motor disturbances and neurotoxicity induced by centrally administered somatostatin and vasopressin in conscious rats: interactive effects of two neuropeptides. Brain. Res. 445: 117-129.

Balaban, C.D., V.P. Starcevic, and W.B. Severs (1989) Neuropeptide modulation of central vestibular circuits. Pharmacol. Rev. 41: 53-90.

Banerjee, S.P., V.K. Sharma, L.S. Kung-Cheung, S.K. Chanda, and S.J. Riggi (1979) Cocaine and d-amphetamine induce changes in central ß-adrenoceptor sensitivity: Effect of acute and chronic drug treatment. Brain Res. 175: 119-130.

Barberis, C. (1983) [3H]vasopressin binding to rat hippocampal synaptic plasma membrane. Kinetic and pharmacological characterization. FEBS. Lett. 162: 400-405.

Baskin, D.G., F. Petracca, and D.M. Dorsa (1983) Autoradiographic localization of specific binding sites for[Arg8]vasopressin in the septum of the rat brain with tritium- sensitive film. Eur. J. Pharmacol. 90: 155-157.

Baudry, M., J. Costentin, H. Marcais, M.P. Martres, P. Protais, and J.C. Schwartz (1977) Decreased responsiveness to low doses of apomorphine after dopamine agonists and the possible involvement of hyposensitivity of dopamine 'autoreceptors'. Neurosci. Lett. 4: 203-207.

Beckwith, B.E., R.E. Till, and V. Schneider (1984) Vasopressin analog (DDAVP) improves memory in human males. Peptides 5: 819-822.

Berg, D.K., R.T. Boyd, S.W. Halvorsen, L.S. Higgins, M.H. Jacob, and J.F. Margiotta (1989) Regulating the number and function of neuronal acetylcholine receptors. Trends. Neurosci. 12: 16-21.

Berridge, M.J. (1987) Inositol trisphosphate and diacylglycerol: low interacting second messengers. Ann. Rev. Biochem. 56: 159-193.

Berridge, M.J., R.M. Dawson, C.P. Downes, J.P. Heslop, and R.F. Irvine (1983) Changes in the levels of inositol phosphates after agonist- dependent hydrolysis of membrane phosphoinositides. Biochem. J. 212: 473-482.

Bevan, J.A., R.D. Bevan, and S.M. Shreeve (1989) Variable receptor affinity hypothesis. FASEB. J. 3: 1696-1704.

Bevan, J.A. and S.M. Shreeve (1989) Will the true receptor affinity please step forward. Trends. Pharmacol. Sci. 10: 63.

Biegon, A., M. Terlou, Th.D. Voorhuis, and E.R. de Kloet (1984) Arginine-vasopressin binding sites in rat brain: a quantitative autoradiographic study. Neurosci. Lett. 44: 229-234.

Bielinski, D., M. McCrory, M. Cahill, P. Polgar, and J.B. Fishman (1988) Differential coupling of smooth muscle and liver vasopressin (V1) receptors to guanine nucleotide binding proteins. Biochem. Biophys. Res. Commun. 151: 1293-1298.

Boakes, R.J., J.M. Ednie, J.A. Edwardson, A.B. Keith, A. Sahgal, and C. Wright (1985) Abnormal behavioural changes associated with vasopressin-induced barrel rotations. Brain Res. 326: 65-70.

Bone, E.A., P. Fretten, S. Palmer, C.J. Kirk, and R.H. Michell (1984) Rapid accumulation of inositol phosphates in isolated rat superior cervical sympathetic ganglia exposed to V1-vasopressin and muscarinic cholinergic stimuli. Biochem. J. 221: 803-811.

Borison, R.L., A. Hitri, H.L. Klawans, and B.I. Diamond (1979) A new animal model for schizophrenia: Behavioral and receptor binding studies. In Catecholamines: basic and clinical frontiers, E. Usdin, I.J. Kopin and J. Barchas, eds., pp. 719-721, Pergamon, Ney York.

Bouvier, M., S. Collins, B.F. O'Dowd, P.T. Campbell, A. de Blasi, B.K. Kobilka, C. MacGregor, G.P. Irons, M.G. Caron, and R.J. Lefkowitz (1989) Two distinct pathways for cAMP-mediated down-regulation of the beta 2-adrenergic receptor. Phosphorylation of the receptor and regulation of its mRNA level. J. Biol. Chem. 264: 16786-16792.

Brinton, R.E., K.W. Gee, J.K. Wamsley, T.P. Davis, and H.I. Yamamura (1984) Regional distribution of putative vasopressin receptors in rat brain and pituitary by quantitative autoradiography. Proc. Natl. Acad. Sci. U. S. A. 81: 7248-7252.

Brinton, R.E., D.R. Gehlert, J.K. Wamsley, Y.P. Wan, and H.I. Yamamura (1986) Vasopressin metabolite, AVP4-9, binding sites in brain: distribution distinct from that of parent peptide. Life. Sci. 38: 443-452.

Brinton, R.E. and B.S. McEwen (1989) Vasopressin neuromodulation in the hippocampus. J. Neurosci. 9: 752-759.

Broekkamp, C.L.E., A.G. Phillips, and A.T. Cools (1979) Stimulants effect of enkephalin injection into the dopaminergic A10 area. Nature 278: 560-562.

Brooks, V.L. (1989) Vasopressin and ANG II in the control of ACTH secretion and arterial and atrial pressures. Am. J. Physiol. 256: R339-R347.

Buijs, R.M. (1983) Vasopressin and oxytocin--their role in neurotransmission. Pharmacol. Ther. 22: 127-141.

Buijs, R.M., G.J. De Vries, F.W. Van Leeuwen, and D.F. Swaab (1983) Vasopressin and oxytocin: distribution and putative functions in the brain. Prog. Brain. Res. 60: 115-122.

Buijs, R.M. and J.J. Van Heerikhuize (1982) Vasopressin and oxytocin release in the brain: A synaptic event. Brain Res. 252: 71-76.

Burbach, J.P., B. Bohus, G.L. Kovacs, J.W. Van Nispen, H.M. Greven, and D. De Wied (1983) Oxytocin is a precursor of potent behaviourally active neuropeptides. Eur. J. Pharmacol. 94: 125-131.

Burbach, J.P., G.L. Kovacs, D. De Wied, J.W. Van Nispen, and H.M. Greven (1983) A major metabolite of arginine vasopressin in the brain is a highly potent neuropeptide. Science. 221: 1310-1312.

Burbach, J.P.H. and J.L.M. Lebouille (1983) Proteolytic conversion of arginine-vasopressin and oxytocin by brain synaptic membranes. J. Biol. Chem. 258: 1487-1494.

Burnard, D.M. (1985) Arginine vasopressin: Its possible role in convulsive disorders, Ph.D. Dissertation, The University of Calgary,

Burnard, D.M., Q.J. Pittman, and W.L. Veale (1983) Increased motor disturbances in response to arginine vasopressin following hemorrhage or hypertonic saline: evidence for central AVP release in rats. Brain. Res. 273: 59-65.

Burnard, D.M., Q.J. Pittman, and W.L. Veale (1985) Brattleboro rats display increased sensitivity to arginine vasopressin-induced motor disturbances. Brain. Res. 342: 316-322.

Burnard, D.M., W.L. Veale, and Q.J. Pittman (1986) Prevention of arginine-vasopressin-induced motor disturbances by a potent vasopressor antagonist. Brain. Res. 362: 40-46.

Burnard, D.M., W.L. Veale, and Q.J. Pittman (1987) Altered sensitivity to arginine vasopressin (AVP) in area CA1 of the hippocampal slice following pretreatment of rats with AVP. Brain. Res. 422: 11-16.

Burt, D.R. (1985) Criteria for receptor identification. In Neurotransmitter receptor binding, Vol. 2nd , H.I. Yamamura, S Enna,J. and M.J. Kuhar, eds., pp. 41-60, Raven press, New york.

Butlen, D., K. Baddouri, R.M. Rajerison, G. Guillon, B. Cantau, and S. Jard (1984) Plasma antidiuretic hormone levels and liver vasopressin receptors in the jerboa, Jaculus orientalis, and rat. Gen. Comp. Endocrinol. 54: 216-229.

Catt, K.J., J.P. Harwood, G Aguilera, and M.L. Dufau (1979) Hormonal regulation of peptide receptors and target cell responses. Nature 280: 109-116.

Changeux, J.P., P. Benoit, A. Bessis, J. Cartaud, A. Devillers Thiery, B. Fontaine, J.L. Galzi, A. Klarsfeld, R. Laufer, C. Mulle, and et al (1990) The acetylcholine receptor: functional architecture and regulation. Adv. Second. Messenger. Phosphoprotein. Res. 24: 15-19.

Chuang, D.M. (1989a) Neurotransmitter receptors and phosphoinositide turnover. Annu. Rev. Pharmacol. Toxicol. 29: 71-110.

Chuang, D.M. (1989b) Introduction to effector-coupled cell surface receptors. Prog. Clin. Biol. Res. 286: 3-9.

Clarke, P.B., D.S. Fu, A. Jakubovic, and H.C. Fibiger (1988) Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. J. Pharmacol. Exp. Ther. 246: 701-708.

Cohn, K.L. and M. Cohn (1975) Barrel rotation induced by somatostatin in the non-lesionned rat. Brain Res. 96: 138-141.

Cooper, K.E., S. Blahser, T.J. Malkinson, G. Merker, J. Roth, and E. Zeisberger (1988) Changes in body temperature and vasopressin content of brain neurons, in pregnant and non-pregnant guinea pigs, during fevers produced by Poly I:Poly C. Pflugers. Arch. 412: 292-296.

Cooper, K.E., N.W. Kasting, K. Lederis, and W.L. Veale (1979) Evidence supporting a role for endogenous vasopressin in natural suppression of fever in the sheep. J. Physiol. Lond. 295: 33-45.

Cooper, K.E., A.M. Naylor, and W.L. Veale (1987) Evidence supporting a role for endogenous vasopressin in fever suppression in the rat. J. Physiol. Lond. 387: 163-172.

Corda, M.G., O. Giorgi, S. Mele, and G. Biggio (1987) Enhanced sensitivity to beta-carboline inverse agonists in rats chronically treated with FG 7142. Brain. Res. Bull. 19: 379-385.

Corda, M.G., O. Giorgi, B. Longoni, and G. Biggio (1988) Decreased sensitivity to diazepam induced by chronic administration of FG 7142. Neurosci. Lett. 86: 219-224.

Cornett, L.E. and D.M. Dorsa (1985) Vasopressin receptor subtypes in dorsal hindbrain and renal medulla. Peptides. 6: 85-89.

Costa, E. and S. Garattini (1970) Amphetamines and Related Compounds, Raven Press, New York.

Costall, B. and R.J. Naylor (1979) Changes in dopamine receptor status after denervation or chronic receptor stimulation. Br. J. Pharmacol. 66: 492p-496p.

Costantini, M.G. and A.F. Pearlmutter (1984) Properties of the specific binding site for arginine vasopressin in rat hippocampal synaptic membranes. J. Biol. Chem. 259: 11739-11745.

Courtney, N. and M. Raskind (1983) Vasopressin affects adenylate cyclase activity in rat brain: a possible neuromodulator. Life sci. 32: 591-596.

Creese, I. and D.R. Sibley (1980) Receptor adaptations to centrally acting drugs. Ann. Rev. Pharmacol. Toxicol. 21: 357-391.

Curtis, A., V. Lyons, and G. Fink (1985) The priming effect of LH-releasing hormone: effects of cold and involvement of new protein synthesis. J. Endocrinol. 105: 163-168.

Dantzer, R., R.M. Bluthe, G.F. Koob, and M. Le Moal (1987) Modulation of social memory in male rats by neurohypophyseal peptides. Psychopharmacology Berlin. 91: 363-368.

Dantzer, R., G.F. Koob, R.M. Bluthe, and M. Le Moal (1988) Septal vasopressin modulates social memory in male rats. Brain Res. 457: 143-147.

Davis, J.L. (1984) Effects of a vasopressin analogue, DDAVP, on chick memory. Med. Sci. 12: 865.

de Kloet, E.R., F. Rotteveel, Th.A.M. Voorhuis, and M. Terlou (1985) Topography of binding sites for neurohypophyseal hormones in rat brain. Eur. J. Pharmacol. 110: 113-119.

de Kloet, E.R., Th.A.M. Voorhuis, Y. Boschma, and J. Elands (1986) Estradiol modulates density of putative 'oxytocin receptors' in discrete rat brain regions. Neuroendocrinology. 44: 415-421.

de Kloet, E.R., T.A. Voorhuis, J.P. Burbach, and D. De Wied (1985) Autoradiographic localization of binding sites for the arginine- vasopressin (VP) metabolite, VP4-9, in rat brain. Neurosci. Lett. 56: 7-11.

de Meyts, P., J. Roth, D.M. Neville, J.R. Gavin, and M.A. Lesniak (1873) Insulin interactions with its receptors: experimental evidence for negative cooperativity. Biochem. Biophys. Res. Comm. 55: 154-161.

De Vries, G.J. and H.A. al Shamma (1990) Sex differences in hormonal responses of vasopressin pathways in the rat brain. J. Neurobiol. 21: 686-693.

De Vries, G.J., W. Best, and A.A. Sluiter (1983) The influence of androgens on the development of a sex difference in the vasopressinergic innervation of the rat lateral septum. Brain. Res. 284: 377-380.

De Vries, G.J. and R.M. Buijs (1983) The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. Brain. Res. 273: 307-317.

De Vries, G.J., R.M. Buijs, and A.A. Sluiter (1984) Gonadal hormone actions on the morphology of the vasopressinergic innervation of the adult rat brain. Brain. Res. 298: 141-145.

De Vries, G.J., W. Duetz, R.M. Buijs, J. van Heerikhuize, and J.T. Vreeburg (1986) Effects of androgens and estrogens on the vasopressin and oxytocin innervation of the adult rat brain. Brain. Res. 399: 296-302.

De Wied, D., O. Gaffori, J.M. Van Ree, and W. De Jong (1984) Central target for the behavioural effects of vasopressin neuropeptides. Nature 308: 276-278.

De Wied, D., O. Gaffori, J.P. Burbach, G.L. Kovacs, and J.M. Van Ree (1987) Structure activity relationship studies with C-terminal fragments of vasopressin and oxytocin on avoidance behaviors of rats. J. Pharmacol. Exp. Ther. 241: 268-274.

Demotes Mainard, J., J. Chauveau, F. Rodriguez, J.D. Vincent, and D.A. Poulain (1986) Septal release of vasopressin in response to osmotic, hypovolemic and electrical stimulation in rats. Brain. Res. 381: 314-321.

Di Paolo, T., C. Rouillard, M. Morissette, D. Levesque, and P.J. Bedard (1989) Endocrine and neurochemical actions of cocaine. Can. J. Physiol. Pharmacol. 67: 1177-1181.

Di Scala-Guenot, D., M.T. Strosser, M.J. Freund-Mercier, and Ph. Richard (1990) Characterization of oxytocin-binding sites in primary rat brain cell cultures. Brain Res. 524: 10-16.

Disturnal, J.E., W.L. Veale, and Q.J. Pittman (1987) Modulation by arginine vasopressin of glutamate excitation in the ventral septal area of the rat brain. Can. J. Physiol. Pharmacol. 65: 30-35.

Dorsa, D.M., M.D. Brot, L.M. Shewey, K.M. Meyers, P. Szot, and M.A. Miller (1988) Interaction of a vasopressin antagonist with vasopressin receptors in the septum of the rat brain. Synapse. 2: 205-211.

Dorsa, D.M., L.A. Majumdar, F.M. Petracca, D.G. Baskin, and L.E. Cornett (1983) Characterization and localization of 3H-arginine8-vasopressin binding to rat kidney and brain tissue. Peptides. 4: 699-706.

Dorsa, D.M., F.M. Petracca, D.G. Baskin, and L.E. Cornett (1984) Localization and characterization of vasopressin-binding sites in the amygdala of the rat brain. J. Neurosci. 4: 1764-1770.

Dreifuss, I.J., M. Mühlethaler, and B.H. Gähwiler (1982) Electrophysiology of vasopressin in normal rats and in rats of the Brattleboro strain. In The Brattleboro rat, Vol 394, H.W. Sokol and H. Valtin, eds., pp. 689-702, The New York Academy of Science, New York.

Dreifuss, J.J., E. Tribollet, M. Dubois Dauphin, and M. Raggenbass (1989) Neurohypophysial hormones: neuronal effects in autonomic and limbic areas of the rat brain. Arch. Histol. Cytol. 52 Suppl: 129-138.

Duff, B.W. and S.K. Durum (1983) The pyrogenic and mitogenic actions of interleukin-1 are related. Nature 304: 449-451.

Elands, J., C. Barberis, and S. Jard (1988a) [3H]-[Thr4,Gly7]OT: a highly selective ligand for central and peripheral OT receptors. Am. J. Physiol. 254: E31-E38.

Elands, J., C. Barberis, S. Jard, E. Tribollet, J.J. Dreifuss, K. Bankowski, M. Manning, and W.H. Sawyer (1988b) 125I-labelled d(CH2)5[Tyr(Me)2,Thr4,Tyr-NH2(9)]OVT: a selective oxytocin receptor ligand. Eur. J. Pharmacol. 147: 197-207.

Ellinwood, E.H. (1967) Amphetamine psychosis: I. Description of the individuals and process. J. Nerv. Mental. Dis. 144: 273-283.

Ellinwood, E.H. (1968) Amphetamine psychosis.II. Theoretical Implications. J. Neuropsychiatry 4: 45-54.

Ellinwood, E.H. (1971) Effect of chronic methamphetamine intoxication in rhesus monkeys. Biol. Psych. 3: 25-32.

Ellinwood, E.H., A. Sudilovsky, and L.M. Nelson (1973) Evolving behavior in the clinical and experimental amphetamine (model) psychosis. Am. J. Psych. 130: 1088-1093.

Ellison, G. and W. Morris (1981) Opposed stages of continuous amphetamine administration: parallel alterations in motor stereotypies and in vivo spiroperidol accumulation. Eur. J. Pharmacol. 74: 207-214.

Ellison, G.D. and M.S. Eison (1983) Continuous amphetamine intoxication: an animal model of the acute psychotic episode. Psychol. Med. 13: 751-761.

Ellison, G.D. and W. Morris (1981) Opposed stages of continuous amphetamine administration: parallel alterations in motor stereotypies and in vivo speroperidol accumulation. Eur. J. Pharmacol. 74: 207-214.

Fahrenholz, F. and R. Gerstberger (1989) Vasopressin receptor subtypes: autoradiographic localization of V1 vasopressin binding sites in rat brain and kidney. J. Protein. Chem. 8: 370-372.

Farago, A. and Y. Nishizuka (1990) Protein kinase C in transmembrane signalling. FEBS. Lett. 268: 350-354.

Ferrier, B.M., S.A. McClorry, and A.W. Cochrane (1983) Specific binding of oxytocin in the female rat brain. Can. J. Physiol. Pharmacol. 61: 989-995.

Ferris, C.F., L. Gold, G.J. De Vries, and M. Potegal (1990) Evidence for a functional and anatomical relationship between the lateral septum and the hypothalamus in the control of flank marking behavior in Golden hamsters. J. Comp. Neurol. 293: 476-485.

Ferris, C.F., E.A. Singer, D.M. Meenan, and H.E. Albers (1988) Inhibition of vasopressin-stimulated flank marking behavior by V1-receptor antagonists. Eur. J. Pharmacol. 154: 153-159.

Fink, G., S.A. Chiappa, and M.S. Aiyer (1976) Priming effect of luteinizing hormone release factor elicited by preoptic stimulation and by intravenous infusion and multiple injections of the synthetic decapeptide. J. Endocrinol. 69: 359-372.

Fliers, E., G.J. De Vries, and D.F. Swaab (1985) Changes with aging in the vasopressin and oxytocin innervation of the rat brain. Brain. Res. 348: 1-8.

Freund-Mercier, M.J., M.E. Stoeckel, M. Dietl, J.M. Palacios and Ph. Richard (1988) Quantitative autoradiographic mapping of neurohypophysial hormone binding sites in the rat forebrain and pituitary gland -I. Characterization of different types of binding sites and their distribution on the Long-Evans strain. Neuroscience 26: 261-272.

Gerstberger, R. and F. Fahrenholz (1989) Autoradiographic localization of V1 vasopressin binding sites in rat brain and kidney. Eur. J. Pharmacol. 167: 105-116.

Giguere, V. and F. Labrie (1982) Vasopressin potentiates cyclic AMP accumulation and ACTH release induced by corticotropin-releasing factor in rat anterior pituitary cells in culture. Endocrinology 111: 1752-1754.

Gillis, B.J. and D.P. Cain (1986) Kindling of the hippocampus and septum in vasopressin-deficient rats (Brattleboro strain). Brain. Res. 363: 386-389.

Goddard, G.V. (1969) Analysis of avoidance conditioning following cholinergic stimulation of amygdala in rats. J. Comp. Physiol. Psychol. 68: 1-18.

Greenwood, R.S., R. Meeker, H. Sullivan, and J.N. Hayward (1989) Kindling in spontaneous hypertensive rats. Brain. Res. 495: 58-65.

Gubitz, G.J., A.M. Naylor, and W.L. Veale (1987) Mechanism of vasopressin induced antipyresis: effects of cool and warm environments on the thermoregulatory actions of central vasopressin. Proc. West. Pharmacol. Soc. 30: 253-257.

Guillon, G., R.C. Gaillard, P. Kehrer, P. Schoenenberg, A.F. Muller, and S. Jard (1987) Vasopressin and angiotensin induce inositol lipid breakdown in rat adenohypophysial cells in primary culture. Regul. Pept. 18: 119-129.

Gunther, S., M.A. Gimbrone,Jr., and R.W. Alexander (1980) Identification and characterization of the high affinity vascular angiotensin II receptor in rat mesenteric artery. circ. res. 47: 278-286.

Hadcock, J.R. and C.C. Malbon (1988) Down-regulation of beta-adrenergic receptors: agonist-induced reduction in receptor mRNA levels. Proc. Natl. Acad. Sci. U. S. A. 85: 5021-5025.

Hamburger Bar, R. and M.E. Newman (1985) Effects of vasopressin on noradrenaline-induced cyclic AMP accumulation in rat brain slices. Pharmacol. Biochem. Behav. 22: 183-187.

Handelman, G.E. and S.C. Sayson (1984) Neonatal exposure to vasopressin decreases vasopressin binding sites in the adult kidney. Peptides 5: 1217-1219.

Hausdorff, W.P., M.G. Caron, and R.J. Lefkowitz (1990) Turning off the signal: desensitization of beta-adrenergic receptor function. FASEB. J. 4: 2881-2889.

Hinkle, P.M. and A.H. Tashjian (1975) Thyrotropin-releasing hormone regulates the number of its own receptors in the GH3 strain of pituitary cells in culture. Biochem. 14: 3845-3851.

Hinko, A., Y. Kim, and A.F. Pearlmutter (1986) Protein phosphorylation in rat hippocampal synaptic plasma membranes in response to neurohypophyseal peptides. Brain Res. 381: 156-160.

Hinko, A. and A.F. Pearlmutter (1987) Effects of arginine vasopressin on protein phosphorylation in rat hippocampal synaptic membranes. J. Neurosci. 17: 71-79.

Hirano, M., H. Uchimura, A. Shiraishi, T. Kuroki, T. Matsumoto, and T. Tsutsumi (1989) [beta-Phenylethylamine and amphetamine: similar aspects in their behavioropharmacological and neurochemical characteristics]. Yakubutsu. Seishin. Kodo. 9: 335-348.

Hoffman, P.L. and B. Tabakoff (1984) Neurohypophyseal peptides maintain tolerance to the incoordinating effects of ethanol. Pharmacol. Biochem. Behav. 21: 535-543.

Hollenberg, M.D. (1990) Receptor triggering and receptor regulation: structure-activity relationships from the receptor's point of view. J. Med. Chem. 33: 1275-1281.

Homcy, C.J., S.F. Vatner, and D.E. Vatner (1991) ß-adrenergic receptor regulation in the heart in pathophysiologic states: abnormal adrenergic responsiveness in cardiac desease. Annu. Rev. Physiol. 53: 137-159.

Horn, A.M. and S.L. Lightman (1988) Vasopressin-stimulated turnover of phosphatidylinositol in the decentralised superior cervical ganglion of the rat. Brain Res. 455: 18-23.

Horwitz, J., C.H. Anderson, and R.L. Perlman (1986) Comparison of the effects of muscarine and vasopressin on inositol phospholipid metabolism in the superior cervical ganglion of the rat. J. Pharmacol. Exp. Ther. 237: 312-317.

Hosey, M.M., M.M. Kwatra, J. Ptasienski, and R.M. Richardson (1990) Regulation of receptor function by protein phosphorylation. Ann. N. Y. Acad. Sci. 588: 155-163.

Howlett, D.R. and S.R. Nahorski (1979) Acute and chronic amphetamine treatments modulate striatal dopamine receptor binding sites. Brain Res. 161: 173-178.

Hurd, Y.L., F. Weiss, G. Koob, and U. Ungerstedt (1990) The influence of cocaine self-administration on in vivo dopamine and acetylcholine neurotransmission in rat caudate-putamen. Neurosci. Lett. 109: 227-233.

Iwamoto, E.T. and E.L. Way (1977) Circling behavior and stereotypy induced by intranigral opiate injections. J. Pharmacol. Exp. Ther. 203: 347-359.

Jard, S. (1983a) Vasopressin: mechanisms of receptor activation. Prog. Brain. Res. 60: 383-394.

Jard, S. (1983b) Vasopressin isoreceptors in mammals: relation to cyclic AMP-dependent or cyclic AMP-independent transduction mechanisms. Curr. Top. Memb. Trans. 18: 255-285.

Jard, S., C. Barberis, S. Audigier, and E. Tribollet (1987) Neurohypophyseal hormone receptor systems in brain and periphery. Prog. Brain. Res. 72: 173-187.

Jard, S., R.C. Gaillard, G. Guillon, J. Marie, P. Schoenenberg, A.F. Muller, M. Manning, and W.H. Sawyer (1986) Vasopressin antagonists allow demonstration of a novel type of vasopressin receptors in the rat adenohypophysis. Mol. Pharmacol. 30: 171-177.

Jenner, P. and C.D. Marsden (1987) Chronic pharmacological manipulation of dopamine receptors in brain. Neuropharmacology. 26: 931-940.

Joëls, M. and I.J.A. Urban (1982) The effect of microiontophoretically applied vasopressin and oxytocin on single neurons in the septum and dorsal hippocampus of the rat. Neurosci. Lett. 33: 79-84.

Johnson, A.E., G.F. Ball, H. Coirini, C.R. Harbaugh, B.S. McEwen, and T.R. Insel (1989) Time course of the estradiol-dependent induction of oxytocin receptor binding in the ventromedial hypothalamic nucleus of the rat. Endocrinology. 125: 1414-1419.

Jolles, J., K.W.A. Wirtz, P. Schotman, and W.H. Gispen (1979) Pituitary hormones influence polyphosphoinositide metabolism in rat brain. FEBS. Lett. 105: 110-114.

Joyce, E.M. and S.D. Iversen (1979) The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neuroscience Letter 14: 207-212.

Junig, J.T., L.G. Abood, and A.M. Skrobala (1985) Two classes of arginine vasopressin binding sites on rat brain membranes. Neurochem. Res. 10: 1187-1202.

Kalivas, P.W. (1985) Sensitization to repeated enkephalin administration into the ventral tegmental area of the rat. II. Involvement of the mesolimbic dopamine system. J. Pharmacol. Exp. Ther. 235: 544-550.

Kalivas, P.W. and P. Duffy (1987) Sensitization to repeated morphine injection in the rat: possible involvement of A10 dopamine neurons. J. Pharmacol. Exp. Ther. 241: 204-212.

Kalivas, P.W., S. Taylor, and J.S. Miller (1985) Sensitization to repeated enkephalin administration into the ventral tegmental area of the rat. I. Behavioral characterization. J. Pharmacol. Exp. Ther. 235: 537-543.

Kalivas, P.W., E. Widerlov, D. Stanley, G.R. Breese, and A.J.Jr. Prange (1983) Enkephalin action on the mesolimbic system: A dopamine-dependent and a dopamine-independent increase in locomotor activity. J. Pharmacol. Exp. 227: 229-235.

Karler, R., L.D. Calder, I.A. Chaudhry, and S.A. Turkanis (1989) Blockade of "reverse tolerance" to cocaine and amphetamine by MK- 801. Life. Sci. 45: 599-606.

Kasting, N.W. (1989) Criteria for establishing a physiological role for brain peptides. A case in point: the role of vasopressin in thermoregulation during fever and antipyresis. Brain. Res. Rev. 14: 143-153.

Kasting, N.W., K.E. Cooper and W.L. Veale (1979) Antipyresis following perfusion of brain sites with vasopressin. Experientia 35: 208-209.

Kasting, N.W., W.L. Veale, and K.E. Cooper (1980) Convulsive and hypothermic effects of vasopressin in the brain of the rat. Can. J. Physiol. Pharmacol. 58: 316-319.

Kasting, N.W., W.L. Veale, K.E. Cooper, and K. Lederis (1981) Vasopressin may mediate febrile convulsions. Brain. Res. 213: 327-333.

Kasting, N.W. and M.F. Wilkinson (1987) Vasopressin functions as an endogenous antipyretic in the newborn. Biol. Neonate. 51: 249-254.

Kelly, J., G.F. Alheid, A. Newberg, and S.P. Grossman (1977) GABA stimulation and blockade in the hypothalamus and midbrain: effects on feeding and locomotor activity. Pharmacol. Biochem. Behav. 7: 537-541.

Khan, C.R., D.M. Neville, and J. Roth (1973) Insulin-receptor interaction in the obese-hyperglycemic mouse. J. Biol. Chem. 248: 244-250.

Kiraly, M., S. Audigier, E. Tribollet, C. Barberis, M. Dolivo, and J.J. Dreifuss (1986) Biochemical and electrophysiological evidence of functional vasopressin receptors in the rat superior cervical ganglion. Proc. Natl. Acad. Sci. U. S. A. 83: 5335-5339.

Kita, T. and T. Nakashima (1989) [Tolerance and reverse tolerance of nicotine on spontaneous motor activity]. Nippon. Yakurigaku. Zasshi. 94: 103-112.

Klawans, H.L., A. Hitri, P.M. Carvey, P.A. Nausieda, and W.J. Weiner (1979) Effect of chronic dopaminergic agonist on striatal membrane dopamine binding. Adv. Neurol. 24: 217-224.

Klawans, H.L., D.I. Margolin, N. Dava, and P. Crosser (1975) Supersensitivity to D-amphetamine and apomorphine-induced stereotyped behavior induced by chronic D-amphetamine administration. J. Neurol. Sci. 25: 283-289.

Klein, W.L., J. Sullivan, A. Skorupa, and J.S. Aguilar (1989) Plasticity of neuronal receptors. FASEB. J. 3: 2132-2140.

Kleinbaum, D.G., L.L. Kupper, and K.E. Muller (1988) . In Applied regression analysis and other multivariable methods, Vol. 2nd , pp. 260-296, PWS-Kent, Boston.

Ko, G.N., B.J. Wilcox, F.M. Petracca, M.A. Miller, M.M. Murburg, D.G. Baskin, and D.M. Dorsa (1989) Localization and measurement of neurotransmitter receptors in rat and human brain by quantitative autoradiography. Comput. Med. Imaging. Graph. 13: 37-45.

Kokkinidis, L. (1984) Effects of chronic intermittent and continuous amphetamine administration on acoustic startle. Pharmacol. Biochem. Behav. 20: 367-371.

Koob, G.F., C. Lebrun, J.L.J. Martinez, R. Dantzer, M. Le Moal, and F.E. Bloom (1985) Arginine vasopressin, stress, and memory. Ann. N. Y. Acad. Sci. 444: 194-202.

Koob, G.F., C. Lebrun, R.M. Bluthe, R. Dantzer, and M. Le Moal (1989) Role of neuropeptides in learning versus performance: focus on vasopressin. Brain Res. Bull. 23: 359-364.

Kordower, J.H. and R.J. Bodnar (1984) Vasopressin analgesia: specificity of action and non-opioid effects. Peptides 5: 747-756.

Kosman, M.E. and K.R. Unna (1968) Effects of chronic administration of the amphetamines and other stimulants on behavior. Clin. Pharmacol. Therap. 9: 240-254.

Kovács, G.L. and D. De Wied (1983) Hormonally active arginine-vasopressin suppresses endotoxin- induced fever in rats: lack of effect of oxytocin and a behaviorally active vasopressin fragment. Neuroendocrinology 37: 258-261.

Kovács, G.L., F. Laczi, M. Vecsernyes, K. Hodi, G. Telegdy, and F.A. Laszlo (1987) Limbic oxytocin and arginine 8-vasopressin in morphine tolerance and dependence. Exp. Brain Res. 65: 307-311.

Kovács, G.L., B. Liu, J.P. Burbach, J.M. Van Ree, and D. De Wied (1989) N alpha-acetyl-[Arg8]vasopressin antagonizes the behavioral effect ofvasopressin-(5-9), but not of vasopressin. Eur. J. Pharmacol. 161: 27-35.

Kovács, G.L., M. Faludi, and G. Telegdy (1985) Oxytocin diminishes heroin tolerance in mice. Psychopharmacology 86: 377-379.

Kovács, G.L., Z. Horváth, Z. Sarnyai, M. Faludi, and G. Telegdy (1985) Oxytocin and a C-terminal derivative (z-prolyl-D-leucine) attenuate tolerance to and dependence on morphine and interact with dopaminergic neurotransmission in the mouse brain. Neuropharmacology 24: 413-419.

Kovács, G.L. and G. Telegdy (1987) ß-endorphin tolerance is inhibited by oxytocin. Pharmacol. Biochem. Behav. 26: 57-60.

Kovács, G.L., H.D. Veldhuis, D.H.G. Versteeg, and D. De Wied (1986) Facilitation of avoidance behavior by vasopressin fragments microinjected into limbic-midbrain structures. Brain Res. 371: 17-24.

Kruse, H., Tj.B. van Wimersma Greidanus, and D. De Wied (1977) Barrel rotation induced by vasopressin and related peptides in rats. Pharmacol. Biochem. Behav. 7: 311-313.

Kruszynski, M., B. Lammek, M. Manning, J. Seto, J. Haldar, and W.H. Sayer (1980) [1-(ß-mercapto-ß,ß-cyclopentamethylenepropionic acid),2-(O-methyl)tyrosine]arginine-vasopressin and [1-(ß-mercapto-ß,ß-cyclopentamethylenepropionic acid)]arginine-vasopressin, two highly potent antagonists of the vasopressor response to arginine-vasopressin. J. Med. Chem. 23: 364-368.

Ksir, C., R.L. Hakan, and K.J. Kellar (1987) Chronic nicotine and locomotor activity: influences of exposure dose and test dose. Psychopharmacology Berlin. 92: 25-29.

Landgraf, R., T. Malkinson, T. Horn, W.L. Veale, K. Lederis, and Q.J. Pittman (1990) Release of vasopressin and oxytocin by paraventricular stimulation in rats. Am. J. Physiol. 258: R155-R159.

Landgraf, R., T.J. Malkinson, W.L. Veale, K. Lederis, and Q.J. Pittman (1990) Vasopressin and oxytocin in rat brain in response to prostaglandin fever. Am. J. Physiol. 259: R1056-R1062.

Landgraf, R., I. Neumann, and H. Schwarzberg (1988) Central and peripheral release of vasopressin and oxytocin in the conscious rat after osmotic stimulation. Brain. Res. 457: 219-225.

Lapchak, P.A., D.M. Araujo, R. Quirion, and B. Collier (1989) Effect of chronic nicotine treatment on nicotinic autoreceptor function and N-[3H]methylcarbamylcholine binding sites in the rat brain. J. Neurochem. 52: 483-491.

Lawrence, D. and Q.J. Pittman (1985) Response of rat paraventricular neurones with central projections to suckling, haemorrhage or osmotic stimuli. Brain. Res. 341: 176-183.

Laycock, J.F. and S.L. Lightman (1989) Cardiovascular interactions between vasopressin, angiotensin and noradrenaline in the Brattleboro rat. Br. J. Pharmacol. 96: 347-355.

Le Moal, M., R. Dantzer, B. Michaud, and G.F. Koob (1987) Centrally injected arginine vasopressin (AVP) facilitates social memory in rats. Neurosci. Lett. 77: 353-359.

Lebrun, C.J., M.G. Gruber, Meister.M., and Th. Unger (1990) Central vasopressin pretreatment sensitizes phosphoinositol hydrolysis in the rat septum. Brain. Res. 531: 167-172.

Lefkowitz, R.J., W.P. Hausdorff, and M.G. Caron (1990) Role of phosphorylation in desensitization of the beta- adrenoceptor. Trends. Pharmacol. Sci. 11: 190-194.

Leidenheimer, N.J. (1991) GABAA receptor phosphorylation: multiple sites, actions and artifacts. TIPS 12: 84-87.

Lewis, D.L., J.D. Lechleiter, D. Kim, C. Nanavati, and D.E. Clapham (1990) Intracellular regulation of ion channels in cell membranes. Mayo. Clin. Proc. 65: 1127-1143.

Little, H.J., D.J. Nutt, and S.C. Taylor (1986) The effects of drugs acting at the GABAA-receptor/ionophore after chemical kindling with the benzodiazepine receptor ligand FG 7142. Br. J. Pharmacol. 88: 507-514.

Loscher, W. and D.N. Stephens (1988) Chronic treatment with diazepam or the inverse benzodiazepine receptor agonist FG 7142 causes differential changes in the effects of GABA receptor stimulation. Epilepsy. Res. 2: 253-259.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Magos, L. (1969) Persistence of the effect of amphetamine on stereotyped activity in rats. Eur. J. Pharmacol. 6: 200-201.

Mahan, L.C. (1987) Metabolism of alpha- and beta-adrenergic receptors in vitro and in vivo. Ann. Rev. Pharmacol. Toxicol. 27: 215-235.

Maiti, A., K. Shahid Salles, S. Grassi, and L.G. Abood (1986) Barrel rotation and prostration by vasopressin and nicotine in the vestibular cerebellum. Pharmacol. Biochem. Behav. 25: 583-588.

Malkinson, T.J., T.E. Bridges, K. Lederis, and W.L. Veale (1987) Perfusion of the septum of the rabbit with vasopressin antiserum enhances endotoxin fever. Peptides. 8: 385-389.

Mannix, S.A., P.L. Hoffman, and C.L. Melchior (1986) Intraventricular arginine vasopressin blocks the acquisition of ethanol tolerance in mice. Eur. J. Pharmacol. 128: 137-141.

Marks, M.J. and A.C. Collins (1985) Tolerance, cross-tolerance, and receptors after chronic nicotine or oxotremorine. Pharmacol. Biochem. Behav. 22: 283-291.

Marks, M.J., J.A. Stitzel, and A.C. Collins (1985) Time course study of the effects of chronic nicotine infusion on drug response and brain receptors. J. Pharmacol. Exp. Ther. 235: 619-628.

Martin Iverson, M.T., S.M. Stahl, and S.D. Iversen (1988) Chronic administration of a selective dopamine D-2 agonist: factors determining behavioral tolerance and sensitization. Psychopharmacology. Berlin. 95: 534-539.

Martin, S.M., T.J. Malkinson, W.L. Veale, and Q.J. Pittman (1985) The action of centrally administered arginine vasopressin on blood pressure in the conscious rabbit. Brain. Res. 348: 137-145.

Matres, M.P., J. Costentin, M. Baudry, H. Marcais, P. Protais, and J.C. Schwartz (1977) Long-term changes in the sensitivity of pre- and postsynaptic dopamine receptors in mouse striatum evidenced by behavioural and biochemical studies. Brain Res. 136: 319-337.

Mattingly, B.A., J.E. Gotsick, and K. Salamanca (1988) Latent sensitization to apomorphine following repeated low doses. Behav. Neurosci. 102: 553-558.

McNamara, J.O. (1988) Pursuit of the mechanisms of kindling. Trends. Neurosci. 11: 33-36.

Merker, G., S. Blähser, and E. Zeisberger (1980) Reactivity pattern of vasopressin-containing neurons and its relation to the antipyretic reaction in the pregnant guinea pig. Cell Tissue Res. 212: 47-61.

Michell, R.H., C.J. Kirk, and M.M. Billah (1979) Hormonal stimulation of phosphatidylinositol breakdown, with particular reference to the hepatic effects of vasopressin. Biochem. soc. trans. 7: 861-865.

Miller, M.A., J.H. Urban, and D.M. Dorsa (1989) Steroid dependency of vasopressin neurons in the bed nucleus of the stria terminalis by in situ hybridization. Endocrinology. 125: 2335-2340.

Mishra, R.K., Y.W. Wong, S.L. Varmuza, and L. Tuff (1978) Chemical lesion and drug induced supersensitivity and subsensitivity of caudate dopamine receptors. Life sci. 23: 443-446.

Mitchell, R., M. Johnson, S.A. Ogier, and G. Fink (1988) Facilitated calcium mobilization and inositol phosphate production in the priming effect of LH-releasing hormone in the rat. J. Endocrinol. 119: 293-301.

Mobbs, C., G. Fink, M. Johnson, W. Welch, and D. Pfaff (1989) Similarity of an estrogen-induced protein and a luteinizing hormone releasing hormone-induced protein. Mol. Cell. Endocrinol. 62: 297-306.

Mobbs, C.V., G. Fink, and D.W. Pfaff (1990a) HIP-70: a protein induced by estrogen in the brain and LH-RH in the pituitary. Science. 247: 1477-1479.

Mobbs, C.V., G. Fink, and D.W. Pfaff (1990b) HIP-70: an isoform of phosphoinositol-specific phospholipase C- alpha. Science. 249: 566-567.

Mobbs, C.V., J.M. Rothfeld, R. Saluja, and D.W. Pfaff (1989) Phorbol esters and forskolin infused into midbrain central gray facilitate lordosis. Pharmacol. Biochem. Behav. 34: 665-667.

Mukherjee, C., M.G. Caron, and R.J. Lefkowitz (1975) Catecholamine-induced subsensitivity of adenylate cyclase associated with loss of ß-adrenergic receptor binding sites. Proc. Nat. Acad. Sci. 72: 1945-1949.

Muller, P. and P. Seeman (1979) Presynaptic subsensitivity as a possible basis for sensitization by long-term dopamine mimetics. Eur. J. Pharmacol. 55: 149-157.

Nathanson, N.M. (1989) Regulation and dvelopment of muscarinic receptor number and function. In The muscarinic receptors, J.H. Brown, ed., pp. 419-454, Humana Press, Inc.,

Naylor, A.M., K.E. Cooper, and W.L. Veale (1987) Vasopressin and fever: evidence supporting the existence of an endogenous antipyretic system in the brain. Can. J. Physiol. Pharmacol. 65: 1333-1338.

Naylor, A.M., G.J. Gubitz, C.A. Dinarello, and W.L. Veale (1987) Central effects of vasopressin and 1-desamino-8-D-arginine vasopressin (DDAVP) on interleukin-1 fever in the rat. Brain. Res. 401: 173-177.

Naylor, A.M., Q.J. Pittman, and W.L. Veale (1988) Stimulation of vasopressin release in the ventral septum of the rat brain suppresses prostaglandin E1 fever. J. Physiol. Lond. 399: 177-189.

Naylor, A.M., W.D. Ruwe, D.M. Burnard, P.D. McNeely, S.L. Turner, Q.J. Pittman, and W.L. Veale (1985a) Vasopressin-induced motor disturbances: localization of a sensitive forebrain site in the rat. Brain. Res. 361: 242-246.

Naylor, A.M., W.D. Ruwe, A.F. Kohut, and W.L. Veale (1985b) Perfusion of vasopressin within the ventral septum of the rabbit suppresses endotoxin fever. Brain. Res. Bull. 15: 209-213.

Neumann, I., H. Schwarzberg, and R. Landgraf (1988) Measurement of septal release of vasopressin and oxytocin by the push-pull technique following electrical stimulation of the paraventricular nucleus of rats. Brain. Res. 462: 181-184.

Nordberg, A., L. Romanelli, A. Sundwall, C. Bianchi, and L. Beani (1989) Effect of acute and subchronic nicotine treatment on cortical acetylcholine release and on nicotinic receptors in rats and guinea-pigs. Br. J. Pharmacol. 98: 71-78.

Norman, A.B., G. Battaglia, and I. Creese (1987) Differential recovery rates of rat D2 dopamine receptors as a function of aging and chronic reserpine treatment following irreversible modification: A key to receptor regulatory mechanisms. J. Neurosci. 7: 1484-1491.

O'Dowd, B.F., R.J. Lefkowitz, and M.G. Caron (1989) Structure of the adrenergic and related receptors. Annu. Rev. Neurosci. 12: 67-83.

Oriowo, M.A., J.A. Bevan, and R.D. Bevan (1989) Variation in sensitivity of six cat and six rat arteries to norepinephrine can be related to differences in agonist affinity and receptor reserve. J. Pharmacol. Exp. Ther. 251: 16-20.

Pearlmutter, A.F., M.G. Costantini, and B. Loeser (1983) Characterization of 3H-AVP binding sites in particulate preparations of rat brain. Peptides. 4: 335-341.

Perez, R., M. Espinoza, R. Riquelme, J.T. Parer, and A.J. Llanos (1989) Arginine vasopressin mediates cardiovascular responses to hypoxemia in fetal sheep. Am. J. Physiol. 256: R1011-R1018.

Petit, P., C. Barberis, and S. Jard (1988) Vasopressin potentiates the noradrenaline-induced accumulation of cyclic AMP in the rat superior cervical ganglion. Brain. Res. 440: 299-304.

Petracca, F.M., D.G. Baskin, J. Diaz, and D.M. Dorsa (1986) Ontogenetic changes in vasopressin binding site distribution in rat brain: an autoradiographic study. Brain. Res. 393: 63-68.

Pickering, A. and G. Fink (1976) Priming effect of luteinizing hormone-releasing factor: in-vitro and in-vivo evidence consistent with its dependence upon protein and RNA synthesis. J. Endocrinol. 69: 373-379.

Pickering, A. and G. Fink (1979) Priming effect of luteinizing hormone releasing factor in vitro: role of protein synthesis, contractile elements, Ca2+ and cyclic AMP. J. Endocrinol. 81: 223-234.

Pittman, Q.J. and L.G. Franklin (1985) Vasopressin antagonist in nucleus tractus solitarius/vagal area reduces pressor and tachycardia responses to paraventricular nucleus stimulation in rats. Neurosci. Lett. 56: 155-160.

Pittman, Q.J., T.J. Malkinson, N.W. Kasting, and W.L. Veale (1988) Enhanced fever following castration: possible involvement of brain arginine vasopressin. Am. J. Physiol. 254: R513-R517.

Pittman, Q.J., A. Naylor, P. Poulin, J. Disturnal, W.L. Veale, S.M. Martin, T.J. Malkinson, and B. Mathieson (1988) The role of vasopressin as an antipyretic in the ventral septal area and its possible involvement in convulsive disorders. Brain. Res. Bull. 20: 887-892.

Pittman, Q.J., C.L. Riphagen, and K. Lederis (1984) Release of immunoassayable neurohypophyseal peptides from rat spinal cord, in vivo. Brain. Res. 300: 321-326.

Pittman, Q.J. and M.F. Wilkinson (1991) Central arginine vasopressin and endogenous antipyresis. Can. J. Physiol. Pharmacol. in press:

Post, R.M. (1980) Intermittent versus continuous stimulation: effect of time interval on the development of sensitization or tolerance. Life. Sci. 26: 1275-1282.

Post, R.M. (1981) Central stimulants: clinical and experimental evidence on tolerance and sensitization. In Research advance in alcohol and drug problems, Vol. 6th , Y. Israel, H. Kalant, R.E. Pophano, W. Schmidt and R.G. Smart, eds., pp. 1-65, Plenum Press, New York.

Post, R.M., N.R. Contel, and P. Gold (1982) Impaired behavioral sensitization to cocaine in vasopressin deficient rats. Life sci. 31: 2745-2750.

Post, R.M. and S.R. Weiss (1989) Sensitization, kindling, and anticonvulsants in mania. J. Clin. Psychiatry. 50 Suppl: 23-30.

Praxinos, G. and C. Watson (1982) The rat brain in stereotaxic coordinates, Academic Press, New York.

Predy, P.A. and L. Kokkindis (1984) Sensitization to the effects of repeated amphetamine administration on intracranial self-stimulation: evidence for changes in reward processes. Behav. Brain. Res. 13: 251-259.

Raff, M. (1976) Self regulation of membrane receptors. Nature 259: 265-266.

Raggenbass, M., E. Tribollet, and J.J. Dreifuss (1987) Electrophysiological and autoradiographical evidence of V1 vasopressin receptors in the lateral septum of the rat brain. Proc. Natl. Acad. Sci. U. S. A. 84: 7778-7782.

Raggenbass, M., E. Tribollet, M. Dubois Dauphin, and J.J. Dreifuss (1989) Vasopressin receptors of the vasopressor (V1) type in the nucleus of the solitary tract of the rat mediate direct neuronal excitation. J. Neurosci. 9: 3929-3936.

Riphagen, C.L. and Q.J. Pittman (1986) Arginine vasopressin as a central neurotransmitter. Fed. Proc. 45: 2318-2322.

Riphagen, C.L. and Q.J. Pittman (1989) Mechanisms underlying the cardiovascular responses to intrathecal vasopressin administration in rats. Can. J. Physiol. Pharmacol. 67: 269-275.

Ritzmann, R.F., K.A. Steece, J.M. Lee, and F.A. DeLeon Jones (1985) Neuropeptides differentially effect various forms of morphine tolerance. Neuropeptides. 6: 255-258.

Robertson, H.A. (1983) Chronic D-amphetamine and phencyclidine: effects on dopamine agonist and antagonist binding sites in the extrapyramidal and mesolimbic systems. Brain Res. 267: 179-182.

Robinson, D. and R.J. McGee (1985) Agonist-induced regulation of the neuronal nicotinic acetylcholine receptor of PC12 cells. Mol. Pharmacol. 27: 409-417.

Robinson, T.E. (1984) Behavioral sensitization: characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. Psychopharmacology. Berlin. 84: 466-475.

Robinson, T.E., J.B. Becker, and S.K. Presty (1982a) Long term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: sex difference. Brain. Res. 253: 231-241.

Robinson, T.E. and J.B. Becker (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain. Res. 396: 157-198.

Roth, R.H. (1979) Dopamine autoreceptors: Pharmacology, function and comparison with postsynaptic dopamine receptors. Comm. Psychopharmacol. 3: 429-462.

Rowland, N., S.M. Antelman, and D. Kocan (1981) Elevated water intake in rats treated chronically with amphetamine: drinking in excess of need. Appetite 2: 51-66.

Ruwe, W.D., A.M. Naylor, and W.L. Veale (1985) Perfusion of vasopressin within the rat brain suppresses prostaglandin E-hyperthermia. Brain. Res. 338: 219-224.

Ruwe, W.D., W.L. Veale, and K.E. Cooper (1983) Peptide neurohormones: their role in thermoregulation and fever. Can. J. Biochem. Cell. Biol. 61: 579-593.

Ryan, K.L., R.M. Thornton, and D.W. Proppe (1989) Vasopressin contributes to maintenance of arterial blood pressure in dehydrated baboons. Am. J. Physiol. 256: H486-H492.

Sakuma, Y. and D.W. Pfaff (1979) Mesencephalic mechanisms for integration of female reproductive behavior in the rat. Am. J. Physiol. 237: R285-R290.

Scatton, B. and P. Worms (1978) Subsensitivity of striatal and mesolimbic dopamine target cells after repeated treatment with apomorphine dipivaloyl ester. Pharmacology 303: 271-278.

Schofield, P.R., B.D. Shivers, and P.H. Seeburg (1990) The role of receptor subtype diversity in the CNS. Trends. Neurosci. 13: 8-11.

Schwartz, J.C., J. Costentin, M.P. Martres, P. Protais, and M. Baudry (1978) Modulation of receptor mechanisms in the CNS: Hyper and hyposensitivity to catecholamines. Neuropharmacology 17: 665-685.

Schwartz, R.D. and K.J. Kellar (1985) In vivo regulation of acetylcholine recognition sites in brain by nicotinic cholinergic drugs. J. Neurochem. 45: 427-433.

Segal, D.S., S.B. Weinberger, J. Cahill, and S.J. McCunney (1980) Multiple daily amphetamine administration: behavioral and neurochemical alterations. Science 207: 904-907.

Shewey, L.M., G.J. Boer, P. Szot, and D.M. Dorsa (1989) Regulation of vasopressin receptors and phosphoinositide hydrolysis in the septum of heterozygous and homozygous Brattleboro rats. Neuroendocrinology. 50: 292-298.

Shewey, L.M., M.D. Brot, P. Szot, and D.M. Dorsa (1989) Enhanced phosphoinositol hydrolysis in response to vasopressin in the septum of the homozygous Brattleboro rat. Brain. Res. 478: 95-102.

Shewey, L.M. and D.M. Dorsa (1986) Enhanced binding of 3H-arginine8-vasopressin in the Brattleboro rat. Peptides. 7: 701-704.

Shewey, L.M. and D.M. Dorsa (1988) V1-type vasopressin receptors in rat brain septum: binding characteristics and effects on inositol phospholipid metabolism. J. Neurosci. 8: 1671-1677.

Sibley, D.R. and R.J. Lefkowitz (1985) Molecular mechanisms of receptor desensitization using the ß-adrenergic receptor-coupled adenylate cyclase system as a model. Nature 317: 124-129.

Snijdewint, F.G., F.W. Van Leeuwen, and G.J. Boer (1989) Ontogeny of vasopressin and oxytocin binding sites in the brain of Wistar and Brattleboro rats as demonstrated by lightmicroscopical autoradiography. J. Chem. Neuroanat. 2: 3-17.

Sofroniew, M.V. (1983) Morphology of vasopressin and oxytocin neurones and their central and vascular projections. In The neurohypophysis: structure, function and control. Progress in Brain Research 60, B.A. Cross and G. Leng, eds., pp. 101-114, Elsevier, Amsterdam.

Sokol, H.W. and H. Valtin (1982) The brattleboro rat, vol. 394, The New York Academy of Science,

Speisky, M.B. and H. Kalant (1986) Vasopressin-like peptides retain ethanol tolerance in the absence of changes in serotonin synthesis in limbic structures. Pharmacol. Biochem. Behav. 25: 797-803.

Spiegel, A.M. (1990) Receptor-effector coupling be G-proteins: implications for neuronal plasticity. In Progress in brain research, Vol. 86th , P. Coleman, G. Higgins and C. Phelps, eds., pp. 269-276, Elsevier Science Publisher,

Stephens, L.R. and S.D. Logan (1986) Arginine-vasopressin stimulates inositol phospholipid metabolism in rat hippocampus. J. Neurochem. 46: 649-651.

Szabo, G., B. Tabakoff, and P.L. Hoffman (1988) Receptors with V1 characteristics mediate the maintenance of ethanol tolerance by vasopressin. J. Pharmacol. Exp. Ther. 247: 536-541.

Thurston, C.L., E.S. Culhane, S.N. Suberg, E. Carstens, and L.R. Watkins (1988) Antinociception vs. motor effects of intrathecal vasopressin as measured by four pain tests. Brain Res. 463: 1-11.

Tribollet, E., S. Audigier, M. Dubois Dauphin, and J.J. Dreifuss (1990) Gonadal steroids regulate oxytocin receptors but not vasopressin receptors in the brain of male and female rats. An autoradiographical study. Brain. Res. 511: 129-140.

Tribollet, E., C. Barberis, J.J. Dreifuss, and S. Jard (1988a) Autoradiographic localization of vasopressin and oxytocin binding sites in rat kidney. Kidney. Int. 33: 959-965.

Tribollet, E., C. Barberis, S. Jard, M. Dubois Dauphin, and J.J. Dreifuss (1988b) Localization and pharmacological characterization of high affinity binding sites for vasopressin and oxytocin in the rat brain by light microscopic autoradiography. Brain. Res. 442: 105-118.

Urban, J.H., M.A. Miller, C.T. Drake, and D.M. Dorsa (1990) Detection of vasopressin mRNA in cells of the medial amygdala but not the locus coeruleus by in situ hybridization. J. Chem. Neuroanat. 3: 277-283.

Van Leeuwen, F.W. (1987) Vasopressin receptors in the brain and pituitary. In Vasopressin, principles and properties, D.M. Gash and G.J. Boer, eds., pp. 477-496, Plenum, New York.

Van Leeuwen, F.W., A.R. Caffe, and G.J. De Vries (1985) Vasopressin cells in the bed nucleus of the stria terminalis of the rat: sex differences and the influence of androgens. Brain. Res. 325: 391-394.

Van Leeuwen, F.W., E.M. Van der Beek, J.J. Van Heerikhuize, P. Wolters, G. Van der Meulen, and W. Yieh-Ping (1987) Quantitative light microscopic autoradiographic localization of binding sites labelled with [3H]vasopressin antagonist d(CH2)5Tyr(Me)VP in the rat brain, pituitary and kidney. Neuroscience Letters 80: 121-126.

Van Leeuwen, F.W. and P. Wolters (1983) Light microscopic autoradiographic localization of arginine-vasopressin binding sites in the rat brain and kidney. Neurosci. Lett. 41: 61-66.

Van Ree, J.M., B. Bohus, D.H.G. Versteeg, and D. deWied (1978) Neurohypophyseal principles and memory process. Biochem. pharmacol. 27: 1793-1800.

Van Ree, J.M. and D. De Wied (1976) Prolyl-leucyl-glycinamide (PLG) facilitates morphine dependence. Life sci. 19: 1331-1340.

Van Wimersma Greidanus, T.B., J.P. Burbach, and H.D. Veldhuis (1986) Vasopressin and oxytocin. Their presence in the central nervous system and their functional significance in brain processes related to behaviour and memory. Acta. Endocrinol. Suppl. Copenh. 276: 85-94.

Vezina, P., P.W. Kalivas, and J. Stewart (1987) Sensitization occurs to the locomotor effects of morphine and the specific mu opioid receptor agonist, DAGO, administered repeatedly to the ventral tegmental area but not to the nucleus accumbens. Brain. Res. 417: 51-58.

Voorhuis, T.A., E.R. de Kloet, and D. De Wied (1988) The distribution and plasticity of vasopressin-labelled specific binding sites in the canary brain. Brain. Res. 457: 148-153.

Wang, X.C., J.P. Burbach, J. Tenhaaf, and D. De Wied (1986) Characterization and quantitation of vasopressin metabolites in the rat brain by specific radioimmunoassay and high-pressure liquid chromatography. Sci. Sin. B. 29: 832-843.

Weiland, G.A. and P.B. Molinoff (1981) Quantitative analysis of drug-receptor interactions: I. Determination of kinetic and equilibrium properties. Life sci. 29: 313-330.

Whittaker, V.P., I.A. Michaelson, and R.J.A. Kirkland (1964) The separation of synaptic vesicles from nerve-ending particles ('synaptosomes'). Biochem. J. 90: 293-303.

Willcox, B.J. (1991) Studies on the centrally-mediated motor effects of arginine vasopressin, Master Dissertation, The University of Calgary, Calgary.

Wilkinson, M.F. and N.W. Kasting (1987a) The antipyretic effects of centrally administered vasopressin at different ambient temperatures. Brain. Res. 415: 275-280.

Wilkinson, M.F. and N.W. Kasting (1987b) Antipyresis due to centrally administered vasopressin differentially alters thermoregulatory effectors depending on the ambient temperature. Regul. Pept. 19: 45-54.

Wilkinson, M.F. and N.W. Kasting (1990a) Centrally acting vasopressin contributes to endotoxin tolerance. Am. J. Physiol. 258: R443-R449.

Wilkinson, M.F. and N.W. Kasting (1990b) Central vasopressin V1-blockade prevents salicylate but not acetaminophen antipyresis. J. Appl. Physiol. 68: 1793-1798.

Wonnacott; S. (1990) The paradox of nicotinic acetylcholine receptor upregulation by nicotine. Trends. Pharmacol. Sci. 11: 216-219.

Wood, P.L., D.L. Cheney, and E. Costa (1979) Modulation of the turning rate of hippocampal acetylcholine by neuropeptides: possible site of action of alpha-melanocyte stimulating hormone, adrenocorticotrophic hormone and somatostatin. J. Pharmacol. Exp. Ther. 209: 97-103.

Wurpel, J.N., R.L. Dundore, Y.R. Barbella, C.D. Balaban, L.C. Keil, and W.B. Severs (1986a) Barrel rotation evoked by intracerebroventricular vasopressin injections in conscious rats. I. Description and general pharmacology. Brain. Res. 365: 21-29.

Wurpel, J.N., R.L. Dundore, Y.R. Barbella, C.D. Balaban, L.C. Keil, and W.B. Severs (1986b) Barrel rotation evoked by intracerebroventricular vasopressin injections in conscious rats. II. Visual/vestibular interactions and efficacy of antiseizure drugs. Brain. Res. 365: 30-41.

Wurpel, J.N., R.L. Dundore, R.M.J. Bryan, L.C. Keil, and W.B. Severs (1988) Regional cerebral glucose utilization during vasopressin-induced barrel rotations or bicuculline-induced seizures in rats. Pharmacology. 36: 1-8.

Wurpel, J.N.D. (1986) Barrel rotation evoked by central administration of arginine vasopressin: neurobiological and pharmacological effects in conscious rats, Ph.D. Dissertation, The Pensylvania State University,

Wurpel, J.N.D., R.L. Dundore, C.D. Balaban, and W.B. Severs (1985) Vasopressin-induced seizures (barrel rotation) after infusions of substantia nigra and basal ganglia. Fed. Proc. 44: 1389.

Yamada, K. and T. Furukawa (1984) Barrel rotation induced by vasopressin and involvement of dopaminergic and cholinergic functions in rats. Arch. Int. Pharmacodyn. 251: 88-94.

Yamamura, H.I., K.W. Gee, R.E. Brinton, T.P. Davis, M. Hadley, and J.K. Wamsley (1983) Light microscopic autoradiographic visualization of-arginine vasopressin binding sites in rat brain. Life. Sci. 32: 1919-1924.

Yehuda, S. (1987) Effects of alpha-MSH, TRH and AVP on learning and memory, pain threshold, and motor activity: preliminary results. Int. J. Neurosci. 32: 703-709.

Zainos, A., R. De Anda, L. Chavez, and M. Garcia-Menoz (1984) Turning behavior, barrel rolling and sensory neglect induced by picrotoxin in the thalamus. Exp. Neurol. 83: 534-547.

Zukin, R.S. and A. Tempel (1986) Neurochemical correlates of opiate receptor regulation. Biochem. pharmacol. 35: 1623-1627.