THE UNIVERSITY OF CALGARY

THE ROLE OF ENDOGENOUS CHOLECYSTOKININ IN THE REGULATION OF FOOD INTAKE

by

GILLIAN SHILLABEER

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

March, 1986

ISBN 0-315-30014-0

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,"The role of endogenous cholecystokinin in the regulation of food intake" submitted by Gillian Shillabeer in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dr. J.S. Davison (Medical Physiology)
Supervisor

Dr. G. Bourne (Biology)

Dr. N. Henderson (Biology)

Dr. Q. Pittman (Medical Physiology)

Dr. R.B. Scott (Paediatrics)

Dr. T. Moran, John Hopkins University

(DATE) March 25, 1986

ABSTRACT

The actions of exogenous cholecystokinin (CCK) and of endogenous CCK, released when food enters the duodenum, have been investigated in the rat, to determine whether CCK has a physiological role in the regulation of short-term food intake.

The injection (i.p.) of CCK reduced food intake inconsistently. The gastrointestinal motility effects of "satiating" doses of CCK were investigated by manometry. A pattern of motility was observed that was unrelated to that seen in the feeding rat. It was concluded that exogenous CCK probably reduced food intake by inducing abnormal gut motility, which was perhaps experienced as malaise.

The role of endogenous CCK in the regulation of food intake was investigated using the specific CCK-antagonist, proglumide. It was postulated that if endogenous CCK acted to reduce food intake, then inhibition of the action of CCK should induce an increase in food intake. Proglumide increased food intake only when injected after a food preload. It was concluded that proglumide must act on a factor released by the preload, and since proglumide is a specific antagonist of CCK, this factor was possibly CCK. Endogenous CCK may, therefore, play a role in the regulation

of food intake. The role of the vagus nerve, in mediating the proglumide-induced increase in food intake, was inconclusive.

Food intake may be limited by gastric distension. Exogenous CCK delays gastric emptying, thus increasing distension, possibly by evoking gastric relaxation. If endogenous CCK reduced food intake, indirectly, by evoking gastric relaxation, then inhibition of CCK with proglumide should induce an increase in intragastric pressure and an acceleration of gastric emptying. However, when proglumide was injected after a food preload, a decrease in intragastric pressure, during a second meal, was observed and was interpreted as indicating a proglumide-induced increase in gastric emptying but achieved by a mechanism other than the inhibition of gastric relaxation. Gastric emptying of labelled liquid food was increased only when proglumide was injected after a food preload, suggesting, as before, that proglumide must act on a factor released by the preload. Endogenous CCK may, therefore, play a physiological role in the regulation of gastric emptying.

Since an increase in food intake, an increase in gastric emptying and a decrease in intragastric pressure all occurred under the same conditions, these effects may be related.

ACKNOWLEDGEMENTS

I wish to thank the following people for their assistance during my graduate programme:

My supervisor, Dr. J.S. Davison, for his good advice, support and enthusiasm, and for allowing me to follow my own research interests.

My committee, Drs. B. Scott, N. Henderson and Q. Pittman for their critical review of my work and their valuable ideas.

Dr. B. Greenwood, for her encouragement and the example of good research and hard work that she set.

Dr. P. Collman, for his knowledge of the literature, Dave Kirk and Louise Tremblay, for assistance with surgery, and Stephanie Diamant, for her illustrations.

The Alberta Heritage Foundation for Medical Research, for providing financial support for this work.

Finally, I wish to thank my family, John, Anna and Holly, for giving me support, encouragement and the time to accomplish this work.

TABLE OF CONTENTS

		PAGE
Chapter 1.	INTRODUCTION	1
Chapter 2.	LITERATURE REVIEW	4
2.1.	INTRODUCTION	4
2.2.	HORMONAL FUNCTION OF CHOLECYSTOKININ	6
2.3.	DISTRIBUTION OF CCK	7
2.3.1.	Endocrine	7
2.3.2.	Neural	7
2.3.2.1.	Vagus nerve	9
2.3.2.2.	Enteric neurones	9
2.4.	CCK SECRETION	10
2.4.1.	Acid-stimulated	10
2.4.2.	Peptone and amino acid-stimulated	11
2.4.3.	Fat-stimulated	11
2.4.4.	Secretion in the rat	12
2.4.5.	Effect of anaesthesia	13
2.4.6.	Inhibition of secretion	13
2.5.	STRUCTURE OF CCK	14
2.6.	STRUCTURAL REQUIREMENTS FOR FUNCTION	16
2.7.	PLASMA CONCENTRATION OF CCK	17
2.7.1.	Correlation of concentration with known functions	21
2.7.2.	Plasma concentration in rats	22
2.7.3.	Degradation and half-life of CCK	23
2.8.	EVIDENCE FOR NEURAL AND PARACRINE FUNCTION	25
2.8.1.	Neural function	25
2.8.2.	Paracrine function	27
2.9.	EVIDENCE FOR SHORT-TERM REGULATION OF FOOD INTAKE	27
2.10.	THE EFFECT OF EXOGENOUS CCK ON FOOD INTAKE	29
2.10.1.	Peripheral effects	29
2.10.2.	Investigations into the mechanism of action of peripheral CCK	32
2.10.2.1.	Synergistic stimuli	32
2.10.2.2.	Endocrine responses	33
2.10.2.3.	Motility effects	34
2.10.2.4.	Gastric emptying	35
2.10.3.	Investigations into the sensory pathway for CCK-induced satiety	37
2.10.3.1.	Vagotomy	37
2.10.3.2.	Afferent vagal fibres and brainstem structures	38
2.10.3.3.	Central monoamines	41

2.10.4. Central effects 43
2.10.4.1. Possible mechanisms of central action....44
2.11. THE BLOOD-BRAIN-BARRIER.....44
2.12. GASTROINTESTINAL MOTILITY EFFECTS OF CCK....47
2.12.1. In vitro 47
2.12.2. In vivo 48
2.12.3. Gastric motility.....49
2.12.4. Gastric emptying.....50
2.13. RELEVANCE OF EXOGENOUS CCK-INDUCED SATIETY....52
2.13.1. Pharmacological or physiological?...53
2.13.2. Satiety or malaise?.....54
2.14. EVIDENCE FOR THE EFFECT OF ENDOGENOUS CCK ON FOOD INTAKE....56
2.14.1. Peripheral.....56
2.14.2. Central.....58
2.15. PROGLUMIDE.....59
2.16. SUMMARY.....64

Chapter 3. THE EFFECT OF EXOGENOUS CCK ON FOOD INTAKE.....66
3.1. EXPERIMENT 1.....68

3.1.1. Methods.....68
3.1.2. Results.....70
3.1.3. Discussion.....74
3.2. EXPERIMENT 2. MOTILITY EFFECTS OF SATIATING DOSES OF CCK....77
3.2.1. Methods.....77
3.2.2. Results.....79
3.3. DISCUSSION.....91

Chapter 4. THE EFFECT OF ENDOGENOUS CCK ON FOOD INTAKE.....100
4.1. METHODS.....102
4.1.1. Experiment 1.....103
4.1.2. Experiment 2.....106
4.2. RESULTS 107
4.2.1. Experiment 1.....107
4.2.2. Experiment 2.....109
4.3. DISCUSSION.....109
4.4. METHODS OF EXPERIMENT 3.....113
4.4.1. Vagotomy.....113
4.4.2. Experimental protocol.....114
4.5. RESULTS.....115
4.6. DISCUSSION.....117
4.7. EXPERIMENT 4.....119

4.7.1. Methods....119
4.7.2. Results....120
4.8. EXPERIMENT 5....120
4.8.1. Methods....120
4.8.2. Results....121
4.9. EXPERIMENT 6....121
4.9.1. Methods....121
4.9.2. Results....123
4.10. DISCUSSION....123

Chapter 5. AN INVESTIGATION OF THE MECHANISM BY WHICH ENDOGENOUS CCK REDUCES FOOD INTAKE....128
5.1. EXPERIMENT 1....130
5.1.1. Methods....130
5.1.1.1. Preparation of catheters....130
5.1.1.2. Surgery....131
5.1.2. Experimental procedure....132
5.1.3. Results....133
5.1.4. Discussion....137
5.2. EXPERIMENT 2....140
5.2.1. Methods....142
5.2.1.1. General....142
5.2.1.2. Gastric emptying....143
5.2.1.3. Calculation....144
5.2.2. Validation of the method....144
5.2.2.1. The effect of acid on the liquid food...144
5.2.2.2. Estimation of recovery....145
5.2.2.3. The effect of ether....146
5.2.2.4. Determination of sensitivity..147
5.2.3. General experimental procedure....149
5.2.4. Experiments....150
5.2.4.1. The effect of proglumide after a preload..150
5.2.4.2. The effect of proglumide before feeding..150
5.2.4.3. The effect of proglumide on a single meal..152
5.2.4.4. The effect of oral proglumide.152
5.2.4.5. The effect of proglumide on gastric acid secretion..153
5.2.5. Results....153
5.2.5.1. The effect of proglumide after a preload...153
5.2.5.2. The effect of proglumide before feeding....155
5.2.5.3. The effect of proglumide on a single meal...155
5.2.5.4. The effect of oral proglumide.156

5.2.5.5. The effect of proglumide on gastric acid secretion..156
5.3. DISCUSSION.....157

Chapter 6. SUMMARY AND DISCUSSION.....161
6.1. SUMMARY.....161
6.2. DISCUSSION.....164

REFERENCES.....178

LIST OF TABLES

TABLE PAGE

1. Plasma CCK values in man.....19
2. Plasma CCK values achieved by infusion of CCK in man.....20
3. Plasma CCK values in the rat.....24
4. Intragastric pressure changes in response to i.p. injection of CCK.....85
5. Intraduodenal pressure changes in response to i.p. injection of CCK.....86

LIST OF FIGURES

FIGURE PAGE

1. The structure of CCK and related peptides.....15
2. The structure of proglumide.....60
3. The effect of CCK administration on meal size when tested on alternate days.....71
4. The effect of CCK administration on meal size when tested only once per week.....73
5. Gastric and duodenal motility responses to 70ng/kg CCK (i.p.).....80
6. Motility responses to bolus i.v. injection of CCK and the inhibition by proglumide.....81
7. Motility responses to i.v. infusion of CCK.....82
8. Motility responses to a large dose of CCK and the inhibition by proglumide.....83
9. Motility responses to very low doses of CCK.....87
10. Motility responses to i.p. CCK in vagotomized rats.....88
11. Motility responses to i.p. CCK when given after proglumide injection.....89
12. Intragastric and intraduodenal pressure responses to the ingestion of food.....93
13. Intragastric and intraduodenal pressure responses to i.p. CCK in the conscious rat.....94
14. Protocol for testing the effect of proglumide on food intake after a preload.....104
15. Protocol for testing the effect of proglumide without a preload.....105
16. The effect of proglumide on food intake with and without a food preload.....108
17. The effect or vagotomy on the proglumide-induced increase in food intake.....116
18. Changes in motility index when proglumide was injected after a food preload.....134
19. Changes in motility index when proglumide was injected before a food preload.....135
20. Changes in motility index when proglumide was injected before a 20min meal.....136
21. A summary of the experimental protocols.....151
22. The effect of proglumide on gastric emptying.....154

Chapter 1.

INTRODUCTION

Obesity is a serious medical problem in our society affecting 12% of men and 18% of women in Canada (306). Yet our understanding of the factors which determine food intake and body weight regulation is minimal. At the other end of the scale, anorexia is also a common problem that occurs as a secondary symptom of many, diverse, diseases such as cancer, depression, rheumatoid arthritis or ulcerative colitis (123). It is not known how to either decrease or increase food consumption by manipulation of physiological signals involved in the control of food intake.

In order to define methods of treatment of pathological eating disorders, the mechanisms for the regulation of normal eating must first be established. Regulation can be considered under several different criteria. Firstly, since feeding is a behaviour, the initiation and the termination of feeding must be determined by the central nervous system (CNS). However, the CNS collects and integrates signals arising from the periphery in addition to signals from possible sensoring mechanisms within the CNS and the integration of the total input results in a behavioural response to the current requirements for energy and

nutrients. Peripheral signals may modulate central signals and vice versa, but they may also operate independently. It is valid therefore to study each possible source of signals separately to determine their input, but the contribution of each to the whole integrated system must also be determined under normal physiological conditions.

In addition to the consideration of central or peripheral signals, the type of regulation being considered must be defined, that is whether regulation is short- or long-term. Short term regulation can be defined as the control of individual meal size, of the length of the intermeal interval, and of the size of the following meal (295). Long-term regulation is the control of day to day and week to week food intake in order to maintain body weight under varying energy balance. There is ample evidence to indicate that such control exists.

In this study, the control of short-term food intake by one specific signal, cholecystokinin, has been investigated. Cholecystokinin (CCK), a long established gastrointestinal hormone, is a putative satiety factor. Injection of CCK causes a decrease in food intake during a single meal in most species studied. The doses of CCK necessary to induce the decrease in meal size are probably pharmacological and there is some evidence to suggest that the decrease in

intake is due to aversive effects of these large doses rather than due to a true satiety signal. The action of exogenous CCK and of endogenous CCK, released when food enters the duodenum under normal physiological conditions, has been investigated to determine whether CCK has a physiological role in the regulation of food intake. The mechanism by which such regulation might be achieved has also been studied.

The evidence to support the postulate, that CCK acts as a satiety factor, is presented in the following chapter. The ability of CCK to regulate food intake depends on the appropriateness of its release in terms of its distribution, the secretory stimuli required, the amount released and the timing of its release relative to a meal. The distribution, secretion and measurement of CCK will, therefore, be discussed first. The evidence for the existence of short-term regulation of food intake, the effect of exogenous CCK on food intake and the mechanisms that may be involved, will then be reviewed. There is evidence to suggest that the possible mechanism may include altered gastrointestinal motility. The effect of CCK on motility will, subsequently, be discussed. Finally, the evidence for the role of endogenous CCK and the availability of a tool, proglumide, by which it may be studied, will be reviewed.

Chapter 2.

LITERATURE REVIEW

2.1. INTRODUCTION

A meal is usually terminated before significant quantities of ingested food have been absorbed (295). Absorbed nutrients therefore can play little part in short-term regulation of food intake. On the other hand, several gastrointestinal peptides are released by food in the gut before absorption has occurred and thus may play a role in regulating food intake. It has been demonstrated that intestinal mucosa contains a substance that induces satiety. Injection of a crude mucosal extract reduced food intake in recipient rats and rabbits (106,173). A partially purified intestinal extract also reduced food intake in mice (250). In 1969, Davis and colleagues injected fasted rats with blood from rats which had just fed to satiation. They found that the "sated" blood reduced food intake in the recipient rats indicating the existence of a humoral satiety factor (57). This factor disappeared if the sated rats were deprived for several hours (56).

The classical gut hormones, known to be contained within the mucosa and released by food entering the

duodenum, were investigated for their possible satiety effect. Secretin was shown to have no effect (250). However, Koopmans and colleagues demonstrated that cholecystokinin/pancreozymin decreased food intake in mice (151) and it was later also found effective in the rat (103).

Since then, many other peptides have been tested for their satiating ability: secretin, glucagon and gastrin reduced food intake in some species, but not in the rat, nor did they inhibit sham-feeding (266). Insulin, pancreatic polypeptide and somatostatin reduced food intake but have not been tested on sham-feeding, while neurotensin and substance P reduced food intake but had toxic effects. Gastric inhibitory peptide was without effect. Bombesin (gastrin releasing peptide) and CCK are the only peptides, as yet examined, that decreased food intake and inhibited sham-feeding and are thus thought to be important factors in the regulation of food intake (100). Whether these peptides have aversive effects is under dispute. Bombesin is contained in nerve fibres of the stomach in rats and in human gastrointestinal mucosa (300) but many attempts to detect its presence in the circulation have failed (111). Bombesin is therefore unlikely to be the satiety hormone described by Davis and coworkers (56,57). CCK induced satiety in most species that have been tested including man

(76,235); it is released from intestinal mucosa (274) and plasma levels of CCK increase in response to a meal (141). The availability of pure, synthetic CCK and the development of radioimmunoassays for CCK, have allowed CCK to become the most thoroughly studied of these putative "satiety" peptides. In addition, the availability of proglumide, a specific, competitive antagonist of CCK, has now made it possible to investigate the actions of endogenous CCK. The role of endogenous CCK in the regulation of food intake is the subject of this study.

2.2. THE HORMONAL FUNCTION OF CHOLECYSTOKININ.

CCK is one of the classical gut hormones. Ivy and Oldberg established the role of CCK in the regulation of gallbladder emptying in 1928 (139). Pancreatic protein secretion was also found to be regulated by a hormone, which was named pancreozymin by Harper and Raper in 1943 (121). Mutt and Jorpes determined that CCK and pancreozymin were the same chemical substance in 1966 and the hormone is now commonly called cholecystokinin since its gallbladder function was discovered first (212). CCK is also known to have a wide spectrum of effects on the motility of the gastrointestinal tract, but the physiological significance of these effects has not been clarified.

2.3. DISTRIBUTION OF CCK

2.3.1. Endocrine distribution

CCK is contained in endocrine cells of the duodenal and jejunal mucosa. By immunohistochemical methods these endocrine cells have been identified as the I cells of the intestine (274). The distal duodenum was shown to contain the highest concentration of CCK in the pig, followed by the proximal jejunum and pylorus (12). Therefore, CCK-containing cells are situated appropriately for stimulation of secretion by food entering the small intestines. Maton and co-workers have shown a differential distribution between structural forms of CCK in the small intestine of man (see page 14) (183). The smaller form of CCK (CCK-8) was found proximally while CCK-33/39 was found more distal in distribution. A high concentration of receptors for CCK was demonstrated to be present in the pylorus of rats, but none were located in the body of the stomach (271).

2.3.2. Neural distribution

CCK has been identified in peripheral and central neurones in man, rat, guinea-pig and pig (75,77,257). The

distribution in the brain has been summarized by Beinfeld (21). There is more CCK in the brain than in the gut and it is found in all regions but the cerebellum. Particularly high concentrations occur in the cerebral cortex and hippocampus and it is found in cells, fibres and terminals. CCK-containing neurones have also been found in intimate association with cerebral blood vessels in rats and monkeys (126) and in dorsal root ganglia of rats (290).

Receptors for CCK were located in the brain, with particular high concentrations in the cerebral cortex, olfactory bulb, amygdala and hippocampus. These locations corresponded to the areas of high concentration of CCK (21). The occurrence of CCK receptors was significantly higher in the cerebral cortex, hippocampus and midbrain of obese rats than in lean but no change in receptor number was observed when obese animals were fasted (89,124) although an increase was seen in male lean rats (124). There is some evidence to suggest that central receptors are not identical to peripheral receptors (246). Tissue and species specific differences have been detected by the observation of variations in binding affinity shown by analogues of CCK and CCK-antagonists (298).

2.3.2.1. The vagus nerve

The vagus nerve was found to contain both CCK-like material and CCK receptors (76,319). These receptors were demonstrated to be transported peripherally (319). There is evidence to show that the CCK in the nucleus of the solitary tract (NTS) in the medulla originated extrinsically (221). The NTS is a major relay centre for vagal, facial and glossopharyngeal nerve afferent fibres. CCK has not yet been demonstrated to be present in the latter two nerves.

2.3.2.2. Enteric neurones

Enteric neurones through most regions of the gut have been shown to contain immunoreactive CCK-like material with species dependent distribution. The concentration of CCK, both neural and endocrine, decreased down the length of the gut in the guinea-pig (77). The rat was found to have a sparse distribution of CCK-containing neurones throughout the gut except in the longtitudinal muscle layer (257). Most of the neurones were found in the myenteric and submucosal plexuses (except in the ileum). CCK-containing fibres were detected in the mucosa in one study (160) and not in another (257). Fibres were also detected around blood vessels in the basal laminar propria and submucosa of

all regions of the gut (257). In the distal colon, only CCK-containing cell bodies have been detected (257). Colchicine pretreatment indicated the presence of CCK-containing fibres that were intrinsic to the myenteric plexus (160). The possibility exists, therefore, that CCK may act as a neural transmitter as well as an hormone in the gut.

2.4. CCK SECRETION

CCK is released from small intestinal mucosa when food enters the duodenum. Sham-feeding does not cause its secretion (4). Acid, fat and amino acids have been reported to release CCK in man, pigs and dogs (165). CCK secretion was also stimulated by gastrin releasing peptide (bombesin) (138) but there is no evidence that this occurs under physiological conditions.

2.4.1. Acid stimulated secretion

A dose-dependent rise in plasma CCK in response to duodenal acidification was observed in dogs (38). Hydrochloric acid (0.2nmoles/min) increased plasma CCK from 8pM basal to 15pM. Conversely, Migata and colleagues reported no change in CCK secretion following duodenal acidification (197).

2.4.2. Peptone and amino acid stimulated secretion

A peptone meal given intraduodenally (i.d.) in dogs produced a rise of plasma CCK in the portal vein within 30 sec with peak secretion occurring 2min after infusion (311). Amino acids, in particular, L-phenylalanine, have also been shown to stimulate CCK secretion since plasma CCK levels rose in response to intrajejunal infusion of amino acids in man (141).

2.4.3. Fat stimulated secretion

Sodium oleate infused i.d. induced a rapid rise in plasma CCK which correlated well with pancreatic protein secretion and gallbladder contraction (93). Bilateral truncal vagotomy, while reducing pancreatic and gallbladder responses, had no effect on fat stimulated CCK secretion indicating that CCK secretion may be independent of the vagus (93). Release of CCK was shown to be dependent on a cholinergic mechanism, however, since atropine inhibited CCK secretion in response to a fat meal in man (182). The fat used in the vagotomy study, discussed above, was in a readily absorbable state. Decreased CCK secretion might have been obtained if triglyceride had been infused under the conditions of reduced pancreatic lipase and bile

secretion. Impaired CCK secretion has been observed in rats following pancreatic and biliary diversion, indicating that CCK is secreted in response to the products of fat digestion (39).

Lilja observed that a mixed meal released CCK faster than i.d. fat in man with peak plasma levels occurring in less than 15min while after the fat, peak CCK was at 20min (165). Long chain fatty acids were more effective than medium chain fatty acids in releasing CCK in man (90,131,132).

Therefore, CCK secretion occurs in response to food entering the small intestine and peaks at approximately 20-30 min after a meal (See Table 1) which is appropriate to a possible role as a regulator of food intake.

2.4.4. Secretion in the rat

It has been assumed that the stimuli to secrete CCK are the same in the rat as in other species. However, recently it was demonstrated that i.d. fat and amino acids were ineffective in inducing CCK secretion or pancreatic protein output in the rat while whole protein caused a significant rise in plasma CCK and pancreatic protein output (164). Several studies on food intake and gastric emptying, used

the response to intraduodenal L-phenylalanine as an indicator of the action of endogenous CCK (42,162, 177,189). In the light of this result, factors other than CCK may have been involved (see page 56).

2.4.5. The effect of anaesthesia on secretion

Anaesthesia may affect CCK secretion (166). Pentobarbital delayed and reduced CCK response to i.d. corn oil while halothane completely inhibited secretion in the dog. Chloralose, on the other hand, only slightly delayed secretion. Data obtained from the anaesthetized animal must be used with caution.

2.4.6. Inhibition of CCK secretion

It has been proposed that trypsin acts as a negative feedback control of CCK secretion (2,261,262). Soybean trypsin inhibitor i.d. induced supranormal plasma concentrations of CCK (2,164). Consistent with this was the observation that pancreatic exocrine impairment was associated with elevated plasma CCK levels (262). Also, after vagotomy, when pancreatic secretion is reduced, elevated CCK levels have been observed (131). Conversely, pancreatic ligation reduced CCK secretion (39). An apparent

inhibition of CCK secretion was observed when CCK-stimulated pancreatic secretion was inhibited by fat infusion into the ileum or colon (249) by a mechanism not involving pancreatic polypeptide (138). But plasma CCK was not measured and inhibition was more likely due to a block of pancreatic secretion by pancreotone or anti-cholecystokinin, a peptide secreted by the colon and ileum, than due to a direct inhibition of CCK secretion (249,119,120).

2.5. STRUCTURE OF CCK.

CCK is a polypeptide which shares the same C-terminal pentapeptide sequence with gastrin (Fig 1) and therefore some of its biological activity (300). Some species specificity involving substitution of specific residues has been demonstrated (36,84,320).

Molecular heterogeneity has been demonstrated. The hormone was first characterized in the pig as a polypeptide with 33 residues (CCK-33) which formed most of the acid extract of intestinal mucosa (212). A smaller variant consisting of the 8 C-terminal residues of CCK-33 (CCK-8) was the major form found in the neutral mucosal extracts. Other variants, that are elongations of the N-terminal, namely CCK-39 and CCK-58 and an intermediate length form (possibly CCK-22) were shown to exist in smaller

concentrations (84,85). CCK-58 is thought to be the precursor of CCK-33 (12). Gastrin and CCK-4 have also been found in the intestines of rats (245).

CCK-39 Tyr-Ile-Gln-Gln-Ala-Arg-
39 34
CCK-33 Lys-Ala-Pro-Ser-Gly-Arg-Val-Ser-Met-Ile-Lys-
33
Asn-Leu-Gln-Ser-Leu-Asp-Pro-Ser-His-Arg-Ile-

Ser-Asp-Arg-
9
CCK-8 Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH
8 | 1
SO_3H

Caerulein Glu-Glu-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH
10 | 1
SO_3H

Gastrin-17 Glp-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-
Tyr-Gly-Trp-Met-Asp-Phe-NH

Fig. 1. The structure of CCK and related peptides. CCK-39,-33 and -8 share the same C-terminal octapeptide. The analogue caerulein has a similar structure. The structure of gastrin-17 is shown for comparison (235,236).

CCK-8 is the main form found in the brain and peripheral nerves, although all other variants have been located there (22). In the rat cerebral cortex, 90% of the CCK was found to be CCK-8 (75), whereas in the gut, less than 20% (13) or 50% (75) was CCK-8. Similar heterogeneity has been demonstrated in man (35,143, 179,180,183).

Plasma concentrations of the different forms of the peptide reflected the pattern of duodenal content (152). CCK-8 was the form of CCK that was first released when food entered the duodenum, and may thus have a more proximal distribution, while CCK-33 mainly contributed to the second peak of the biphasic response to a meal and may thus have a more distal distribution in the intestines (179,183). The first peak, consisting mainly of CCK-8, occurred 20-30min after the start of a meal.

2.6. STRUCTURAL REQUIREMENTS FOR FUNCTION.

The biological potencies of the CCK variants has been determined. Lamers and colleagues found that CCK-33 and CCK-8 were equipotent on a molar basis for gallbladder contraction (157). CCK-8, CCK-10 and CCK-14 were also found to be equipotent for exocrine and endocrine pancreatic stimulation while CCK-4 was without effect (11). The minimal structure for full biological activity in the guinea-pig pancreas has been shown to be the sulphated C-terminal heptapeptide (145). Deamination of the C-terminal abolished the biological activity (11,63,95) while desulphation of the tyrosine residue at position 7 from the C-terminal greatly reduced the potency in the guinea-pig gallbladder (63), canine pancreas (63), the mouse

central nervous system (CNS) (41) and diminished its satiety effect (104).

The structural requirements for binding at CNS receptors were apparently the same as for peripheral receptors (63) although some C-terminal fragments of CCK showed a higher affinity at central sites than at peripheral sites (150). Recently, the cholecystokinic and pancreozymic activities of CCK were separated by substituting a glycine residue for either methionine or tryptophan residues at the 3 or 4 position respectively. The resulting analogues retained pancreatic activity in rats and dogs, but the gallbladder response was greatly reduced indicating a receptor specificity for function (317). Variations in potency of the different forms of CCK at receptors in different tissues indicated that there may be additional structural requirements at individual receptor populations (205).

2.7. PLASMA CONCENTRATION OF CCK

The heterogeneity of CCK and its homology with gastrin have made the development of specific and accurate radioimmunoassays (RIA), for the quantitation of CCK, very difficult. Antibodies specific for the sulphated tyrosine at position 7 and the active C-terminal have now been

developed that allow the separation of biologically active CCK moieties from gastrin and desulphated CCK. However, differential binding affinities for the different types of CCK and the existence of CCK fragments, that still retain some biological activity but are not detected by some antibodies, have led to an inconsistency in the data between laboratories. Examples of these data are presented in table 1.

It can be seen from Table 1 that few comparisons can be made between data obtained from different laboratories. Within laboratories, however, results can be interesting, in particular the differences between plasma levels postprandially and those resulting from infusion of exogenous CCK. Examples of these data are shown in Table 2. A comparison of these values is pertinent to the question of whether the functions assigned to CCK, on the basis of the effects of exogenous CCK, are in fact physiological or pharmacological effects. The dose of CCK that induces 50% (D50) of maximal pancreatic protein secretion has been considered to be a "physiological dose" (64,113) but, as will be discussed in the next section, this may not be the case.

PLASMA CCK VALUES IN MAN

Basal pmol/l	Stimulus	Peak pmol/l	Time min	Ref
41	Breakfast Lunch Dinner	103 106 142	< 10	33
-	High fat meal	30		182
8.3	Mixed meal	24.4	20-30min	34
8	High fat meal	18	15 and 90	32
<0.2	Mixed meal Na Oleate,i.d.	1.1 6.2		301
	LCT,Lundh meal or breakfast MCT	26.4 8.6		90
6.2	Liquid food Breakfast	24.5 24.5	30 45-60	18
2.1	High fat meal	4.8		131
2.0	Mixed meal	4.6-7.3		147
-	Mixed meal	Basal+2.4		19
27.4	Na oleate, i.d.	69		92
9.5 12.7	Amino acids,i.j. High fat meal	16.5 19.0		141

Table 1. Plasma CCK data, obtained by radioimmunoassay, have been converted to the same units for easier comparison. Time - represents the time of peak postprandial CCK concentration. The two times shown for ref. 32 refer to 2 observed peaks. i.d. - intraduodenal, i.j. - intrajejunal.

PLASMA CCK VALUES ACHIEVED BY INFUSION OF CCK IN MAN.

CCK	Dose	Plasma conc.	Ref.
	pmol/kg/h	pmol/l	
Caerulein	9.2	Basal+2.6	19
	18.5	Basal+6.1	
	37	Basal+20.8	
CCK-8	35	Basal+13.5 - 27.4	141
CCK-8	5	5.6	5
	20	11.4	
	50	17.1	
CCK-8	0.75	< 3.0	184
	6.8	6.6	
	23.8	13.3	

Table 2. Data, obtained by RIA, have been converted to the same units for easier comparison. Basal + values are presented where data was only given as an increase in plasma concentration.

2.7.1. Correlation with known hormonal function

The D50 for pancreatic secretion was thought to be 125ng/kg/h or 109.5pmol/kg/h (64,113), but this rate of infusion is now known to produce plasma levels of CCK greatly in excess of postprandial concentrations. For example, a mixed meal caused a plasma CCK increase of 2.4pmol/l (Ref.19, Table 1) which was comparable to an infusion rate of only 9.2pmol/kg/h (Ref.19, Table 2). Similarly, a high fat meal induced a plasma level of 19pmol/l (Ref.141. Table 1) while an infusion rate of 35pmol/kg/h resulted in a plasma level of 27.4pmol/l (Ref.141, table 2). However, following a meal, CCK acts synergistically with signals arising from other stimulatory mechanisms, such as vagal reflexes, local reflexes or other hormonal mechanisms, to induce pancreatic secretion (193), while during i.v. infusion of CCK, no other such input is stimulated. Alternatively or additionally, local CCK levels, for example in the duodenal muscle, may be much higher than plasma levels indicate.

Anagnostides and colleagues determined that half maximal pancreatic trypsin secretion occurred when plasma CCK was 4.7pmol/l while maximal secretion occurred at 17.1pmol/l (5). The biliary system was less sensitive to CCK, the plasma concentration being 18pmol/l for half

maximal gallbladder output.

Some data have indicated that there is sufficient CCK released to induce the putative functions of CCK without synergistic input from other stimuli. Maton and colleagues determined that infusion of CCK-8 to produce a plasma concentration of 25pmol/l induced gallbladder emptying equal to that following a fat meal when plasma levels reached 30pmol/l (184). Similarly, Kerstens and co-workers found that infusion of 2.5pmol/kg/h CCK which resulted in a plasma concentration of 3.9pmol/l induced significant pancreatic protein secretion and gallbladder emptying (147). This plasma concentration was in the same range as those seen after a mixed meal (4.6-7.3pmol/l).

2.7.2. Plasma concentrations in the rat

Fewer data are available for the rat. These are presented in Table 3. The D50 for pancreatic secretion in the rat was shown to be 333.8pmol/kg/h for CCK-33 and 69.7pmol/kg/h for caerulein (158).

While secretion of biologically active CCK has been shown to occur at a time appropriate to a role in regulating meal size, the secretion of an adequate amount of the peptide is still questionable.

2.7.3. Degradation of CCK

CCK is rapidly cleared from the circulation. Early studies showed that it has a half life of less than 3min (122,233). However, more recent studies, with perhaps improved detection methods, observed a longer half life: 50min in man and 17 min in rats (153). The desulphated CCK-8 and CCK-4 were less stable, with half-lives of 18 and 13min in man and 5 and 1min in rat, respectively. The use, in this study, of synthetic CCK-8 based on the structure of porcine CCK, however, may be misleading, since there may be some species variation in the CCK of man and rat. The half-life of cerultide (caerulein), a CCK-10 variant occurring in the frog, was found to be 10min (3).

Hydrolysis of CCK-8, by four different proteases, has been detected in the kidney of the rat (214). Clearance of plasma CCK by the kidney has not yet been reported. The liver has been shown to preferentially clear plasma CCK-8 during a single pass, while CCK-39 was mainly unaffected (27). Conversely, Doyle and colleagues found, when perfusing rat livers <u>in situ</u>, that only CCK peptide fragments smaller than CCK-8 were appreciably degraded by the liver (79). Several CCK-degrading peptidases have been located in synaptic membranes in most regions of the brain (70.71,279) and in the liver (278) while the pancreas

PLASMA CCK VALUES IN THE RAT.

Basal	Stimulus	Peak	Time	Ref.
62.2pmol/l	Rat chow	166pmol/l	30min	39
*0.31pmol/l	Liquid food	6.2pmol/l		163
	Trypsin-	10.1pmol/l		
	inhibitor			
	ethanol 10%	2.0pmol/l		
	ethanol 40%	4.7pmol/l		
	bolus, i.v.			
	CCK-8			
	40.3pmol	96.3pmol/l		141
	20.1pmol	61.3pmol/l		
	10.1pmol	<detection		
	bolus, i.p.			
	1750pmol/Kg	0.5-1.2pmol/l	10min	268
	3502pmol/Kg	1.2-4.2pmol/l		
	High CHO meal	0.5-3.2pmol/l		

Table 3. Data, obtained from RIA methods, have been converted to the same units for easier comparison. * Data obtained by bioassay. Time - represents the time at which plasma samples were taken after the administration of the stimulus to secrete.

contained little degrading activity (278). Degradation resulted in the formation of CCK fragments that retained some biological activity (70,71). These fragments, for example CCK-3, may antagonize CCK-8 at the CCK receptor (144). The distribution of peptidases generally followed the distribution of CCK receptors in the brain. CCK was found to compete with the enkephalins for these enzymes (70).

2.8. EVIDENCE FOR NEURAL OR PARACRINE FUNCTION.

2.8.1. Neural function

Since CCK and CCK receptors have been located in central and peripheral nerves, it is possible that CCK may act as a neurotransmitter. Most of the evidence to support this hypothesis has come from studies of central mechanisms since CCK-containing neurons in the gut are sparse and, therefore, difficult to study.

Synthesis of CCK was demonstrated in the CNS using incorporation of radioactive [35S]-methionine injected intracerebroventricularly (i.c.v.) in the rat (108). The peptide was isolated in the synaptosomal fraction of brain extracts and appeared to be in vesicles (21). Synaptosomal release of CCK from cortical and hypothalamic <u>in vitro</u>

preparations was observed, in response to KCl (149, 196) and to dopamine and acetylcholine (149). Release from neurones in the spinal cord was also demonstrated (314). Re-uptake of CCK by enteric neurones was not detected (77) but rapid degradation of the peptide in several tissues (see page 23) has been described (27,70,71, 79,278,279).

CCK may act as a neurotransmitter in enteric neurones. In the guinea-pig ileum, the local peristaltic reflex, evoked by distension, was blocked completely by the specific CCK-antagonists, proglumide and dibutyryl cyclic guanosine monophosphate, as well as by atropine and tetrodotoxin. This suggested that CCK was a transmitter in a neurone of the reflex arc (60).

CCK induced a rapid, reversible depolarization of pyramidal neurones, accompanied by a decrease in membrane resistance, in an <u>in vitro</u> preparation of the hippocampus. In no case was inhibition seen (78) which is the opposite of the effect seen in the nucleus of the solitary tract in cats (206).

Therefore, much evidence has already been collected to suggest that CCK may function as a neurotransmitter.

2.8.2. Paracrine function.

The possibility that CCK is acting locally, close to its site of release, has not been investigated. The indirect evidence that there is probably insufficient CCK in the peripheral circulation to achieve its hormonal effects, nevertheless, suggests that a paracrine mode of action may be important for some functions. For example, there may be insufficient circulating CCK, postprandially, to delay gastric emptying. But release of CCK, by endocrine cells of the duodenum, may raise local concentrations within the duodenal muscle sufficiently to cause duodenal contraction. This contraction could then reflexly inhibit gastric emptying by inducing gastric relaxation or pyloric contraction (6,116,242).

2.9. EVIDENCE FOR SHORT-TERM REGULATION OF FOOD INTAKE.

Since the subject of this thesis is the role of CCK in the regulation of short-term food intake, it is appropriate to examine the evidence that such regulation does occur.

It is generally believed that a pre-absorptive signal(s) is responsible for the termination of a meal, as a meal usually ends before a significant quantity of nutrients has been absorbed (295). When absorption of a meal is

prevented by the removal of gastric contents via an open gastric fistula, as in sham-feeding, an animal shows no satiety and will eat continuously (55,104). Negative feedback signals arising postgastrically or from gastric distension, were eliminated in this model. Conversely, gastric distension was shown to contribute significantly to the termination of the meal since a free-feeding rat compensated for a volume of the meal removed from the stomach by re-ingesting a similar volume (55,72,74). Deutsch demonstrated that this occurred even when the pylorus was ligated to prevent gastric emptying suggesting that distension was sufficient to inhibit further food intake (72,74). Compensatory eating was not immediate (55), however, implying that distension was not the only signal, since the animal would start to refeed as soon as some gastric emptying had occurred. A rapid drop in distension might be a signal to refeed whereas a slow fall due to normal emptying was not (55).

Pappas and colleagues found that gastric distension is a short-term satiety signal in the dog (222). Using an intragastric water-filled balloon and intragastric infusions of inert liquid or nutrients in sham-fed dogs, these researchers demonstrated that volume alone determined the size of the meal. Pretreatment with atropine had no effect. They concluded that distension caused a dose-dependent

inhibition of food intake by a non-cholinergic mechanism that was independent of the nutrient properties of the meal. Nutrients infused intraduodenally in the sham-fed rat caused an inhition of sham-feeding in the absence of gastric distension, indicating that postgastric stimuli for the termination of a meal were also sufficient to act independently of gastric distension (104). Therefore, there is evidence for short-term regulation of food intake that may involve both gastric distension and postgastric signals.

2.10. THE EFFECT OF EXOGENOUS CCK ON FOOD INTAKE

The inhibition of food intake by the administration of CCK has been extensively studied. The effects of peripheral and central injection will be considered separately.

2.10.1. Peripheral effects.

The administration of CCK intraperitoneally (i.p.) or i.v. reduced food intake in a dose-dependent manner in lean (8,26) and obese rats (201), lean and obese mice (186,282,288), golden hamsters (194), pigs (14), rhesus monkeys (101), dogs (223) and in lean (260,276,277,285) and obese man (229). A few studies found no change in food intake in response to CCK in man (3) and an increase in

intake was found in cats (192) and in man when very low doses were infused (285). The effect was specific for food intake since water intake was unaffected (8,26,103,282). However, in pigs, CCK injection reduced operant responding for water intake as well as for sucrose or food, but did not affect responding for heat (14). Inhibition of intake for both solid and liquid foods has been observed (26,103,276) however, the inhibition was more pronounced for liquid foods (26,103). Inhibition of food intake was more pronounced in rats when feeding was tested during daylight hours than during the night when rats normally feed (155). CCK has also been shown to inhibit stress-induced feeding (209).

In addition to reducing food intake, CCK induced the complete behavioural sequence associated with satiety, that is resting, grooming and exploratory behaviours (9).

Caerulein was found to be equipotent on a molar basis with CCK-8, while desulphated CCK-8 was without effect (104). Impure CCK (20%) was more potent than CCK-8 in limiting food intake, but the increase in potency was not due to the contaminants gastrin, secretin or gastric inhibitory peptide (171).

In free feeding rats, meal-contingent i.p. infusion of CCK induced a change in feeding pattern over 4 days. Meal frequency increased as meal size decreased so that by the

end of the 6 day experiment total food intake had returned to basal levels (304). Hsiao and colleagues, in contrast, found a decreased meal frequency and total amount of food eaten, but meal size and rate of feeding were unaffected (136). These authors concluded that CCK prolonged satiety rather than decreased meal size. However, infusion of CCK during the intermeal interval did not prolong the interval (305) as might be predicted from Hsiao's conclusion, but indicates, rather, the ineffectiveness of CCK in the absence of food intake.

The peptide, bombesin, also reduced food intake and induced CCK secretion. But the effects of bombesin and CCK on food intake were found to be independent and probably achieved by different mechanisms (135) since their effects were additive (280).

CCK injection inhibited the hyperphagia induced by sham-feeding in rats but the doses required to achieve inhibition were 4-fold higher than those necessary in the intact animal (104). Slow i.v. injection was more potent than i.p. injection in sham-fed rats (171).

CCK was shown to be acting peripherally since bilateral subdiaphragmatic vagotomy abolished the induced reduction in food intake in most experiments (169,208,269) but not in others (8,156). The effect of vagotomy will be further

discussed in a later section (page 37).

Houpt attempted to locate the peripheral site of action of CCK on satiety (134). Infusion of CCK into several venous and arterial sites in the pig revealed that the major site of action of CCK in reducing meal size was in the bed of the mesenteric and coeliac arteries. These arteries supply the stomach, liver, duodenum and jejunum and are thus the potential sites of action of CCK. However, infusion into the portal vein or the gastric branch of the splenic artery was less effective in producing satiety, thus indicating that the stomach and liver were not the site of action for CCK's satiety effect. This correlates with the absence of CCK receptors in the body of the stomach, noted by Smith and colleagues (271). The peripheral site of action of CCK would thus appear to be the small intestine. In addition, infusions into the carotid artery and jugular vein were equally effective, indicating that CCK was not acting centrally.

2.10.2. Investigations into the mechanism of action of peripheral CCK.

2.10.2.1. Synergistic stimuli.

CCK may interact with orosensory signals since the magnitude

of the CCK-induced inhibition of intake was observed to be proportional to the concentration of sucrose feeding solutions (16). CCK was shown to act synergistically with pregastric food to inhibit sham-feeding in rats (10). CCK was most effective when injected 12min after the start of sham-feeding. Food infused into the small intestines also inhibited sham-feeding and was more potent when infused in close temporal association with orogastric food (10,102). CCK was found to have no effect on feeding motivation, estimated by runway performance, unless injection followed consumption of a food preload (46). The threshold for this effect was between one-third to two-thirds of a full sucrose meal.

2.10.2.2. Endocrine responses

CCK caused an increase in blood glucose and suppressed the feeding stimulated by insulin-induced hypoglycemia. It was, therefore, hypothesized that CCK may cause satiety in rats, indirectly, by inducing hyperglycemia (161). On the other hand, the satiety effect of CCK was shown to be independent of plasma insulin levels since CCK was equally effective in streptozotocin-induced diabetic rats, with or without insulin, as in normal rats (296).

Both CCK and bombesin, another putative satiety agent, stimulated the release of pancreatic polypeptide (PP) (81). Vagotomy impaired the release of PP in response to CCK. It was, therefore, postulated that CCK and bombesin were acting via PP to induce satiety. However, PP was found to have no effect on food intake, and vagotomy failed to abolish bombesin-induced satiety, although CCK-induced satiety was reduced. CCK and bombesin thus do not act on food intake via release of PP, but an interaction between these peptides and PP remains a possibility.

2.10.2.3. Motility effects of CCK

CCK has a stimulatory effect on the smooth muscle of most regions of the gastrointestinal tract. It could be hypothesized that the changes in motility that delay intestinal transit or gastric emptying might act to reduce food intake.

2.10.2.4. Gastric emptying

Gastric distension is a function of the rate of feeding, the rate of gastric emptying and gastric tone (137) and has been shown to affect food intake (203,265). Distension signals are carried centrally by the vagus nerve which has also been

found essential for CCK's effect on satiety. Compensatory food intake in response to partial withdrawal of a liquid meal was demonstrated in rats with pyloric ligation (55,72,74), an effect abolished by subdiaphragmatic vagotomy (109). Distension was shown to be a short-term satiety factor in the dog (222).

CCK may affect distension directly by altering gastric tone or indirectly by delaying gastric emptying. However, earlier in this review (p.32), the site of action of CCK on satiety, was shown to be the small intestine rather than the stomach (134). In addition, no gastric CCK receptors were detected (271). An indirect effect seems more likely, therefore. CCK's role in gastric emptying will be discussed below and later on page 50. Some attempts have been made to correlate the satiety effect of CCK with delayed gastric emptying.

Infusion of CCK inhibited food intake and delayed gastric emptying in man (260) and in neonatal rats (241), but the correlation between these changes was weak. Lorenz (170) and Phifer and colleagues (228) reported that gastric emptying was directly correlated with food intake in rats. Moran and McHugh observed, in the rhesus monkey, that doses of CCK that effectively delayed gastric emptying of a saline meal, were ineffective in reducing food intake unless the

stomach was filled with saline (203). These authors suggested that CCK-evoked satiety depended upon gastric distension that was secondary to CCK-induced inhibition of gastric emptying.

No delay in emptying was observed, however, following CCK-8 injection in suckling rats, even though the same dose of CCK was effective in reducing food intake (133). In addition, Houpt and Houpt found no correlation between the inhibition of gastric emptying by various intragastric loads and the decrease in intake induced by these loads. However, the total gastric volumes being measured in this study were less than 0.4ml and, therefore, may have been subject to considerable error. Collins and coworkers argued that gastric emptying played no part in the satiety effect of CCK, since satiety was induced by CCK in sham-fed rats (44). Conclusions negating the role of gastric distension in CCK-induced satiety, based on sham-feeding experiments, however, may not be valid. Kraly observed that high doses of CCK still inhibited sham-feeding even after vagotomy (156). From this it was deduced that, since vagotomy abolished CCK's inhibition of normal feeding, the mechanism of action of CCK on normal and sham-feeding was not the same. The use of the sham-feeding paradigm was thus inappropriate for the study of the mechanism of action of CCK. Nevertheless, this researcher had found earlier that

since normal and vagotomized rats ate the same meal size whether pyloric nooses were open or closed, gastric distension, signalled by vagal afferents, was not a necessary stimulus for satiety (154).

2.10.3. Investigations of the sensory pathway for CCK-induced satiety.

2.10.3.1. Vagotomy.

The satiety effect induced by administration of CCK is dependent on the integrity of the vagus nerve. While a few studies found that vagotomy had no effect (8,102,156) most studies in rats demonstrated that the CCK-induced reduction of food intake was abolished by bilateral subdiaphragmatic vagotomy thus indicating that exogenous CCK was acting at a peripheral site (169,208,267,269). Total or gastric vagotomies were effective while hepatic or coeliac vagotomies failed to abolish the CCK effect (269). Atropine blockade was ineffective indicating that vagal cholinergic efferent fibres were not involved in CCK's satiety effect (269). Spinal cordotomy was also ineffective (169). In the rat, vagotomy reduced food intake and weight gain (204). Vagotomy abolished the effect of exogenous CCK in golden hamsters but did not affect their body weight or normal food

intake as in rats (195). Very large doses of CCK (>8ug/kg), however, were still effective in reducing intake, suggesting the existence of extravagal CCK receptors (195).

2.10.3.2. Afferent fibres.

Bilateral afferent fibre vagotomy combined with unilateral efferent fibre vagotomy abolished the CCK-induced reduction in food intake while the reverse surgery was not effective (270). Capsaicin is a neurotoxin that destroys small -diameter sensory neurones (174,240). The destruction of vagal sensory fibres in neonatal (174) or adult rats (240) by capsaicin pretreatment attenuated the satiety effect of exogenous CCK but did not abolish it. Capsaicin delivered perivagally, i.c.v. and i.p. was effective but not when delivered intrapylorically (275). Therefore, the CCK-induced reduction of food intake was partly dependent on capsaicin-sensitive fibres. These data indicated that afferent fibres and not efferent fibres were essential for CCK's induction of satiety although long-term weight regulation was not affected by the treatment (174). Furthermore, the capsaicin-induced attenuation of CCK-evoked satiety was shown to be a direct effect on CCK-sensitive afferent fibres and was not secondary to an impairment of gastromechanoreceptor function or gastric emptying, since

capsaicin was effective in sham-fed animals (318).

There is evidence that large doses of CCK stimulate afferent vagal fibre firing. Close arterial infusion of CCK in sheep caused an increase in duodenal motor activity that was associated with an increase in impulse frequency from tension receptor units, measured by recording from single unit afferent fibres in the vagus nerve (45). In 22/24 units responding to CCK, increased impulses corresponded to increased phasic activity, while the remaining 2 units responded to increased tone. CCK-8 was applied iontophoretically to neurones in the dorsal vagal motor nucleus, that responded to gastric distension in rats (83). CCK-8 enhanced both "on" and "off" responses, an effect that was not inhibited by the specific CCK-antagonist, proglumide. Brainstem neurones responding to gastric distension have also been recorded, extracellularly, in the NTS with the same results (96,232). Furthermore, Davison demonstrated an increase in frequency of vagal afferent firing in response to gastric mechanoreceptor stimulation by systemic CCK-8 (58). This response was not secondary to contraction of the stomach since relaxation occurred. Therefore, CCK appeared to act directly on the gastromechanoreceptors.

Recording the responses to click stimuli in rats, decreases in activity were obtained in the anterior (AH) and ventromedial hypothalamus (VMH) and hippocampus after i.p. CCK administration, while an increase was observed in the lateral (LH) hypothalamus (53). No response was obtained in the amygdala or caudate nucleus. The LH and the VMH areas correspond to the "feeding" and "satiety" centres believed to play a role in the control of food intake (218).

Vagotomy abolished the inhibitory effect of CCK on the exploratory behaviours that are thought to be an integral part of the satiety response (49,50). Vagal afferent fibres terminate in the NTS in the medulla. Lesions of the parvocellular subdivision of the NTS blocked the exploratory behavioural effects of peripheral CCK administration (52) whereas lesions in other areas of the NTS or in the DVMN were ineffective. Both vagal fibres (76,319) and the NTS (221) contained CCK suggesting the possibility that CCK might act as the neural transmitter in its satiety action. However, injection of CCK directly into the NTS did not induce satiety, but injection of carbachol, an acetylcholine agonist, mimicked the peripheral action of CCK on both food intake and behaviour (47). Therefore, it seems unlikely that CCK is the transmitter in the NTS. On the other hand, local application of CCK to neurones in the NTS of cats inhibited spike discharge including those to respiratory

receptors (206).

Thermal lesion of the area postrema in the medulla attenuated the reduction of food intake induced by peripheral CCK (294) without affecting the sense of taste. The area postrema contains a high concentration of CCK receptors and is located at a site where the blood-brain-barrier is "leaky" (294). It is also considered to be an area associated with nausea and vomiting. Lesions in this region may, however, have resulted in damage to the NTS. To support this, Edwards and colleagues found no attenuation of CCK-evoked satiety by lesion of the area postrema, but lesions of the adjacent areas of the NTS (commissural and medial subnuclei) were effective (80). Tracing the ascending sensory pathway beyond the NTS, Crawley and Kiss demonstrated that ablation of the paraventricular nucleus of the hypothalamus, which receives fibres from the NTS, abolished the actions of CCK on feeding (51).

2.10.3.4. Central monoamines

Peripheral CCK may induce satiety by modulation of central monoamine levels as fasting followed by feeding caused a fall in dopamine, norepinephrine and serotonin levels in specific sites in the brain compared to non-deprived animals

(146). CCK injected i.p. before feeding caused a decrease in hypothalamic dopamine and a significant correlation between norepinephrine in the hypothalamus and the amount of food eaten. Norepinephrine, infused into the pre-optic and medial regions of the hypothalamus, induced spontaneous feeding in rats (185). Prior CCK infusion peripherally or at the specific hypothalamic site attenuated the norepinephrine response thus indicating that CCK could act directly or indirectly via vagal afferents to affect the noradrenergic feeding system. Push-pull perfusion experiments revealed that norepinephrine release increased in medial and rostral hypothalamic sites during the CCK-induced inhibition of feeding (213). Since norepinephrine is an inhibitory neurotransmitter in the CNS, it was proposed that NE inhibited satiety, and that the CCK-induced fall in NE thus caused an increase in satiety. Food in the duodenum caused a similar increase in NE efflux in lateral hypothalamic sites (213). However, prior depletion of catecholamines by 6-hydroxydopamine treatment in the LH, caudate putamen and olfactory tubercle, did not affect the satiety response to peripheral injection of CCK (310).

2.10.4. Central effects of CCK on feeding.

CCK and CCK receptors are widely distibuted throughout the brain and although research into the central role of CCK is only just beginning, evidence suggests that it may be involved in many different systems such as analgesia, sedation, serum glucose regulation, temperature regulation, and anterior pituitary hormone release (208). It is possible that central effects of CCK on feeding are the indirect results of action in other systems. In the chick, both temperature regulation and water intake were affected by central CCK administration, in addition to a decrease in food intake (69).

Continuous intracerebroventricular (i.c.v.) infusion of CCK in sheep induced a decrease in total daily food intake (66). Increasing doses of CCK were required to block food intake following longer periods of fasting. This effect on feeding was accompanied by a fall in plasma insulin without a change in plasma glucose (67). CCK also reduced food intake in pigs after i.c.v. infusion (225). The data on the central action of CCK in rats is variable, showing: no effect (65,169); an increase in intermeal interval, and a decrease (255) or no change in meal size (175); a decrease in food intake following injection into the paraventricular nucleus (88,310); a decrease (310) or no effect (88) in the

ventromedial hypothalamus, but a decrease in both water and food intake when injection was made into the cerebral aqueduct (310). Inhibition of food intake was also seen following CCK injection (100ng/kg) into the lateral hypothalamus and olfactory tubercle, while injection of 200ng/kg i.p. was without effect (118).

2.10.4.1. Mechanism of central effects of CCK

Central injection of CCK in the rat induced hyperglycemia without affecting insulin levels. The increase in plasma glucose was not due to a decrease in glucose uptake (207). Adrenalectomy abolished the effect but not via catecholamine release, since glucagon levels were not elevated. CCK may act centrally by antagonizing endogenous opiate-induced feeding (87). The CCK content of the PVN is high (87) and this region is known to be involved in opiate-induced feeding (128). Injection of CCK into the PVN reduced food intake while injection of proglumide, a specific antagonist of CCK (62), into the same area, induced an increase in feeding (128).

2.11. THE BLOOD-BRAIN-BARRIER.

Since CCK is present in the brain and since CCK receptors

are also present a question to be considered is whether peripheral CCK could act in the brain and vice versa. The blood-brain-barrier effectively blocks the uptake of small water soluble compounds from the blood unless there is a specific carrier mechanism (224). The circumventricular organs, including the median eminence and the ventral lobe of the pituitary and part of the hypothalamus, have no blood brain-barrier. However there is a resistance to diffusion of solutes on the ventricular side of the ependymal cells surrounding the capillaries (191). Specific receptors have been identified for some peptides, but none have been reported for CCK. In addition, a very low "uptake index" for CCK after a single passage of labelled CCK through the brain of the rat was reported (217). Therefore, it seems unlikely that peripheral CCK enters the brain, but the converse may be true. Labelled CCK, injected i.c.v., appeared rapidly in the peripheral circulation (half time 13mins) (224), while following doses a 1000-fold larger in the peripheral circulation, no label was detected in the cerebrospinal fluid (226). Thus it is possible that CCK released centrally or injected into the brain might act peripherally. But the administered doses required to produce central effects are usually at least an order of magnitude less than those required for peripheral effects.

Grovum showed that pentagastrin probably acted centrally to decrease gastric motility in sheep by comparing the responses to carotid or jugular injection (115). However, using the same technique, Grovum was unable to show that peripheral CCK could act in the brain to reduce feeding (114), despite evidence to show that CCK in the CNS reduced food intake in sheep (65,66), pigs (225) and rats (310). In contrast, Cottrell noted a differential effect on duodenal motility between CCK-8, -33 and -39 in sheep (45). Intracarotid infusion of CCK-8 induced an initial brief increase in amplitude and frequency of contraction and an increase in tone, followed by a decrease in these parameters and then a prolonged increase in amplitude and frequency of contraction. Whereas, CCK-33 produced a decrease in amplitude of contraction, but no change in rate or tone, and CCK-39 was without effect. From these data, it was deduced that CCK-8 might be acting centrally, while CCK-33, due to the size of the peptide, was probably only acting peripherally. On the other hand, a heterogeneous population of duodenal receptors with varying affinities for CCK-8 and CCK-33 could be argued.

In an attempt to determine whether systemic CCK was exciting neurones in the substantia nigra by acting peripherally or whether it was crossing the blood-brain -barrier to act centrally, Hommer performed a series of

neural lesions in the rat (130). High cervical spinal cord transection, thoracic and abdominal vagotomies were ineffective, whereas bilateral intramedullary lesion between the NTS and the substantia nigra significantly attenuated the stimulation of the central neurones in response to systemic CCK. It was concluded that most of the CCK effect was on neurones in the NTS, while some direct action on the dopaminergic neurones of the substantia nigra was indicated and that therefore, some CCK must cross the blood brain barrier. Systemic CCK might reach the NTS via the area postrema, since this region, adjacent to the NTS, is known to be "leaky" (294). However, other neural input into the NST was not excluded, such as the superior laryngeal nerve via the nodose ganglion. The residual excitation of the substantia nigra neurones may constitute evidence of central action for systemic CCK, but the existence of other convergent pathways that may signal peripheral action of CCK has not been eliminated.

2.12. GASTROINTESTINAL MOTILITY EFFECTS OF CCK

2.12.1. In vitro.

There are regional variations in action of CCK on gastrointestinal motility; CCK can act directly on the

muscle or indirectly by modulation of neural activity. Behar and Biancani found that CCK caused gallbladder contraction by acting both directly on the muscle and by stimulating cholinergic neurones. In contrast, at the sphincter of Oddi, CCK induced relaxation by inhibiting non-adrenergic, non-cholinergic neurones, but induced contraction in the presence of the nerve blocker, tetrodotoxin (20). Similarly, the lower oesophageal sphincter of the cat was found to have inhibitory (neural) and excitatory (muscle) receptors for CCK (231). The specific CCK-antagonists, proglumide (62) and dibutyrylcyclic guanosine monophosphate (59) were only effective in inhibiting CCK at the muscle receptor (231).

In the guinea-pig ileum (125,160,216,298) and feline jejunum (303), CCK evokes an increase in motor activity indirectly by releasing acetylcholine *in vitro*. Release of substance P by CCK has also been demonstrated (37) but this was not confirmed (216). On the other hand, feline colonic muscle was excited directly by CCK (272).

2.12.2. *In vivo.*

Endogenous CCK, released during a meal, may modulate gut motor activity. Infusion (i.v.) of CCK at doses lower than the D50 for pancreatic secretion, evoked an increase in

motor activity in the human colon (273). Infusion of CCK (125ng/kg/h or 109pmol/kg), in fasting dogs, disrupted the migrating myoelectic complex (127,211), but the activity pattern produced was not identical with a fed pattern, whereas L-tryptophan, a secretagogue for CCK, infused intraduodenally, did induce a fed pattern (211). The difference, however, may have been due to the duodenal distension in the latter case. CNS infusion (i.c.v.) of CCK also disrupted the migrating myoelectric complex (28). Low doses of caerulein (0.06 µg/kg/h) evoked an increase in motor activity accompanied by an increase in intestinal transit in the rat, while high doses (60µg/kg/h) delayed transit (258).

2.12.3. CCK effects on gastric motility.

Using an *in vitro* whole stomach/duodenal preparation from the rat, CCK was shown to increase the base line pressure in antrum, pylorus and duodenum (253). Contractions of decreased amplitude but increased frequency were seen in the pylorus and antrum, while the reverse was observed in the duodenum. The increase in tone was accomplished by a neural, non-cholinergic mechanism, and the phasic contractions were a result of direct action on the muscle (254). Similar results were obtained with muscle from the

guinea-pig stomach (98). Conversely, only pyloric muscle strips from rats contracted in response to CCK *in vitro*, whereas fundic, antral, duodenal and ileal sections were unaffected (178). This result was consistent with the observation that CCK receptors were detectable only in the pylorus in the rat (271). CCK induced contraction of pyloric muscle strips *in vitro* in a dose-dependent manner (24,25). No difference in response was obtained between muscle from lean or obese Zucker rats.

2.12.4. CCK effects on gastric emptying

Infusion of CCK delays gastric emptying in dogs (64), monkeys (203) and man (260,292). Since the effective dose of CCK was equal to the D50 for pancreatic secretion, the regulation of gastric emptying was thought to be a physiological function of CCK (64). However, "physiological" doses of CCK were ineffective in man although intraduodenal oleate was effective (292). In addition, intraduodenal infusion of soybean trypsin inhibitor induced secretion of endogenous CCK in rats resulting in significantly elevated plasma CCK concentrations, but gastric emptying of a saline meal was not delayed (86). But the trypsin inhibitor may have effects unrelated to CCK secretion, that have, as yet, not

been identified. In contrast, i.v. infusion of CCK which produced plasma levels 3-fold lower than those seen after the trypsin inhibitor infusion, induced a significant delay in emptying.

CCK delayed emptying by evoking both relaxation of the proximal stomach and contraction of the pylorus in the dog (315). The proximal stomach only responded to doses higher than the D50 for pancreatic secretion, while the pylorus was more sensitive, responding to doses 4-fold lower than the D50. This is consistent with the demonstration of the presence of CCK receptors in the pylorus but not in the fundus of the rat (271). However, species difference in the distribution of CCK receptors probably exists. Fundic pressure decreased (293) while pyloric pressure in the dog increased in response to infused CCK (threshold dose 40ng/kg/h) and to infusions of secretin and glucagon at doses thought to be pharmacological (227). The threshold dose of CCK was probably also pharmacolgical, based on the data discussed earlier (page 21).

Humorally-evoked relaxation of the proximal stomach was demonstrated using autotransplanted proximal gastric pouches in dogs. Food in the small intestine or infusion of low doses of CCK (D50=62ng/kg/h) induced a fall in luminal pressure in the transplanted pouch (251,252).

Hormonally-induced relaxation has not been demonstrated in the rat. CCK may also induce fundic relaxation by vago-vagal reflexes in the intact animal induced, for example by duodenal or jejunal activation (6,116,242).

Intraduodenal tryptophan, which stimulates the secretion of CCK, but not gastrin or secretin, also delayed gastric emptying, suggesting that endogenous CCK acts to control emptying (64). Exogenous CCK and intraduodenal tryptophan or phenylalanine similarly delayed gastric emptying of a saline meal in rats (7,177). The effectiveness of the amino acids was incompatible with the evidence that only whole protein caused the secretion of CCK in the rat (164).

2.13. RELEVANCE OF EXOGENOUS CCK-INDUCED SATIETY.

There are three important questions arising from experiments involving the injection of exogenous CCK. Firstly, what is the relationship between the effective administered dose of CCK and the amount of CCK released endogenously when food enters the duodenum. Are the effects seen physiological or pharmacological? Secondly, is the reduction in food intake the result of satiety or due to malaise? Thirdly, what is the role of endogenous CCK, released by food entering the duodenum, in the regulation of food intake? These

experiments using exogenous CCK cannot answer this question.

2.13.1. Pharmacological or physiological?

Plasma concentrations of CCK following a meal and after CCK administration have been discussed earlier (page 21). Generally, the concentrations resulting from CCK administration were higher than those seen postprandially, although some evidence exists to suggest that sufficient CCK is released by a meal to elicit pancreatic secretion and gallbladder emptying in the absence of other synergistic input. Recently, Smith and colleagues have specifically addressed this problem. Injection of 2μg/kg or 4μg/kg i.p. in rats produced plasma concentrations in the range of 0.5-1.25 or 1.17-4.25 pmol/l respectively whereas plasma levels 10 min after a high carbohydrate liquid food meal were 0.5-3.2 pmol/l (268). It was concluded that these doses of CCK elicited plasma levels within the physiological range. Since measurement was made only 10min after i.p. injection, low plasma concentrations may indicate slow absorption (or rapid degradation). The uptake of CCK from the peritoneal fluid has not been shown to be essential for the induction of the satiety effect. If not necessary, the prolonged presence of CCK in the peritoneal fluid, undiluted by absorption into the circulation, might act to enhance the

CCK effect.

2.13.2. Satiety or malaise?

When a decrease in food intake is the experimental variable, the possibility exists that the observed decrease is due to malaise caused by the experimental procedure and not due to satiety. The use of doses of CCK that are probably pharmacological rather than physiological makes this more likely.

In rats, the presence of malaise is determined by one- or two-bottle aversion tests. A one-bottle test showed no aversion to CCK (103) whereas the more sensitive two-bottle test showed an aversion to the flavour associated with CCK injection (73). Aversion to a feeding place associated with CCK administration has also been demonstrated (286). Large doses of CCK (12μg/kg) produced behavioural effects similar to those of a known toxin, lithium chloride (297). It is possible that CCK may evoke nausea since an anti-emetic (trimethobenzamide) inhibited the satiety effect of CCK (200). Nausea is presumed not to be a normal part of satiety. Consistent with this conclusion was the observation of abnormal gastrointestinal motility patterns after CCK injection, that were unlike those following ingestion of a meal (72).

In man, a subjective evaluation of malaise can be obtained from the subjects involved in the study, but even so, no consensus can be reached. On the one hand, Sturdevant and colleagues reported nausea and cramps following i.v. bolus injection of a low dose of 20% pure CCK (approximately equivalent to 22ng/kg CCK-8) (285) while on the other, Stacher and coworkers reported no ill effects following i.v. infusion of CCK-8 (30-60ng/kg/15min)(276,271,). A few subjects in a study by Akner complained of malaise after CCK doses of 83-117ng/kg (3) and in another study by Shaw after a 2µg i.v. bolus and 5µg/45min infusion (260). CCK injection (0.3µg/kg intramuscularly) used routinely in biliary function tests, was reported to cause nausea and other side effects in 60% of patients (54).

Other evidence suggesting the pharmacological nature of the CCK-elicited satiety comes from data showing tolerance or habituation to CCK. When CCK was infused into rats on a meal-contingent basis, the initial decrease in total daily food intake gradually declined and had returned to normal over 6 days (198,304). Meal size remained depressed but compensation by increased meal frequency occurred (304). Inconsistency in the response to repeated doses of CCK have been reported (198). Acute challenges of high doses of CCK i.p. were ineffective in reducing food intake in rats chronically infused with CCK , nor was there a decrease in

body weight or daily food intake over a two week period (48).

Evidence of tolerance, inconsistency and aversion might be considered incompatible with the role of CCK as a physiological regulator of food intake. But the unphysiological nature of the exogenously-evoked satiety, does not preclude a physiological role for endogenous CCK in the regulation of food intake.

2.14. EVIDENCE FOR THE EFFECT OF ENDOGENOUS CCK ON FOOD INTAKE.

2.14.1. Peripheral evidence.

A few studies have attempted to determine if the CCK, released by food entering the duodenum, acts to regulate food intake. Sham-feeding in rats was inhibited by i.d. infusion of L-phenylalanine and not D-phenylalanine (162). This was interpreted as a evidence for a role for endogenous CCK in food intake regulation since L- and not D-phenylalanine stimulates CCK secretion. Enhanced CCK secretion stimulated by phenylalanine or trypsin inhibitor, induced a decrease in meal size but an increase in meal frequency in lean and obese Zucker rats (189). Conversely, trypsin inhibitor infusion, while causing a rise in plasma

CCK, did not induce a reduction in food intake (111). Pancrease, a concentrate of pancreatic enzymes increased meal size and decreased meal frequency (189). Pancreatic enzymes are thought to act as a negative feed-back signal for CCK secretion (See page 13). Modulation of CCK secretion by feeding rats a glucose or liquid food preload resulted in a small suppression (2ml) of testmeal size only when a liquid food preload preceded a liquid food testmeal by 20min (210). Gastric volume was greatest at 20min after the preload.

Lean Zucker rats, injected with antibodies against CCK, increased their daily food intake by 9% and their weight gain by 17% over a 3 month period while obese rats were unaffected (187). The CCK-antibodies were also shown to inhibit the satiety effect of exogenous CCK.

Collins and coworkers inhibited sham feeding in the rat by infusing (i.d.) food or L-phenylalanine (44). Proglumide, a specific competitive antagonist of CCK and related peptides (62) significantly reduced the inhibition of sham-feeding induced by both food and L-phenylalanine (42) thus suggesting the involvement of endogenous CCK. When proglumide was administered orally to lean and obese Zucker rats, following a fast, the size of the first meal was the same as the control, but the second meal was

significantly larger (190). Both 3hr and 18hr intakes were elevated in response to 590mg/kg proglumide, and inconsistently to 420mg/kg, but a 250 mg/kg dose was ineffective. There was no difference between obese and lean rats.

Evidence against the involvement of endogenous CCK in the regulation of food intake in the dog was demonstrated by Pappas and coworkers (223). The D50 for the satiety effect of CCK in sham-fed dogs was 500ng/kg/h, whereas the D50s for pancreatic protein secretion and gallbladder contraction were 135 and 25ng/kg/h respectively. The D50 for satiety induced by central CCK administration was 0.02ng. In addition, duodenal fat infusion, as a stimulus for CCK secretion, did not inhibit sham-feeding. These researchers concluded that, in the dog, the D50 for CCK's satiety effect was not in the physiological range of CCK secretion, a conclusion that has recently been corroborated (237).

2.14.2. Central evidence.

Intraventricular injection of CCK-antibody induced feeding in sheep (68) suggesting that CCK in the brain acted to reduce food intake. A meal-stimulated rise in CCK in some regions of the hypothalamus was demonstrated in lean and obese Zucker rats (188). Increases in CCK were observed in

ventromedial, lateral and suprachiasmatic nuclei of the hypothalamus in lean animals, and ventromedial, dorsomedial and anterior hypothalamus in obese rats. Levels were generally higher in obese rats than lean, indicating that while CCK may play a role in satiety in the CNS, a deficiency in CCK was not the cause of obesity in Zucker rats. Consistent with these data was the observation that CCK concentrations in the cerebral cortex decreased on fasting (281) although this was not confirmed (256). Fasting evoked a significant increase in CCK-binding within the olfactory bulb and hypothalamus, but not within other areas of the brain of rats (247). Proglumide and benzotript, another CCK-antagonist (117), injected into the PVN of the hypothalamus in rats induced an increase in feeding, suggesting that endogenous CCK within the PVN might be involved in regulation of food intake (128). This study was preliminary only and the results need to be confirmed.

2.15. PROGLUMIDE.

The availability of antagonists, specific for CCK and related peptides, has made it possible to study the physiological role of endogenous CCK. Proglumide (62,117), benzotript (117) dibutyryl cyclic guanosine monophoshate (59) and more recently, analogues of CCK (95,144) have been found to inhibit the actions of CCK. Of these antagonists,

proglumide has been most widely used and investigated, and is the only one that has been used to study the satiety effect of CCK. Since proglumide was the antagonist used in the present study, it is the only one to be discussed here. The structure of proglumide, a glutaramic acid derivative, is shown in Fig. 2.

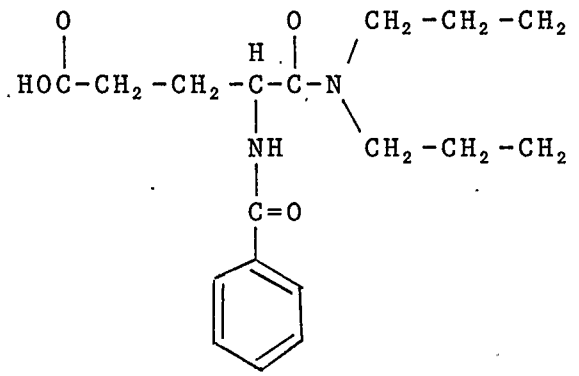

Fig.2. The structure of proglumide, (DL-4-benzamido-N,N-dipropylglutaramic acid) (117).

Proglumide shifted the dose response curve of dispersed pancreatic acini (117,140) and ileal muscle (62) to CCK to the right. The slope of the Schild plot was approximately 1.2, thus indicating that proglumide was a competitive antagonist of CCK. The inhibition was specific to CCK and the response to cholinergic stimulation was unaffected in

the intact perfused pancreas of rabbit (215). In addition, insulin binding to receptors, insulin stimulation of glucose transport and protein synthesis were unaffected by CCK at isolated mouse pancreatic cells (308).

Proglumide is a weak antagonist. The affinity constant (Kb) was found to be 10^{-5}M in the guinea-pig ileum (62) and the D50 for its inhibitory action was found to be 3mM for amylase secretion, and 0.1nM for trypsin secretion in the intact perfused canine pancreas (92). In the conscious dog, the dose (i.v.) of proglumide resulting in optimal inhibition of pentagastrin-stimulated gastric acid secretion was 300mg/kg/h which produced plasma concentrations of approximately 1mmol/l. Infusion of 150mg/kg/h was ineffective (168). The D40 for pentagastrin-stimulated acid secretion in the rat was 198mg/kg when injected i.v.(243). Injection (i.v.) of 100mg/kg proglumide was effective in reducing pancreatic secretion in rats in response to CCK infusion (1µg/kg) and in dogs, 300mg/kg/h infusion of proglumide almost completely blocked the pancreatic response to intraduodenal fat (284). Proglumide did not affect gastric acid secretion in reponse to histamine or bethanechol, nor pancreatic secretion in response to secretin, demonstrating its specificity to the CCK and gastrin family of peptides (243).

Proglumide consistently inhibited the action of CCK *in vitro*, but inconsistent results have been observed *in vivo*. Proglumide inhibited CCK-stimulated gallbladder contraction in the dog when infused at 150mg/kg/10min or injected as an i.v. bolus of 5-40mg/kg (94). Whereas, infusion of 500mg/kg following a bolus i.v. injection of 400mg/kg of proglumide did not inhibit pancreatic protein secretion in response to caerulein in man (220). Proglumide (40mg/kg/hr) significantly inhibited the secretion of pancreatic polypeptide in response to CCK infusion (15-240ng/kg/h) but did not block pancreatic protein secretion (91).

Proglumide increased the antral response to food whereas gastrin infusion inhibited antral motility (29). Proglumide also blocked pentagastrin-stimulated rumination but not gastric motor effects in sheep (31). Whereas, the antagonist (400mg bolus + 500mg/h i.v.infusion) partially reversed the pentagastrin inhibition of the interdigestive migrating myoelectric complex in man (82). However, the resulting pattern of motility was neither the normal fed or fasting pattern. Neural CCK receptors were shown to be more sensitive to proglumide than myoreceptors (112).

Collins and colleagues have used proglumide (50-150mg/kg i.p.) to inhibit CCK-induced satiety in the rat. The antagonist had no effect on food intake when

injected before feeding, nor did it reduce the satiety effect of low doses of bombesin (42,43). Proglumide (150mg/kg) also abolished the CCK-suppression (10.9µg/kg CCK) of sham-feeding (44). Proglumide and CCK were delivered i.p. at the same time.

Proglumide blocked the trophic effect on the pancreas of low doses but not of high doses of CCK. On the other hand, proglumide alone acted as a weak agonist on pancreatic growth (316).

Proglumide failed to inhibit CCK-induced excitation of neuronal activity in the rat dorsal vagal motor nucleus (83). In vitro binding of CCK-8 to cortical receptors of the guinea-pig (167) and mouse (41) was also not inhibited by the antagonist at a concentration of 10^{-3} M. But in the intact mouse, while systemic proglumide was only a weak antagonist of CCK-induced analgesia, more potent inhibition was obtained following i.c.v. administration (15). In addition, proglumide potentiated opiate -induced analgesia (302) and hypokinesia (23) when administered intrathecally whereas CCK antagonized the opiates (87). Proglumide also blocked CCK-induced excitation of dopaminergic neurones in the rat midbrain (40).

2.16. SUMMARY

It has been established that the administration of CCK reduces food intake, but it has not yet been established if this is achieved by inducing normal satiety or by causing an aversive response induced by malaise. Nor has it been demonstrated that CCK reduces food intake at concentrations comparable to those seen postprandially. Is it a physiological or pharmacological effect?

The main question, arising from the review, is whether endogenous CCK, released by food entering the duodenum, acts in a similar manner to exogenous CCK, to reduce food intake? It has been shown that the distribution and secretion of CCK are appropriate to those of a short-term satiety factor, but the secretion of sufficient CCK to cause a reduction in meal size has not been demonstrated. An attempt to answer these questions forms the basis for this study and is presented in the following two chapters.

A further area of considerable dispute, that is apparent from this review, is that of the mechanism by which CCK acts to reduce food intake. Peripherally administered CCK has been shown to act by signals that arise peripherally, but the specific signals have not been identified. Vagally-mediated gastric distension signals

have been implicated by some researchers, while the stomach has been shown to be unimportant for CCK-induced satiety by others. If peripheral, endogenous CCK acts to regulate food intake, is the effect also mediated by a peripheral mechanism and does this mechanism involve gastric distension or some other source of signals, such as the small intestine? These questions have been examined in the second part of this study and are presented in chapter 5.

Chapter 3.

THE EFFECT OF EXOGENOUS CCK ON FOOD INTAKE.

The administration of exogenous CCK induces a decrease in food intake (103). It is not known whether the effective doses of CCK result in plasma concentrations that are comparable to those seen after a normal meal or are pharmacological in nature. In man, i.v. infusion of 262 or 525pmol/kg/h CCK-8 (units have been converted to be consistent) induced a 17 or 50% reduction of food intake respectively (276). A recent study demonstrated that a mixed meal produced a plasma concentration equivalent to an infusion rate of CCK-8 of only 9.2pmol/kg/h (19). Similarly, infusion of only 35pmol/kg/h resulted in plasma levels (27.4pmol/l) greater than those following a high fat meal (19pmol/l) (141). These data suggest that the doses effective for satiety in man produce pharmacolgical concentrations of plasma CCK.

In the rat, a liquid food meal produced a plasma concentration of 6.2pmol/l whilst a bolus i.v. injection of 23ng/kg (20.1pmol/kg) CCK-8 resulted in 61.3pmol/l plasma concentration (163). The minimum dose of CCK-8 required to significantly reduce food intake was 500ng/kg (437pmol/kg) but administered i.p. (185,280). No investigation of plasma levels following i.p. administration of CCK had been

made, until very recently, when Smith and coworkers found that i.p. injection of CCK (1748-3496pmol/kg) resulted in plasma levels in the same range as following a high carbohydrate meal (0.5-4.25pmol/l postinjection compared to 0.5-3.2pmol/l postprandially) (268). However plasma samples were taken only 10min after injection or beginning the meal and it is not at all clear how significant the timing is to the results, since absorption of CCK from the peritoneal fluid has not been studied. The low plasma levels at 10min after the very large i.p. doses would suggest that absorption is slow (or that rapid degradation occurs). Nor is it clear whether absorption of the CCK from the peritoneal fluid into the circulation, is necessary for CCK's effect on food intake.

It has thus not yet been demonstrated that exogenous CCK induces a decrease in food intake at physiological levels of the hormone. Therefore, the first study was undertaken to determine:

1) If the satiety induced by CCK administration is a physiological or pharmacological effect.
2) The mechanism by which exogenous CCK acts to reduce food intake.

Since CCK levels could not be measured with accuracy,

particularly when this study was started, an indirect approach was necessary. The aim, initially, was to attempt to reproduce the satiety effect of CCK shown by others (103) and then attempt to reduce the minimum effective dose to levels closer to those thought to be physiological.

3.1. EXPERIMENT 1.

3.1.1.Methods

Thirty male Sprague-Dawley rats, with intitial weights 350-400g, were housed in individual wire-bottomed cages. The rats were maintained at 20 C on a reverse 12h light-dark cycle and were fed during the dark period. Animals were weighed on the same day each week at the end of an overnight fast.

Testing was carried out on a 5 day-per-week schedule. On test days, the rats were allowed access to liquid food (Sustagen, kindly donated by Mead Johnson, diluted to 1 kcal/ml, protein 18%, carbohydrate 67%, fat 6.8%) or standard rat chow (Purina) for 6 hours, while on the remaining 2 days, the chow was available continuously. Water was available at all times.

The rats were trained to drink the liquid food from 25ml pipettes that were modified as drinking tubes, designed to prevent dripping. After an 18h fast, the rats were handled to accustom them to the experimental procedure, and were then given liquid food. Each rat received the same drinking tube each day to eliminate variability due to slight differences in the tubes. Intake of the food was recorded at 2min intervals for 20min. Spillage was measured by means of disposable weigh-dishes placed under the tubes. After the experiment, the weigh-dishes were weighed, the volume of the spilt food calculated and deducted from the measured volume of the meal. The liquid food was then removed and the rats were allowed unlimited rat chow for the remainder of the 6h feeding period. This procedure was continued until a steady baseline intake was achieved, about 3 weeks in most cases.

On experimental days, after an 18h fast, the animals were injected i.p. with either 1ml 0.9% saline as control or 0.25-4.0µg/kg (219pmol-35nmol/kg) CCK-8 (Sincalide, Squibb) made up to 1ml in saline. Fifteen minutes later, the rats were given liquid food and intake measured for 20min. CCK was given on alternate days except in one case (4.0µg/kg).

Data were analysed by Student's t-test for paired data, with each rat acting as its own control.

3.1.2.Results

One rat consistently refused to drink the liquid food and was eliminated from the study. The rats continued to gain weight during the study (mean initial weight 382±4g [S.E.M.], mean weight at end of study, 6 weeks later, 425±6g) thus indicating that they were receiving sufficient food despite the restricted access to the food. There was a tendency for the rats to eat progressively more during the 5 day testing period, however, suggesting that they became progressively hungrier on this fasting schedule, but compensated on the 2 non-test days. The volume consumed in 10min is illustrated in Fig.3.

It was observed that rats ate maximally during the first few minutes of a meal and had usually finished eating by 10min. For example, on 2 days of the baseline week, rats (n=10) had eaten 18.1±1.6 and 19.3±1.4ml at 10min. By 20min these volumes had only increased to 19.7±1.5 and 19.9±1.5ml respectively. Any further increase in food intake after 10min, occurred towards the end of the 20min period, which perhaps reflected compensation for gastric emptying of the meal. We thus considered the volume at 10min to be the size

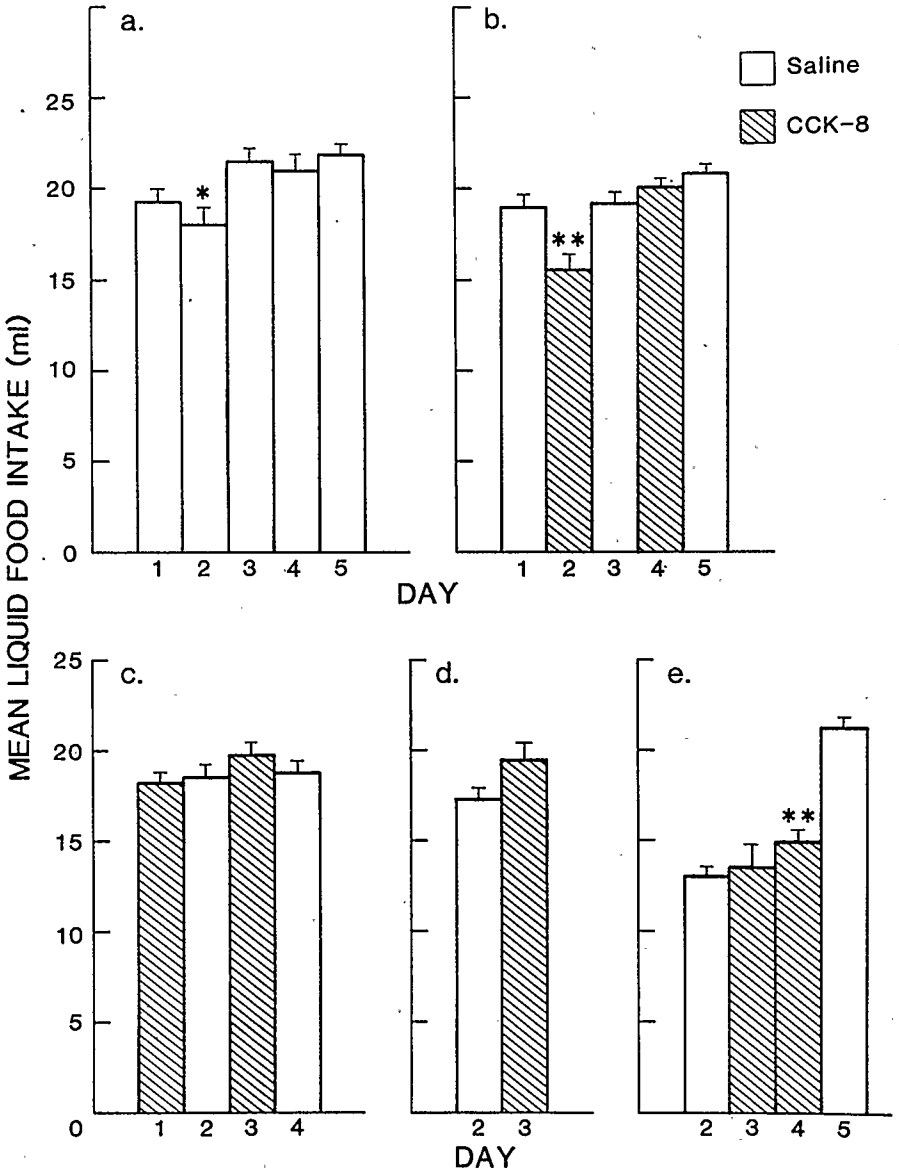

Fig.3. The effect of CCK administration (i.p.) on the volume (mean ± S.E.M.) of liquid food eaten in 10min following an 18h fast (n=10). a) Control week: saline injections only. b) and c) Test weeks: injections of CCK (500ng/kg) or saline on alternate days. d) CCK (1µg/kg) or saline injection. e) CCK (4µg/kg) or saline injection. ** $p < .005$, * $p < .05$.

of the meal consumed. All data have been analysed and presented on this basis.

On the first occasion that rats (n=10) received 0.5µg/kg (438pmol/kg) CCK, the previous minimum effective dose, food intake was significantly reduced compared to both the control days before and after the test day ($p<0.005$) (Fig. 3B). Intake on the same day of the week during the previous baseline week, however, was also significantly reduced compared to the following day, although not to the day before. Nevertheless, when intake during the test week was compared to the same day of the baseline week, only the day when rats were given 0.5µg/kg for the first time was there a significant difference ($p<0.025$).

When the rats received 0.5µg/kg CCK for the second time, no reduction in intake was obtained, nor was there any reduction when the order of presentation was reversed during the second test week (Fig.3 B and C). When the dose of CCK, given to this same group of rats, was increased to 1.0 and 4.0µg/kg on subsequent weeks, no significant differences were observed except between the last 2 days tested (Fig.3 D and E). Subjectively, these rats became erratic in their behaviour and increasingly hard to handle, particularly on a control day following CCK injection. Because of this assessment, a second (n=10) and third group (n=9) of rats

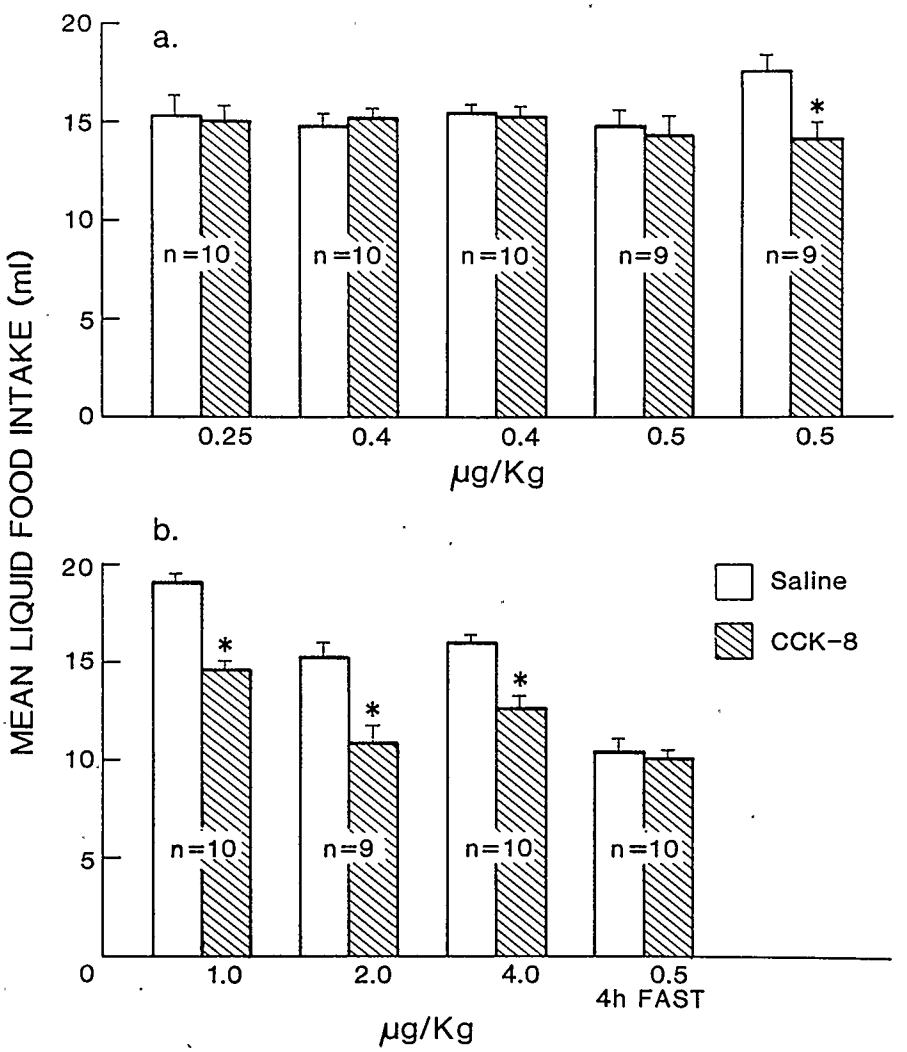

Fig.4. The effect of CCK, injected i.p., on the volume (mean ± S.E.M.) of liquid food eaten in 10min after an 18h fast (except where indicated). Rats were tested only once per week. * $p < .005$.

were injected with CCK only once per week. A significant reduction ($p<0.005$) in food intake was obtained following injection of 0.5, 1.0, 2.0 and 4.0µg/kg CCK but not after 0.25 and 0.4µg/kg (Fig.4A). Retesting one group of rats with 0.5µg/kg CCK was also ineffective although the prior fasting time was decreased to 4h in order that the rats would be less hungry (Fig.4B).

3.1.3.Discussion

Intraperitoneal injection of CCK-8 at doses of 0.5µg/kg (438pmol/kg) or greater induced a decrease in liquid food intake. Doses smaller than 0.5µg/kg were ineffective. This agrees with the minimum effective dose established in an earlier study (280). A study by Collins and colleagues found a decrease in meal size in response to a dose of 70ng/kg but the statistical significance of the decrease was not reported (43).

The smaller decrease (10%) in meal size obtained with 500ng/kg CCK-8 compared to that reported elsewhere (26%) (280) may reflect the difference between day and night-time feeding. The present study was performed during the dark period when rats normally feed. A greater response to CCK was reported when rats were tested during daylight hours when most other studies were performed (155).

The dose of 0.5µg/kg CCK-8 can be compared to known values. In man, this dose would be approximately 4-fold greater than the D50 for pancreatic secretion, if infused i.v. over an hour (64,113). The bolus injection may result in transient plasma levels much higher than those during infusion. In the rat, intravenous injection of 20.1pmol/kg produced a plasma concentration 10-fold higher than that following a mixed meal (163), which would suggest that the administered minimum effective dose in the present study would result in pharmacological plasma levels of CCK. But the different route of delivery of the CCK in the two studies does not permit a conclusion to be drawn. The data reported by Smith and coworkers (268) suggest that plasma levels 10min after i.p. injection of 2µg/kg CCK-8 were within the physiological range. In the present study, the CCK was administered 15min before feeding, and thus conclusions about the plasma concentration at this time are still hypothetical.

Normal satiety is experienced after every meal with no development of habituation or tolerance and with no adverse side-effects. A putative satiety factor must mimic normal satiety in a consistent manner, also with no adverse side-effects.

The failure of repeated doses of CCK to consistently reduce food intake when administered more than once per week and the resultant erratic intake and behaviour, however, suggest the induction of an adverse response rather than true satiety, even though the dose used was at the lowest end of the effective range. A similar inconsistency has previously been reported (198).

The presence of aversion to CCK has been tested by standard methods (See p.42) with varying results (73,103) yet an anti-emetic, trimethobenzamide, inhibited the satiety effect of CCK (200). This suggests that, despite the inconsistent development of detectable aversion, the presence of nausea may be a possible cause of reduced food intake. Nausea is associated with gastointestinal motility changes, in particular, a pronounced inhibition of gastric tone (1,199, 172,234). Since CCK is known to induce motility effects, it is possible that pharmacological doses of CCK act on food intake by inducing motility changes that cause nausea or other symptoms of malaise.

The aim of the next experiment, therefore, was to examine the motility effects, on the stomach and duodenum, induced by doses of CCK effective for reducing food intake.

3.2. EXPERIMENT 2. MOTILITY EFFECTS OF SATIATING DOSES OF CCK.

3.2.1.Methods

Male Sprague-Dawley rats (weight 350-450gm), after an overnight fast, were anaesthetized by i.p. injection of pentothal (40mg/kg) and were placed supine on a heating pad regulated to 37°C by means of a rectal probe. A tracheotomy was routinely performed in case of respiratory problems during the experiment. A midline abdominal incision was made and the stomach and duodenum exposed with minimal handling. A small incision was made in the duodenum approximately 1cm below the pylorus and 2 saline-filled tubes (PE-190, Intramedic) were inserted: the first was directed into the body of the stomach and was tied in place by ligating the pylorus; the second tube was directed distally along the duodenum and was secured by ligating the duodenum at the site of entry. The oesophagus was ligated in the neck, below the tracheotomy site, to prevent gastric reflux. The duodenum was ligated a second time to achieve an approximately 10cm closed segment. The abdomen was sutured closed. In some rats, an intravenous catheter (PE-50) was inserted into a jugular vein to facilitate i.v. injection or infusion. The catheter was connected to a

Harvard infusion pump when necessary, and infusion rates of 0.034 or 0.068ml/min were used.

The gastric and duodenal tubes were connected to saline-filled pressure transducers (Gould) which were fixed at the same height as the rat. Output from the transducers was recorded on a curvilinear Grass Polygraph recorder (Grass 79D). Volumes of saline were introduced into the system to distend the stomach or duodenum by means of three-way taps at the transducers. In most cases, the stomach was distended with 10ml and the duodenum with 2ml saline.

Baseline motility was recorded. The changes in motility were then recorded in response to bolus i.p. or i.v. injection or i.v. infusion of various doses of CCK-8 (range 25ng-5.6µg/kg or 21.9-4902pmol/kg). All doses for injection were made up to 1ml with 0.9% saline warmed to 37 C. A minimum of 20min was allowed between bolus injections. Similar experiments were also performed on a few rats that had received bilateral subdiaphragmatic vagotomy (see chapter 5 for surgical methods). The effect of the specific CCK-antagonist, proglumide (kindly donated as the sodium salt of the amino acid by Rotta Research Laboratorium, Milan), on CCK-induced motility, was also investigated in intact rats. The proglumide was prepared by dissolving it

in 0.9% saline.

The pressure transducer responses were calibrated by connecting the gastric and duodenal tubes to a manometer, with zero set at the same height as the rat. Pressure was measured as cms H_2O above atmospheric pressure. The gastric pressure measured by the method employed here is dependent on gastric volume and on the tone of the gastric musculature. But since the volume is constant in a closed compartment, the pressure was a direct measure of tone.

3.2.2.Results

Changes in motility of the stomach and duodenum were observed following most doses of CCK administered. These responses were consistent in the direction of change but very variable in pattern and degree (Fig. 5). Precise quantitation and statistical analysis was thus not possible. The data are therefore presented mainly in a qualitative manner; however, the ranges of magnitude and duration of the effect obtained are given where possible (Tables 4 and 5).

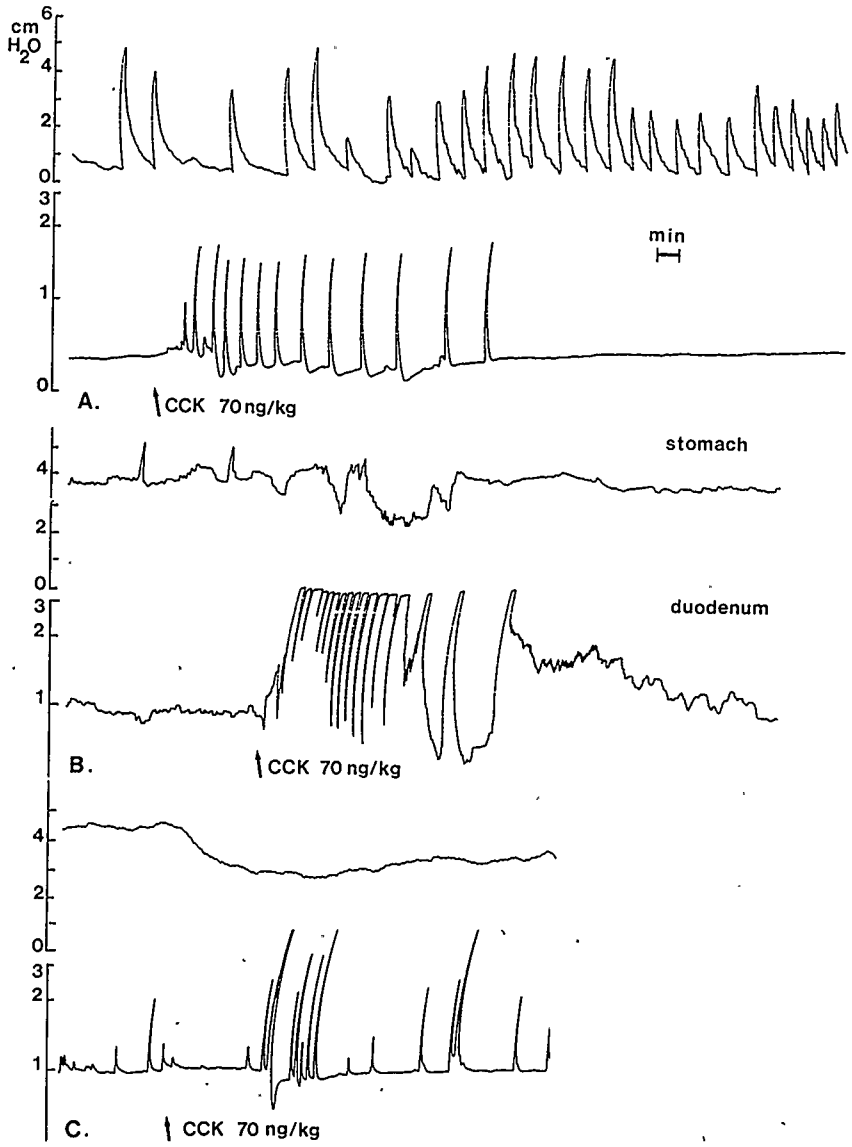

Fig.5. This figure illustrates the range of responses obtained to the same dose of CCK-8 in 3 anaesthetized rats. Pressure recordings of stomach (top trace of each panel) and duodenum (lower trace of each panel) in response to injection i.p. of 70ng/kg CCK-8 at the time indicated by the arrows. (Recordings were made on a curvilinear recorder which gives the pressure changes a curved appearence).

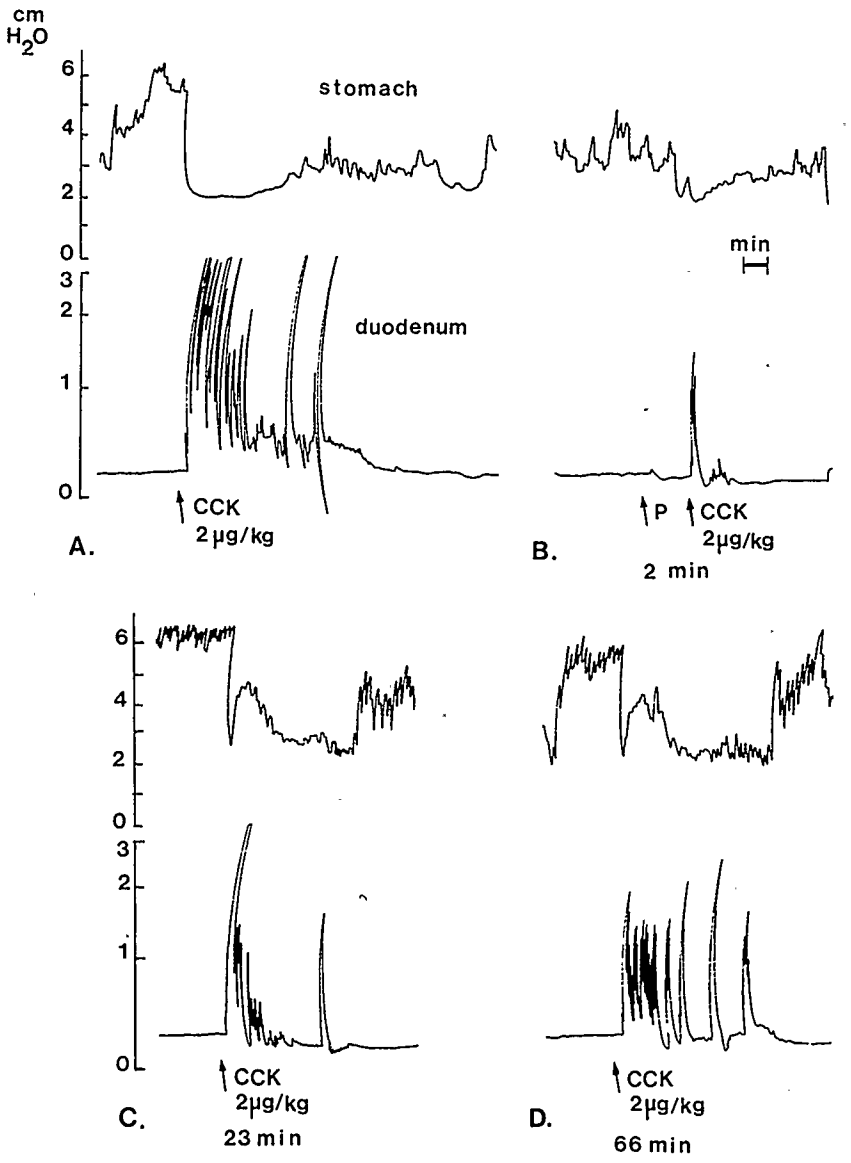

Fig.6. The motility responses to a bolus i.v. injection of 2µg/kg CCK-8 are shown in panel A. Panels B-D: segments of a continuous recording made in the same rat as shown in panel A, at the indicated times after proglumide (P) injection (150mg/kg, i.p.). All CCK-8 doses were made by bolus i.v. injection.

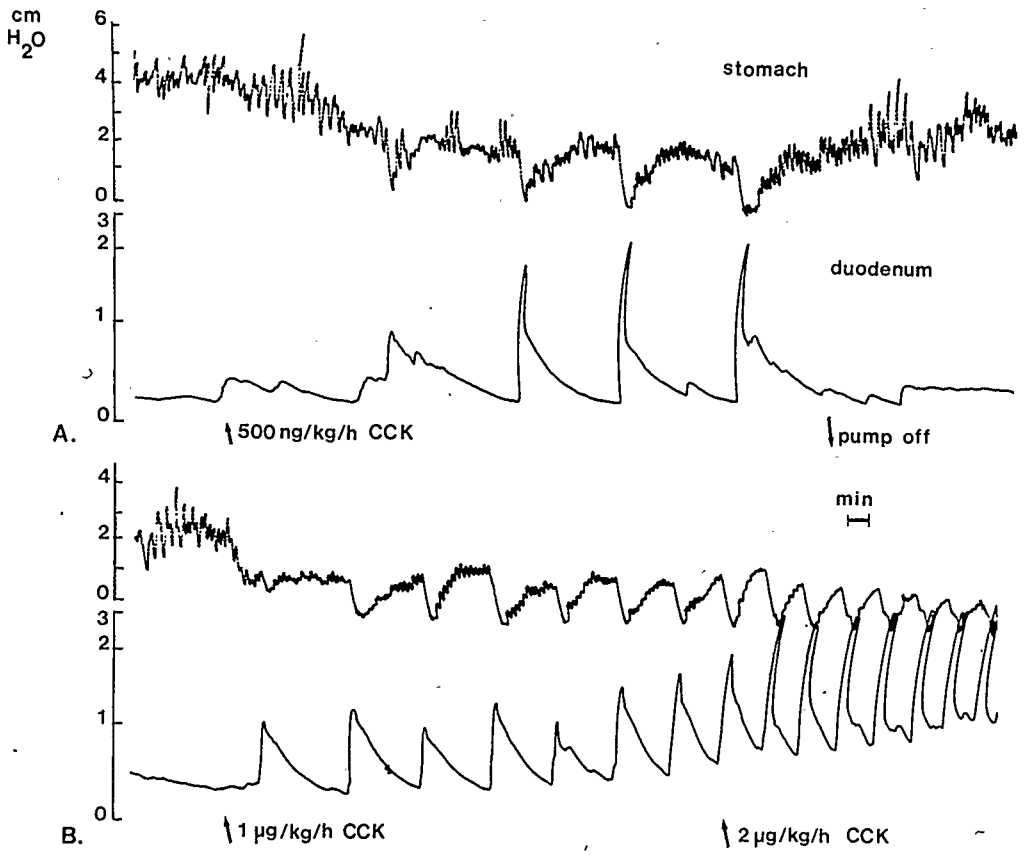

Fig.7. The motility responses to i.v. infusion of increasing doses of CCK-8 in the same rat are shown in panels A and B. The gradual onset of the response and the rapid return to basal pressures when the infusion pump was turned off, are illustrated in panel A.

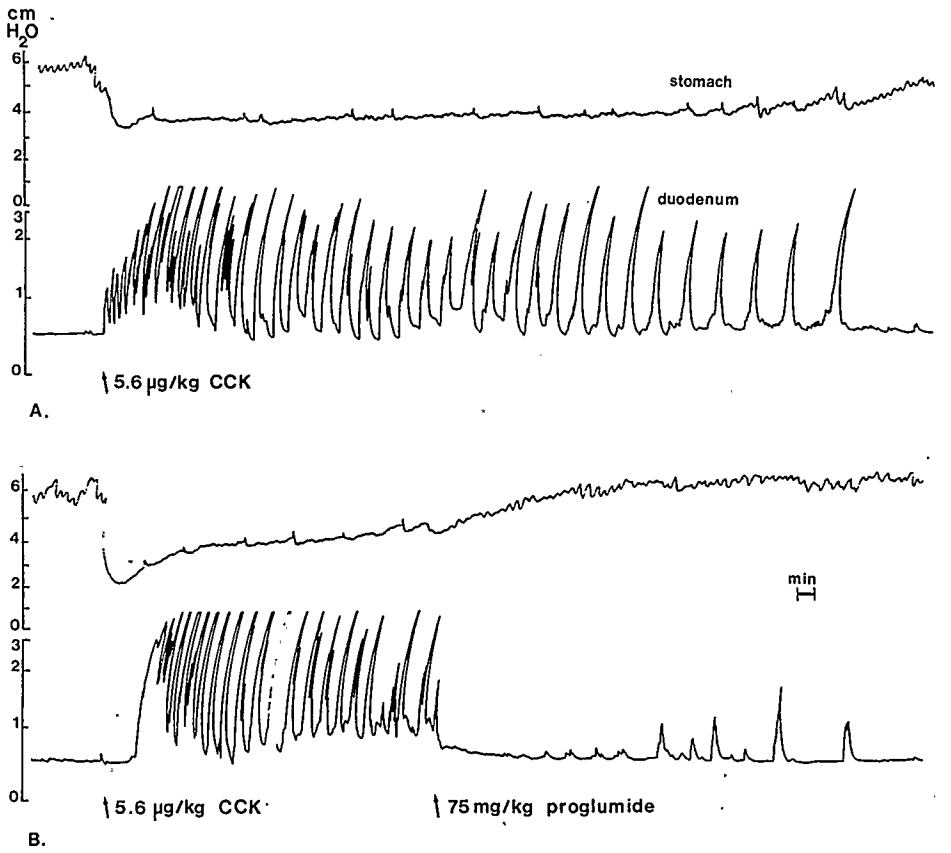

Fig.8. Motility responses in the anaesthetized rat to i.p. injection of a dose of CCK-8 (5.6µg/kg) that induced a 35% reduction of food intake (44) are shown in panel A. Injection of proglumide caused an immediate inhibition of CCK-stimulated duodenal motility (Panel B). The recording in panel B is continuous with that of panel A.

An immediate excitation of duodenal motility with a simultaneous inhibition of gastric motility was observed in almost all cases following i.p. injection of CCK (Fig 6A). Bolus i.v injections (Fig. 6 A, C and D) also caused similar rapid responses, whereas infused doses produced a gradual onset of the same effects (Fig.7). In some cases, gastric excitation was seen as duodenal activity decreased (Fig. 5A).

Duodenal excitation consisted of an increase in phasic activity which was usually not accompanied by a rise in basal pressure. Gastric inhibition, on the other hand, consisted mainly of a steep fall in pressure, or tone, and a decrease in phasic activity. This was succeeded by a gradual rise in pressure and phasic activity to basal levels. The fall in gastric pressure was only observed if the stomach was inflated (10ml) with saline (n=5, doses 100-250ng/kg or 87-219pmol/kg).

There was a quantitative and qualitative difference in the response to CCK when administered by i.p. injection compared to i.v. infusion. The response to i.p. injection was immediate, usually irregular in the duodenum and, at the higher doses, very intense initially, then fading to basal levels (Fig.8A). On the other hand, i.v. infusion induced a gradual response resulting in a rhythmic activity which

Dose	Route	n	Pressure Change	Phasic activity	Duration min
5.6ug/kg (4902)	i.p.	3	↓ 3-4	↓	8-11
4ug/kg (3500)	i.p.	6	↓ 1.5-6.0	↓	2-16
2ug/kg (1750)	i.p.	2	↓ 3.0-6.0	↓	13->20
1ug/kg (875)	i.p.	3	↓ 3-10	↓	20-45
500ng/kg (438)	i.p.	4	↓ 2-3	↓	9-50
250ng/kg (219)	i.p.	4	↓ <1-2	↓	<1-15
100ng/kg (87)	i.p.	3	↓ 1-4	↓	9-15
70ng/kg (61)	i.p.	1	↓ 2	↓	6
		1	-	delayed ↑	9
		1	-	-	-
50ng/kg (44)	i.p.	2	↓ 1-2	↓	4-20
		1	-	-	-
		1	↑	-	<1
25ng/kg (22)	i.p.	1	↓ 1	↓	5
		4	-	-	-
2ug/kg (1750)	i.v. bolus	1	-	↓	19
3ug/kg/h (2626)	i.v.	1	↓ 3	↓	Contin.
2ug/kg/h (1750)	i.v.	1	↓ 3	↓	Contin.
1ug/kg/h (875)	i.v.	2	↓ 1.5-2.5	↓	Contin.
		2	-	-	-
500ng/kg/h (438)	i.v.	5	↓ <1-4	↓	Slow, contin.
		1	-	-	-
250ng/kg/h (219)	i.v.	3	↓ 1.5-2	↓	slow, contin.
		3	-	↓ or -	-

Table 4. GASTRIC RESPONSES TO CCK-8 administered by 3 routes is shown. The pmol/kg equivalents of the dosages used are given in parentheses. n is the number of animals tested. The direction of the change in intragastric pressure is indicated by an arrow and the magnitude of the change is shown as a range in cmH_2O. The duration of the response is expressed as a range of times.

Dose	Route	n	Phasic activity	Frequency peaks/min	Duration min
5.6ug/kg (4902)	i.p.	3	↑	1-4	18-55
2ug/kg (1750)	i.p.	2	↑	>10	3-6
1ug/kg (875)	i.p.	4	↑	1.5->10	3.5-45
500ng/kg (438)	i.p.	3	↑	3->10	25-45
		1	-	-	-
250ng/kg (219)	i.p.	3	↑	1-2.2	<1-14
		1	-	-	-
100ng/kg (87)	i.p.	3	↑	<1	9-20
70ng/kg (61)	i.p.	3	↑	<1-2.7	<1-15
		1	-	-	-
50ng/kg (44)	i.p.	3	↑ or ?↑	<1-3.5	<1-5
		2	delayed ↑	<1-4	8-16
25ng/kg (22)	i.p.	7	delayed ↑	<1-1.8	<1-12
1ug/kg (875)	i.v. bolus	1	? ↑	? >10	4
2ug/kg (1750)	i.v bolus	2	↑	1.6-3.1	5-7
3ug/kg/h (2626)	i.v.	1	↑	2.8	Contin.
2ug/kg/h (1750)	i.v.	1	↑	1	Contin.
1ug/kg/h (875)	i.v.	5	↑	<1-3.4	Contin.
500ng/kg/h (438)	i.v.	5	slow ↑	0.2-2	Contin.
		2	-	-	-
250ng/kg/h (219)	i.v.	4	slow ↑	0.2-0.6	Contin.
		2	-	-	-
125ng/kg/h (87)	i.v.	2	slow ↑	0.2-0.4	Contin.
		1	-	-	-

Table 5. DUODENAL RESPONSES TO CCK-8, administered by 3 routes, is shown. The pmol/kg equivalents of the dosages used are given in parentheses. n is the number of animals. The direction of change of phasic activity is indicated by an arrow. The range of maximum frequency of contraction seen during the responses is given as peaks/min. The duration of the response is also presented as a range. ?↑ indicates a single, sustained increase in pressure.

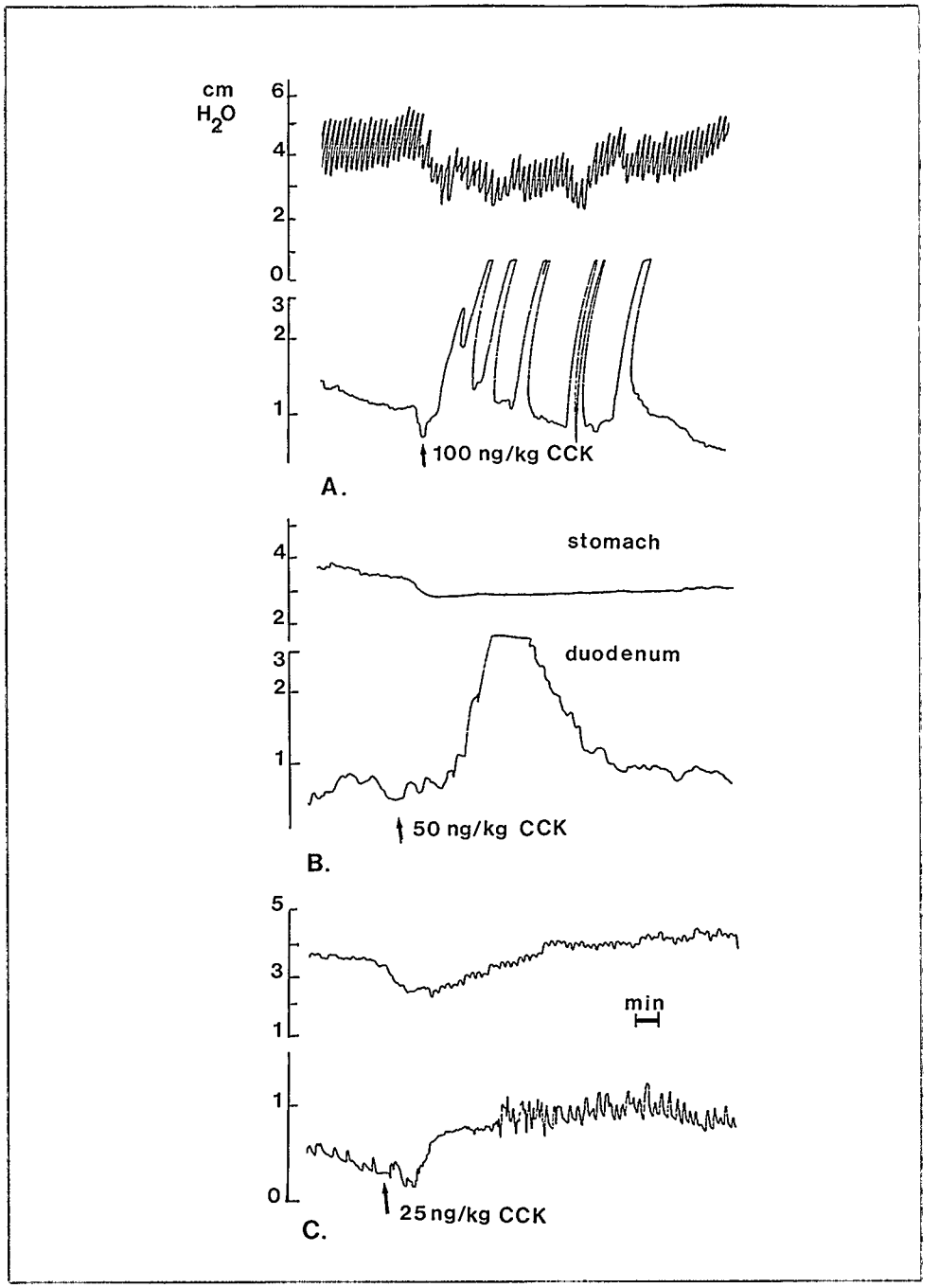

Fig.9. Motility responses in 3 anaesthetized rats to very low i.p. doses of CCK-8 that were ineffective for inducing satiety.

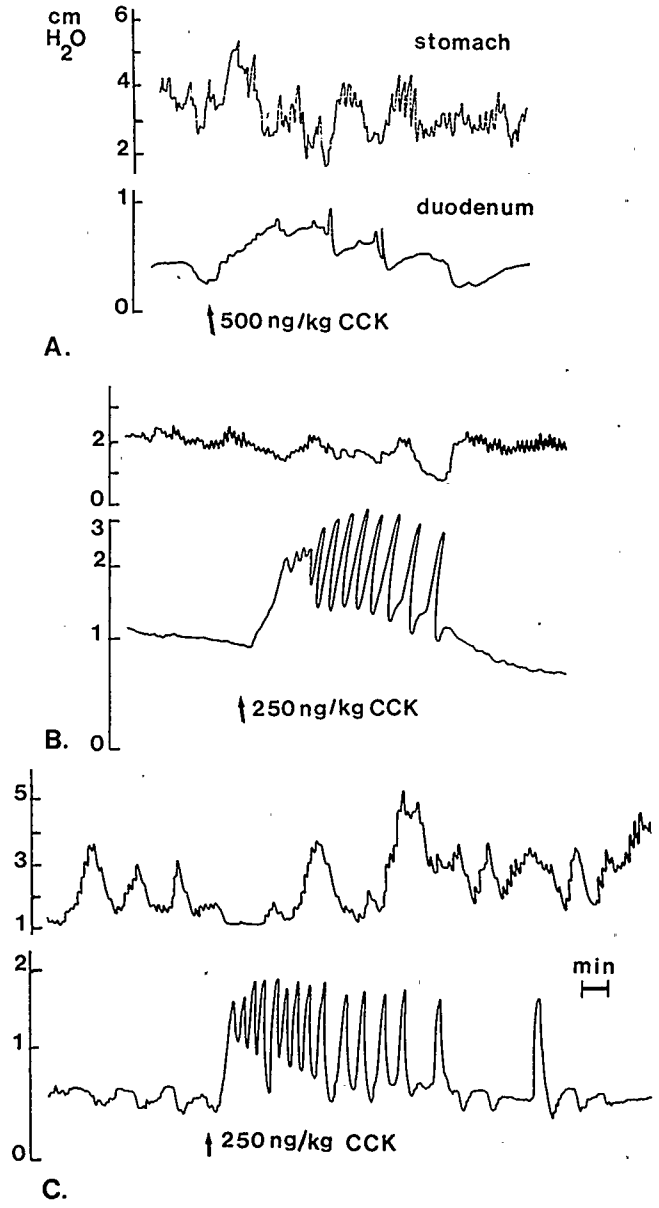

Fig.10. Motility responses to i.p. injection of CCK-8 in 3 vagotomized, anaesthetized rats. This figure illustrates the absence of the fall in gastric pressure that parallels the increase in duodenal phasic activity seen in intact rats (Figs.5-9).

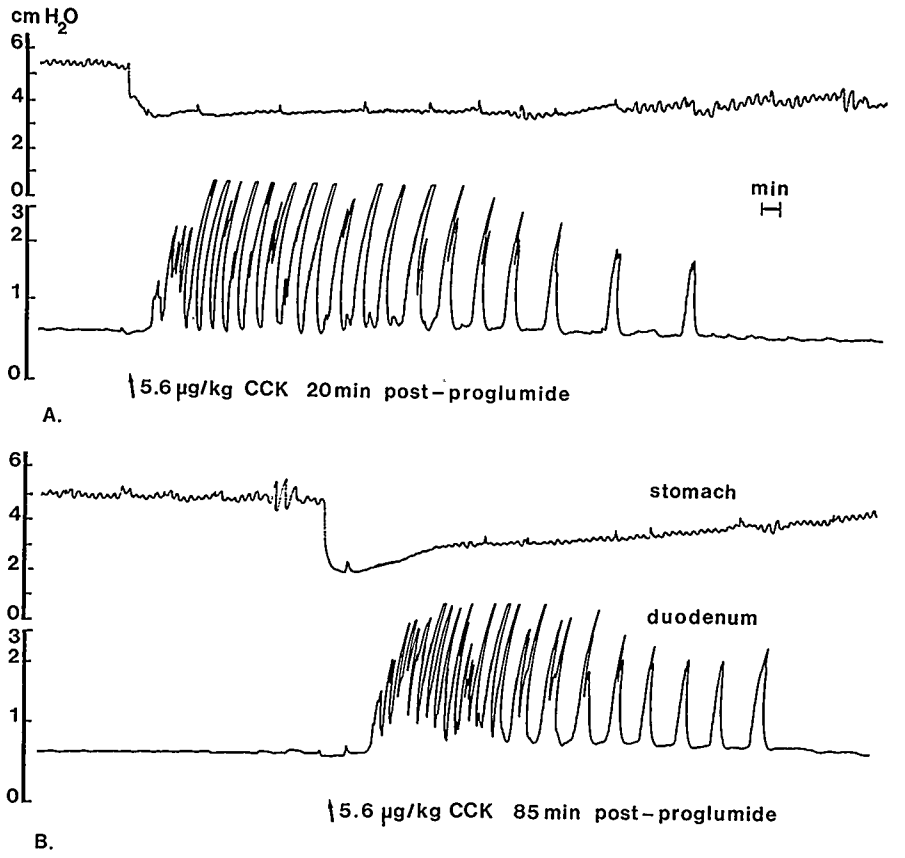

Fig. 11. Motility responses to i.p. injection of CCK-8 at 20 and 85min after i.p. injection of 150mg/kg proglumide. These recordings followed those shown in Fig. 8 in the same rat. Little inhibition of the motility effect, by proglumide, is apparent when these responses are compared to those obtained before proglumide injection shown in Fig.8A.

continued for the duration of the infusion, but which was less profound than an i.p. response. Recovery to basal levels occurred rapidly (within 1-2min) after infusion ceased (Fig.7).

Motility changes were induced by doses of CCK up to 20-fold smaller than those necessary to induce a decrease in meal size (Fig.9) although responses were not obtained on every occasion at these low doses. The effects were also more variable in nature and shorter in duration (Tables 4 and 5). In vagotomized rats (n=8), the gastric inhibitory response was absent and the duodenal excitation appeared to be reduced (Fig.10). Increased irregularity of basal motility and the presence of bezoars in the stomachs of some animals were observed.

Injection (i.p.) of the CCK-antagonist, proglumide, during an ongoing CCK-induced motility response caused a rapid block of the response (n=4) (Fig. 8). However, this inhibition was brief, since a second injection of CCK at 20min post-proglumide (Fig.11A) caused a response of almost the same dimensions as the pre-proglumide response (Fig.8 A). (Figs. 8 and 11 are recorded from the same rat, but the dose of proglumide was increased to 150mg/kg before the start of recording in Fig.11). An inhibition of the response to CCK infused i.v. (n=2) and to a bolus i.v.

dose of CCK by i.p. proglumide was also obtained (n=1) (Fig.6): the inhibition by proglumide was almost total at 2min after injection, but by 20min, the block was only partial.

3.3. DISCUSSION

Administration of CCK-8 i.p. at doses required to induce satiety, caused profound duodenal and gastric motility effects. Immediate duodenal excitation of phasic activity was paralleled by an intense gastric inhibition of both phasic and tonic activity. Such effects were seen at doses (25-50ng/kg) upto 20-fold lower than the minimum effective dose for evoking a reduction in food intake (500ng/kg). However, gastric inhibition was less pronounced at these low doses. Infusion (i.v.) of CCK-8 produced a slower onset of similar changes but the effects were less intense and more regular in nature.

Inhibition of gastric tone was, at least in part, probably initiated by an intestinally-evoked reflex. This is clearly illustrated by the reciprocal changes in pressure seen in Fig.7. Each increase in duodenal pressure, representing a contraction, slightly preceded each fall in gastric tone. The existence of such enterogastric reflexes, responding to distension (6,116,242) and mechanical or

chemical nociceptive stimuli (107), is well established. The reflex is effected by the activation of vagal non-adrenergic, non-cholinergic inhibitory nerve fibres to the stomach (107). In this study, vagotomy abolished the gastric component of the CCK-evoked response, indicating that a vagal pathway might also be involved in this peptide-induced effect (Fig.10).

In a few cases, gastric inhibition appeared slightly before duodenal excitation (Fig.9B). It can be speculated that gastric tone was reflexly inhibited by the preceding stimulation of a more distal region of the small intestine, rather than the measured duodenal segment. The apparent delay in duodenal activity might have been due to the variable distribution of each 1ml injected dose within the peritoneal cavity and the time taken for the dose to mix throughout the peritoneal fluid.

The contrast between the motility changes observed during normal feeding and those produced by CCK administration can be seen by comparing Fig.12 with Figs.5-11. The pressure recordings shown in Fig.12 were obtained from chronically implanted intragastric and intraduodenal catheters in the conscious rat. The methods used will be described in chapter 5. Several methodological differences exist between the data shown in Fig.12 and those

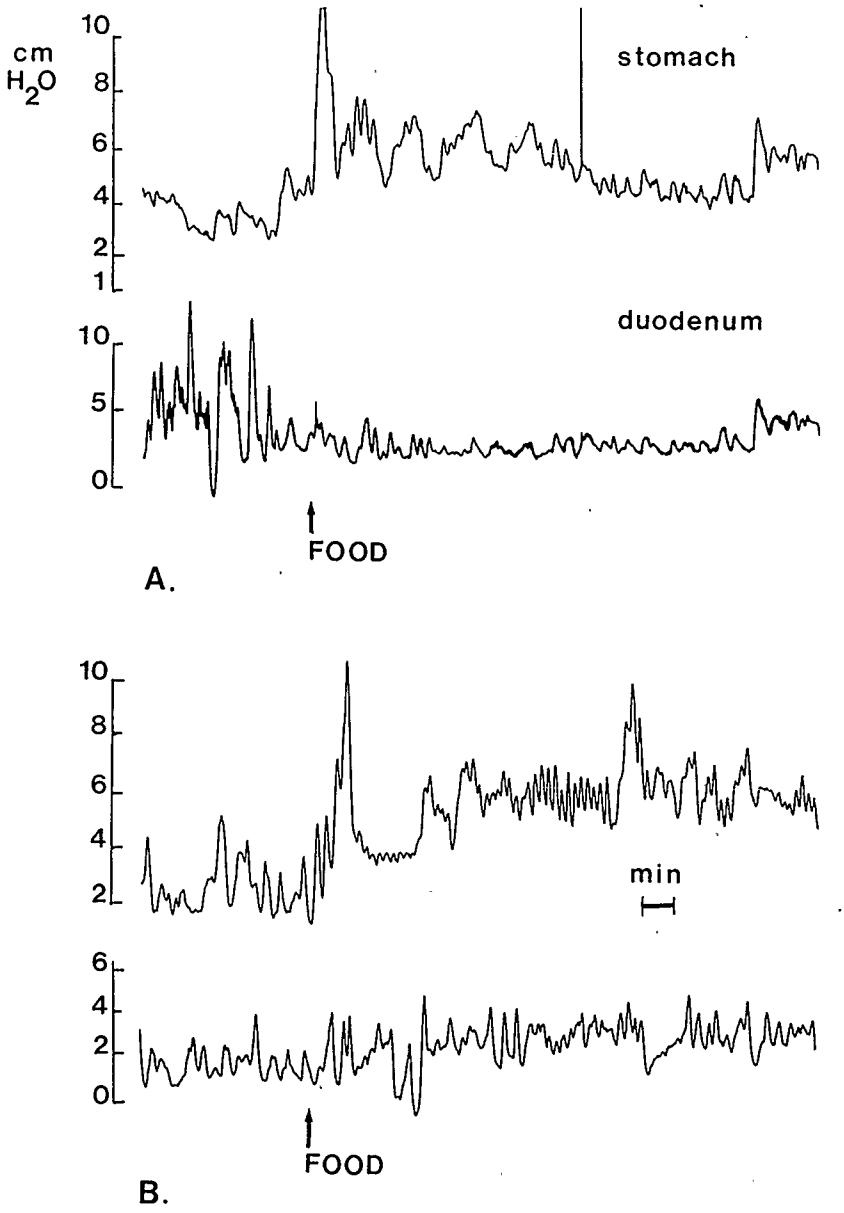

Fig.12. Gastric and duodenal pressure responses to the intake of liquid food by fasted, conscious rats, A and B.

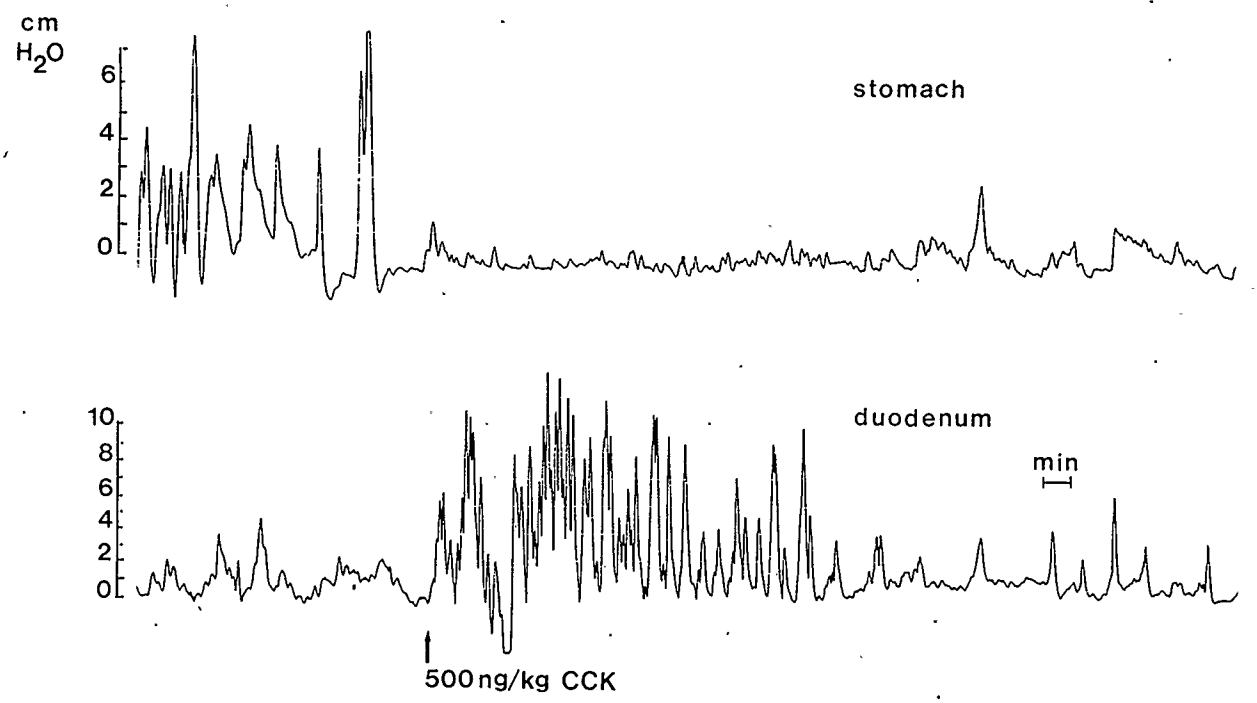

Fig.13. Intragastric and intraduodenal pressure responses to the i.p. administration of CCK-8 in a conscious fasted rat.

discussed in this chapter: the rats were in their normal posture and not supine; they were conscious and not anaesthetized; pressure was measured in open and not closed segments of gut, and therefore measured the pressure and not tone, and a constant perfusion system was used rather than the static method applied here. The same volume of liquid food (10ml) was eaten by the conscious rats as was used to inflate the stomachs of the anaesthetized rats.

Therefore, to confirm that the responses to exogenous CCK seen in the anaesthetized rat were comparable to those seen in the normal, conscious, fasted rat, a few animals were injected with 500ng/kg (438pmol/kg) CCK-8 via an indwelling intraperitoneal cannula and pressure was measured in the same manner as for the feeding rats shown in Fig.12. A typical result is shown in Fig.13. Intense duodenal excitation and gastric inhibition was observed on each occasion (n=6). A fall in basal intragastric pressure was not seen as in the anaesthetized animal, probably since the stomach was empty, but inhibition of phasic activity is clearly present. The fall in intragastric pressure was also absent in the anaesthetized rats when the stomach was uninflated. Therefore, the data obtained in the anaesthetized rats is representative of the effects seen in the normal, conscious rat.

The differences between the fed motility pattern and the CCK-induced pattern are so pronounced that there can be little doubt that the CCK-evoked motility bears no resemblance to the normal pattern of motility obtained in the feeding rat seen in Fig. 12 or reported by others using other methods (30,244,259). Under physiological conditions during feeding, there was no evidence of profound gastric inhibition or intense duodenal excitation, nor was there any evidence of the reciprocity of these two effects. It was concluded that the pattern of motility evoked by CCK injection did not mimic those observed during normal feeding when satiety signals would be operative.

These data agree with a report by Deutsch (73) that food- and CCK-induced patterns of motility were considerably different. But the method used by Deutsch was unconventional, indirect and difficult to interpret. The methods used here, on the other hand, were standard, direct and semi-quantifiable.

The immediate responses to injected CCK suggest that absorption of CCK into the systemic circulation is not essential for the peptide's effect on gut motility. The concentration of CCK in the plasma 10min after i.p. injection of large doses of CCK (2-4μg/kg or 1750-3502pmol/kg) demonstrated that less than 0.005%,

approximately, of the dose had been absorbed (268), assuming that little degradation had occured within that time, which suggests that uptake was very slow and, furthermore, that plasma concentrations of CCK are not a good indicator of the bioavailability of CCK. These same doses reduced food intake by 23.3% and 37.9% respectively. The plasma concentrations arising from the smallest doses used here to stimulate motility (25-50ng/kg or 21.9-43.8pmol/kg), based on the absorption data above (268), would have been negligible yet produced measurable changes in gastric and duodenal pressure (Fig.9). The possibility must be considered that the biological responses to i.p. injections, including behavioural changes in eating pattern, may be induced by purely local, topical effects of CCK, which do not mimic normal physiological states.

Proglumide, injected i.p., caused an immediate block of ongoing CCK-stimulated duodenal motility (Fig.8B) thus confirming *in vivo* its antagonistic action previously reported for *in vitro* preparations (62,117). However, the inhibition was brief: it had nearly disappeared within 20min when CCK was administered i.p. (Fig.11A) or was considerably reduced by 23min when CCK was administered by bolus i.v. injection (Fig.6). Plasma proglumide levels have been shown to remain elevated for at least 2h after oral administration, suggesting that it is not rapidly

cleared from the circulation (190) and it should thus be able to inhibit the actions of CCK for a prolonged period. But, the decrease in antagonism only 20min after i.p. injection suggests a decreased bioavailability of proglumide at its site of action. Uptake into the circulation, from the peritoneal cavity, would tend to dilute the antagonist, thus decreasing its effectiveness. Alternatively, rapid degradation within the peritoneal fluid might occur. Irrespective of the process by which the availability of proglumide decreases, the evidence suggests that since the actions of both CCK and proglumide are very rapid, they may act topically on the gut muscle and may not require prior uptake into the circulation.

Nausea has been associated with gastric inhibition in man (172,234), cat (1) and dog (199). Duodenal contraction was also seen in man (234). The profound gastric relaxation seen following CCK administration in the rats in the experiment described here, may be similar to that seen in nausea. Whether nausea is present or not, the doses of CCK effective for inducing satiety, evoked abnormal gastric and duodenal motility responses which may have been experienced by the rats in the food intake experiment (Expt.1) as malaise. The abolition of the CCK-induced reduction in food intake by an anti-emetic (200) reinforces this argument.

Reports of malaise in man (3,54, 260,285) and of the development of aversion to flavours and feeding place in rats (286) have been associated with the administration of CCK. Behavioural effects, similar to those produced by a toxin, have also been observed (297). Further evidence demonstrating inconsistency of response to CCK (198) and habituation (48,198, 304), together with the evidence of aversion have led to the hypothesis that the reduction in food intake evoked by CCK administration, is achieved at pharmacological levels of the hormone and is indicative of malaise and not satiety. The data obtained in this study, in the first experiment, suggesting inconsistency of response to CCK accompanied by erratic behaviour and, in the second experiment, suggesting the induction of abnormal gastrointestinal motility, support this hypothesis and furthermore, provide evidence of the mechanism by which malaise might be induced by CCK.

But the fact that pharmacological doses of exogenous CCK are required to induce satiety and that the decrease in meal size is probably achieved by non-physiological mechanisms, does not exclude the possibility that endogenous CCK may act to regulate food intake under normal physiological conditions. This postulate will be addressed in the next chapter.

Chapter 4.

THE EFFECT OF ENDOGENOUS CCK ON FOOD INTAKE.

A candidate for the role of intestinal satiety factor should satisfy certain criteria: the factor must be released by ingested food; the administration of the factor exogenously must mimic the effect of the endogenous release of the factor; a specific antagonist must block both the endogenous and exogenous factor equally; plasma concentration of the endogenous factor must be sufficient to induce satiety under physiological conditions (264).

In the case of CCK, several of these criteria have been satisfied. It is known that CCK is released rapidly by food in the duodenum and that plasma levels reach a peak within 20-30min of starting a meal (18,33, 34,141). The administration of exogenous CCK has been shown to reduce food intake (103). Proglumide, a specific antagonist of CCK and related peptides (62,117), has been shown to inhibit the satiety effect of exogenous CCK (43). However, the effect of the endogenous release of the peptide on food intake and its inhibition by an antagonist have not yet been demonstrated. In addition, plasma concentrations of endogenous CCK following a meal are probably much lower than those resulting from i.p.injection of the hormone (see tables 1-3) which has been discussed earlier (17-22).

The fact that plasma concentrations of CCK appear to be inadequate for its putative effects, does not eliminate the possibility that CCK is involved in the regulation of food intake. CCK may achieve high concentrations locally in the duodenum and act directly to stimulate duodenal muscle without entering the general circulation. Reflex inhibition of the stomach is known to occur in response to duodenal stimulation (107,116,242). Secondly, it is possible that CCK could act neurally since the peptide has been detected in enteric nerves (257). Thirdly, it is possible that low levels of CCK are potentiated by other prandial signals, neural or hormonal, in a similar manner to pancreatic secretion (176,193) or ileal contraction (61). Subthreshold doses of CCK were shown to be effective in reducing food intake only in the presence of gastric distension (203). Hence, testing the effects of exogenous CCK in an animal with an empty stomach might not mimic the effect of endogenous CCK released during the digestive phase.

The role of CCK in satiety has, therefore, been investigated by a method that allowed any or all of these possible mechanisms to operate, by permitting normal rats to eat under normal, physiological conditions. Proglumide was used to block the actions of endogenous CCK released during a mixed meal. Since postprandial plasma CCK levels are very low compared to those following CCK administration, the

effect of endogenous CCK would likely be difficult to detect. It could be argued that a proglumide-induced effect could only be seen under optimal conditions and that these would probably coincide with peak CCK secretion. An experiment was, therefore, designed to test this hypothesis by stimulating CCK secretion, under normal, physiological conditions, with a food preload and by then attempting to inhibit the actions of the secreted CCK when plasma levels were known to be reaching their maximum (141). As a control, proglumide was also tested at a time when CCK release had not been stimulated.

It was postulated that if duodenal CCK is a satiety factor, inhibition of the actions of CCK should induce an increase in food intake.

4.1.METHODS

Thirty-four male Sprague-Dawley rats (initial weights 300-400 g) were housed and maintained as in chapter 3. Food was presented, measured and spillage assessed also as previously. The rats were maintained on a 7 day-per-week schedule to avoid the tendency, noted in the results of chapter 3, of the animals to eat more as the week progressed. A more stable baseline was observed on the 7-day schedule, although on occasional days, intake was

still erratic. During the 8 days prior to testing, mean test meal intake was 9.0±1.2, 9.4±1.2, 9.6±1.1, 11.9±1.2, 9.2±1.1, 9.3±1.1, 8.0±1.2, 9.4±1.0ml (n=14).

Proglumide (kindly donated as the pure amino acid by Rotta Research Laboratorium) was prepared, freshly each test day, as the sodium salt by dissolving it in the minimum amount of 2N NaOH at 60°C, and then back-titrating with HCl to an end pH of 7.8. The sodium salt was made up to volume with 0.9% saline.

4.1.1.Experiment 1.

To examine the action of proglumide on the satiating effect of a food preload (protocol A)(Fig 14), after an 18h fast, 34 rats were handled as if for an injection and then given a preload of 10ml liquid food (Sustagen). They were allowed 10min to finish the preload. Failure to do so eliminated them from the experiment. 20min after the completion of the preload period, the rats were again allowed access to the liquid food. Intake was measured for 10min and this volume constituted the test meal. They received solid rat chow for the remainder of the 6h feeding period.

When liquid food intake had stabilized (at least 3 weeks), the rats were tested on two consecutive days. 15min

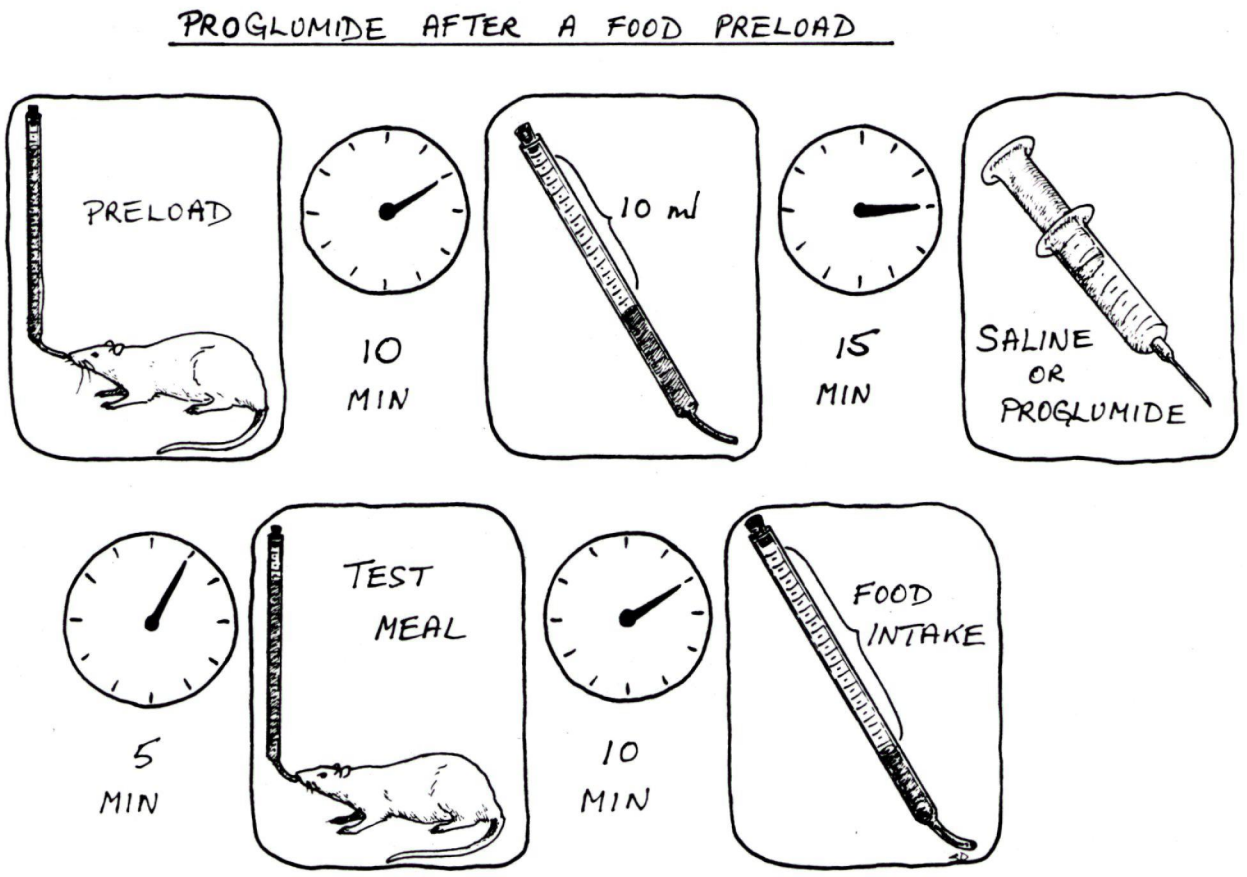

Fig.14. An illustration of the experimental protocol for testing the effect of proglumide on food intake, after a food preload.

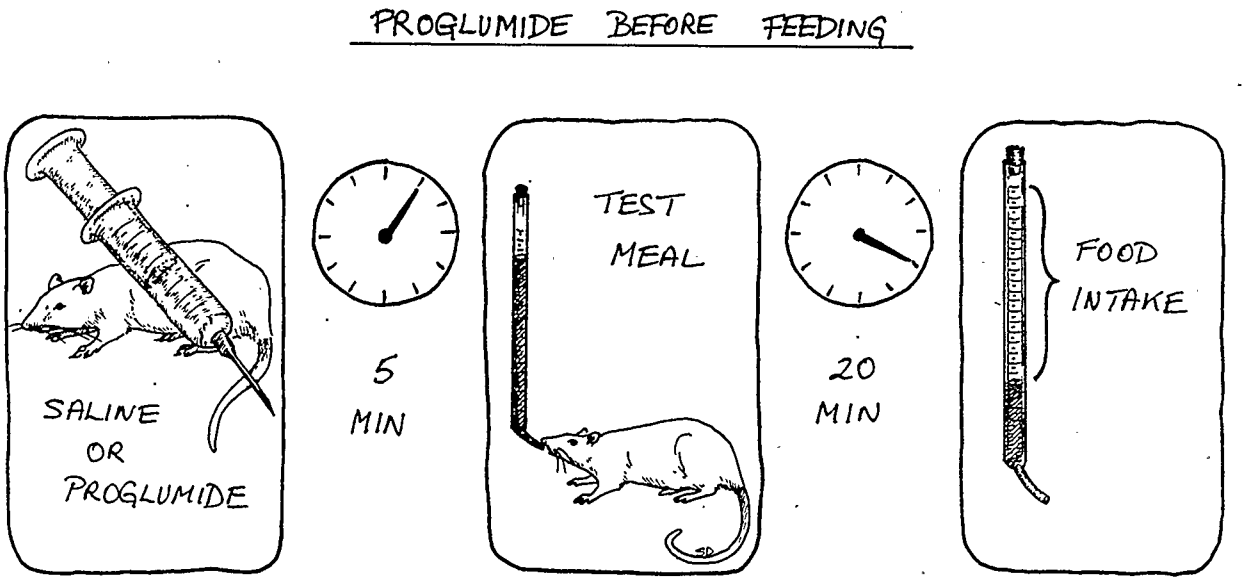

Fig.15. An illustration of the experimental protocol for testing the effect of proglumide on food intake, without a food preload.

after the end of the preload period, each of the rats was injected (i.p.) with either 1ml saline or proglumide sodium salt (150mg/kg) in 1ml saline and fed the test meal 5min later. To eliminate day to day variability, half of the rats received saline and half received proglumide on the first day. On the following day, the treatments were reversed.

4.1.2.Experiment 2.

To examine the effect of proglumide without a food preload (protocol C), that is, at a time when CCK secretion had not been stimulated, 14 rats were fasted for 18h, handled as if to receive an injection, then given liquid food (Sustagen, 1kcal/ml) for 20min and intake measured at 10 and 20min (Fig.15). When intake had stabilized (at least 3 weeks), the rats were tested by the following procedure. On the first test day, 5min before feeding commenced, the rats were injected i.p. with 1ml 0.9% saline or 150mg/kg proglumide sodium salt in 1ml saline. On the following day, the control and proglumide treatments were reversed.

The results of both experiments were analyzed using Student's t-test for paired data, with each rat acting as its own control.

4.2.RESULTS

4.2.1.Experiment 1

All but one of the 34 rats finished the 10ml preload of liquid food in the allocated time. Food intake following i.p. administration of proglumide was 11.0±0.8ml compared to 8.9±0.5ml following saline injection. This represents an increase in food consumed of 24% over control intake ($p<0.01$). Closer examination of the data (Fig.16), however, revealed that animals which showed some degree of satiety following the preload, demonstrated by a low test meal intake on control days (Group I), were more responsive to proglumide than animals which showed no satiety after the preload (group II). Rats with a control intake less than 8ml increased the meal size by 158% (3.7±0.6ml control and 9.0±1.0ml after proglumide) following proglumide ($p<0.001$) whereas rats with a control intake greater than 8ml ate the same amount after proglumide (12.7±0.6ml control and 12.6±1.1ml after proglumide). The control intake in these animals is nearly as large as food intake in hungry rats allowed free access, which is as fast as these animals can eat.

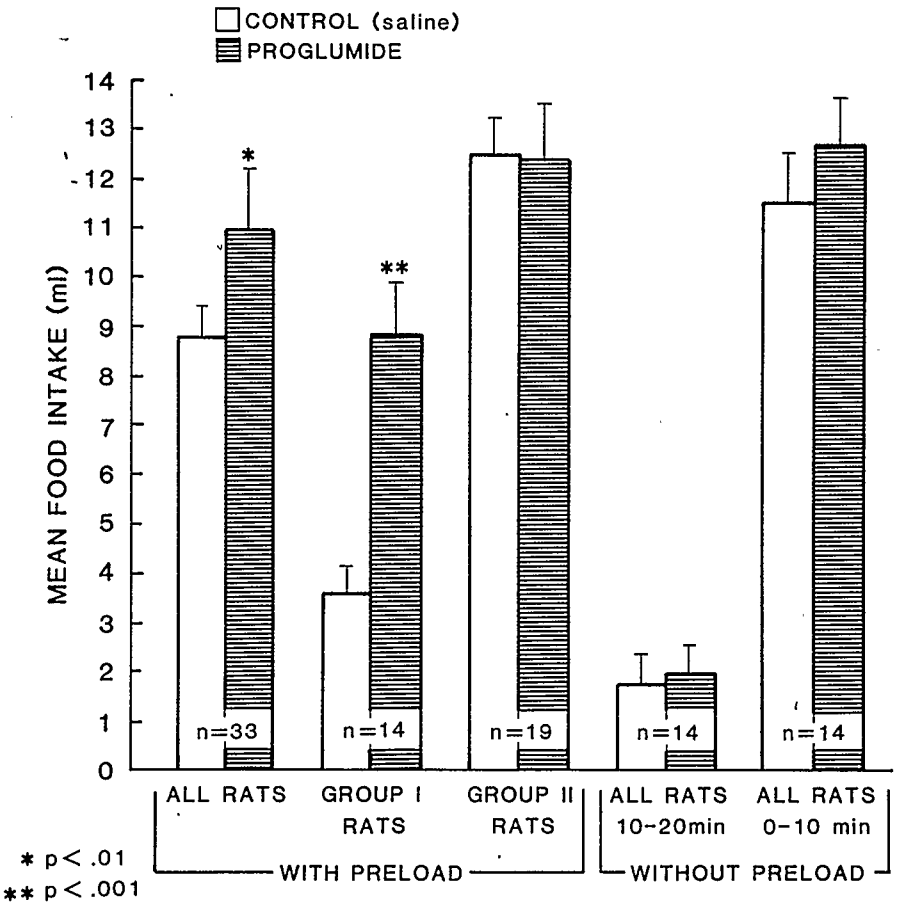

Fig.16. Mean (±S.E.M.) liquid food intake (ml) during the 10min test period in the presence (shaded bars) or absence (open bars) of proglumide (150mg/kg) injected i.p. 5min before the testmeal commenced. Group I rats were those which ate < 8ml on control day; group II rats were those which ate > 8ml on control day. Experiment I with preload. Experiment II without preload. * P < 0.01, ** P< 0.001.

4.2.2.Experiment 2

During the baseline period, it was noted that rats eat maximally during the first 10min following a long fast and this hunger drive might overcome any effect due to proglumide. An increase in intake might only be seen after this initial time. However, no increase in food intake following proglumide administration was observed during either the first 10min of the meal (11.7±0.9ml control and 12.9±0.9ml after proglumide) nor during the second 10min of the meal (1.9±0.6ml control and 2.2±0.6ml after proglumide) (Fig 16).

4.3.DISCUSSION

Proglumide, a specific competitive antagonist of CCK and related peptides, increased food intake in rats provided the test meal was preceded by a food preload and sufficient time was allowed between the preload and testmeal. Moreover, the effect was only seen in those animals which showed evidence of satiety following the preload. This result suggests that proglumide acts on a factor released by the preload and is consistent with the data from a sham-feeding experiment reported by Collins et al (43). It was concluded that proglumide must act on a factor released by the preload to

affect intake and since proglumide is a specific antagonist of CCK, that factor is probably CCK. The failure of proglumide to increase meal size when administered before feeding is consistent with other studies (43,190) and indicates that proglumide has no non-specific effect on food intake. When proglumide was injected before feeding, the release of CCK had not been stimulated whereas when injection was at 25min, following a food preload, CCK secretion was nearly maximal (17,32, 34,181).

Plasma levels of proglumide remain elevated for at least 2-h after oral administration (190) and thus might be expected to increase food intake when injected before feeding. However, there is no evidence that the effects of proglumide are systemic. Proglumide is only effective in high concentrations (62) and the observed effects of proglumide on gastric acid secretion (see chapter 5) and gastric motility (see chapter 3) indicate that it acts topically. Hence, if the site of action of CCK is the muscle wall of the stomach or duodenum, then uptake into the circulation would act to dilute the proglumide and reduce the duration of the CCK blockade. Whatever the reason, it would appear that proglumide is only effective when injection coincides with peak CCK secretion.

Both gastrin and CCK are released by food (300) and the actions of both are inhibited by proglumide (62,243). But gastrin was shown to have little effect on food intake in the rat (171). In addition, pentagastrin-stimulated gastric acid secretion was not inhibited by the dose of proglumide used in this study when administered by the intraperitoneal route (See next chapter). It seems unlikely that inhibition of gastrin was responsible for the observed increase in meal size and thus CCK is the most probable candidate.

Earlier studies have reported, and the results of this experiment have confirmed, that proglumide has no effect on the size of the first meal after its administration (43,190). During the first stages of eating, a powerful hunger drive may override any effect of proglumide and this phase of the meal would not act as an adequate control. In the later stages (10-20min) when food intake was reduced, proglumide was found to be still without effect. Hence it appeared to have no non-specific action and moreover appeared to be effective only when given at a time and under conditions when circulating CCK levels were likely to be near maximal. McLaughlin et al postulated that the lack of effect on the first meal might be due to slow absorption of proglumide (190). However, their own data suggests fairly rapid absorption with plasma levels peaking 20min after intragastric administration. In the present study, the

proglumide was injected 5min before feeding in both protocols. Therefore, it is suggested, rather, that the antagonistic effect of proglumide can be demonstrated only when sufficient CCK has been released to produce its satiety effect as in the preloading experiment. Plasma CCK has been shown to peak 20-30min after starting a meal (18,32-34,181).

Proglumide increased food intake consistently in rats that were mildly hungry as suggested by the level of control test meal intake whilst having a variable effect on very hungry rats (Fig.16). It is possible that the very hungry rats ate a volume of test meal that equalled their maximal capacity and therefore could not, by the action of proglumide, be induced to eat more within the time allowed. This implies that the satiety signal induced by CCK was a weak one which could be easily overcome by a strong hunger drive. A greater degree of inhibition by proglumide might be detected, therefore, after a shorter fasting time or a longer refeeding time.

In conclusion, proglumide, which has been shown previously to block the satiety effect of exogenous CCK, has now been shown to block an endogenous satiety factor. Therefore, sufficient satiety factor must be released under the physiological conditions of this experiment to induce satiety. Because of the established specificity of

proglumide, this study supports a role for endogenous CCK as a satiety factor.

The satiety effect of exogenous CCK is abolished by bilateral subdiaphragmatic vagotomy, suggesting that exogenous CCK decreases food intake by acting at a peripheral site (169,208, 269). If the CCK released by food entering the duodenum acts by the same mechanism as administered CCK, then vagotomy should abolish the proglumide-induced increase in food intake. Therefore, to test this hypothesis, the study was repeated using vagotomized and sham-operated rats.

4.4.METHODS OF EXPERIMENT 3.

Twelve male Sprague-Dawley rats (initial weights 300-400 g) were housed, maintained and fed as previously described.

4.4.1.Vagotomy

Bilateral subdiaphragmatic vagotomy was performed on 6 rats. Under halothane anaesthesia, a longtitudinal midline incision was made, the stomach retracted and the liver gently moved to expose the esophagus. Using an operating microscope, the anterior and posterior trunks of the vagus nerve were identified and each was ligated twice,

approximately 1cm apart. The segment of nerve between the ligatures was transected and removed. All connective and neural tissue surrounding the esophagus in this segment was stripped. The abdomen was closed with resorbable sutures. The vagotomized rats were maintained, for the first 3 days after surgery, on intraperitoneal glucose and saline infusions. Six rats received sham vagotomy in the same manner except that the vagi were not ligated or sectioned. The animals were allowed at least 3 weeks to recover before the experiments were started.

After the last experiment, the animals were killed by decapitation, the esophagus was exposed and examined under the microscope to assess the completeness of the vagotomy. The loss of weight, low food intake, and the presence of a bezoar in the stomach at autopsy were further indications of the completeness of vagotomy.

4.4.2.Experimental protocol.

The sham-operated and vagotomized rats were tested by the same procedures described in experiments 1 and 2. Each animal was tested once without a preload and twice with a preload, and a week was allowed between each test.

Differences between saline and proglumide treatments were tested for significance by Student's t-test for paired data, with each rat acting as its own control.

4.5.RESULTS

The initial weights of vagotomized and sham-operated rats were 346±10gm and 349±27gm respectively. One week after surgery the weights were 293±23gm and 385±28gm respectively indicating the effectiveness of the vagotomy. At the time of the first test, the vagotomized rats were gaining weight (307±23gm and 322±24gm one week later when the experiment was repeated) thus indicating the recovery of these animals. Sham-operated rats weighed 447±35gm and 457±34gm at the time of the first experiment. No rat was eliminated for failure to complete the preload on any of the test days. Baseline test meal intake (no injection) was 4.1±1.56ml for vagotomized rats and 12.4±1.1ml for sham-operated rats.

When proglumide was injected following a food preload, food intake in the sham-operated rats was 14.6±1.0ml compared to 12.2±1.3ml after saline injection. This represents an increase in meal size of 19.7% over the control intake ($n=12$, $p<0.05$) confirming the results

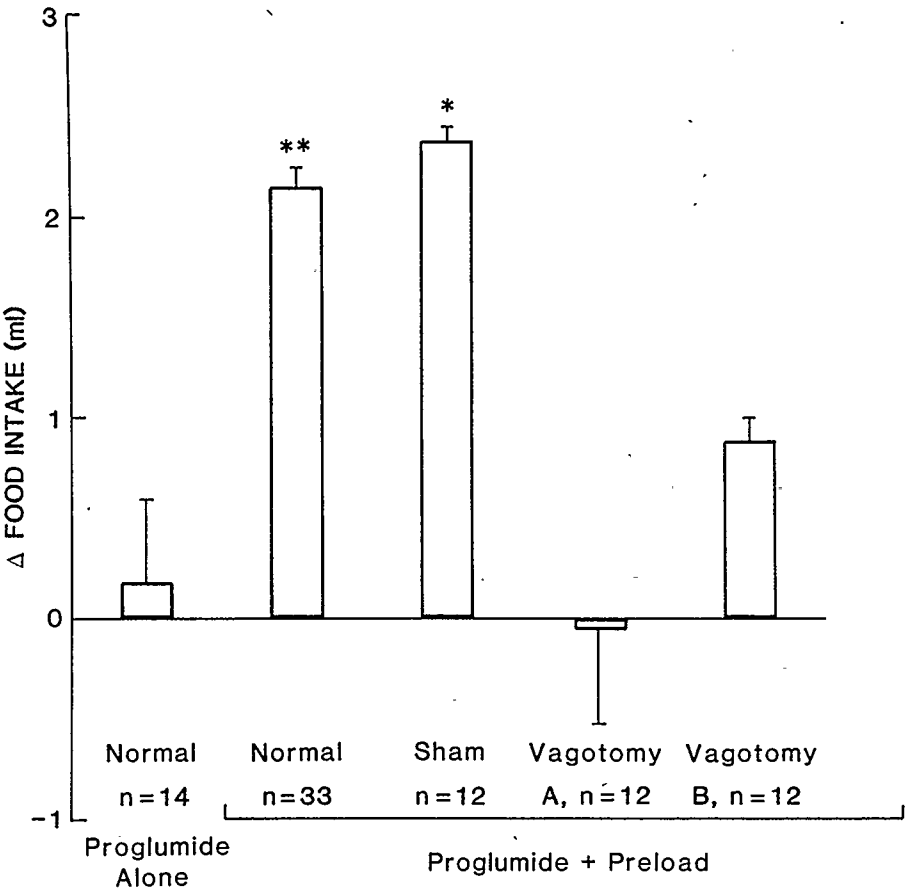

Fig.17. The change in food intake (test meal volume, ml) induced by proglumide in normal, sham-operated and vagotomized rats (mean ± S.E.M.). Data for normal rats is taken from experiments 1 and 2. Proglumide before feeding: proglumide injected 5min before 2 consecutive 10min meals. Proglumide after preload: proglumide injected at 25min after a preload. N: normal; S: sham-operated; VA: vagotomized, test meal given at 30min; VB: vagotomized, test meal given at 70.3min (mean). n values shown are the number of observations. Only 6 vagotomized and 6 sham-operated rats were tested. The change for normal rats after a preload represents a 24% increase in meal size (**P < 0.01) while that for sham-operated rats represents a 19.7% increase (*P < 0.05).

obtained in experiment 1 (Fig.17). However, no change in meal size was seen for the vagotomized rats following proglumide injection (5.2±1.5ml after saline vs. 5.2±2.1ml after proglumide).

When proglumide was injected before feeding, no increase in food intake was observed. Sham-operated rats ate 13.7±1.7ml compared to 13.5±1.3ml after saline during the first 10min and 2.8±0.8ml compared to 1.8±1.1ml after saline during the second 10min period (n=6). Vagotomized rats consumed 10.9±1.3ml after proglumide and 10.1±1.7ml after saline during the first 10min and 0.7±0.4ml after proglumide and 0.6±0.4ml after saline during the second 10min period (n=6).

4.6. DISCUSSION

The results of experiments 1 and 2 have been confirmed in the sham-operated rats showing that proglumide, injected after a food preload, increased food intake. Proglumide injected before feeding, that is, without a preload, had no effect on intake as predicted from the data of experiment 2.

No change in food intake was seen in vagotomized rats when proglumide was injected either before or after the preload. The lack of effect in these rats suggests that

endogenous CCK acts via the same mechanism as administered CCK. However, several alternative interpretations of this result can be made. Firstly, the lack of effect could be an artifact of delayed gastric emptying and hence delayed cholecystokinin secretion. The emptying of solid foods is known to be delayed after vagotomy, whereas liquids are thought to empty faster than normal (307). Yamagishi and Debas, conversely, have demonstrated that in dogs, liquid emptying is also delayed (315). In the experiments described here, a liquid food which clots in the presence of acid, was used. However, acid secretion is reduced by vagotomy (105). It is thus not known whether the meal would be handled as a liquid or solid by the stomach. Secondly, delayed gastric emptying might directly inhibit an increase in food intake. The stomach after vagotomy is known to lose the ability to relax to accomodate a meal (242). Distension due to the preload may continue to inhibit further intake signalled by pathways other than the vagus nerve. Food intake has been shown to be dependent on gastric distension (203). Thirdly, the lack of effect of proglumide could be due to very rapid emptying of the liquid food which might cause "dumping" symptoms (159). In which case, the rat would feel sick and thus not increase its intake.

The following experiments were undertaken to investigate some of these possibilities, initially using the

feeding protocol of experiment 1 but varying the conditions and then by investigating gastric emptying of the liquid food.

4.7. EXPERIMENT 4.

The following hypothesis was examined: if CCK secretion was delayed by late gastric emptying, then proglumide should be effective in inducing an increase of food intake at a time when gastric emptying had occured.

4.7.1. Methods

The vagotomized rats were fed a 10ml liquid food preload after an 18-h fast. The food was removed at 10 min and returned to the animal 20 min later. For 10 days the rats were observed and the time at which each rat started to refeed was recorded. The mean time for each rat was calculated and this time was used as the test meal time (mean time 70.3±7.7min, n=6). Experiment 1 was repeated with saline or proglumide injection 5 min before the test meal given at the individual mean time for each rat. The experiment was repeated one week later.

4.7.2. Results

Food intake was not altered by proglumide. After saline injection test meal volume was 8.6±0.7ml compared to 9.5±1.0ml after proglumide (n=12). However, the rats ate significantly more ($p < .01$) when fed at 70.3min than when fed at 30min as in experiment 1, regardless of the treatment given.

4.8. EXPERIMENT 5

Cholecystokinin is thought to delay gastric emptying (64). Inhibition of CCK with proglumide might increase gastric emptying and allow the vagotomized rats to eat the test meal earlier. The effect of proglumide on the the time at which the rats chose to refeed, was investigated.

4.8.1. Methods

The rats were given a 10ml preload as before, injected with saline or proglumide at 25 min and the time at which they started to refeed was recorded.

4.8.2. Results

No change in the time at which the rats consumed the test meal was observed (77±2min after saline vs. 79±8 min after proglumide, n=6).

4.9. EXPERIMENT 6

The results of the previous two experiments still apparently demonstrated that vagotomized rats do not respond to proglumide. The inability of proglumide to induce earlier refeeding suggested that delayed gastric emptying was not a factor. The possibility of rapid gastric emptying was, therefore, explored.

4.9.1. Methods

The method used to measure gastric emptying will be described in greater detail in the next chapter. The sham-operated rats were maintained on a totally liquid diet for one week before testing, while the vagotomized rats had no solid food for at least 3 weeks. On test days, after an 18-h fast, rats were fed a 10ml liquid food meal labelled with the non-absorbable marker, 14C-polyethylene glycol (14C-PEG), which had to be completed within 10 min. At 28

min, the rats were lightly anaesthetized with ether and at 30 min. an intraoral tube was passed into the stomach and the stomach contents withdrawn. (An infant feeding tube 8 Fr., Medicraft, with extra holes was used). The residual volume was estimated by flushing the stomach with 2ml water, mixing by withdrawing and re-injecting 4 times, and then sampling. The concentration of 14C-PEG in both volumes was measured using a β-counter (Beckman) and the amount of the meal remaining in the stomach was calculated by the method of George (97):

$$\text{Volume remaining} = \frac{V1.C2}{C1} + \frac{V2.C3}{C2 - C3}$$

where V1 = volume withdrawn; C1 = concentration of marker in initial meal; V2 = volume added (2ml); C2 = concentration in V1; C3 = concentration in final sample.

The vagotomized animals were also tested at 15 min. To test how effective this method was for estimating the volume remaining in the stomach after a liquid food meal, the experiment was repeated but instead of sampling by orogastric tube, the rats were killed by decapitation while under ether, the stomach rapidly clamped at the pylorus and lower esophageal sphincter and removed. The stomach contents were removed, the stomach washed thoroughly and the volume of the meal remaining was calculated and compared with the volume obtained on intubation.

4.9.2. Results

The data obtained from the intubation method compared to those from the terminal experiment were not statistically different for sham-operated rats. The volume of the meal remaining in the stomach at 30min was 5.4±0.22ml by intubation (the mean of 3 experiments) compared to 5.4±0.57ml in the terminal experiment. In the vagotomized rats, on 3 attempts to sample gastric contents at 30 min, in only 9/18 cases could a significant volume be withdrawn (2.4±0.11 ml, n=9). We repeated the experiment twice sampling at 15 min. 9/12 rats had a significant volume (3.6±0.28 ml) remaining in the stomach at this time. The liquid food, therefore, emptied faster in vagotomized than in sham-operated rats. The terminal experiment revealed a mean volume of 3.3±1.2ml for 4/6 rats while the remaining 2 animals had only a trace of the labelled meal remaining. The stomachs from vagotomized animals all contained large bezoars consisting mainly of hair.

4.10. DISCUSSION

The results of experiments 1 and 2 in normal rats, have been confirmed in the sham-operated rats. Proglumide induced an increase in food intake only when injection was preceded by

a food preload and sufficient time was allowed for CCK secretion to reach peak levels (18,32-34, 181). No change in food intake was observed when proglumide was administered before feeding. Proglumide must act on a factor released by the preload, and this factor is probably CCK. CCK may thus play a role in regulating meal size.

The finding that vagotomy eliminated the increase in food intake induced by proglumide suggests that the effect of endogenous CCK as well as that of exogenous CCK is dependent on the integrity of the vagus nerve. If these results are interpreted as others have interpreted the effects of vagotomy on exogenous CCK-induced satiety (169,208, 269), then they would imply that the endogenous CCK was acting peripherally to modulate vagal signals. The vagotomized rats did not respond to proglumide when tested at the same time as the sham-operated rats, nor did they respond when tested at a time more appropriate to their individual feeding pattern (70 min). However, in the discussion of experiment 3 (p.118), alternative explanations for the lack of response were suggested: delayed gastric emptying and thus delayed CCK secretion; delayed gastric emptying resulting in prolonged gastric distension; and very rapid emptying causing dumping symptoms. In order to eliminate these alternatives and to understand why these animals do not eat the test meal at 30 min the gastric

emptying of the same liquid food, used in the feeding studies, was studied. The liquid food was found to empty faster in vagotomized rats than in control animals. Therefore, delayed CCK secretion as a result of delayed gastric emptying can be eliminated. CCK secretion has been shown to be unaffected by vagotomy in the dog (93). Therefore, it is probable that adequate CCK secretion had occured in the vagotomized rats since a greater amount of food had emptied into the duodenum than in the control rats.

Prolonged gastric distension due to the delayed emptying of the preload can also be eliminated, but distension due to the presence of large bezoars may contribute to the rats' failure to eat at 30 min. Sympathetic pathways are known to exist which may signal gastric or abdominal distension (242). Hormonal mechanisms, activated by this distension, cannot be excluded.

Since gastric emptying was found to be faster than normal, symptoms of dumping such as nausea, epigastric fullness and diarrhoea, as a cause of failure to eat the test meal remain a distinct possibility. Dumping is often seen in humans following vagotomy particularly after liquid food (159). Significant increases were observed in both control and test groups when test meal intakes at 70min were compared to those at 30min ($p < .01$). These differences were

not due to the volume of the meal remaining in the stomach at 30min since it was demonstrated that the vagotomized stomach was almost empty by that time. Rather, the differences may be evidence of dumping. It is possible that a proglumide-induced effect might be observed if the vagotomized rats were tested at a even later time. It is not known, however, how long plasma CCK levels remain elevated in these animals.

Many other factors, such as low acid secretion (105) and hypergastrinemia (129), are induced by vagotomy which complicate the interpretation of feeding studies. From observations made in this study and those of other investigators (204,312) food intake by vagotomized rats is substantially reduced compared to normal. It is questionable whether a further reduction in intake by exogenous CCK administration could be observed. Moreover, for the reasons discussed above, in particular, the evidence for dumping, the lack of a proglumide-induced response in vagotomized rats cannot be interpreted as an interruption of vagal satiety signals. The effects of vagotomy on exogenous and endogenous CCK mediated satiety must be interpreted with caution. Evidence for the involvement of the vagus in endogenous CCK-induced satiety must be sought by methods less perturbing to the animal than total vagotomy.

The possible mechanisms by which endogenous CCK might act, to regulate food intake, will be discussed in the next chapter.

Chapter 5.

AN INVESTIGATION OF THE MECHANISM BY WHICH ENDOGENOUS CCK REDUCES FOOD INTAKE.

The possible role of endogenous CCK in the regulation of food intake has been demonstrated by the experiments described in the previous chapter. There are several possible mechanisms whereby such regulation might be achieved.

Endogenous CCK might act directly on CNS structures known to be involved in satiety, since central administration of CCK has been shown to reduce food intake in some species (66,225) although not consistently in the rat (65,88,118,169, 175,255,309). However, this seems unlikely since the ability of CCK to cross the blood-brain-barrier has not been demonstrated (217,224,226). In addition, the abolition of exogenous CCK-induced satiety by vagotomy indicates the peripheral origin of the satiety signal (169,208,269). The inconclusive evidence for the involvement of the vagus in the endogenous CCK-induced satiety, discussed in the previous chapter, does not exclude the possibility that this satiety signal might also be of peripheral origin. The vagus nerve remains the most likely route by which the signal may be transported centrally.

CCK may stimulate the vagus nerve directly since CCK-receptors have been identified within the vagus (76,319). Alternatively, CCK may stimulate the vagus indirectly by affecting the tone or motility of the stomach or duodenum. The evidence presented in chapter 3 suggested abnormal gastrointestinal motility to be the mechanism by which exogenous CCK acted to decrease meal size. While it can be assumed that abnormal motility is not a part of normal satiety, endogenous CCK may cause a reduction in food intake by affecting gut motility.

The motility effects of endogenous CCK were therefore investigated in the normal, conscious rat using the same experimental conditions under which the reduction of food intake by endogenous CCK was demonstrated. It was postulated that if endogenous CCK acted to reduce food intake by causing gastric relaxation and duodenal excitation, as observed when exogenous CCK was administered, then inhibition of the action of endogenous CCK, with proglumide, should induce an increase in intragastric pressure and a decrease in duodenal motor activity.

5.1. EXPERIMENT 1.

5.1.1. Methods

Male Sprague-Dawley rats were maintained as described in chapter 3, and were trained as before to eat liquid food at prescribed times. When the rats would reliably eat the food preload, surgery was performed to implant indwelling gastric and duodenal catheters for chronic measurement of intraluminal pressure.

5.1.1.1. Preparation of catheters.

The catheters were prepared from polyvinyl tubing (I.D. 1.40mm, Dural Plastics, Australia). Duodenal and gastric tubes were cut to premeasured lengths and fastened together approximately 1cm from one end, for approximately 2cm, using the solvent tetrahydrofuran. A third tube (I.D. 0.75mm), for making i.p. injections without disturbing the animal while feeding, was attached in the same manner. A small section of tubing was attached at the same place to act as a means of securing the combined catheters to the skin. Several side holes were made at the free end of the 2 catheters for pressure recording.

5.1.1.2. Surgery

Under halothane anaesthesia, a mid-line abdominal incision was made through the skin. A second small incision was made though the skin at the back of the neck. Forceps were used to tunnel under the skin from neck to abdomen and the combined catheters were passed through so that the joined ends protruded at the neck. The small length of attached tubing was sutured to the outside of the skin with non-absorbable silk sutures (2-0) and the incision closed leaving approximately 1cm of tube protruding.

A mid-line abdominal incision through the muscle was made, the stomach and duodenum exposed. The 2 catheters were inserted into the body of the stomach and into the duodenum, 1cm below the pylorus, via small incisions and secured by purse-string sutures (silk, 4-0). The end of the third tube was placed freely in the peritoneal cavity. All 3 tubes were sutured to the abdominal wall to prevent them from being pulled out. The abdomen was closed using interrupted sutures (silk, 2-0). The tubes were flushed with sterile 0.9% saline and plugged with short pieces of wire of suitable diameter.

The rats were allowed to recover for a week before the experiment was started. During pressure recording, the rats

were loosely restrained, to prevent twisting of the catheters, but able to maintain a normal posture during feeding.

5.1.2. Experimental procedure.

The rats were fasted for 18h, then placed in the restrainer. The catheters were connected to disposable pressure transducers (Medex Inc.) which were connected, in turn, to 10ml glass syringes mounted in an infusion pump (Harvard), all previously filled with saline. The pump was briefly turned on at medium speed to ensure the catheters were filled with saline. The speed was then reduced to deliver 0.0051ml/min saline. Constant infusion prevented blockage of the small holes in the catheters by food particles. Output from the pressure transducers was recorded on a Gould 2400S recorder and was calibrated as described in chapter 3.

Baseline motility was recorded. Movement and respiratory artifacts were filtered out using the Gould averager, so that only the slow changes in pressure, associated with gut motility, were recorded. The rats were fed and injected with proglumide (150mg/kg) or saline (via the i.p. tube) according to the protocols described in experiments 1 (protocol A) and 2 (protocol C) of chapter 4. In addition, the protocol of experiment 1 was modified by

injecting saline or proglumide 5min before the preload instead of after the preload at 25min (protocol B).

At the end of each experiment, the recording was traced, the area underneath the curve was cut out and weighed in 5min units. The average change in pressure from basal (weight/5min basal) during a 5min experimental period (weight/5min expt.) was calculated from the following equation:

$$\text{Average change in pressure cm H O/5min} = \frac{\text{Weight/5min expt.} - \text{Weight/5min basal}}{\text{Weight of 1cm pressure change/5min}}$$

This represents an index of the motility recorded and includes both tonic and phasic changes. Results were analysed using Student's t-test for paired data, with each rat acting as its own control.

5.1.3. Results

The gastric motility index during the testmeal was significantly reduced when proglumide was injected after a food preload (protocol A) compared to that following saline injection ($p<.025$, $n=6$). No difference in duodenal motility index was observed between treatments (Fig.18).

There were no differences in gastric or duodenal motility indices between treatments when injection preceded

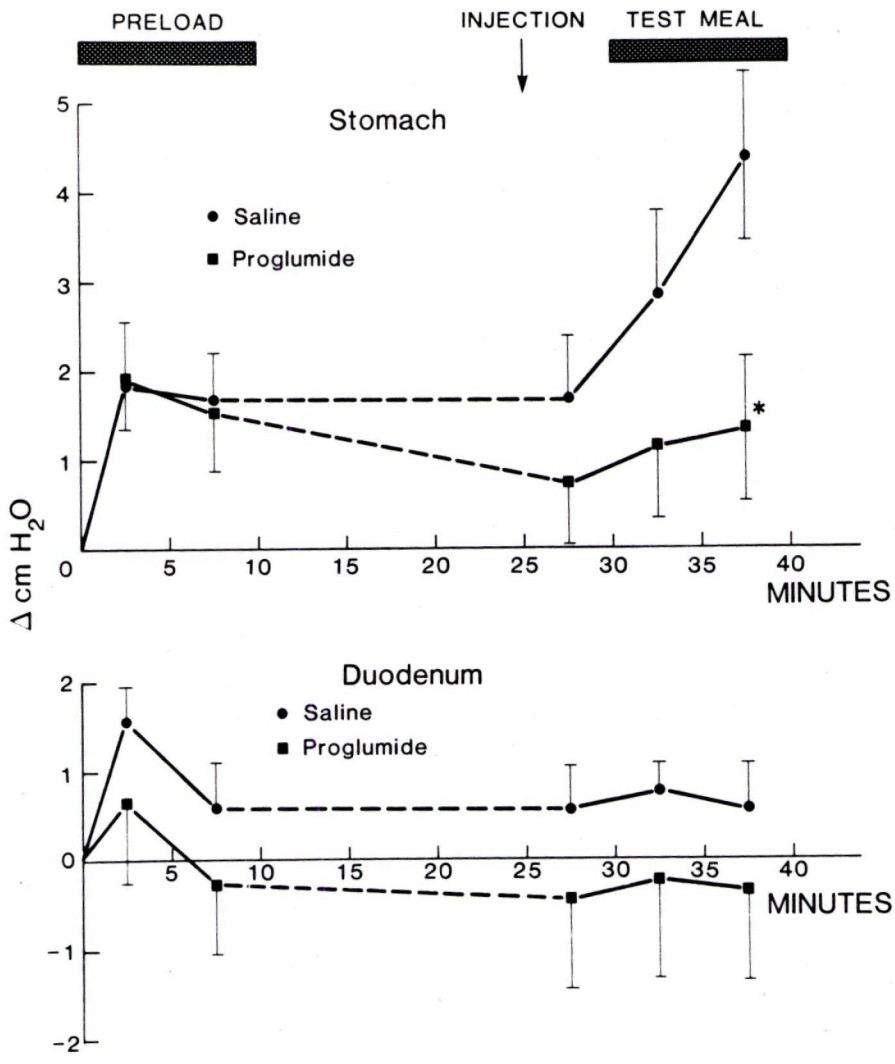

Fig.18. The changes in gastric and duodenal motility indices during feeding (Protocol A) in the conscious rat in response to saline or proglumide injection (n=6). Each point represents the mean (± S.E.M.) of the intraluminal pressure changes over a 5min period and is placed at the centre of the respective 5min period. * $p < .025$.

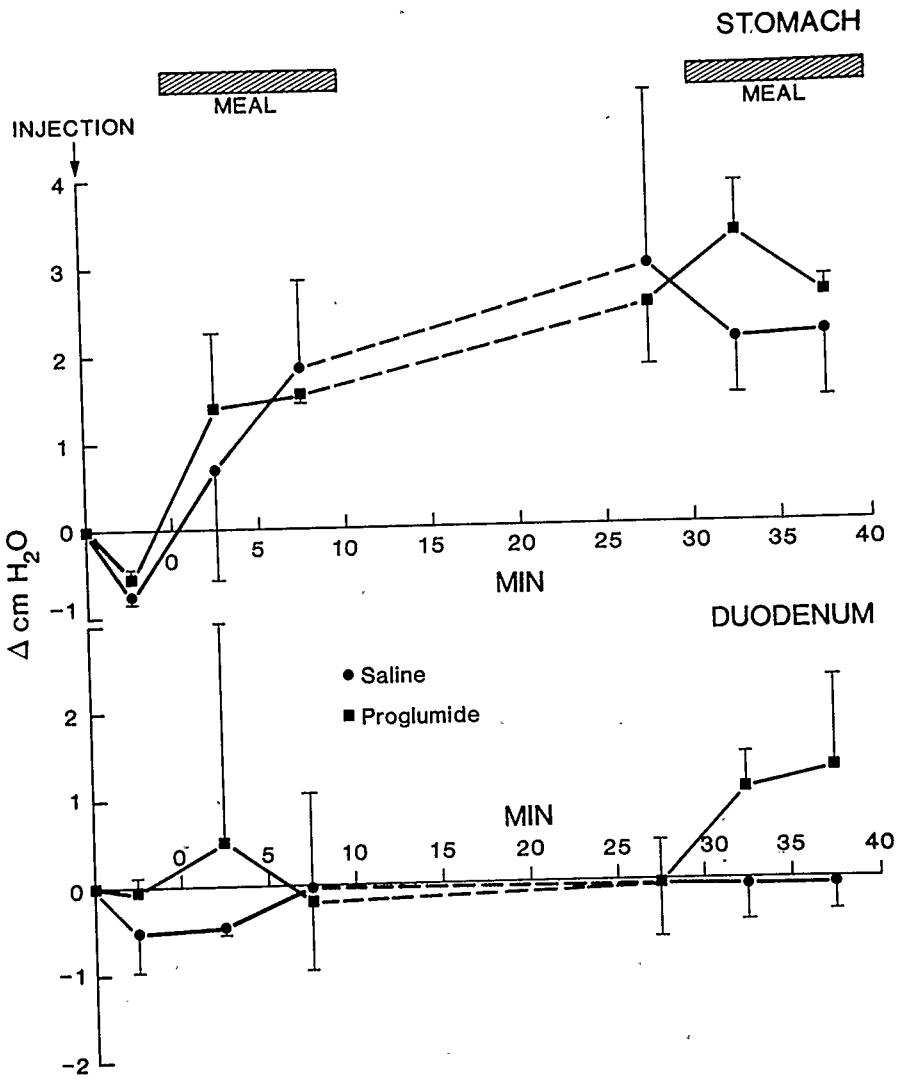

Fig.19. The changes in gastric and duodenal motility indices during feeding (Protocol B) in response to saline or proglumide injection. Points were calculated and placed as in Fig.18.

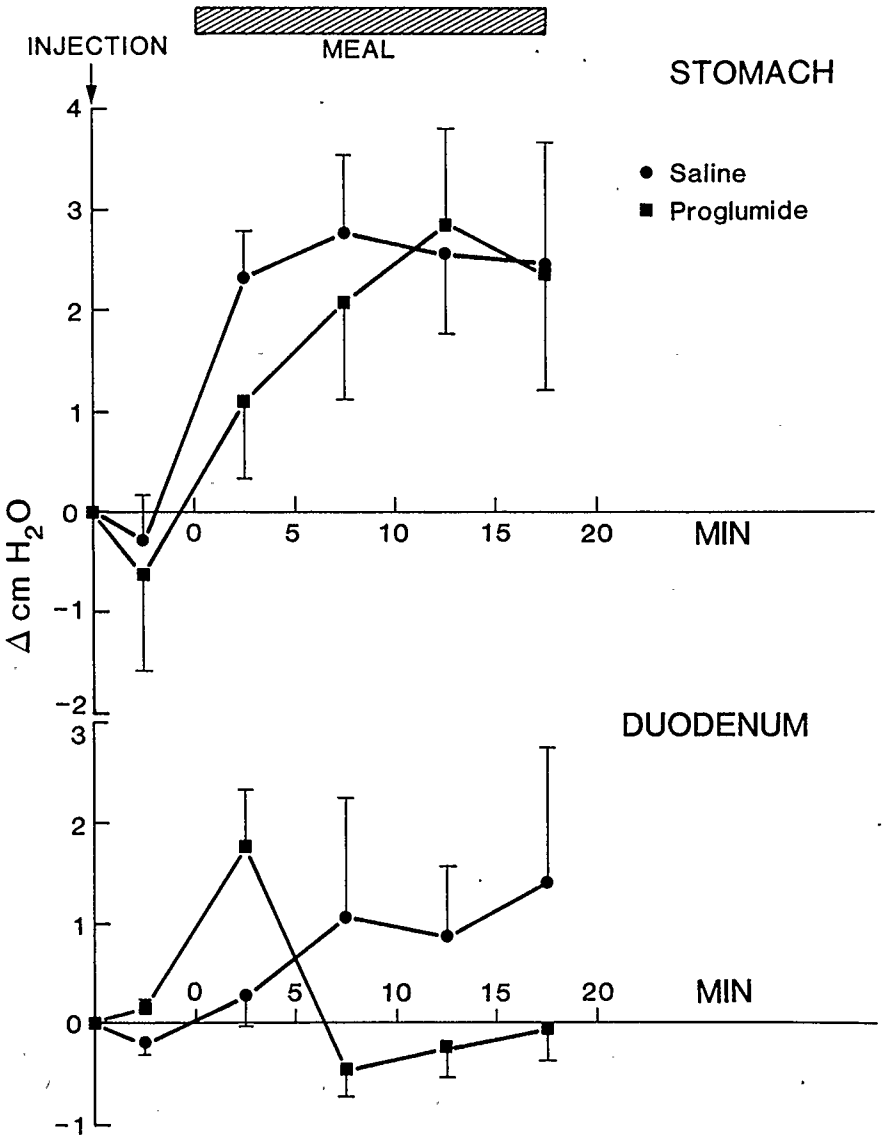

Fig.20. The changes in gastric and duodenal motility indices during feeding (Protocol C) in response to saline or proglumide injection. Points were calculated and placed as in Fig.18.

feeding by either protocol (protocol B, n=2, protocol C, n=3) tested (Figs.19,20).

5.1.4. Discussion

A proglumide-induced increase in gastric motility with a decrease in duodenal activity was predicted based on the data obtained in experiment 2 of chapter 3. In that experiment, exogenous CCK induced an increase in duodenal phasic activity and an inhibition of both gastric tone and phasic activity. Inhibition of endogenous CCK with proglumide might be expected to cause the reverse. CCK has been shown to induce gastric relaxation in auto-transplanted gastric pouches of the dog (251,252), thus indicating an hormonal mechanism. In the rat, a direct hormonally-induced relaxation has not been demonstrated and the gastric inhibition seen in chapter 3 may be of reflex origin only (1,6,116,242). Whichever mechanism of gastric relaxation operates in the rat, however, inhibition by proglumide would cause an increase in gastric activity.

In the present study, the opposite of the predicted effect was obtained: proglumide induced a decrease in gastric motility index when injected after a food preload. Endogenous CCK, therefore, induced an increase in gastric motility index.

A proglumide-induced increase in duodenal activity was not detected, thus suggesting that the observed decrease in gastric motility was not due to the activation of an enterogastric inhibitory reflex (242). In one study, high concentrations (0.1μmol/l) of CCK have been shown to evoke gastric muscle contraction *in vitro* (253,254), but this concentration is unlikely to be achieved physiologically in gastric muscle. Another study demonstrated that only rat pyloric, and not fundic or antral, muscle contracted in response to CCK *in vitro* (178). Antagonism of CCK-induced gastric contraction is thus unlikely to be the mechanism by which proglumide decreased gastric pressure.

How, then, did endogenous CCK induce an increase in gastric motility index? The vagally-mediated receptive relaxation known to occur (242) was only transiently apparent in some rats, and was rapidly masked by the increase in pressure as the rat continued to eat. This suggests that the volume of the meal contained within the stomach was the main determinant of the intragastric pressure recorded in this system. It is postulated, therefore, that the decrease in gastric motility index induced by proglumide, might be the result of the inhibition of an endogenous CCK-induced delay in gastric emptying. An increase in gastric emptying would result in a lower intragastric volume, and thus a lower motility index.

It was decided to discontinue these experiments (which accounts for the small n values), since insufficient information was supplied by this manometric technique. Monitoring intraluminal pressure does not give a direct measure of muscle tone since the pressure obtained is the net effect of tone and the volume contained within the lumen. It is therefore not possible to determine, from these recordings, what mechanism is responsible for the observed difference in pressure between saline and proglumide treatments. However, the data obtained suggest that gastric emptying might be increased by proglumide, possibly acting on the pylorus, indicating that endogenous CCK may delay gastric emptying by acting at the same site.

Gastric emptying of liquids has been shown to be dependent on the pressure gradient between stomach and duodenum, and on the pylorus. Relaxation of the body of the stomach increases the reservoir capacity of the stomach and lowers the pressure gradient (137,283). Contraction of the pylorus would slow gastric emptying. In the experiment described here (protocol A), an increase in pressure was observed when CCK was not inhibited. In the observed absence of a rise in duodenal pressure, this would increase the pressure gradient and faster gastric emptying would follow. But exogenous CCK has been observed to delay gastric emptying (64,177). The only way this could be

achieved under the conditions observed here, would be by contraction of the pylorus. CCK has been shown to cause pyloric contraction *in vitro* (24,98,178,253) and *in vivo* (227,315).

These speculations could be confirmed by additional experiments in which fundic, pyloric and duodenal tone was measured directly by means of tension transducers sutured to the serosal surface of the musculature, or by myoelectric recording. Alternatively, the postulate, that proglumide lowers intragastric pressure by enhancing gastric emptying, could be tested by measuring gastric emptying in the presence and absence of proglumide. It was decided to proceed by the latter route, initially, since the role of endogenous CCK in the regulation of gastric emptying has not yet been established. A study of gastric emptying, under the same conditions used in the satiety study, might thus contribute to the establishment of such a role, as well as suggesting an explanation of how CCK might reduce food intake.

5.2.EXPERIMENT 2.

Regulation of gastric emptying may be a physiological function of cholecystokinin. It may also be the mechanism whereby endogenous CCK achieves the reduction in food intake

observed in the experiments described in chapter 4. Infusion of 125ng/kg/hr in dogs induced a 50% inhibition of emptying (64). As has been discussed earlier, physiological plasma concentrations are probably considerably lower than those resulting from infusion of CCK. One study found that infusion of 7.5ng/kg/hr CCK produced a plasma concentration equivalent to that seen after a mixed meal (147), yet infusion of 90ng/kg/hr failed to delay gastric emptying in man (292).

As for CCK's putative role in satiety, the fact that plasma concentrations of CCK appear to be too low to delay gastric emptying, does not eliminate the possibility that CCK is involved in the regulation of gastric emptying. The same arguments apply. High concentrations of CCK may exist locally in the duodenum and act directly to stimulate duodenal muscle without entering the general circulation. This, however, does not seem likely, since duodenal activity was not affected by proglumide treatment in experiment 1. Secondly, it is possible that CCK could act neurally since CCK has been detected in enteric nerves (257). Thirdly, it is possible that low levels of CCK are potentiated by other prandial signals, neural or hormonal, in a similar manner to pancreatic secretion (176) or ileal contraction (61).

If a delay in gastric emptying was the mechanism by which CCK acted to reduce food intake, then such a delay should be detectable under the same conditions used to detect the reduction in food intake. The role of endogenous CCK in gastric emptying was therefore investigated using the same protocols described in chapter 4, with only minimal modifications for the measurement of gastric volume.

Unlike standard methods for measuring gastric emptying, the method used here allowed the rats to eat as normally as possible. The use of a liquid food under free feeding conditions introduced several complications. The first part of this study was therefore to validate the method.

Having established the method, the hypothesis was then tested. It was postulated that if CCK released during a normal meal slows gastric emptying, then inhibition of the actions of CCK with proglumide should induce an increase in gastric emptying.

5.2.1. Methods

5.2.1.1. General

Twelve (except in the metoclopramide experiment) male Sprague-Dawley rats (initial weights 350-450gm) were housed

and maintained as described in chapter 1. The animals were allowed food for 6 hours each day and for at least one week before any experiment were fed only liquid food to prevent the possibility of any solid particles of food remaining in the stomach during testing.

5.2.1.2. Gastric emptying

The rats were trained to drink liquid food (Sustagen) as before. Some variation in consistency of the food was observed. To avoid possible errors that this might cause, the same batch of food was used for both control and test days of an experiment. The results of different experiments may not be directly comparable. The food was labelled with the non-absorbable marker 14C-polyethylene glycol (14C-PEG) to a concentration of approximately 4μCi/150ml (C1). Following a meal, rats were lightly anaesthetized with ether (2min were allowed for this) and an intraoral tube (infant feeding tube, 8 Fr., Medicraft, with extra holes cut) was passed into the stomach and the stomach contents were withdrawn using a 10ml syringe (V1). The residual volume, after withdrawal was estimated by infusing the stomach with 2ml water, mixing and withdrawing (V2). The concentration of 14C-PEG in V1 (C2) and V2 (C3) was measured, after mixing thoroughly, by pipetting 100ul aliquots into 5ml

scintillation fluid (Readisol, Fisher,) and counting in a β-counter after allowing the scintillant to stabilize overnight.

5.2.1.3. Calculation

The volume of liquid food remaining in the stomach was calculated by the method of George(97):

$$\text{Volume remaining} = \frac{V1.C2}{C1} + \frac{V2.C3}{C2-C3}$$

Where V1=Volume withdrawn C1=initial concentration
V2=Washout volume(2ml) C2=concentration of V1
C3=concentration in final sample

5.2.2. Validation of the method.

The use of a milk-type food and a free feeding experimental design in conscious rats introduced several complications which were first examined in detail.

5.2.2.1. The effect of acid on the liquid food.

Since the milk-like food would be exposed to acid conditions in the stomach, the effect of acid on the food was tested in

vitro. Acid was found to clot the liquid food. The labelled PEG was found to distribute nearly equally between phases (51% in the liquid phase, 46% in the semi-solid phase, n=5). An initial drop in counts per minute was observed when 0.1N HCl was added to the milk, but no further decrease was seen as acid concentration was increased.Mean recovery of 14C was 97% (n=10) over a range of acid concentration 0.004N - 0.2N HCl.

5.2.2.2. Estimation of recovery by the intubation method

Semi-solid food is more difficult to sample by orogastric tube than is liquid food. Phase separation might lead to an error in determining the volume of the meal remaining in the stomach. The volume of the test meal recovered by the intubation method was, therefore, compared to the volume recovered by a terminal experiment. On the first day, the rats were fed a 10ml liquid food meal, and the stomach contents were removed by orogastric tube under ether anaesthesia at 30min. On the second day, the same procedure was followed except that the rat was decapitated while under ether, and the stomach was rapidly clamped and removed. The stomach was opened and the contents were thoroughly flushed out. The percent recovery by the intubation method was 77% of that by the terminal method.

The volume measured in the terminal method may have been an overestimate as it contained material, such as mucus and cell debris, not seen in aspirated samples. The recovery by intubation would thus be underestimated. Nevertheless, it was recognized that the intubation method led to a loss of the solid phase, and measurement of changes in gastric emptying reflected changes occurring mainly in the liquid phase. However, the error in estimation was consistent since the correlation between the intubation and terminal methods was $r=0.89$ $(n=15)$. Furthermore, acid secretion, and hence phase separation, would only be potentially altered in the oral proglumide experiment (see below). Therefore, the intubation method provided a satisfactory estimate of gastric contents.

5.2.2.3. The effect of ether on gastric emptying.

Intubation of the rats was performed under ether to reduce the stress caused by passing the large calibre tube. Ether is known to alter gut motility (313). Phasic activity in the gastrointestinal tract has been shown to be increased by ether anaesthesia, but this activity was uncoordinated and probably non-propulsive (313). In one study, ether was observed to delay transit in the rat (239) while in another no effect was seen (219). Faster transit due to ether has

not been reported. The effect of ether on gastric emptying under the conditions of these experiments was investigated. After a 10ml preload, animals were anaesthetized at 18min and intubated at 20min, or were intubated while conscious at 20min. The anaesthetized animals had 5.0±0.3ml remaining in the stomach at 20min while in conscious rats 6.9±0.5ml remained ($n=11$, $p<.005$). Gastric emptying in the anaesthetized rat was thus faster than in the conscious animal which may have been an effect of the ether. On the other hand, the stress of intubation in the conscious rat may have delayed gastric emptying (263,289). The use of ether in these experiments reduced the stress to the rats and since both test and control experiments were performed under the same conditions, valid comparisons could be made.

5.2.2.4. Determination of the sensitivity of the method.

To determine the ability of the intubation method to demonstrate an increase in gastric emptying, the response to 5mg/kg metoclopramide (Reglan, A.H.Robins Canada Inc.), a drug known to accelerate gastric emptying in the rat was tested (142,238). Eighteen rats were injected i.p. with 1ml saline or metoclopramide at 20 min immediately after 2 consecutive 10ml meals and sampled at 50min. Metoclopramide reaches peak plasma concentration at 30min after oral

administration (230). The rats refused to eat the liquid food if the drug was mixed with it, so that i.p. administration was necessary. The time for absorption of the drug injected i.p. is not known. If the drug was injected 30min before the preload, to allow an adequate time for absorption, the rats refused to eat the second meal. Therefore, 30 min was allowed after the second meal before sampling to permit maximal plasma levels to be attained. Metoclopramide significantly increased gastric emptying of the 2 consecutive 10ml meals from 9.4$\pm$0.3ml remaining after saline injection to 8.3$\pm$0.4ml remaining after metoclopramide ($n=16$, $p<.025$). Therefore the intubation method used to test the hypothesis was sufficiently sensitive to detect a small change in gastric emptying of the combined preload and test meal.

To summarize, these preliminary studies indicated that phase separation would not prevent the measurement of emptying with accuracy. The method was sufficiently sensitive to detect the expected changes in emptying rate induced by metoclopramide. Since changes in acid secretion following proglumide administration would be the only potential factor in the experiments which might influence phase separation, an experiment to control for such a possibility was included.

5.2.3. General experimental procedure.

Although the methods and protocols have been described in chapter 3, repetition here, including the minor modifications made for measurement of gastric emptying instead of food intake, will make these experiments easier for the reader to follow.

After an 18hr fast, rats were fed a 10ml meal at time zero. This meal constituted a food preload to stimulate CCK secretion. Failure to finish the meal within 10min eliminated the rat from the study. In experiments which included a second 10 ml meal, the same criterion was applied. Proglumide in 1ml saline (150mg/kg) or 1ml saline as control was injected i.p.. An experiment was performed on 2 consecutive days. To eliminate day to day variability, half the rats received proglumide on the first day while the other half received saline. The treatments were reversed on the second day. At least two days were allowed between experiments and no rat received proglumide on consecutive days. Results were analysed by Student's t-test for paired data with each rat acting as its own control in all procedures. All results are reported as the volume remaining in the stomach at sampling time.

5.2.4. Experiments

The protocols described below are summarized in Fig.21

5.2.4.1. The effect of proglumide when injected after a food preload.

It was hypothesized that a food preload was required to stimulate CCK secretion in order to observe a proglumide-induced effect on gastric emptying. To test the combined effect of a proglumide and a food preload, rats were injected at 25 min. A second 10ml meal was given at 30min which was completed by 40min. At 43min the rats were anaesthetized and gastric contents were sampled at 45min. (Protocol A).

5.2.4.2. The effect of proglumide injected before feeding.

To determine whether proglumide could affect gastric emptying if injected when levels of CCK were basal, rats were injected 5min before the preload (-5min) and then tested as in protocol A (Protocol B). To test the effect of proglumide plus a preload when insufficient time was allowed for peak CCK secretion to occur, the emptying of 2 consecutive 10 ml meals was examined by injecting saline or

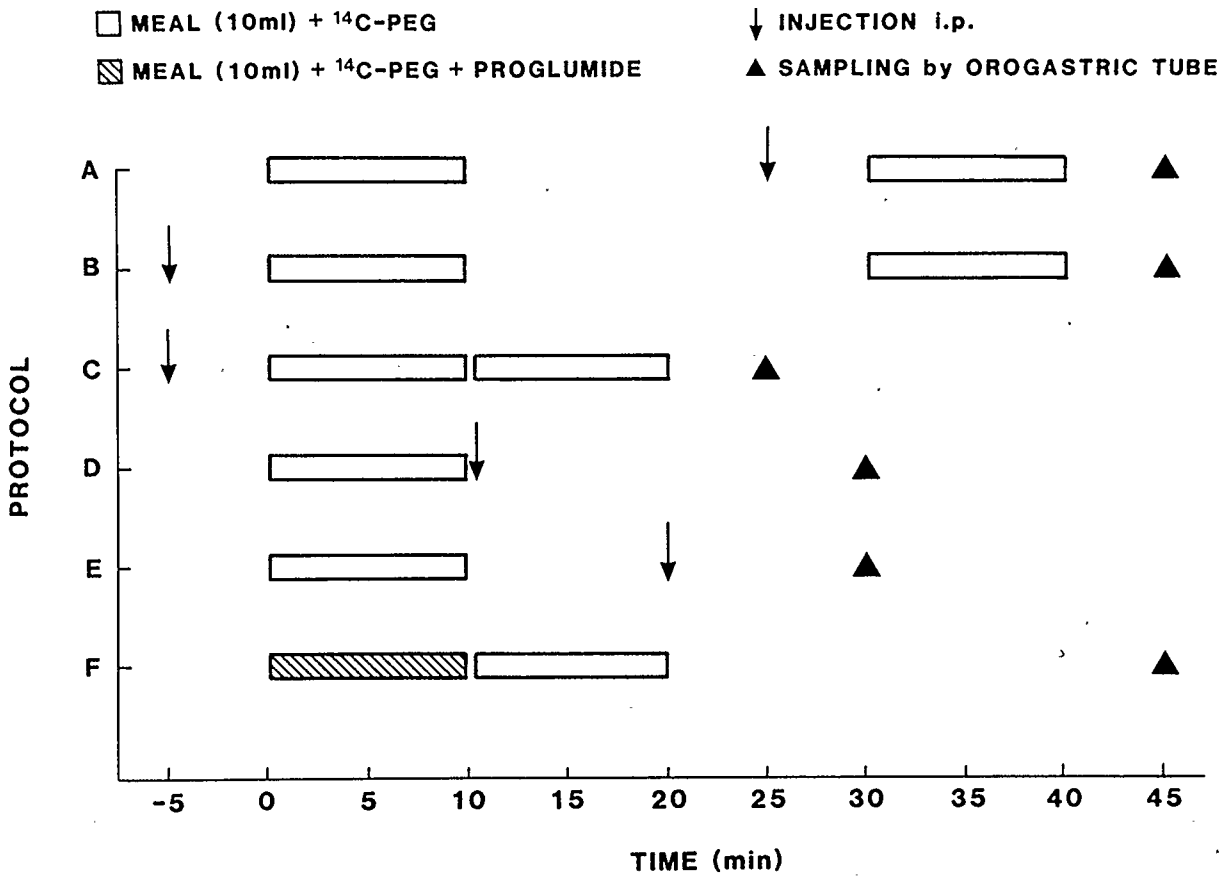

Fig.21. Summary of protocols used to study the effect of proglumide on gastric emptying. A description of protocols A - F is given in the text.

proglumide 5min before the start of the preload and sampling at 25min (Protocol C).

5.2.4.3. The effect of proglumide on the emptying of a single meal.

To determine whether proglumide had an effect on gastric emptying of the preload alone, the rats were injected at 10min (protocol D) or 20min (protocol E) after the start of a 10ml meal. At 28min rats were anaesthetized and at 30min gastric contents were withdrawn.

5.2.4.4. The effect of oral proglumide on gastric emptying.

Proglumide is an antagonist of CCK-related peptides and therefore inhibits the actions of gastrin. Proglumide inhibits gastrin-stimulated gastric acid secretion when given orally (243). The effect of oral proglumide on gastric emptying of liquid food was tested. The proglumide (150mg/kg) was mixed with the preload and given as usual at time zero. A second meal was given at 30min and gastric contents sampled at 45min (Protocol F).

5.2.4.5. The effect of proglumide on gastric acid secretion

Since proglumide inhibits the actions of gastrin, the possibility that proglumide might affect gastric emptying by blocking gastrin-stimulated gastric acid secretion was investigated in a preliminary study on anaesthetized rats (sodium pentobarbital, 65mg/kg). Bilateral cervical vagotomies were performed on the rats to stabilize acid secretion. A method of continuous automatic acid titration based on that of Ghosh and Schild was used (99). Pentagastrin (Peptavlon, Ayerst Laboratories) was infused i.v. to achieve a steady sub-maximal acid output (dose range 2-16 µg/kg/h) and the effect of i.p., i.v. and oral proglumide (bolus 150mg/kg) was tested. Acid was measured in 5min periods and the mean of several periods before administration of proglumide was compared using Student's paired t-test to the mean of the periods after administration of proglumide.

5.2.5.Results

5.2.5.1.The effect of proglumide injected after a food preload (protocol A).

When proglumide was injected at 25min between the preload

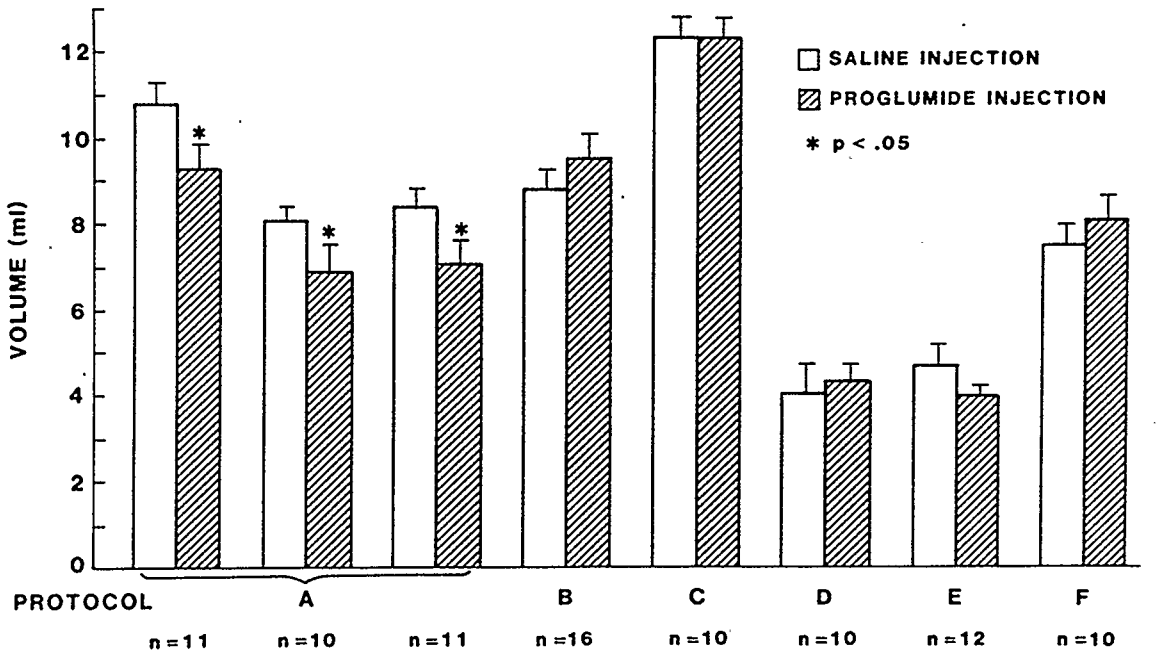

Fig.22. The volume of liquid food remaining in the stomach after saline or proglumide (150mg/kg) administration under several conditions. Protocols A - F are described in detail in the text and summarized in Fig. 21.

and second meal, a significant increase in gastric emptying was observed (8.1±0.3ml remaining after saline compared to 6.9±0.6ml after proglumide, n=10, $p<.05$). This experiment was repeated twice with the same result in each case : 10.8±0.5ml control vs. 9.3±0.5ml after proglumide, n=11, $p<.05$; 8.4±0.4ml after saline vs. 7.1±0.5ml after proglumide, n=11, $p<.05$). The mean of these 3 trials (only 2 trials for 4 rats) calculated for each rat individually, was 9.1±0.3ml saline compared to 7.7±0.3ml after proglumide (n=12, $p<.005$).

5.2.5.2.The effect of proglumide injected before feeding (protocols B and C).

Proglumide had no effect on the emptying of preload plus a second meal fed at 30 min (8.8±0.4ml after saline compared to 9.5±0.5ml after proglumide, n=12). Proglumide, injected 5min before the preload, also had no effect on the emptying of 2 consecutive 10ml meals (12.3±0.4ml control compared to 12.3±0.4ml after proglumide, n=10).

5.2.5.3.The effect of proglumide on the emptying of a single meal (protocols D and E).

Proglumide injected at either 10 or 20min had no effect on

emptying of the meal. The volumes remaining in the stomach were 4.1±0.7ml after saline and 4.4±0.4ml after proglumide injected at 10min (n=10), and 4.8±0.5ml after saline and 4.1±0.2ml after proglumide injected at 20min (n=12).

5.2.5.4.The effect of oral proglumide on gastric emptying (protocol F).

Proglumide dissolved in the preload had no effect on the emptying of 2 consecutive 10ml meals. The volume remaining following saline injection was 7.5±0.5ml and after proglumide was 8.1±0.6ml, n=10.

5.2.5.5.The effect of proglumide on gastric acid secretion

No decrease in acid output was seen following i.p. proglumide (13.5±2.5 μmoles acid before vs. 13.2±3.3 μmoles after, n=6) or i.v. proglumide (10.3±1.3 μmoles before vs. 10.9±2.1 μmoles after, n=4). After oral administration of the antagonist a 49% decrease in acid output was seen (14.4±3.3 μmoles before vs. 7.3±1.0 μmoles after, n=5, $p<.05$). A further decrease (60%) was seen following an oral dose of 300mg/kg proglumide (12.6±3.8 μmoles before vs. 5.1±1.4 μmoles after, n=4, $p<.05$).

5.3.DISCUSSION

The effect of proglumide on gastric emptying under several different conditions has been studied. Proglumide increased gastric emptying of the preload plus a second meal when injected at 25min after a food preload at a time when circulating CCK levels were expected to be high. Proglumide did not change the emptying of combined meals when injection preceded the first meal, nor did it affect the emptying of a single meal. This suggests that the antagonist acted on a factor released by the food preload to affect the emptying of the subsequent meal. This factor could be gastrin or CCK since proglumide is a specific antagonist of the CCK/gastrin family of peptides. Gastrin could delay emptying directly by modifying antral motility or indirectly by stimulating acid secretion (300). Acid is a powerful stimulant of secretin release, and secretin, in turn, is a potent inhibitor of gastric emptying (300). The failure of i.p. proglumide to change acid output suggests that gastrin-stimulated acid secretion was not involved in the proglumide-induced effect. Also oral proglumide, which inhibited gastric acid secretion, did not affect gastric emptying. The involvement of gastrin-stimulated motility was not eliminated; however, an earlier study found gastrin to have little effect on gastric emptying in the rat (287).

The factor released by the first meal was, therefore, probably CCK.

The time of the effective dose of proglumide (25min) coincided with peak CCK concentrations following a meal (18,33, 34,141). Injection before the preload was at a time when no stimulus to secrete CCK had occurred. Injection at 10 min after a single meal was perhaps before adequate concentrations of CCK had been achieved to affect gastric emptying. Alternatively, since the emptying rate is dependent on volume (137), any change in the rate of emptying of the small volume of a single meal remaining would be more difficult to detect. On the other hand, injection at 20min after the preload would be expected to coincide with rising levels of CCK secretion. A small but insignificant increase in emptying was seen at this time. An explanation of this may be that CCK may interact with gastric distension to induce its effect on emptying and, in this case, distension would be at a low level compared to that after the rat was fed a second meal.

The ineffectiveness of proglumide when injected before the first meal compared to injection at 25min suggests firstly that proglumide is only effective when sufficient CCK has been released to cause its actions and, secondly, that the injection coincided with the activity of other

factors, such as distension, stimulated by the preload. Plasma proglumide levels remain elevated for at least 2hr after oral administration (190). Injection before the first meal might thus be expected to affect the emptying of the second meal at 30min. Rapid absorption of proglumide from the peritoneal cavity into the circulation would act to dilute the antagonist while a high concentration of proglumide is required to inhibit CCK (62). A concentration of proglumide sufficient to competitively antagonize CCK may only be present immediately after i.p. injection. The effectiveness of proglumide in blocking gastrin-stimulated acid secretion when given orally compared to its ineffectiveness when given i.p. or i.v. supports the hypothesis that the action of proglumide is topical rather than systemic. It is possible that an increase in gastric emptying, induced directly or indirectly by a reduction in acid output, may have been partly masked by the improved recovery of the meal due to this reduction in acid. But it is more likely that the ineffectiveness of oral proglumide in changing gastric emptying was due to the application of the antagonist at an inappropriate target site.

Since the CCK inhibited by proglumide was released by normal feeding in the intact rat, a physiological role for CCK in the regulation of gastric emptying is implied. This result is consistent with studies in which endogenous CCK

was released by phenylalanine or tryptophan administration (64,177). The mechanism by which CCK acts to delay gastric emptying cannot be determined by this experimental protocol. The involvement of neuronal CCK seems unlikely since administration of CCK during the emptying of a single meal was without effect and a rapid response would be expected. Both hormonal and paracrine mechanisms are compatible with this data. Local accumulation of CCK, acting via a paracrine mechanism may coincide with peak plasma levels.

In conclusion, it has been demonstrated that proglumide, a specific competitive antagonist of CCK-like peptides, when injected after a food preload, increases gastric emptying of the following meal probably by the inhibition of CCK. These data support the hypothesis that CCK plays a physiological role in the regulation of gastric emptying and since proglumide induced both an increase in gastric emptying and food intake under the same experimental conditions, these functions may be related.

Chapter 6.

SUMMARY AND GENERAL DISCUSSION

6.1. SUMMARY

The actions of exogenous CCK and of endogenous CCK, that is released when food enters the duodenum under normal physiological conditions, have been investigated in the rat, to determine whether CCK has a physiological role in the regulation of short-term food intake.

The administration (i.p.) of exogenous CCK significantly reduced food intake on the first occasion, but retesting, using the minimum effective dose, in the same rats, produced inconsistent results and erratic behaviour, suggesting an aversive reaction to the CCK. The gastrointestinal motility effects of "satiating" doses of CCK were investigated using manometric methods in the anaesthetized rat. Profound gastric inhibition and duodenal excitation were observed following i.p. or i.v. administration of the peptide. This pattern of motility bore no resemblance to established fed motor patterns or to those seen here in the conscious feeding rat. It was concluded that exogenous CCK probably reduced food intake at pharmacological doses, by inducing abnormal gut motility, which may have been experienced, by the rats, in the feeding

study, as malaise.

The role of endogenous CCK in the regulation of food intake was investigated using the specific CCK-antagonist, proglumide. It was postulated that if endogenous CCK acted to reduce food intake, then inhibition of the action of CCK should induce an increase in food intake. Proglumide was found to increase food intake only in satiated animals when injection was preceded by a food preload and at a time when CCK secretion is known to reach its peak following a meal. It was concluded that proglumide must act on a factor released by the preload, and since proglumide is a specific antagonist of CCK and related peptides, this factor was possibly CCK. Endogenous CCK may, therefore, play a role in the regulation of short-term food intake.

To investigate whether the putative satiety effect of endogenous CCK is mediated by the vagus nerve, the experiment, using proglumide, was repeated in vagotomized and sham-operated rats. The results obtained in the sham-operated rats confirmed the earlier data obtained in normal rats. However, proglumide failed to increase food intake in the vagotomized rats, which suggested that the integrity of the vagus nerve was essential for the putative satiety effect of endogenous CCK. But these animals were found to have abnormally rapid gastric emptying, which, in

man, has been associated with symptoms of dumping. Therefore, no conclusion could be made as to the involvement of the vagus nerve in the proglumide-induced increase in meal size, since symptoms of dumping could, as well as vagotomy, prevent the rats from increasing their food intake. Nevertheless, a role for the vagus nerve in mediating the proglumide-induced increase in food intake, was not eliminated.

The regulation of short-term food intake has been associated with gastric distension. Exogenous CCK has been shown to delay gastric emptying which would effectively increase distension. CCK may delay gastric emptying by evoking relaxation of the body of the stomach. Therefore, it was proposed that if endogenous CCK reduced food intake by evoking gastric relaxation, which would act to delay gastric emptying, then inhibition of the action of CCK with proglumide should induce an increase in intragastric pressure and an acceleration of gastric emptying. Using manometric methods in the conscious feeding rat, a decrease in intragastric pressure during a second meal was observed, only when proglumide was injected after a food preload. This result was the opposite of the expected change in pressure, and was interpreted as indicating a proglumide-induced increase in gastric emptying but achieved by a mechanism other than gastric relaxation. This result

suggested that endogenous CCK might act to delay gastric emptying.

Gastric emptying was determined using the same experimental design used to test the effect of endogenous CCK on feeding and motility, but with the addition of a label, 14C-PEG, to the liquid food. Once again, proglumide induced an increase in gastric emptying only when injected after a food preload, at a time when peak CCK secretion is known to occur. It was concluded that proglumide must act on a factor released by the food preload, and since proglumide is a specific antagonist of CCK, this factor was probably CCK. Endogenous CCK may, therefore, play a physiological role in the regulation of gastric emptying.

Since an increase in food intake, an increase in gastric emptying and a decrease in intragastric pressure all occurred under the same conditions and at the same time relative to the preload and proglumide injection, these effects may be related.

6.2.DISCUSSION.

In the investigation of endogenous CCK, it has been concluded that proglumide acted on a factor, released by a food preload, to achieve an increase in food intake and

gastric emptying, and to cause a decrease in intragastric pressure. Proglumide is known to inhibit the actions of CCK and gastrin (62,117, 243), both of which are released by food in the gut (300). The factor could thus be CCK or gastrin.

Gastrin could delay gastric emptying directly, and thus reduce food intake, by modifying antral motility or indirectly, by the stimulation of acid secretion. Oral administration of proglumide has been shown to increase the antral motor response to food (29). Inhibition of gastrin-stimulated acid secretion might also inhibit the release of secretin, a gastrointestinal hormone thought to delay gastric emptying (300). The data presented, in this study, however, support the results of a previous study which demonstrated that gastrin had no effect on gastric emptying in the rat (287). Oral proglumide, which inhibited gastrin-stimulated gastric acid secretion, failed to accelerate gastric emptying of the liquid food. In addition, i.p. injection of proglumide, instead of oral administration, failed to inhibit the gastrin-stimulated acid output, thus suggesting that inhibition of gastric acid secretion and an increase in antral activity were not factors in the observed proglumide-induced increase in gastric emptying. An increase in antral activity might have made a significant contribution to an increase in gastric

emptying if solid food had been used, since the antrum is important in the emptying of solids, but not of liquids (307). In addition, gastrin has been shown to have no effect on food intake (171). On the other hand, i.p. injection of proglumide caused immediate inhibition of duodenal motor activity, induced by i.p. or i.v. administration of large doses of CCK. While possible motility effects of gastrin have not been eliminated, it seems probable that the factor, released by the food preload, was CCK. Therefore, endogenous CCK may have a physiological role in the regulation of food intake and gastric emptying.

The proglumide-induced increases in food intake and gastric emptying were observed under the same conditions and only when injection of proglumide was preceded by a food preload. Therefore, proglumide seems to act on both mechanisms simultaneously. The proglumide-evoked decrease in intragastric pressure was also observed under identical conditions, thus suggesting that the 3 events might be interrelated. There is some evidence that gastric distension plays a role in the satiety effect of exogenous CCK and since gastric emptying affects distension, the proglumide-evoked increase in food intake might be the consequence of the proglumide-induced acceleration of gastric emptying.

Sham-feeding experiments, in which pregastric stimuli were present, but gastric distension was absent, have shown a decreased efficiency of exogenous CCK in evoking a reduction in food intake (104). Furthermore, distension of the stomach with a saline preload, combined with a subthreshold dose of CCK, caused a significant decrease in food intake, although neither stimulus alone was effective (203). These data imply that the CCK satiety signal is potentiated by gastric distension. A recent study on rat pups, in which liquid food is the normal diet, demonstrated a high degree of correlation between gastric volume (and thus distension), gastric emptying and the suppression of ingestion (170). In a study that bears some resemblance to the present work, Mueller and Hsiao found that intake of a liquid food test meal was maximally suppressed by a preload of the same food at 20min after the preload, but by 30min, after the preload, intake had increased (210). It can be argued that the satiety effect of CCK may be dependent, in part, on gastric distension and since distension was reduced at 30min compared to that at 20min, due to greater gastric emptying, the satiating action of CCK was less effective. Synergism between the satiety effects of endogenous CCK and gastric distension might thus explain the discrepancy between the apparent efficacy of very low postprandial plasma concentrations of CCK and the high doses of exogenous

CCK required to induce satiety. In most studies, exogenous CCK has been injected before feeding, at a time when gastric distension is absent.

Distension signals are carried to the brain by the vagus nerve and could thus be the "satiety signals" involved in the reduction of food intake by endogenous CCK. Crawley and Kiss have traced the sensory pathway for satiety signals from the gut to the brain and found that the pathway apparently terminates in the paraventricular nucleus (PVN) of the hypothalamus (51). Lesion of this nucleus blocked exogenous CCK-induced satiety (148). Injection of CCK into the PVN reduced food intake in rats (88), while electrical stimulation of the PVN inhibited gastric motility (248). It is possible, then, that both food intake and gastric motility are modulated by signals from the same hypothalamic nucleus.

The distension signals arise from "in series" tension receptors within the gastric muscle. CCK could affect these vagal signals directly by binding to the CCK-receptors contained within the fibres (319) or indirectly by increasing the tone of the stomach wall (45). The tone could also be increased by CCK in two ways: directly, by stimulation of the muscle, or indirectly by an increase in intragastic volume caused by a delay in gastric emptying.

Thus these vagal mechanoreceptors could provide the common pathway for the combined satiety effects of gastric distension and circulating CCK. Preliminary studies in this laboratory have shown an increase in single vagal afferent fibre firing in response to gastric mechanoreceptor stimulation by systemic injection of CCK even though gastric relaxation occurred (58). This suggests that CCK can act directly on gastric vagal mechanoreceptors. The vagal response was achieved with very high doses of CCK (2.3-4.6μg/kg or 2-4nmol/kg) that probably resulted in plasma levels greatly in excess of physiological levels. However, this route of action remains a possibility for endogenous CCK's action in the present study.

A direct increase in rat antral muscle tone has been observed in vitro with high concentrations of CCK (0.1μmol/l) (253,254) but not in fundic muscle (178). In fact, the opposite effect is thought to occur: that is a decrease in gastric tone in the intact animal. In the dog, CCK has been shown to directly decrease the intragastric pressure of the intact stomach (291,293) and of an auto-transplanted gastric pouch (251,252) but similar inhibition by an hormonal route has not been demonstrated in the rat. A direct hormonal inhibition of gastric motor activity cannot be eliminated here in the intact animal, but if present, it was completely masked by the increase in

intragastric pressure, probably due to an increase in volume. Gastric inhibition, however, was not observed following large doses of exogenous CCK (i.p.) in vagotomized rats, in the acute motility experiments of this study. This suggests that gastric relaxation, in the rat, may be of reflex origin only and is consistent with the lack of CCK receptors in the body of the stomach (271).

Vagally-mediated, reflex inhibition of gastric tone by duodenal activation is known to exist (6,116,242) and was observed here (although not proved) in the experiments using exogenous CCK (Chapter 3). A direct increase in gastric tone by CCK, therefore, seems unlikely. It seems probable that the observed proglumide-induced decrease in gastric pressure was the result of the inhibition of a CCK-induced increase in gastric pressure caused indirectly by a delay in gastric emptying. In support of this hypothesis, proglumide was shown to accelerate gastric emptying. This data also provided evidence to support a role for endogenous CCK in the regulation of gastric emptying. But, the mechanism by which proglumide induced the acceleration of gastric emptying has not been determined.

Gastric emptying of liquids can be delayed by relaxation of the body of the stomach or by contraction of the pylorus (315). In this study, no gastric relaxation was

detected. In addition, gastric emptying was observed to be slower when, under the same conditions, intragastric pressure was found to be higher. Proglumide reduced intragastric pressure and accelerated gastric emptying. The only explanation for this apparent reversal of the accepted mechanics of gastric emptying (137,283) is that proglumide evoked relaxation of the pyloric sphincter, thus accelerating gastric emptying which reduced gastric volume and therefore, intragastric pressure. If so, it would suggest that endogenous CCK might delay gastric emptying by causing pyloric contraction. Further studies, in which pyloric motility is measured, are needed to confirm this point.

The pylorus has been shown to contract in response to infusion of CCK at doses 4-fold lower than the D50 for pancreatic secretion in the dog (293,315). While this demonstrates a high degree of sensitivity to CCK, this dose (40ng/kg/h or 35pmol/kg/h) may still produce circulating levels of the peptide higher than those seen after a meal (19,141). However, a high concentration of CCK, second only to that of the distal duodenum, has been reported to be present in the pylorus of the pig (12) and a high concentration of CCK receptors has been found in the pylorus of rats (271). These observations suggest that, if a similar high concentration of CCK exists in the pylorus of

the rat, as in the pig, levels of CCK might be much higher locally within the pylorus, than is reflected by plasma levels. CCK may thus act by a neurocrine and/or paracrine mechanism in this tissue and may act directly on the muscle or by stimulating the intrinsic nerves. The basal pressure of the rat pylorus increased in response to CCK by a neural, non-cholinergic mechanism, whereas, phasic activity was increased by direct action on the muscle, in a whole stomach-duodenal preparation *in vitro* (253,254). Rat pyloric muscle strips contracted in a dose dependent manner to CCK *in vitro* (24). A pyloric site of action for CCK in the regulation of gastric emptying, and thus, maybe, of food intake also, is therefore feasible.

Proglumide has been a useful tool in the investigation of endogenous CCK in this work. Evidence collected throughout this study suggests that it acts topically and not systemically. Firstly, in the acute motility studies in the anaesthetized rat, injection of proglumide i.p. caused immediate, almost total inhibition of the duodenal motility induced by i.p. or i.v. administration of CCK. A release of the gastric inhibition was sometimes seen. The effect was much too fast for appreciable absorption of the proglumide to have taken place. The inhibition lasted about 20min. Similarly, if proglumide was injected immediately before i.p. or i.v. administration of CCK, inhibition of

duodenal motility was nearly total, but if CCK was given 15min after proglumide, inhibition was greatly attenuated and by 20min there was almost no inhibition. This suggests either that proglumide is rapidly degraded or that it is rapidly absorbed from the peritoneal cavity into the circulation, which acts to dilute it. A very high concentration of the antagonist has been shown to be necessary to inhibit CCK (62). A concentration of proglumide sufficient to competitively antagonize CCK may only be present immediately after i.p. injection.

The concept of a topical mode of action for proglumide was further supported by the data obtained in the experiments investigating the role of endogenous CCK. In each case, proglumide only induced a significant effect when injected i.p. at the time known to coincide with peak CCK secretion following a food preload. Proglumide failed to induce an increase in food intake or gastric emptying when injected before the preload, even when the second meal was delayed to allow time for peak CCK secretion to occur, as in protocol B (chapter 5). Plasma proglumide levels are known to remain elevated for at least 2h following oral administration (190) and would thus be expected to be elevated at the optimal time of peak CCK secretion and peak gastric distension caused by the second meal in protocol B, but there was a small decrease in emptying if anything

(volume remaining in the stomach 8.8+0.4ml after saline v. 9.5+0.5ml after proglumide).

Oral proglumide did not increase gastric emptying. Conversely, in the acute experiments on pentagastrin-stimulated gastric acid secretion, oral proglumide caused a significant decrease in acid output, whereas i.p. or i.v. administration did not inhibit acid secretion. These data, taken together, indicate that the route of administration of proglumide relative to its target site of action is of critical importance. In other words, to effectively antagonize gastrin-stimulated acid secretion, proglumide must be delivered directly to the gastrin receptor in the stomach, and for the inhibition of CCK-induced motility, proglumide must be delivered intraperitoneally. In both cases, it is obvious that proglumide was not acting systemically.

The discrepancies observed between the gastrointestinal motility pattern, elicited by injection of CCK and those seen on feeding, emphasize the problems associated with experiments which involve the administration of biologically active peptides. Data obtained by this method should only be used as a rough indicator of possible physiological action. In this case, if the reslts of the present study have been interpreted correctly, the evidence suggesting a

satiety effect for exogenous CCK, did, in fact, lead to a possible physiological role for CCK in the regulation of food intake. It would appear, however, that this role is of far less significance than suggested by the magnitude of the exogenously-induced effect. Proglumide induced a 24% increase in meal size in normal rats and a 19.7% increase in sham-operated rats, which suggests that, if CCK is the satiety signal inhibited by proglumide, then it is only a minor one and may be one of several such signals as in the regulation of pancreatic secretion. In this study, no attempt was made to demonstrate the effect of endogenous CCK in isolation in contrast to a study by Collins and coworkers (42). Indeed, it was shown to have little effect when other stimuli, such as gastric distension, were absent (44). All other possible factors affecting food intake and gastric emptying were operative. Gastric distension may be the major signal for the termination of a meal. In the dog, gastric distension has been shown to be sufficient to reduce sham-feeding (222). Furthermore, CCK infusion, in conjunction with gastric distension, did not augment the reduction in sham-feeding. A species difference may explain the ineffectiveness of CCK in this study, since introduodenal infusion of fat was also without effect on sham-feeding, but inhibited sham-feeding in the rat (10,102,104).

If endogenous CCK acts to regulate food intake by delaying gastric emptying, the question must be asked as to whether the observed change in emptying can fully account for the observed change in food intake, measured under the same experimental conditions. Proglumide induced a 12.8% increase in gastric emptying (1.4ml) and a 24% increase in food intake (2.1ml). It is possible that CCK may act by more than one mechanism. CCK may directly stimulate gastric mechanoreceptors, independently of gastric distension, as has been discussed earlier. Also, while central administration of CCK in the rat has produced inconsistent results (88,169,175,255,309), and despite the fact that CCK has not been shown to cross the blood-brain-barrier, it is possible that very small amounts of circulating CCK could modulate activity in a part of the brain that has no blood-brain-barrier. The area postrema, which overlies the NTS, is such a region. The NTS has previously been associated with CCK's regulation of food intake (52,221) and gastric motility (96,232). It receives input from the vagus nerve, and projects to the PVN, which has also been associated with CCK and feeding (128). Optimal conditions, as have been used in the present study, would be essential to demonstrate a possible central effect of peripheral, endogenous CCK. The lack of a conclusive result in the vagotomy experiment, does not permit the elimination of a

central site of action for peripheral, endogenous CCK.

In summary, it has been demonstrated that proglumide induced an increase in both food intake and gastric emptying under the same conditions. Since proglumide is a specific competitive inhibitor of CCK and related peptides, the active agent in both these functions was probably CCK. Therefore, CCK, released by food entering the duodenum, may play a role in regulating food intake and gastric emptying. It is proposed that a CCK-induced reduction in food intake may be dependent on the prolongation of gastric distension caused by the delay of gastric emptying and furthermore, that the delay in gastric emptying may be achieved by an increase in pyloric tone.

REFERENCES

1. Abrahamsson, H., Janson, G. and Martinson, J., Vagal relaxation of the stomach induced by apomorphine in the cat. Acta Physiol. Scand., 390 (Suppl) (1973) 1-38.

2. Adrian, T.E., Pasquali, C., Pescosta, F., Bacarese-Hamilton, A.J. and Bloom, S.R., Dramatic plasma CCK elevation results from dietary enzyme inhibition, Regul. Pept., Suppl. 7 (1983) S64.

3. Akner, G. and Rossner, S., Intramuscular cerultide does not affect food intake in obese and non-obese human subjects, Appetite, 6 (1985) 21-24.

4. Anagnostides, A., Chadwick, V.S., Seldon, A.C. and Maton, P.N., Sham feeding and pancreatic secretion: evidence for direct vagal stimulation of enzyme output, Gastroenterol., 87 (1984) 109-114.

5. Anagnostides, A.A., Chadwick, V.S., Selden, A.C., Barr, J. and Maton, P.N., Human pancreatic and biliary responses to physiological concentrations of cholecystokinin octapeptide, Clin. Sci., 69 (1985) 259-262.

6. Andrews, P.L.R. and Lawes, I.N.C. The role of vagal and intramural inhibitory reflexes in the regulation of intragastric pressure in the ferret, J. Physiol., 326 (1982) 435-451.

7. Anika, M.S., Effects of cholecystokinin and caerulein on gastric emptying, Eur. J. Pharm., 85 (1982) 195-199.

8. Anika, S.M., Houpt, T.R. and Houpt, K.A., Satiety elicited by cholecystokinin in intact and vagotomized rats, Physiol. Behav., 19 (1977) 761-766.

9. Antin, J., Gibbs, J., Holt, J., Young, R.C. and Smith, G.P., Cholecystokinin elicits the complete behavioural sequence of satiety in rats, J. comp. Physiol. Psychol., 89 (1975) 784-790.

10. Antin, J., Gibbs, J. and Smith, G.P., Cholecystokinin interacts with pregastric food stimulation to elicit satiety in the rat, Physiol. Behav., 20 (1978) 67-70.

11. Baba, S., Otsuli, M., Ohki, A., Okabayashi, Y., Sakamoto, C. and Yanaihara, N., Structural requirements of COOH-terminal fragments of cholecystokinin for pancreatic juice, amylase and insulin secretion from the isolated perfused rat pancreas, Biomed. Res., 5(Suppl) (1984) 33-40.

12. Bacarese-Hamilton, A.J., Adrian, T.E. and Bloom, S.R., Distribution and heterogeneity of immunoreactive cholecystokinin (CCK) in the mucosa of the porcine gastrointestinal tract, Regul. Peptides, 9 (1984) 289-298.

13. Bacarese-Hamilton, A.J., Adrian, T.E., Chohan, P. and Bloom, S.R., Rat immunoreactive cholecystokinin (CCK): characterization using two chromatagraphic techniques, Regul. Peptides, 11 (1985) 149-158.

14. Baldwin, B.A., Cooper, T.R. and Parrott, R.F., Effect of cholecystokinin octapeptide on food intake in pigs, Proc. Nutr. Soc., 41 (1982) 119-121.

15. Barbaz, B.S., Autry, W.L., Ambrose, F.G., Gerber, R. and Liebman, J.M., Antagonism of cholecystokinin by naloxone and proglumide in mice, Ann. N.Y. Acad. Sci., 448 (1985) 573-574.

16. Bartness, T.J. and Waldbillig, R.J., Cholecystokinin -induced suppression of feeding: an evaluation of the generality of gustatory-cholecystokinin interactions, Physiol. Behav., 32 (1984) 409-415.

17. Becker, H.D., Werner, M. and Schafmayer, A., Release of radioimmunologic cholecystokinin in human subjects, Am. J. Surg., 147 (1984) 124-128.

18. Becker, J.M. and Kelly, K.A., Antral control of canine gastric emptying of solids, Am. J. Physiol., 245 (1983) G334-338.

19. Beglinger, C., Fried, M., Whitehouse, I., Jansen, J.B., Lamers, C.B. and Gyr, K., Pancreatic enzyme response to a liquid meal and to hormonal stimulation, J. Clin. Invest., 75 (1985) 1471-1476.

20. Behar, J. and Biancani, P., Effect of cholecystokinin and the octapeptide of cholecystokinin on the sphincter of Oddi and gallbladder, J. Clin. Invest., 66 (1980) 1231-1239.

21. Beinfeld, M.C., Cholecystokinin in the central nervous: a minireview, Neuropeptides, 3 (1983) 411-427.

22. Beinfeld M.C., The distribution and characterization of an amino-terminal fragment of cholecystokinin (CCK) -58 in rat brain, Biochem. Biophys. Res. Comm., 127 (1985) 720-725.

23. Ben-Horin, N., Ben-Horin, E. and Frenk, H., The effects of proglumide on morphine induced motility changes, Psychopharmacol., 84 (1984) 541-543.

24. Berk, T., Crochelt R.F. and Peikin, S.R., Neurohumoral control of the rat pylorus, Gastroenterol., 88 (1985) 1322.

25. Berk, T., Crochelt, R.F. and Peikin, S.R., Role of the pylorus in mediating satiety caused by cholecystokinin in the rat, Gastroenterol., 88 (1985) 1322.

26. Bernstein, I.L., Lotter, E.C. and Zimmerman, J.C., Cholecystokinin-induced satiety in the weanling rat, Physiol. Behav., 17 (1976) 541-543.

27. Bottcher, W.,Eysselein, V., Kauffman, G.L. and Walsh, J.H., Hepatic uptake of porcine CCK-39 and CCK-8 in dogs, Gastroenterol., 84 (part 2) (1983) 1112.

28. Bueno, L. and Ferre, J.-P., Central regulation of intestinal motility by somatostatin and cholecystokinin octapeptide, Science, 216 (1982) 1427-1429.

29. Bueno, L., Ferre, J.P. and Ruckesbusch, M., Control of the antral motor response to feeding by gastric acid secretion in rats, J. Physiol., 325 (1982) 43-50.

30. Bueno, L., Ferre, J.P., Ruckebusch, M., Genton, M. and Pascaud, X., Continuous electrical and mechanical activity recording in the gut of the conscious rat, J. Pharmacol. Method, 6 (1981) 129-136.

31. Bueno, L., Honde, C. and Fioramonte, J., Proglumide: selective antagonism of the rumination but not gastric motor effects induced by pentagastrin in sheep, Life Sci., 34 (1984) 475-481.

32. Burhol, P.G., Jensson, T.G., Jorde, R., Lygren, I. and Johnson, J.A., Plasma cholecystokinin (CCK) before and

after jejunoileal-bypass operation in obese patients with reference to appetite regulation, Int. J. Obesity, 8 (1984) 233-236.

33. Burhol, P.G., Rayford, R., Waldum, H.L., Schulz, T.B. and Thompson, J.C., Radioimmunoassay of plasma cholecystokinin (CCK), duodenal release of CCK, diurnal variation of plasma CCK, and immunoreactive plasma CCK components in man, Hepato-gastroenterol., 27 (1980) 300-309.

34. Byrnes, D.J., Henderson, L., Borody, T. and Rehfeld, J.F., Radioimmunoassay of cholecystokinin in human plasma, Clin. Chem. Acta, 111 (1981) 81-89.

35. Calam, J., Ellis, A. and Dockray J.G., Identification and measurement of molecular variants of cholecystokinin in duodenal mucosa and plasma. Diminished concentrations in patients with coeliac disease, J. Clin. Invest., 69 (1982) 218-225.

36. Carlquist, M., Mutt, V. and Jornvall, H., Characterization of two novel forms of cholecystokinin isolated from bovine upper intestine, Regul. Peptides, 11 (1985) 27-34.

37. Chang, R.S.L.,Lotti, V.J. and Chen, T.-B., Further evidence that substance P partly mediates the action of cholecystokinin octapeptide (CCK-8) on the guinea-pig ileum but not gallbladder: studies with a substance P antagonist, Neurosci. Lett., 46 (1984) 71-75.

38. Chen, Y.F., Chey, W.Y., Chang, T.-M. and Lee, K.Y., Duodenal acidification releases cholecystokinin, Am. J. Physiol., 249 (1985) G29-G33.

39. Chen, Y.F., Chey, W.Y., Park, H.J. and Chang, T.-M., Postprandial plasma cholecystokinin in normal and pancreatic duct ligated rats, Gastroenterol., 84 (1983) 1122.

40. Chiodo, L.A. and Bunney, B.S., Proglumide: selective antagonism of excitatory effects of cholecystokinin in the central nervous system, Science, 219(4591) (1983) 1449-1451.

41. Clark, C.R., Daum, P. and Hughes, J., Lack of competition between two reputed peripheral

cholecystokinin receptor antagonists for central cholecystokinin binding sites, Ann. N.Y. Acad. Sci., 448 (1985) 581-582.

42. Collins, S.M., Conover, K.L., Forsyth, P. and Weingarten, H., Endogenous cholecystokinin and intestinal satiety, Am. J. Physiol. 249 (1985) R667-R680.

43. Collins, S.,Walker, D., Forsyth, P. and Belbeck, L., The effects of Proglumide on cholecystokinin-, bombesin-, and glucagon -induced satiety in the rat, Life Sci., 32 (1983) 2223-2229.

44. Collins, S.M. and Weingarten, H.P., Inhibition of satiety by a cholecystokinin antagonist is independent of gastric emptying, Peptides, 5 (1984) 481-484.

45. Cottrell, D.F. and Iggo, A., The responses of duodenal tension receptors in sheep to pentagastrin, cholecystokinin and some other drugs, J. Physiol., 354 (1984) 477-495.

46. Cox, J.E., CCK-8 interacts with prefeeding to impair runway performance. Soc. Neurosci. Abstr., 11 (1985) 39.

47. Crawley, J.N., Neurochemical investigation of the afferent pathway from the vagus nerve to the nucleus tractus solitarius in mediating the "satiety syndrome" induced by systemic cholecystokinin, Peptides, 6(suppl 1) (1985) 133-137.

48. Crawley,J.N. and Beinfeld, M.C., Rapid development of tolerance to the behavioural actions of cholecystokinin, Nature, 302 (1983) 703-706.

49. Crawley, I., Hays, S.E. and Paul, S.M., Vagotomy abolishes the inhibitory effects of cholecystokinin on rat exploratory behaviours, Eur. J. Pharm., 73 (1981) 379-380.

50. Crawley, J.N., Hays, S.E., Paul, S.M. and Goodwin, F.K., Cholecystokinin reduces exploratory behaviour in mice, Physiol. Behav., 27 (1981) 407-411.

51. Crawley, J.N. and Kiss, J.Z., Tracing the sensory pathway from the gut to brain regions mediating the actions of cholecystokinin on feeding and exploration,

Ann. N.Y. Acad. Sci., 448 (1985) 586-588.

52. Crawley, J.N. and Schwaber, J.S., Abolition of the behavioural effects of cholecystokinin following bilateral radionfrequency lesions of the parvocellular subdivision of the nucleus tractus solitarius, Brain Res., 295 (1984) 289-299.

53. Dafny, N., Jacob, R.H. and Jacobson, E.D., Gastrointestinal hormones and neural interaction within the central nervous system, Experientia, 31 (1975) 658-660.

54. Davidsen, D. and Jorgensen, J., Gallbladder emptying with cerultide in oral cholecystography, Acta Radiol. Diag., 22 (1981) 165-169.

55. Davis, J.D. and Campbell, C.S., Peripheral control of meal size in the rat. Effect of sham feeding on meal size and drinking rate, J. comp. Physiol. Psychol., 84 (1972) 379-387.

56. Davis, J.D., Campbell, C.S., Gallagher, R.J. and Zurakov, M.A., Disappearance of a humoral satiety factor during food deprivation, J. comp. Physiol. Psychol., 75 (1971) 476-482.

57. Davis, J.D., Gallagher, R.J. and Ladooc, R.V., Inhibition of food intake by a humoral factor, J. comp. Physiol. Psychol., 67 (1969) 407-414.

58. Davison, J.S., Response of gastric vagal mechanoreceptors to circulating exogenous CCK octapeptide in the rat, Physiologist, 28 (1985) 295.

59. Davison, J.S. and Najafi-Farashah, A., Dibutyryl cyclicGMP, a competitive inhibitor of cholecystokinin/ pancreozymin and related peptides in gallbladder and ileum, Can. J. Physiol. Pharmacol., 59 (1981) 1100-1104.

60. Davison, J.S. and Najafi-Farashah, A., The possible role of cholecystokinin/pancreozymin (CCK/Pz) in the peristaltic reflex - studies with competitive antagonists to CCK/Pz and related peptides, In: M. Wienbeck (Ed.), Motility of the Digestive Tract, Raven Press, N.Y., pp.79-84, 1982.

61. Davison, J.S. and Najafi-Farashah, S.A., Potentiation

of transmural electrical stimulation-evoked contraction of ileum by persistent occupation of cholecystokinin receptors, IRCS Med. Sci., 10 (1982) 503-504.

62. Davison, J.S. and Najafi-Farashah, A., Proglumide: a specific antagonist to the actions of cholecystokinin -like peptides in guinea-pig gallbladder and ileum, IRCS Med. Sci., 10 (1982) 409-410.

63. de Castiglione, R., Structural requirements of cerultide-like peptides for activity on gut and brain, Peptides, 2(suppl 2) (1981) 61-63.

64. Debas, H.T.,Farooq, O. and Grossman, M.I., Inhibition of gastric emptying is a physiological action of cholecystokinin, Gastroenterol., 68 (1975) 1211-1217.

65. Della-Fera, M.A. and Baile, C.A., Cholecystokinin -octapeptide: continuous picomole injections into the cerebral ventricles of sheep suppress feeding, Science, 206 (1979) 471-473.

66. Della-Fera, M.A., and Baile, C., CCK-octapeptide injected in CSF decreases meaL size and daily food intake in sheep, Peptides, 1 (1980) 51-54.

67. Della-Fera, M.A. and Baile, C.A., CCK-octapeptide injected into cerebral ventricles of sheep decreases plasma insulin levels, Physiol. Behav., 34 (1985) 283-289.

68. Della-Fera, M.A., Baile, C.A., Schneider, B.S. and Grinker, J.A., Cholecystokinin-antibody injected in cerebroventricles stimulates feeding in sheep, Science, 212 (1981) 687-689.

69. Denbow, D.M. and Myers, R.D., Eating, drinking and temperature responses to intracerebroventricular cholecystokinin in the chick, Peptides, 3 (1982) 739-743.

70. Deschodt-Lanckman, M. and Bui, N.D., Cholecystokinin octa- and tetrapeptide degradation by synaptic membranes. I. Evidence for competition with enkephalins for in vitro common degradation pathways, Peptides, 2 (Suppl 2) (1981) 113-118.

71. Deschodt-Lanckman, M., Bui, N.D., Koulischer, D.,

Paroutaud, P. and Strosberg, A.D., Cholecystokinin octa- and tetrapeptide degradation by synaptic membranes. II. Solubilization and separation of membrane-bound CCK-8 cleaving enzymes, Peptides, 4 (1983) 71-78.

72. Deutsch, J.A., Dietary control and the stomach, Prog. Neurobiol., 20 (1983) 313-332.

73. Deutsch, J.A. and Hardy, W.T., Cholecystokinin produces bait shyness in rats, Nature, 266 (1977) 196.

74. Deutsch, J.A., Young, W.G. and Kalogeris, T.J., The stomach signals satiety, Science, 201 (1978) 165-167.

75. Dockray, G.J., Cholecystokinins in rat cerebral cortex: identification, purification and characterization by immunochemical methods, Brain Res., 188 (1980) 155-165.

76. Dockray, G.J. The physiology of cholecystokinin in brain and gut, Brit. Med. Bull., 38 (1982) 253-258.

77. Dockray, G.J. and Hutchison, J.B., Cholecystokinin octapeptide in guinea-pig ileum myenteric plexus: localization and biological action, J. Physiol. 300 (1980) 28P.

78. Dodd, J. and Kelly, J.S., The action of cholecystokinin and related peptides on pyramidal neurons of the mammalian hippocampus, Brain Res., 205 (1981) 337-350.

79. Doyle, J.W., Wolfe, M. and McGuigan, J.E., Hepatic clearance of gastrin and cholecystokinin peptides, Gastroenterol., 87 (1984) 60-68.

80. Edwards, G.L., Ladenheim, E.E. and Ritter, R.C., Dorsal hindbrain participation in cholecystokinin -induced satiety, Soc. Neurosci. Abstr., 11(1985) 37.

81. Elashoff, J., Garcia, R. and Taylor, I.L., Effects of vagotomy on satiety induced by gastrointestinal hormones in the rat, J. Physiol., 354 (1984) 78P.

82. Erckenbrecht, J.F., Caspari, J. and Wienbeck, M., Pentagastrin induced motility pattern in the human upper gastrointestinal tract is reversed by

proglumide, Gut, 19 (1984) 953-956.

83. Ewart, W.R. and Wingate, D.L., Cholecystokinin octapeptide and gastric mechanoreceptor activity in rat brain, Am. J. Physiol., 244 (1983) G613-G617.

84. Eysselein, V.E., Bottcher, W., Kauffman, G.L. and Walsh, J.H., Molecular heterogeneity of canine cholecystokinin (CCK) in portal and peripheral plasma, Gastroenterol., 84 (1983) 1147.

85. Eysselein, V.E., Deveney, C.W., Sankaran, H., Reeve, J.R. Jr. and Walsh, J.H., Biological activity of canine intestinal cholecystokinin-58, Am. J. Physiol., 245 (1983) G313-G320.

86. Falasco, J.D., Avilion, A.A., Greenberg, D., Smith, G.P., Gibbs, J., Liddle, R.A. and Williams, J.A., Soybean trypsin inhibitor increases plasma cholecystokinin but does not decrease gastric emptying in the rat, Soc. Neurosci. Abstr., 11 (1985) 556.

87. Faris, P.L., Opiate antagonistic function of cholecystokinin in analgesia and energy balance systems, Ann. N.Y. Acad. Sci., 448 (1985) 437-447.

88. Faris, P.L. and Olney, J.W., Suppression of food intake in rats by microinjection of cholecystokinin (CCK) to the paraventricular nucleus (PVN), Soc. Neurosci. Abstr., 11 (1985) 39.

89. Finkelstein, J.A., Steggls, A.W., Martinez, P.A. and Praissman, M., Cholecystokinin receptor binding levels in the genetically obese rat brain, Peptides, 5 (1984) 11-14.

90. Forgacs, I.C., Isaacs, P.E.T., Ladas, S., Murphy, G.M.and Dowling, G.H., Meal composition influences plasma immuno-reactive cholecystokinin (IR-CCK) levels and gallbladder (GB) contraction, Gastroenterol., 84 (1983) 1157.

91. Fried, M.,Beglinger, C.,Koehler, E., Whitehouse, I., Varga, L. and Gyr, K., Effect of proglumide, a cholecystokinin receptor antagonist, on caerulein-stimulated pancreatic enzyme secretion and pancreatic polypeptide release in the dog, Regul. Peptides, 8 (1984) 117-122.

92. Fried, M., Beglinger, C., Whitehouse, I. and Gyr, K., Effect of proglumide, a CCK-receptor antagonist, on caerulein-onduced pancreatic enzyme secretion and plasma PP release, Dig. Dis. Sci., 28 (1983) 934.

93. Fried, G.M., Ogden, W.D., Greeley, G.,and Thompson, J.C., Correlation of release and actions of cholecystokinin in dogs before and after vagotomy, Surgery, 93 (1983) 786-791.

94. Fujimura M., Beauchamp R.D., Lluis F., Sakamoto T., Townsebd C.N.,Jr, and Thompson J.C., Effect of cholecystokinin(CCK)-antagonist, proglumide on CCK-8-induced gallbladder contraction in conscious dogs, Gastroenterol., 88 (1985) 1389.

95. Gardner, J.D., Knight, M., Sutliff, V.E., Tamminga, C.A. and Jensen, R.T., Derivatives of CCK-(26-32) as cholecystokinin receptor antagonists in guinea pig pancreatic acini, Am. J. Physiol., 246 (1984) G292-G295.

96. Gayton, R.J. and Raybould, H.E., Effect on intravenous cholecystokinin octapeptide on brain-stem neurones responding to gastric distension in the rat, J. Physiol., 357 (1984) 84P.

97. George, J.D., New clinical method for measuring the rate of gastric emptying: the double sampling test meal, Gut, 9 (1968) 237-242.

98. Gerner, T., Maehlumshagen, P. and Haffner, J.F.W., The effect of cholecystokinin on the in vitro motility of the guinea-pig stomach, Scand. J. Gastroenterol., 10 (Suppl) (1975) 48-50.

99. Ghosh, M.N. and Schild, M.O., Continuous recording of acid gastric secretion in the rat, Brit. J. Pharmacol. Chemother., 13 (1958) 54-61.

100. Gibbs, J., Effect of bombesin on feeding behaviour, Life Sci., 37 (1985) 147-153.

101. Gibbs, J., Falacso, J.D. and McHugh, P.R., Cholecystokinin-decreased food intake in rhesus monkeys, Am. J. Physiol., 230 (1976) 15-18.

102. Gibbs, J. and Smith, G.P., Gut peptides and food in the gut produce similar satiety effects, Peptides, 3

(1982) 553-557.

103. Gibbs, J., Young, R.C. and Smith, G.P., Cholecystokinin decreases food intake in rats, J. comp. Physiol. Psychol., 84 (1973) 488-495.

104. Gibbs, J., Young, R.C. and Smith, G.P., Cholecystokinin elicits satiety in rats with open gastric fistulas, Nature, 245 (1973) 323-325.

105. Gillespie, I.E., Clark, D.H., Kay, A.W. and Tankel, H.I., Effect of antrectomy, vagotomy with gastrojejunostomy and antrectomy with vagotomy on the spontaneous and maximal gastric acid output in man, Gastroenterol., 38 (1960) 61-367.

106. Glick, Z. and Mayer, J., Preliminary observations on the effect of intestinal mucosa extract on food intake of rats, Fed. Proc., 27 (1968) 485.

107. Glise, H. and Abrahamsson, H., Reflex vagal inhibition of gastric motility by intestinal nociceptive stimulation in the cat, Scand. J. Gastroenterol., 15 (1980) 769-774.

108. Golterman, N.R., In vivo synthesis of cholecystokinin in the rat cerebral cortex: Identification of COOH-terminal peptides with labelled amino acids, Peptides, 3 (1982) 733-737.

109. Gonzalez M.F. and Deutsch J.A., Vagotomy abolishes cues of satiety produced by gastric distension, Science, 212 (1981) 1283-1284.

110. Greenberg, D., Smith, G.P., Gibbs, J., Falasco, J.D., Liddle, R.A. and Williams, J.A., Soybean trypsin inhibitor increases plasma cholecystokinin (CCK) but does not decrease food intake, Soc. Neurosci. Abstr., 11 (1985) 557.

111. Greenberg, G.R., Chan, B., McDonald, T.J. and Alleyne, J., The role of vagal integrity in gastrin-releasing peptide stimulated gastroenteropancreatic hormone release and gastric acid secretion, Regul. Peptides, 10 (1985) 179-187.

112. Grider, J.R., Loewe, C.J. and Maklouf, G.M., Characterization of myoreceptors and neuroreceptors for CCK in the gut with the CCK/gastrin antagonist,

proglumide, Gastroenterol., 86 (1984) 1099.

113. Grossman, M.I., Physiological effects of gastrointestinal hormones, Fed. Proc., 36 (1977) 1930-1932.

114. Grovum, W.L., Cholecystokinin administered intravenously did not act directly on the central nervous system or on the liver to suppress food intake by sheep, J. Physiol., 326 (1982) 55-56P.

115. Grovum, W.L. and Chapman, H.W., Pentagastrin in the circulation acts directly on the brain to depress motility of the stomach in sheep, Regul. Peptides, 5 (1982) 35-42.

116. Grundy, D. and Scratcherd, T., A splanchno-vagal component of the inhibition of gastric motility by distension of the intestines, In: M. Wienbeck (Ed.), Motility of the digestive tract, Raven Press, New York, 1982, pp.39-43.

117. Hahne, W.F., Jensen, R.T.,Lemp, G.F. and Gardner, J.D., Proglumide and benzotript: members of a different class of cholecystokinin receptor antagonists, Proc. Natl. Acad. Sci., 78 (1981) 6304-6308.

118. Hansky, J., Willis, G., Pavey, G. and Smith, G., Effect of intracerebral injection of cholecystokinin, bombesin and gastrin on short term satiety in the rat, Gastroenterol., 84 (1983) 1182.

119. Harper, A.A., Hood, A.J.C., Mushens, J. and Smy, J.R., Inhibition of external pancreatic secretion by intracolonic and intraileal infusion of the cat, J. Physiol., 292 (1979) 445-454.

120. Harper, A.A., Hood, A.J.C., Mushens, J. and Smy, J.R., Pancreotone, an inhibitor of pancreatic secretion in extracts of ileal and colonic mucosa, J. Physiol., 292 (1979) 455-467.

121. Harper, A.A. and Raper, H.S., Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine, J. Physiol., 102 (1943) 115-125.

122. Harvey, R.I., Oliver, J.M., Read, A.E., Edele, A.,

Vantini, I., Scuro, L.A., Groarke, J.F. and Fitzgerald, J., Half-life, clearance, and endogenous secretion rates of cholecystokinin-pancreozymin in man, Gut, 18 (1977) A982.

123. Hawkins, C., Diseases of the alimentary system. Anorexia and loss of weight, Brit. Med. J., 2 (1976) 1373-1375.

124. Hays, S.E., Goodwin, F.K. and Paul, S.M., Cholecystokinin receptors in brain: effects of obesity, drug treatment, and lesions, Peptides, 2(Suppl 2) (1981) 21-26.

125. Hedner, P. and Rorsman, G., Structures essential for the effect of cholecystokinin on the guinea-pig small intestine in vitro, Acta Physiol. Scand., 74 (1968) 58-68.

126. Hendry, S.H.C., Jones, E.G. and Beinfeld, M.C., Cholecystokinin-reactive neurons in rat and monkey cerebral cortex make symmetric synapses and have intimate associations with blood vessels, Proc. Natl. Acad. Sci., 80 (1983) 2400-2404.

127. Heppell, J., Blinks, S., Kelly, K.A. and Go, V.L.W., Inhibition of small intestinal interdigestive motility by cholecystokinin-octapeptide (CCK-OP), In: M. Wienbeck (Ed.), Motility of the digestive tract., Raven Press, New York, 1980, pp. 207-214.

128. Hoebel, B.G., Integrative peptides, Brain Res. Bull., 14 (1985) 525-528.

129. Hollinshead, J.W., Debas, H.T., Yamada, T., Elashoff, J., Osadchey, B. and Walsh, J.S., Hypergastrinemia develops within 24 hours of truncal vagotomy in dogs, Gastroenterol., 88 (1985) 35-40.

130. Hommer, D.W., Palkovits, M., Crawley, J.N., Paul, S.M. and Skirboll, L.R., Cholecystokinin-induced excitation in the substantia nigra : evidence for peripheral and central components, J. Neurosci., 5 (1985) 1387-1392.

131. Hopman, W.P.M., Jansen, J.B.M.J. and Lamers, C.B.H.W., Plasma cholecystokinin response to a liquid fat meal in vagotomized patients, Ann. Surg., 200 (1984) 693-697.

132. Hopman, W.P.M., Jansen, J.B.M.J., Rosenbusch, G. and Lamers, C.B.H.W., Effect of equimolar amounts of long chain triglycerides and medium chain triglycerides on plasma cholecystokinin and gallbladder contraction, Am. J. Clin. Nutr., 39 (1984) 356-359.

133. Houpt, K.A. and Houpt, T.R., Gastric emptying and cholecystokinin in the control of food intake in suckling rats, Physiol. Behav., 23 (1979) 925-929.

134. Houpt, T.R., The sites of action of cholecystokinin in decreasing meal size in pigs, Physiol. Behav., 31 (1983) 693-698.

135. Hsiao, S. and Deupree, D., Cholecystokinin and bombesin effects on rewarded and nonrewarded operants, Peptides, 4 (1983) 1-3.

136. Hsiao, S. and Wang, C.H., Continuous infusion of cholecystokinin and meal pattern in the rat, Peptides, 4 (1983) 15-17.

137. Hunt, J.N. and Spurrell, W.R., The pattern of emptying of the human stomach, J. Physiol., 113 (1951) 157-168.

138. Inoue, K., McKay, D., Yajima, H. and Rayford, P.L., Effect of synthetic porcine gastrin-releasing peptide on plasma levels of immunoreactive cholecystokinin, pancreatic polypeptide and gastrin in dogs, Peptides, 4 (1983) 153-157.

139. Ivy, A.C. and Oldberg, E., A hormone mechanism for gall-bladder contracting and evacuation, Am. J. Physiol., 86 (1928) 599-613.

140. Iwamoto, Y., Nakamura, R. and Akanuma, Y., In vitro and in vivo effects of proglumide on CCK-stimulated amylase release and glucose uptake in mouse pancreatic acini, Gastroenterol., 84 (1985) 1196.

141. Izzo, R.S., Brugge, W,R, and Praissman, M., Immunoreactive cholecystokinin in human and rat plasma: correlation of pancreatic secretion in response to CCK, Regul. Peptides, 9 (1984) 21-34.

142. Jacoby, H.I. and Brodie, D.A., Gastrointestinal actions of metoclopramide , Gastroenterol., 52 (1967) 676-684.

143. Jansen, J.B.M.W. and Lamers, C.B.H.W., Molecular forms of cholecystokinin in human plasma during infusion of bombesin, Life Sci., 33 (1983) 2197-2205.

144. Jensen, R.T.,Jones, S.W. and Gardner, J.D., COOH-terminal fragments of cholecystokinin. A new class of cholecystokinin receptor antagonists, Biochim. Biophys. Acta, 757 (1983) 250-258.

145. Jensen, R.T., Lemp, G.F. and Gardner, J.D., Interactions of COOH-terminal fractions of cholecystokinin with receptors on dispersed acini from guinea-pig pancreas, J. Biol. Chem., 257 (1982) 5554-5559.

146. Kadar, T., Varszegi, M., Sudakov, S.K., Penke, B. and Telegdy, G., Changes in brain monoamine levels of rats during cholecystokinin octapeptide-induced suppression of feeding, Pharmacol. Biochem. Behav., 21 (1984) 339-344.

147. Kerstens, P.J.S.M., Lamers, C.B.H.W., Jansen, J.B.M.J., de Jong, A.J.L., Hessels, M. and Hafkenscheid, J.C.M., Physiological plasma concentrations of cholecystokinin stimulate pancreatic enzyme secretion and gallbladder contraction in man, Life Sci., 36 (1985) 565-569.

148. Kiss, J.Z. and Crawley, J.N. In press, cited in ref. 124.

149. Klaff, L.J., Hudson, A.M., Paul, M. and Miller R.P., A method for studying synaptosomal release of neurotransmitter candidates, as evaluated by studies on cortical cholecystokinin octapeptide release, Peptides, 1 (1982) 155-161.

150. Knight, M., Tamminga, C.A., Steardo, L., Beck, M.E., Barone, P. and Chase, T.N., Cholecystokinin -octapeptide fragments: binding to brain cholecystokinin receptors, Eur. J. Pharmacol., 105 (1984) 49-55.

151. Koopmans, H.S., Deutsch, J.A., Branson, P.J., The effect of cholecystokinin pancreozymin on hunger and thirst in mice, Behav. Biol., 7 (1972) 441-444.

152. Kothary, P.C., Vinik, A.I., Owyang, C. and Fiddian-Green, R.G., Immunochemical studies of

molecular heterogeneity of cholecystokinin in duodenal perfusates and plasma in humans, J. Biol. Chem., 258 (1983) 2856-2563.

153. Koulischer, D., Moroder, L. and Deschodt-Lanckman, M., Degradation of cholecystokinin octapeptide, related fragments and analogs by human and rat plasma in vitro, Regul. Peptides, 4 (1982) 127-139.

154. Kraly, F.S. and Gibbs, J., Vagotomy fails to block the satiating effect of food in the stomach, Physiol. Behav., 24 (1980) 1007-1010.

155. Kraly, F.S., A diurnal variation in satiating potency of cholecystokinin in the rat, Appetite, 2 (1981) 177-191.

156. Kraly, F.S., Vagotomy does not alter cholecystokinin's inhibition of sham feeding, Am. J. Physiol., 246 (1984) R829-R831.

157. Lamers, C.B.H.W., Poitras, P., Jansen, J.B.M.J. and Walsh, J.H., Relative potencies of cholecystokinin-33 and cholecystokinin-8 measured by radioimmunoassay and bioassay, Scand. J. Gastroenterol., 18 (suppl.82) (1983) 191-192.

158. Laugier, R., Papp, A., Demol, P., Charbit, J.-J. and Sarles, H., Pancreatic secretory response to cholecystokinin-pancreozymin and caerulein in the conscious rat, Pflugers Arch., 384 (1980) 83-92.

159. Lawaetz, O.,Aritas, Y., Blackburn, A.M. and Ralphs, D.N.L., Gastric emptying after peptic ulcer surgery, Scand. J. Gastroenterol., 17 (1982) 1065-1072.

160. Leander, S., Ekman, R., Uddman, R., Sundler, F. and Hakanson, R., Neuronal cholecystokinin, gastrin -releasing peptide, neurotensin and B-endorphin in the intestine of the guinea-pig, Cell Tiss. Res., 235 (1984) 521-531.

161. Levine, A.S. and Morley, J.E., Peptidergic control of insulin-induced feeding, Peptides, 2 (1981) 261-264.

162. Lew, M.F., Gibbs, J. and Smith, G.P., Intestinal satiety is elicited by L- but not by D-phenylalanine, Soc. Neurosci. Abstr., 9 (1983) 900.

163. Liddle, R.A., Goldfine, I.D. and Williams, J.A., Bioassay of plasma cholecystokinin in rats: effects of food, trypsin inhibitor, and alcohol, Gastroenterol., 87 (1984) 542-549.

164. Liddle, R.A., Green, C.K., Conrad, C.K. and Williams, J.A., CCK response to food is species specific: fat and amino acids do not stimulate CCK release in the rat, Gastroenterol., 88 (1985) 1476.

165. Lilja, P., Wiener, I., Inoue, K., Fried, G.M., Greeley, G.H. and Thompson, J.C., Release of cholecystokinin in response to food and intra-duodenal fat in pigs, dogs and man, Surg. Gyn. Obs., 159 (1984) 557-561.

166. Lilja, P., Wiener, I., Inoue, K.,Priano, L.L., Greeley, G.H. and Thompson, J.C., Influence of different anesthetic agents on the release of cholecystokinin in dogs, Surgery, 97 (1985) 415-419.

167. Lin, C.W. and Miller, T., Craracterization of cholecystokinin receptor site in guinea-pig cortical membranes using [125I] Bolton Hunter- cholecystokinin octapeptide, J. Pharmacol. Exp. Ther., 232 (1985) 775-780.

168. Loewe, C.J., Grider, J.R., Gardiner, J. and Vlahcevic, Z.R., Selective inhibition of pentagastrin- and cholecystokinin-stimulated exocrine secretion by proglumide, Gastroenterol., 89 (1985) 746-51.

169. Lorenz, D.N. and Goldmanm S.A., Vagal mediation of the cholecystokinin satiety effect in rats, Physiol. Behav., 29 (1982) 599-604.

170. Lorenz, D.N., Gastric emptying of milk in rat pups, Am. J. Physiol., 248 (1985) R732-R738.

171. Lorenz, D.N., Kreielsheimer, G. and Smith, G.P., Effect of cholecystokinin, gastrin, secretin and GIP on sham feeding in the rat, Physiol. Behav., 23: (1979) 1065-1072

172. Lumsden, K. and Holden, W.S., The act of vomiting in man, Gut, 10 (1969) 173-9.

173. MacLagan, N.F., The role of appetite in the control of body weight, J. Physiol., 90 (1937) 385-394.

174. MacLean, D.B., Abrogation of peripheral cholecystokinin -satiety in the capsaicin treated rat, Regul. Peptides, 11 (1985) 321-333.

175. Maddison, S., Intraperitoneal and intracranial cholecystokinin depress operant responding for food, Phyiol. Behav., 19 (1977) 819-824.

176. Malagelada, J.-R., Gastric, pancreatic and biliary response to a meal In: L.R. Johnson, (Ed.), Physiology of the Gastrointestinal tract, Raven Press, New York, 1981, pp.893-924.

177. Mangel, A.W. and Koegel, A., Effects of peptides on gastric emptying, Am. J. Physiol., 246 (1984) G342-G345.

178. Margolis, R.L., Moran, T.H. and McHugh, P.R., The contractility of rat gastrointestinal tissue in response to bombesin and cholecystokinin, Physiologist, 28 (1985) 295.

179. Maton, P.N., Seldon, A.C. and Chadwick, V.S., Measurement of plasma cholecystokinins (CCKs) using high pressure liquid chromatography (HPLC) and radioimmuoassay (RIA): response to oral fat and effect of atropine, Regul. Peptides, 3 (1982) 76.

180. Maton, P.N., Seldon, A.C. and Chadwick, V.S., Differential distribution of cholecystokinin (CCK)-8 and CCK 33/39 in human intestinal mucosa, Regul. Pept., 4 (1982) 373.

181. Maton, P.N., Seldon, A.C. and Chadwick, V.S., Large and small forms of cholecystokinin in human plasma: measurement using high pressure liquid chromatography and radioimmunoassay, Regul. Peptides, 4 (1982) 251-260.

182. Maton, P.N., Selden, A.C. and Chadwick, V.S., Atropine inhibits meal-stimulated release of cholecystokinin, Scand. J. Gastroenterol., 19 (1984) 831-834.

183. Maton, P.N., Seldon, A.C. and Chadwick, V.S., Differential distribution of molecular forms of cholecystokinin in human and porcine small intestinal mucosa, Regul. Peptides, 8 (1984) 9-19.

184. Maton, P.N., Seldon, A.C., Fitzpatrick, M.L. and

Chadwick, V.S., Infusion of cholecystokinin octapeptide in man: relation between plasma cholecystokinin concentration and gallbladder emptying rates, Eur. J. Clin. Invest., 14 (1984) 37-41.

185. McCaleb, M.L. and Myers, R.D., Cholecystokinin acts on the hypothalamic "noradrenergic system" involved in feeding, Peptides, 1 (1980) 47-49.

186. Mclaughlin, C.L. and Baile, C.A., Obese mice and the satiety effects of cholecystokinin, bombesin and pancreatic polypeptide, Physiol. Behav., 26 (1981) 433-437.

187. McLaughlin, C.L., Baile, C.A. and Buonomo, F.C., Effect of CCK antibodies on food intake and weight gain in Zucker rats, Physiol. Behav., 34 (1985) 277-282.

188. McLaughlin, C.L., Baile, C.A., Della-Fera, M.A. and Kasser, T.G., Meal-stimulated increased concentrations of CCK in the hypothalamus of Zucker obese and lean rats, Physiol. Behav., 35 (1985) 215-220.

189. McLaughlin, C.L., Peikin, R. and Baile, C.A., Food intake response to modulation of secretion of cholecystokinin in Zucker rats, Am. J. Physiol., 244 (1983) R676-685.

190. McLaughlin, C.L., Peikin, S.R. and Baile, C.A., Feeding behaviour response of Zucker rats to proglumide, a CCK receptor antagonist, Pharm. Biochem. Behav., 18 (1983) 879-883.

191. Meisenberg, G. and Simmons, W.H., Minireview: peptides and the blood brain barrier, Life Sci., 32 (1983) 2611-2623.

192. Mendel, V.E., Sturdevant, R.A.L. and Elashoff, J., The effects of caerulein on food intake in the cat, Physiol. Behav., 24 (1980) 629-631.

193. Meyer, J.H., Control of pancreatic exocrine secretion, In: L.R. Johnson, (Ed.), Physiology of the gastrointestinal tract, Raven Press, New York, 1981, pp.821-829.

194. Miceli, M.O. and Malsbury, C.W., Feeding and drinking responses in the golden hamster following treatment

with cholecystokinin and angiotensin II. Peptides, 4 (1983) 103-106.

195. Miceli, M.O., Abdominal vagus and regulation of ingestive behaviour and body weight in golden hamsters, Am. J. Physiol., 248 (1985) R686-R697.

196. Micevyeh, P.E., Go, V.L.W., Yaksh, T.G. and Finkelstein, J., In vitro releases of cholecystokinin from hypothalamus and frontal cortex of Sprague-Dawley, Zucker lean (Fa/-) and obese (fa/fa) rats, Peptides, 5 (1984) 73-80.

197. Migata, M., Rayford, P.L. and Thompson, J.C., Hormonal (gastrin, secretin, cholecystokinin) and secretory effects of bombesin and duodenal acidification in dogs, Surgery, 87 (1980) 209-215.

198. Mineka, S. and Snowdon, C.T., Inconsistency and possible habituation of CCK-induced satiety, Physiol. Behav., 21 (1978) 65-72.

199. Monges, H., Salducci, J. and Naudy, B., Electrical activity of the gastrointestinal tract in dog during vomiting, In: E.E. Daniel (Ed.), IVth International symposium on Gastrointestinal motility, Mitchell Press, Vancouver, 1974, pp.479-488.

200. Moore, B.O. and Deutsch, J.A., An antiemetic is antidotal to the satiety effects of cholecystokinin, Nature, 315 (1985) 321.

201. Moos, A.B., McLaughlin, C.L. and Baile, C.A., Effects of CCK on gastrointestinal function in lean and obese Zucker rats, Peptides, 3 (1982) 619-622.

202. Moran, T.H., Robinson, P.H. and McHugh, P.R., The pyloric cholecystokinin receptor, Ann. N.Y. Acad. Sci., 448 (1985) 621-623.

203. Moran, T.H. and McHugh, P.R., Cholecystokinin suppresses food intake by inhibiting gastric emptying, Am. J. Physiol., 242 (1982) R491-R497.

204. Mordes, J.P., el Lozy, M., Herrera, G. and Silen, W., Effects of vagotomy with and without pyloroplasty on weight and food intake in rats, Am. J. Physiol., 236 (1979) R61-R66.

205. Morgan, K.G., Schmalz, P.F., Go, V.W.L. and Szurszewski, J.H., Electrical and mechanical effects of molecular variants of CCK on antral smooth muscle, Am. J. Physiol., 235 (1978) E324-E329.

206. Morin, M.P., deMarchi, P., Champagnat, J., Vanderhaeghen, J.J., Rossier, J. and Denavit-Saubi, M., Inhibitory effect of cholecystokinin-octapeptide on neurones in the nucleus tractus solitarius, Brain Res., 265 (1983) 333-338.

207. Morley, J.E., Minireview: the ascent of cholecystokinin (CCK) - from gut to brain, Life Sci., 30 (1982) 479-493.

208. Morley, J.E., Levine, A.S., Kneip, J. and Grace, M., The effect of vagotomy on the satiety effects of neuropeptides and naloxone, Life Sci., 30 (1982) 1943-1947.

209. Morley, J.E., Levine, A.S., Murray, S.S., Kneip, J. and Grace, M., Peptidergic regulation of stress-induced eating, Am. J. Physiol., 243 (1982) R159-R163.

210. Mueller, K. and Hsiao, S., Oral preloads of liquid diet and hormonal satiety mechanisms, Physiol. Behav., 24 (1980) 667-673.

211. Mukhopadhyay, A.K., Thor, P.J., Copeland, E.M., Johnson, L.R. and Weisbrodt, N.W., Effect of cholecystokinin on myoelectric activity of small bowel of the dog, Am. J. Physiol., 232 (1977) E44-E47.

212. Mutt, V. and Jorpes, J.E., Isolation of aspartyl-phenylalanine amide from cholecystokinin / pancreozymin, Biochem. Biophys. Res. Comm., 26 (1967) 392-397.

213. Myers. R.D. and McCaleb. M.L., Peripheral and intrahypothalamic cholecystokinin act on the noradrenergic "feeding circuit" in the rat's diencephalon, Neurosci., 6 (1981) 645-655.

214. Najdovski, T., Collette, N. and Deschodt-Lanckman, M., Hydrolysis of the C-terminal octapeptide by rat kidney membranes: characterization of the cleavage by solubilized endopeptidase-24.11, Life Sci., 37 (1985) 827-834.

215. Niederau, C., Grendell, J.H. and Rothman, S.S., Effects of proglumide on ductal and basolateral secretion of pancreatic digestive enzymes, Am. J. Physiol., 249 (1985) G100-G107.

216. Nilsson, S.,Leander, S.,Vallgren, S. and Hakanson, R., Gastrins and cholecystokinins release acetylcholine but not substance P from neurons in the guinea-pig taenia coli, Eur. J. Pharmacol., 90 (1983) 245-250.

217. Oldendorf, W.H., Blood-brain barrier permeability to peptides: pitfalls in measurement, Peptides, 2 (suppl 2) (1981) 109-111.

218. Oomura, Y., Input-output organization in the hypothalamus relating to food intake behaviour, In: P.J. Morgane and J. Panksepp (Eds.), Handbook of the Hypothalamus, Vol. 2, Marcel Dekker Inc., New York, 1980, pp.557-620.

219. Orhn, P.G.,Johansson, H. and Nilsson, F., Influence of ether anaesthesia and oro-gastric intubation on gastric evacuation and small bowel propulsion after laparotomy, Acta Chir. Scand., (Suppl) 461 (1976) 5-18.

220. Otte, M., Dann, T. and Lambrecht, A., Proglumide, a CCK-PZ receptor antagonist? Digestion, 27 (1983) 53-54.

221. Palkovits, M., Kiss, J.Z., Beinfeld, M.C. and Williams, T.H., Cholecystokinin in the nucleus of the solitary tract of the rat: evidence for its vagal origin, Brain Res., 252 (1982) 386-390.

222. Pappas, T.N., Melendez, R.L. and Debas, H.T., Gastric distension is a physiological peripheral satiety signal in the dog, Gastroenterol., 88 (1985) 1530.

223. Pappas, T.N., Melendez, R.L., Strah, K.M. and Debas, H.T., Cholecystokinin is not a peripheral satiety signal in the dog, Am. J. Physiol., 249 (1985) G733-G738.

224. Pardridge, W.M., Neuropeptides and the blood-brain-barrier, Ann. Rev. Physiol., 45 (1983) 73-82.

225. Parrott, R.F. and Baldwin, B.A., Operant feeding and drinking in pigs following intracerebroventricular

injection of synthetic cholecystokinin-octapeptide, Physiol. Behav., 26 (1981) 419-422.

226. Passano, E., Debas, H., Oldendorf, W. and Yamada, T., Rapid appearence of intraventricularly administered neuropeptide in the peripheral circulation, Brain Res., 241 (1982) 338-340.

227. Phaosawasdi, K. and Fisher, R.S., Hormonal effects on the pylorus, Am. J. Physiol., 243 (1982) G330-G335.

228. Phifer, C.B., Sikes, C.R. and Hall, W.G., Satiety in 6-day old rat pups: is gastric distension the only cue? Soc. Neurosci. Abstr., 11 (1985) 59.

229. Pi-Sunyer, X., Kissileff, H.R., Thornton, J. and Smith, G.P., C-terminal octapeptide of cholecystokinin decreases food intake in obese men, Physiol. Behav., 29 (1982) 627-630.

230. Pinder, R.M., Brogden, R.N., Sawyer, P.R., Speight, T.M. and Avery, G.S., Metoclopramide: a review of its pharmacological properties and clinical use, Drugs, 12 (1976) 81-131.

231. Rattan, S. and Goyal, R.K., Pharmacological differences between neural (inhibitory) and muscle (excitatory) CCK receptors in the cat lower esophageal sphincter, Gastroenterol., 84 (part 2) (1983) 1281.

232. Raybould, H., Gayton, R.J. and Dockray, G.J., CNS effects of circulating CCK8: involvement of brainstem neurones responding to gastric distension, Brain Res., 342 (1985) 187-190.

233. Rayford, P.L., Miller, T.A. and Thompson, J.C., Secretin, cholecystokinin and new gastrointestinal hormones, N. Eng. J. Med., 294 (1976) 1093-1101.

234. Read, A.E., Harvey, R.F. and Naish, J.M., In: Basic Gastroenterology, J.Wright and Sons, Bristol, U.K., 1981.

235. Rehfeld, J.F., Cholecystokinin, Clin. Gastroenterol., 6 (1980) 593-607.

236. Rehfeld, J.F., How to measure cholecystokinin in plasma, Gastroenterol., 87 (1984) 434-438.

237. Reidelberger, R.D., Kalogeris, T.J. and Solomon, T.E., Comparative effects of caerulein on food intake and pancreatic secretion in dogs, Soc. Neurosci. Abstr., 11 (1985) 38.

238. Rentzhog, L. and Wikstrom, S., Pharmacological effects on gastric emptying following laparotomy in the rat, Acta Chir. Scand., 141 (1975) 649-653.

239. Reynell, P.C. and Spray, G.H., The effect of ether and pentobarbitone sodium on gastrointestinal function in the intact rat, Brit. J. Pharmacol., 12 (1957) 104-106.

240. Ritter, R.C. and Ladenheim, E.E., Capsiacin pretreatment attenuates suppression of food intake by cholecystokinin, Am. J. Physiol., 248 (1985) R501-R504.

241. Robinson, P.H., Moran, T.H. and McHugh, P.R., Gastric cholecystokinin receptors and the effect of cholecystokinin on feeding and gastric emptying in the neonatal rat, Ann. N.Y. Acad. Sci., 448 (1985) 627-629.

242. Roman, C. and Gonella, J., Extrinsic control of digestive tract motility, In: L.R. Johnson (Ed.), Physiology of the gastrointestinal tract, Raven Press, New York, 1981, pp.289-333.

243. Rovati, A.L., Inhibition of gastric secretion by anti-gastrinic and H2-blocking agents, Scand. J. Gastroenterol., 11 (suppl.42) (1976) 113-118.

244. Ruckebusch, M. and Fioramonte, J., Electrical spiking activity and propulsion in small intestine in fed and fasted rats, Gastroenterol., 68 (1975) 1500-1508.

245. Ryder, S., Eng, J., Strauss, E. and Yalow, R.S., Alkaline extraction and characterization of cholecystokinin-immunoreactivity from rat gut, Gastroenterol., 81 (1981) 267-275.

246. Saito, A., Goldfine, I.D. and Williams, J.A., Characterization of receptors for cholecystokinin and related peptides in mouse cerebral cortex, J. Neurochem., 37 (1981) 483-490.

247. Saito, A., Williams, J.A. and Goldfine, I.D.,

Alterations in brain cholecystokinin receptors after fasting, Nature, 289 (1981) 599-600.

248. Sakaguchi, T. and Ohtake, M., Inhibition of gastric motility induced by activation of the hypothalamic paraventricular nucleus, Brain Res., 335 (1985) 365-367.

249. Sarles, H., Hage, G., Laugier, R., Demol, P. and Bataille, D., Present status of the anticholecystokinin hormone, Digestion, 19 (1979) 73-76.

250. Schally, A.V., Redding, T.W. and Lucien, H.W., Enterogastrone inhibits eating by fasted mice, Science, 157 (1967) 210-211.

251. Schang, J.-C. and Kelly, K.A., Inhibition of canine interdigestive proximal gastric motility by cholecystokinin octapeptide, Am. J. Physiol., 240 (1981) G217-G220.

252. Schang, J.-C., Kelly, K.A. and Go, V.L.W., Postprandial hormonal inhibition of canine interdigestive gastric motility, Am. J. Physiol., 240 (1981) G221-224.

253. Scheurer, U., Varga, L., Drack, E., Burki, H.-R. and Halter, F., Measurement of cholecystokinin octapeptide-induced motility of rat antrum, pylorus, and duodenum in vitro, Am. J. Physiol., 244 (1983) G261-G265.

254. Scheurer,U., Varga, L., Drack, E., Burki, H.-R. and Halter, F., Mechanism of action of cholecystokinin octapeptide on rat antrum, pylorus, and duodenum, Am. J. Physiol., 244 (1983) G266-G272.

255. Schick, R.R., Yaksh, T.L. and Go, V.L.W., Pharmacological characterization of the suppressive effect of cholecystokinin octapeptide (CCK-8) on feeding in rats, Soc. Neurosci. Abstr., 11 (1985) 1574.

256. Schneider, B.S., Monahan, T.W. and Hirsch, J., Brain cholecystokinin and nutritional status in rats and mice, J. Clin. Invest., 64 (1979) 1348-1356.

257. Schultzberg, M., Hokfelt, T., Nilsson, G., Terenius,

L., Rehfeld, J.F., Brown, M., Elde, R.,Goldstein, M. and Said, S., Distribution of peptide- and catecholamine- containing neurons in the gastrointestinal tract of rat and guinea-pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin / cholecystokinin, neurotensin and dopamine-B-hydroxylase, Neurosci., 5 (1980) 689-744.

258. Scott, L.D. and Summers, R.W., Correlation of contractions and transit in the rat small intestine, Am. J. Physiol., 230 (1976) 132-137.

259. Scott, R.B., Diamant, S. and Gall, D.G., Intestinal anaphylaxis and gut motility in the rat, Gastroenterol., 90 (1986) In press.

260. Shaw, M.J., Hughes, J.J., Morley, J.E., Levine, A.S., Silvis, S.E. and Shafer, R.B., Cholecystokinin octapeptide action on gastric emptying and food intake in the normal and vagotomized man, Ann. N.Y. Acad. Sci., 448 (1985) 640-641.

261. Slaff, J.I., Wolfe, M.M. and Toskes, P.P., Acute and chronic inhibition of plasma cholecystokinin (CCK) levels by pancreatic proteases, Gastroenterol., 88 (1985) 1590.

262. Slaff, J.I., Wolfe, M.M. and Toskes, P.P., Elevated fasting cholecystokinin levels in pancreatic exocrine impairment: evidence to support feedback regulation, J. Lab. Clin. Med., 105 (1985) 282-285.

263. Slanghellini, V., Malagelada, J.R., Zinsmeister, A.R., Go, V.L.W. and Kao, P.C., Stress-induced gastroduodenal motor disturbances in humans: possible humoral mechanisms, Gastroenterol. 85 (1983) 83-91.

264. Smith, G.P., The role of the gut in the control of food intake, Viewpoints on Digestive Diseases 15 (1983) 1-4.

265. Smith, G.P. and Gibbs, J., Postprandial satiety, In: J.M. Sprague and A.N. Epstein (Eds.), Progress in psychobiology and physiological psychology, Vol.8, Academic Press, New York, 1979, pp.179-242.

266. Smith, G.P. and Gibbs, J., Brain-gut peptides and the

control of food intake, In: J.B. Martin, S. Reichlin and K.L. Bick (Eds.), Neurosecretion and brain peptides, Raven Press, New York, 1981, pp. 389-395.

267. Smith, G.P., Gibbs, J., Jerome, C., Pi-Sunyer, F.X., Kissileff, H.R. and Thirnton, J., The satiety effect of Cholecystokinin: a progress report, Peptides 2 (suppl 2) (1981) 57-59.

268. Smith, G.P.,Greenberg, D., Falasco, J.D., Gibbs, J., Liddle, R.A. and Williams, J.A., Plasma levels of cholecystokinin produced by satiating doses of exogenous CCK-8, Soc. Neurosci. Abstr., 11 (1985) 557.

269. Smith, G.P., Jerome, C., Cushin, B.J., Eterno, R. and Simansky, K.J., Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat, Science, 213 (1981) 1036-1037.

270. Smith, G.P., Jerome, C. and Norgren, R., Vagal afferent axons mediate the satiety effect of CCK-8, Soc. Neurosci. Abstr., 9 (1983) 902.

271. Smith, G.T., Moran, T.H., Coyle, J.T., Kuhar, M.J., O'Donahue, T.L. and McHugh, P.R., Anatomic localization of cholecystokinin receptors to the pyloric sphincter, Am. J. Physiol., 246 (1984) R127-R130.

272. Snape, W.J., Interaction of the octapeptide of cholecystokinin and gastrin I with bethanechol in the stimulation of feline colonic smooth muscle, Gastroenterol., 84 (1983) 58-62.

273. Snape, W.J., Matarazza, S.A. and Cohen, S., Effect of eating and gastrointestinal hormones on colonic myoelectric and motor activity, Gastroenterol., 75 (1978) 373-378.

274. Solcia, E., Capella, C., Buffa, R., Usellini, L., Fiocca, R. and Sessa, F., Endocrine cells of the digestive system, In: L.R.Johnson (Ed.), Physiology of the Gastrointestinal Tract, Raven Press, New York, 1981, pp. 39-58.

275. South, E.H. and Ritter, R.C., Attenuation of CCK-induced satiety by systemic, intraventricular or

perivagal capsaicin administration, Soc. Neurosci. Abstr., 11 (1985) 37.

276. Stacher, G.,Steinringer, H., Schmierer, G., Schneider, C. and Winklehner, S., Cholecystokinin octapeptide decreases intake of solid food in man, Peptides, 1 (1980) 133-136.

277. Stacher, G.,Steinringer, H., Schmierer, G., Schneider, C. and Winklehner, S., Cerultide decreases food intake in non-obese man, Peptides, 3 (1982) 607-612.

278. Steardo, L., Knight, M., Tamminga, C.A., Barone, P., Kask, A.M. and Chase, T.N., CCK26-33 degrading activity in brain and nonneural tissue: a metalloendopeptidase, J. Neurochem., 45 (1985) 784-790.

279. Steardo, L., Knight, M., Tamminga, C.A. and Chase, T.N., Products of cholecystokinin (CCK)-octapeptide proteolysis interact with central CCK receptors, Neurosci. Lett., 54 (1985) 319-325.

280. Stein, L.J. and Woods, S.C., Cholecystokinin and bombesin act independently to decrease food intake in the rat, Peptides, 2 (1981) 431-436.

281. Strauss, E. and Yalow, R.S., Brain cholecystokinin in fasted and fed mice, Life Sci., 26 (1980) 969-970.

282. Strohmayer, A.J. and Smith, G.P., Cholecystokinin inhibits food intake in genetically obese (C57BL/6J ob) mice, Peptides, 2 (1981) 39-43.

283. Strunz, U.T. and Grossman, M.I., Effect of intragastric pressure on gastric emptying and secretion, Am. J. Physiol., 234 (1978) E552-E555.

284. Stubbs, R.S. and Stabile, B.E., Role of cholecystokinin in pancreatic exocrine response to intraluminal amino acids and fat, Am. J. Physiol., 248 (1985) G347-G352.

285. Sturdevant, R.A.L. and Goetz, H., Cholecystokinin both stimulates and inhibits human food intake, Nature, 261 (1978) 713-715.

286. Swerdlow, N.R., van der Kooy, D., Koob, G.F. and Wenger, J.R., Cholecystokinin produces conditioned

place aversions, not place preferences, in food -deprived rats: evidence against involvement in satiety, Life Sci., 32 (1983) 2087-2093.

287. Tani, S. and Muto, N., Effects of pentagastrin, histamine, carbamylcholine and catecholamines on gastric secretion, motility and emptying in the rat, Biochem. Pharmacol., 31 (1982) 3475-3481.

288. Taylor, I.L. and Garcia, R., Effects of pancreatic polypeptide, caerulein, and bombesin on satiety in obese mice, Am. J. Physiol., 248 (1985) G277-G280.

289. Thompson, D.G., Richelson, E. and Malagelada, J.R., Perturbation of gastric emptying and duodenal motiltity through the central nervous system, Gastroenterol., 83 (1982) 1200-1206.

290. Tuchscherer, M.M. and Seybold, V.S., Immunohistochemical studies of substance P, cholecystokinin-octapeptide and somatostatin in dorsal root ganglia of the rat, Neurosci., 14 (1985) 593-605.

291. Valenzuela, J.E., Effect of intestinal hormones and peptides on intragastric pressure in dogs, Gastroenterol., 71 (1976) 766-769.

292. Valenzuela, J.E. and Defilippi, C., Inhibition of gastric emptying in humans by secretin, the octapeptide of cholecystokinin, and intraduodenal fat, Gastroenterol., 81 (1981) 898-902.

293. Valenzuela, J.E. and Grossman, M.I., Effect of pentagastrin and caerulein on intragastric pressure in the dog, Gastroenterol., 69 (1975) 1383-1384.

294. van der Kooy D., Area postrema: site where cholecystokinin acts to decrease food intake, Brain Res., 295 (1984) 345-347.

295. Van Itallie, T.B. and Vanderweele, D.A., The phenomenon of satiety, In: P. Bjorntorp, M.Cairella and A.N. Howard (Eds.), Rec. Adv. in Obesity: 111, John Libbey, London, 1980, pp.278-289.

296. Vanderweele, D.A., CCK, endogenous insulin condition and satiety in free-fed rats, Physiol. Behav., 29 (1982) 961-964.

297. Vanderweele, D.A., Oetting, R.L., Jones, R.E. and Deems, D.A., Sham feeding, flavour associations and diet self-selection as indicators of feeding satiety or aversive effects of peptide hormones, Brain Res. Bull., 14 (1985) 529-535.

298. Vigna, S.R., Szeecowka, J. and Williams, J.A., Do antagonists of pancreatic cholecystokinin receptors interact with central nervous system cholecystokinin receptors, Brain Res., 343 (1985) 394-397.

299. Vizi, S.E., Bertaccini, G., Impicciatore, M. and Knoll, J., Evidence that acetylcholine released by gastrin and related polypeptides contributes to their effect on gastrointestinal motility, Gastroenterol., 64 (1973) 268-277.

300. Walsh, J.H., Gastrointestinal hormones and peptides, In: L.R. Johnson (Ed.), Physiology of the Gastrointestinal tract, Raven Press, New York, 1981, pp.59-144.

301. Walsh, J.H., Lamaers, C.B. and Valenzuela, J.E., Cholecystokinin-octapeptidelike immunoreactivity in human plasma, Gastroenterol., 82 (1982) 438-444.

302. Watkins, L.R., Kinschecki, B. and Mayer, D.J., Potentiation of opiate analgesia and apparent reversal of morphine tolerance by proglumide, Science, 224 (1984) 395-396.

303. Weems, W.A., Seidel, E.R. and Johnson, L.R., Induction in vitro of a specific pattern of jejunal propulsive behaviour by cholecystokinin, Am. J. Physiol., 248 (1985) G470-G478.

304. West, D.B., Fey, D. and Woods, S.C., Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats, Am. J. Physiol., 246 (1984) R776-R788.

305. West, D.B., Prescod, L. and Triscari, J., Infusion of CCK-8 between meals into free-feeding rats fails to prolong the intermeal interval, Soc. Neurosci. Abstr., 11 (1985) 37.

306. White, F.M.M. and Pereira, L.H., Obesity: epidemiology and the problem of measurement, Can. J. Surg., 27 (1984) 120-123.

307. Wilbur, B.G. and Kelly, K.A., Effect of proximal gastric, complete gastric, and truncal vagotomy on canine gastric electric activity, motility, and emptying, Ann. Surg., 178 (1973) 295-303.

308. Williams, J.A., Bailey, A. and Steigerwalt, R.W., Effects of proglumide on pancreatic acinar cell function, Digestion, 27 (1983) 227-233.

309. Willis, G.L., Hansky, J. and Smith, G.C., The role of some central catecholamine systems in cholecystokinin-induced satiety, Peptides, 5 (1984) 41-46.

310. Willis, G.L., Hansky, J. and Smith, G.C., Ventricular, paraventricular and circumventricular structures involved in peptide-induced satiety, Regul. Peptides, 9 (1984) 87-99.

311. Wolfe, M.M. and McGuigan, J.E., Immunochemical characterization of gastrinlike and cholecystokininlike peptides released in dogs in response to a peptone meal, Gastroenterol., 87 (1984) 323-334.

312. Woods, S.C., West, D.B., Stein, L.J., McKay, L.D., Lotter, E.C., Porte, S.G., Kenney, N.J. and Porte Jr., D., Peptides and the control of meal size, Diabetologia, 20 (1981) 305-313.

313. Wright, J.W., Healy, T.E., Balfour, T.W. and Hardcastle, J.D., Effects of inhalation anaesthetic agents on the electrical and mechanical activity of rat duodenum, Brit. J. Anaesth., 54 (1982) 1223-1230.

314. Yaksh, T.L., Abay II, E.O. and Go, V.L.W., Studies on the location and release of cholecystokinin and vasoactive intestinal polypeptide in rat and cat spinal cord, Brain Res., 242 (1982) 279-290.

315. Yamagishi, T. and Debas, H.T., Cholecystokinin inhibits gastric emptying by acting on both proximal stomach and pylorus, Am. J. Physiol., 234 (1978) E375-E378.

316. Yamaguchi, T., Tabata, K. and Johnson, L.R., Effect of proglumide on rat pancreatic growth, Am. J. Physiol., 249 (1985) G294-G298.

317. Yanaihara, C., Suguira, N., Kashimoto, K., Kond, M., Kawamura, M., Narise, S.,Yasui, A. and Yanaihara, N., Dissociation of pancreozymin (PZ) activity from cholecystokinin (CCK) activity by N -carboxyacyl CCK-7 and CCK-8 analogues with a substituted glycine, Biomed. Res., 6 (1985) 111-115.

318. Yox, D. and Ritter, R.C., Capsaicin pretreatment attenuates CCK-induced suppression of sucrose intake during sham feeding and real feeding, Soc. Neurosci. Abstr., 11 (1985) 37.

319. Zarbin, M.A., Walmsley, J.K., Innis, R.B. and Kuhar, M.J., Cholecystokinin receptors: presence and axonal flow in the rat vagus nerve, Life Sci., 29 (1981) 697-705.

320. Zhou, Z.-Z., Eng, J., Pan, Y.-C. E., Chang, M., Hulmes, J.D., Raufman, J.-P. and Yalow, R.S., Unique cholecystokinin peptides isolated from guinea pig intestine, Peptides, 6 (1985) 337-341.