THE UNIVERSITY OF CALGARY

Adenosine, not the Endogenous Opioids, Mediates the "Regulated" Decrease in Core Temperature that Occurs in Both Newborn and Older Guinea Pigs During Acute Hypoxemia.

by

Kim Carolyn Crisanti

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ABSTRACT

In guinea pigs, acute hypoxemia produces a "regulated" decrease in core temperature, the mechanism of which is unknown. Both the opioids and adenosine have been shown to influence thermoregulation, and to increase in the interstitial and cerebrospinal fluid during periods of hypoxemia in an age dependent manner. Considering this, experiments were conducted to test two hypotheses: 1) the opioids and 2) adenosine plays an age dependent role in mediating the decrease in core temperature during hypoxemia. We studied 185 chronically instrumented guinea pigs in a thermocline during normoxemia and hypoxemia (10% O_2) following saline, 1, 2, or 4 mg/kg naloxone or 10 mg/kg aminophylline. Naloxone did not significantly alter the core temperature response to hypoxemia. Alternatively, aminophylline significantly attenuated the core temperature response to hypoxemia. Our data support the hypothesis that adenosine plays a role in mediating the core temperature response to hypoxemia, however, the response was not age-dependent.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

In 1668 Robert Boyle recognized that air was essential to life when he evacuated a chamber and noted that neither flame nor small animal could survive (Graubard, 1964). Over one hundred years later it was realized that oxygen was the component of air that was required for animal survival. The need for oxygen has been outlined in a review by Rudolph (1996) and is related to basic cellular functions such as moving ions across membranes, to mandatory functions such as myocardial contraction and the movement of the muscles used in breathing, and to the cellular processes of metabolism which are crucial for the maintenance of body temperature in homeothermic species.

1.1. HYPOXEMIA

Since the conception of the idea that it was indeed oxygen that was the essential component of air, numerous attempts have been made to demonstrate what would occur in the absence or presence of a limited amount of oxygen. Classically the term anoxemia was used for many years to describe the condition of oxygen want by the body (Barcroft, 1920). However, the term anoxemia actually means "without oxygen" and therefore the more appropriate term that should be used to describe situations where there is less than the adequate supply of oxygen is hypoxia (Van Liere and Stickney, 1963). Although not the

earliest, one of the best definitions of hypoxia has been provided by Jones (1996) in which it was defined as a condition in which the oxygen supply to the cells, tissues, organs or the whole animal is insufficient to sustain normal function.

When defining hypoxia it is important to consider the way in which the oxygen deficiency is incurred. Barcroft (1920) outlined three ways in which hypoxia may arise: 1) anoxic hypoxia (hypoxemia), 2) anemic hypoxia and, 3) stagnant hypoxia. Anoxic hypoxia or hypoxemia occurs when there is a lack of oxygen in the arterial blood and this could be caused by decreasing the fraction of inspired oxygen in the air. In contrast, anemic hypoxia occurs when there is a reduced ability of the blood to carry oxygen which could arise from a shortage of functioning hemoglobin, despite there being an adequate supply of oxygen. Stagnant or circulatory hypoxia occurs when there is a problem in efficiently delivering the oxygen which is stored in the blood to the tissues possibly due to reduced blood flow. In addition to these three, Peters and Van Slyke (1932) described a fourth way in which hypoxia may arise and termed this histotoxic hypoxia to indicate the presence of a toxic substance, such as cyanide which in effect poisons tissue cells rendering them unable to utilize the oxygen which is presented to them. Hence once again the necessary oxygen is available, but the body is just unable to make use of it.

A further classification of the types of hypoxia involves distinguishing between either acute or chronic hypoxia. Acute hypoxia is a brief, extremely

rapidly induced type of hypoxia, and is usually initiated by breathing a decreased fraction of inspired oxygen (Van Leire and Stickney, 1963). In contrast, chronic hypoxia is induced by long periods at high altitudes or repeated exposures to inadequate supplies of oxygen (Van Liere and Stickney, 1963). Acute hypoxia may be subdivided further based on the degree of hypoxia (ie. mild, moderate, or severe) to which the subject is exposed.

With these definitions in mind the remainder of this thesis will use the term hypoxemia to refer to situations in which the animals underwent a period of anoxic hypoxia incurred by breathing a decreased fraction of inspired oxygen (ie. FiO2 10%). As well, the type (ie. chronic or acute) and the level (i.e. mild, moderate or severe) of the hypoxic exposure will also be indicated.

1.2. RESPONSES TO HYPOXEMIA

1.2.1. Cardiorespiratory responses

Perhaps the most well defined response to hypoxemia is the ventilatory response to breathing a decreased fraction of inspired oxygen. It has been well established that hypoxemia produces an initial increase in ventilation. Early reports in pre and full term human infants illustrated that this initial rapid increase in ventilation was followed by a return towards or below control levels (Cross and Warner, 1951; Cross and Oppe, 1952; Rigatto et. al., 1975). This typical biphasic response has since been observed in neonates of a number of other species including rabbits (Grunstein et. al. 1981) and piglets (Moss et. al., 1987) and is commonly referred to as "hypoxic ventilatory decline" (Bisgard and Neubauer, 1995). For years it was believed that the biphasic ventilatory response to hypoxemia was a neonatal phenomenon. However, the characteristic biphasic ventilatory response has been observed to occur in adult humans (Easton et. al., 1986) and cats (Vizek et. al. 1987) as well. Therefore more recently it has been generally well accepted that hypoxemia produces a biphasic ventilatory response in most animal species irrespective of the animals age.

In essence almost shadowing the ventilatory response to hypoxemia is the heart rate response. In a series of papers Resnik (1925a; 1925b; 1925c)

described the heart rate response to hypoxemia in dogs and noted that the SA (sinoatrial) node was particularly sensitive to hypoxemia resulting in an initial increase in impulse formation which was quickly followed by a progressive slowing of the heart rate. This series of papers also illustrated that hypoxemia produced an initial increase, followed by a decrease in atrioventricular conduction time which was thought to be attributed to the decrease in heart rate. These observations in dogs were also noted by Rahn and Otis (1947) in humans when they demonstrated that the elevation of heart rate which occurs during hypoxic exposure was not sustained but rather fell towards resting levels. In a comparative study on the effects of hypoxemia on isolated perfused rat, rabbit and guinea pig hearts, Froldi and Belardinelli (1990) showed that the main effect of hypoxia was a slowing of ventricular rate which was caused by either a decrease in atrial slowing or an elongation of atrioventricular conduction time in a species dependent manner. More recently Thomas and Marshall (1994) have demonstrated a biphasic heart rate response in anesthetized rats exposed to 8% oxygen. In an almost identical pattern to the ventilatory response outlined previously, heart rate increased upon initial exposure to a decreased fraction of inspired oxygen which wanned within two to three minutes. Thus the cardiorespiratory responses to hypoxemia seem to be interdependent, both illustrating a biphasic pattern.

1.2.2. Thermoregulatory responses

1.2.2.1. Core temperature and oxygen consumption

Core temperature decreases during acute hypoxemia in both newborns and adults of a number of species including humans (Cross et. al., 1955; Mortola, 1993, Behauge et. al., 1927; Gellhorn and Janus, 1936; Kottke et. al., 1948; Hill, 1959; Gautier et. al., 1987).

This decrease in core temperature was originally thought to be the result of an accompanying decrease in oxygen consumption. Cross et. al. (1955) demonstrated a significant decrease in oxygen consumption and a significant decrease in rectal temperature in newborn humans exposed to 15% oxygen. This was also demonstrated in newborns of a number of other animal species including lambs (Acheson, 1957), puppies (Moore, 1956) and kittens (Hill, 1959). Alternatively, oxygen consumption in adult animals including humans has been shown to be maintained during a modest decrease in oxygen availability and only decreased during severe hypoxemia (Dempsey and Forster, 1962). As a result of the overwhelming evidence of a decrease in oxygen consumption that accompanied the decrease in core temperature in newborn animals during hypoxemia, much like the biphasic ventilatory response, the decrease in oxygen consumption during hypoxemia was at first thought to principally occur in neonates.

The decrease in oxygen consumption was soon realized to be a phenomenon of resting metabolic rate prior to exposure to hypoxemia rather than a newborn characteristic. This was achieved in part by the work of McCance and Widdowson (1957) who demonstrated that small adult rodents (ie. mice and rats) had a similar decrease in oxygen consumption during hypoxemia as newborns. As well in 1959, in an experiment that compared the effects of acute hypoxemia (10% O_2) in newborn kittens and adult guinea pigs, Hill showed that a reduction in oxygen consumption occurred in both newborns and adults. Thus, it was concluded that the decrease in oxygen consumption that occurred during acute moderate hypoxemia was not a newborn phenomenon, but rather it was dependent on the size of the animal and its resting metabolic rate (Hill, 1959). This early work is supported by more recent work by Frappell *et. al.* (1992) who noted that the decrease in oxygen consumption during hypoxemia was greater the higher the resting metabolic rate of the animal. Therefore, if an animal has a low resting metabolic rate no change in oxygen consumption may occur during acute moderate hypoxemia. This was demonstrated by Blatteis (1964) in newborn rabbits who showed the characteristic decrease in core temperature without a concomitant decrease in oxygen consumption.

A decrease in core temperature during hypoxemia would be a logical solution to a limited oxygen supply. In fact, it has been widely demonstrated over the past 50 years that hypothermia increases survival rates during hypoxemia in a number of species including rats (Davidovic and Wesley, 1959),

mice (Adolph and Goldstein, 1959; Kottke et.al., 1948), puppies (Miller and Miller, 1961) and newborn guinea pigs (Miller, 1949; Miller et. al. 1954).

Exactly how hypothermia increases survival rates during acute moderate hypoxemia is unknown. However, what is known, is that decreasing core temperature has an effect on the oxygen hemoglobin dissociation curve and hence oxygen transfer to the tissues. Brown and Hill (1923) demonstrated that decreasing temperature causes a shift of the oxygen hemoglobin dissociation curve to the left, thus causing an increase in percent hemoglobin saturation at the same partial pressure of oxygen. This leftward shift is notable, but perhaps more important is that the decrease in core temperature during acute moderate hypoxemia prevents or minimizes the rightward shift of the oxygen hemoglobin dissociation curve that would normally occur during hypoxemia due to a lowering pH. As a result the blood maintains a higher total oxygen level despite a low partial pressure of oxygen (Carlsson et. al., 1976). Thus the shift of the oxygen hemoglobin dissociation curve to the left, or more appropriately the prevention of the rightward shift that occurs with a decrease in core temperature during hypoxemia, would be beneficial in maintaining oxygen supply.

Decreasing core temperature is also known to have an effect on oxygen consumption in resting animals (Krogh, 1914), such that a 1°C decrease in core temperature causes an 11% reduction in metabolic rate (Dupre et. al., 1988). Because of this, hypothermia would be beneficial to an animal exposed to a limited amount of oxygen because it would slow the metabolic rate. As alluded

to previously, oxygen consumption has indeed been demonstrated to either stay unchanged or decrease during hypoxemia, and a low oxygen consumption during hypoxemia would maintain low oxygen demands. Therefore, a decrease in core temperature during acute moderate hypoxemia would be protective because it may represent a practical solution to the problem of matching oxygen supply to oxygen demand during periods of limited oxygen availability (Dupre et. al, 1988).

1.2.2.2. Selected ambient temperature

Homeotherms employ both autonomic (ie. metabolism, cutaneous blood flow, evaporation, and piloerection) and behavioural mechanisms to maintain a stable body core temperature around a set point. The IUPS (International Union of Physiological Sciences, Committee on Thermal Physiology) (1987) defined set point as the value of a regulated variable which a healthy organism tends to stabilize by the process of regulation. Gordon (1993) tailored the above definition to thermoregulation, stating that "set point" is a threshold temperature such that when core temperature deviates from this point a corrective behavioural and autonomic response will be elicited.

Five thermoregulatory states have been outlined by Gordon (1983): 1) normothermia, 2) forced hypothermia, 3) regulated hypothermia, 4) forced

hyperthermia, and 5) regulated hyperthermia. During normothermia skin blood flow is the principle mechanism controlling core temperature as autonomic and behavioural responses are at a minimum since core temperature and set point approximate one another. In forced hypothermia, the core temperature is below the set point and as a result the animal employs both autonomic and behavioural mechanisms to bring core temperature towards set point. In contrast, in a condition of forced hyperthermia the core temperature is above set point and the animal would use both autonomic and behavioural means to reduce core temperature. Regulated hypothermia is a thermoregulatory condition in which the set point is below the core temperature and hence the animal uses both autonomic and behavioural methods to initiate and enhance heat loss. The opposite would occur in a state of regulated hyperthermia when the set point is above the core temperature.

The above thermoregulatory states may be summarized as either "forced" (ie., core temperature is forced by internal or external conditions to deviate from the central nervous system set point temperature) or "regulated" (ie., core temperature follows a change in the central nervous system set point temperature) thermoregulatory responses. Investigators have attempted to classify alterations in core temperature as either forced or regulated thermoregulatory responses in order to determine if the deviation in core temperature is a result of a change in set point and thus centrally mediated such as during pregnancy in rats, or whether the deviation in core temperature is one

that has in essence been forced upon the animal, such as during lactation in rats (Eliason and Fewell, 1997).

Behavioural thermoregulation, a response that involves both positional and postural changes (Hicks and Wood, 1985) is employed by poikiolothermic animals such as lizards to control body core temperature. In a landmark study, Hicks and Wood (1985) demonstrated that lizards exposed to a hypoxic gas mixture would decrease their core temperature and this was facilitated by the animals moving to a cooler ambient temperature. In summary, the animals enlisted behavioural means to enhance heat loss, instead of attempting to correct for the decrease in core temperature that occurred during hypoxemia. Therefore, according to the definitions outlined above the decrease in core temperature during hypoxemia in lizards is a "regulated" rather than a "forced" thermoregulatory response. A similar decrease in selected ambient temperature has been observed to accompany the decrease in core temperature during hypoxemia in a number of other species from invertebrates to rats, mice and hamsters (Wood, 1991; Dupre and Owen, 1992; Gordon and Fogelson, 1991). Given that the decrease in core temperature that occurs during acute moderate hypoxemia has been demonstrated to be a "regulated" thermoregulatory response, it may be considered to be an example of "rheostasis" (Mrosovsky, 1990) -- or regulation around a shifted set point -- rather than a failure of "homeostasis" (Cannon, 1929).

Recently Clark and Fewell (1996) have shown that the decrease in core temperature during acute moderate hypoxemia in both newborn and older guinea pigs also results from a "regulated" rather than a "forced" thermoregulatory response. Of particular interest, is that newborn but not older guinea pigs actually selected a cooler ambient temperature during acute moderate hypoxemia which would aid heat loss and facilitate the decrease in core temperature that was observed. Despite the behavioural responses of the newborn animals, the decrease in core temperature that occurred during acute moderate hypoxemia was accentuated in the older verses the newborn guinea pigs (Clark and Fewell, 1996). This provides evidence that there may be a postnatal maturation influence on the thermoregulatory responses to acute moderate hypoxemia in guinea pigs.

1.3. MECHANISMS OF THE "REGULATED" DECREASE IN CORE TEMPERATURE

Several mechanisms have been proposed for the "regulated" decrease in core temperature that occurs during acute hypoxemia including 1) a direct effect of hypoxemia on the thermosensitive neurons in the preoptic area of the anterior hypothalamus (Tamaki and Nakayama, 1987), 2) a possible role of the cerebral cortex (Shibata et. al., 1985), as well as 3) the release of various neurotransmitters and/or neuromodulators in the central nervous system that influence thermoregulation [e.g. arginine vasopressin (Wood, 1991), adenosine (Laudignon et. al., 1991; Wager-Srdar et. al., 1983; Van Wylen et. al., 1986; Winn et. al., 1981), histamine (Brezenoff and Lomax, 1970; Sudhakaran et. al., 1979) and the endogenous opioids (Mayfield et. al., 1994)].

Tamaki and Nakayama (1987) have shown that under hypoxemic conditions certain thermosensitive neurons in the preoptic area of the anterior hypothalamus increase their activity. The warm sensitive neurons increased their firing rate which would in effect stimulate both autonomic and behavioural effectors responsible for heat dissipation, resulting in heat loss and a decrease in core temperature. Therefore, if hypoxemia directly stimulates or inhibits the firing rate of temperature sensitive neurons in the preoptic area of the anterior hypothalamus it would eliminate the role of a mediator in producing the

"regulated" decrease in core temperature that occurs during acute moderate hypoxemia.

In addition to the preoptic area of the anterior hypothalamus, other brain regions have been implicated in the role of decreasing core temperature during acute moderate hypoxemia. Shibata et. al. (1985) demonstrated that electrical stimulation of the sulcal prefrontal cortex of the rat brain increased the firing rate of warm sensitive neurons in the preoptic area of the anterior hypothalamus. As discussed earlier, an increase in the firing rate of warm sensitive neurons would stimulate effector mechanisms that would result in heat loss and a decrease in core temperature. Therefore, if the prefrontal cortex is stimulated by hypoxemia, this could in turn activate the warm sensitive neurons, enhance heat loss, and decrease core temperature. However, experiments in our laboratory have shown that in rats an intact cerebral cortex does not appear to be an important factor in mediating the core temperature response to hypoxemia (Rollins and Fewell, 1997).

If hypoxemia is not having a direct effect on neurons within the preoptic area of the anterior hypothalamus (ie. the central site of temperature regulation) then a neurotransmitter and/or neuromodulator may mediate the "regulated" decrease in core temperature during acute moderate hypoxemia.

Upon exposure to a decreased fraction of inspired oxygen there is an elevation in plasma and cerebrospinal arginine vasopressin levels in rats and dogs (Forsling and Aziz, 1983; Forsling and Ullmann, 1974). Arginine

vasopressin is an endogenous antipyretic peptide in mammals that has an effect on thermoregulatory control (Naylor et. al, 1986). Due to the increase in arginine vasopressin during hypoxemia and its role in thermoregulation, Wood (1991) has suggested that this peptide may be involved in mediating the "regulated' decrease in core temperature in reptiles which rely on behavioural thermoregulation to control their core temperature. However, previous work by Clark and Fewell (1994) with Brattleboro rats - which are deficient in central arginine vasopressin - held at a constant ambient temperature, has shown that there was no attenuation of the core temperature response and in some instances this decrease was accentuated (Clark and Fewell, 1994). Although the results may have been different if the animals were allowed to behaviourally thermoregulate, it is logical to speculate that at least in rats, arginine vasopressin does not seem to play a role in mediating the "regulated" decrease in core temperature that occurs during acute moderate hypoxemia.

Histamine is another peptide with hypothermic thermoregulatory effects (Brezenoff and Lomax, 1970) which has also been shown to increase in plasma during periods of hypoxemia (Sudhakaran et. al., 1979). Therefore, it is possible that the "regulated" decrease in core temperature that occurs during acute moderate hypoxemia may be due to the central release of histamine. Despite this obvious relationship, the role of histamine as a mediator of this response remains to be investigated.

Many recent investigations have provided evidence that the endogenous opioids are involved in thermoregulation. Experiments carried out on adult rats in a thermocline by Spencer et. al. (1990) have shown that intracerebroventricular administration of a specific mu receptor agonist (DAMGO) resulted in a "regulated" increase in core temperature, whereas administration of a specific kappa receptor agonist (U-50,488H) and a specific delta receptor agonist (DPDPE) produced a "regulated" decrease in core temperature. As well, Adler et. al. (1991) have demonstrated that in adult guinea pigs, where there is an abundance of kappa receptors located centrally (Robson et. al., 1985), a subcutaneous injection of the specific kappa receptor agonist U-50,488H resulted in a prolonged "regulated" hypothermia.

In addition to a thermoregulatory role, an endogenous opioid, β-endorphin has been shown to be released into the plasma and cerebrospinal fluid of piglets during hypoxemia. Moss and Inman (1989) have shown a greater release of β-endorphin into the plasma and cerebrospinal fluid of young piglets during both normoxemia and hypoxemia in comparison to older piglets. This increase in endogenous opioids during acute moderate hypoxemia has led investigators to determine whether the endogenous opioids are involved in the characteristic biphasic ventilatory response to hypoxemia. Grunstein et. al. (1981) illustrated a role for endogenous opioids in the biphasic ventilatory response to hypoxemia in newborn rabbits. As well, DeBoeck et. al., (1984) demonstrated that in a healthy full-term human infant, the decrease in ventilation that normally occurs during

hypoxemia was significantly less following an injection of a general opioid antagonist when compared to the response following an injection of saline. This would suggest a role for the endogenous opioids in the ventilatory response to acute moderate hypoxemia.

Given that the endogenous opioids seem to have a thermoregulatory role, a ventilatory role, and have been shown to be released during periods of stress; such as stress-induced hyperthermia (Blasig et. al., 1978) and under hypoxemic conditions (Moss and Inman, 1989); it is not surprising that Mayfield et. al. (1994) and the preliminary results of Young and Malvin (1996) have provided evidence that the endogenous opioids participate in mediating the decrease in core temperature following acute severe hypoxemia (FiO_2 0.045) in adult mice, and during acute moderate hypoxemia (FiO_2 0.11) in adult rats, respectively.

The purine nucleoside, adenosine, has been shown to influence thermoregulation both in the central nervous system (Yarbrough and McGuffin-Clineschmidt, 1981; Anderson et. al., 1994) and peripherally, by inhibiting nonshivering thermogenesis in brown adipose tissue (Vernon et. al., 1991; Bruns, 1990). In a similar fashion to the endogenous opioids, adenosine levels in brain interstitial fluid have been shown to increase during acute moderate hypoxemia in adult rats (Winn et. al., 1981; Phillis et. al., 1987) and in newborn piglets (Park et. al., 1987). In addition, Laudignon et. al. (1991) have provided evidence of an age-dependent release of adenosine into the cerebrospinal fluid of piglets during both normoxemic and hypoxemic conditions. Darnall (1985)

has shown that pretreatment with aminophylline (an adenosine antagonist) decreased the amount of ventilatory depression during acute moderate hypoxemia in piglets by preventing a decrease in respiratory frequency. Despite the evidence that 1) adenosine may play a role in the biphasic ventilatory response to hypoxemia, 2) the demonstrated increase in adenosine during hypoxemia, and 3) it's thermoregulatory influence, the role of adenosine as the mediator of the "regulated" decrease in core temperature during acute moderate hypoxemia remains to be investigated.

1.4. HYPOTHESES

Given the role of both the endogenous opioids and the purine nucleoside adenosine on thermoregulation and their release into the interstitial and cerebrospinal fluid during acute moderate hypoxemia in an age-dependent manner, experiments were conducted to test two separate hypotheses.

1.4.1. Endogenous Opioids Hypothesis

The endogenous opioids play an age-dependent role in mediating the "regulated" decrease in core temperature that occurs in guinea pigs during exposure to acute moderate hypoxemia.

1.4.2. Adenosine Hypothesis

Adenosine plays an age-dependent role in mediating the "regulated" decrease in core temperature that occurs in guinea pigs during exposure to acute moderate hypoxemia.

MATERIALS AND METHODS

2.1. GUINEA PIGS

One hundred and eighty five Hartley strain guinea pigs were studied between both 5 to 10 days of age, and 25 to 30 days of age. Each pup, born by spontaneous vaginal delivery, was housed with its mother and siblings in the animal health care facility at an environmental temperature of 22 ± 1°C , 20-30% relative humidity and a 12:12 light / dark cycle. Although 22 ± 1°C is below the thermoneutral zone of a newborn guinea pig (Hull, 1973), each pup was able to modify its environmental temperature by huddling with its siblings and mother (i.e. behavioural thermoregulation).

Most guinea pig sows were obtained from Charles Rivers Laboratories (Quebec, Canada) as nonpregnant virgins which were subsequently bred within the animal health care facility. The remaining guinea pig sows were obtained from Charles Rivers Laboratories (Quebec, Canada) between 40 to 45 days of gestation. The guinea pig gestation period is between 62 to 74 days depending on litter number [ie. the greater the number of pups in a litter the shorter the gestation period (Goy et. al., 1957; Sisk, 1976)]. All sows were housed in the animal health care facility at the University of Calgary under the constant conditions outlined above. As the due date approached, the pregnant sows were checked repeatedly, and upon delivery of the pups the date of birth and the

number of pups in the litter were recorded. If the litter was born after 1700 hours, the date of birth was recorded as the next day and this was held constant throughout the collection of the animals. Each guinea pig pup was allowed to adjust to its "new" surroundings for at least one day before undergoing surgical preparation.

2.2. SURGICAL PROCEDURES

Each guinea pig underwent one operation prior to experimentation. Each pup was anesthetized by inhalation of halothane (2% for induction and maintenance) in oxygen. The guinea pig was prepped for surgery by shaving the abdominal wall and right scapular region, and then wiping the shaved area with a savlon solution followed by a Povidone/Iodine Scrub solution for disinfection purposes. Once the animal no longer demonstrated a paw withdrawal reflex it was considered to be in the surgical plane of anesthesia and suitable for surgery.

A midline incision was done so as to perform a paramedian laporotomy for insertion of a battery operated biotelemetry device (PhysioTel TA10ETA - F20, Data Sciences International, St. Paul MN) into the peritoneal cavity for later measurement of core temperature. One of the electrocardiogram (ECG) leads was tunneled subcutaneously through to the right scapular region and sutured in place by two stay sutures (4.0 silk on a cutting needle; Johnson and Johnson, Peterborough, Ontario) and the second ECG lead was inserted into the peritoneal cavity for later measurement of heart rate.

The wound was then sutured closed using an interrupted blanket stitch for both the abdominal muscle wall (4.0 or 3.0 silk on a taper needle; Johnson and Johnson; Peterborough, Ontario) and the skin (4.0 silk on a cutting needle; Johnson and Johnson; Peterborough, Ontario). Prior to closing the skin the

muscle layer was sprayed with an antibiotic solution (Gentocin; Schering Canada Inc.; Pointe - Claire, Quebec), and following closure of the abdominal skin, the area was covered with a spray adhesive bandage (New-skin; Medtech Labs, Inc.; Jackson, WY) to provide further protection from infection.

After surgery the pups were allowed to recover in a designated recovery area within the laboratory, then they were returned to their mother and siblings to allow for a full recovery from the surgical procedures. Each animal underwent several post - operative examinations to ensure a proper recovery.

All surgical procedures were carried out in accordance with the "Guide to the Care and Use of Experimental Animals" provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

2.3. EXPERIMENTAL APPARATUS

A thermocline has been used as a measure of behavioural thermoregulatory effector mechanisms. Gordon (1993) has divided behavioural thermoregulatory effector mechansims into either natural (ie. can be observed in natural conditions) or instrumental (ie. requires equipment for the observation) behaviour. Linear temperature gradients such as the thermocline are useful for recording instrumental behaviour. The thermocline used in these experiments consisted of a sealed plexiglass cylinder 200 centimeters in length and an internal diameter of 11.5 centimeters with a plastic grid along the bottom (Figure 2.1.). The thermocline was equipped with both an air inflow and outflow port which allowed the appropriate gas mixture to flow into the thermocline and at the same time allowed for gas outflow so as to determine oxygen consumption. A linear temperature gradient which was set from 10 °C to 40 °C in these experiments was produced by circulating hot and cold water (Neslab- Endocal Refrigerated Circulating Bath RTE - 8DD, Newington, NH) through two copper coils which were wrapped around the cylinder. Twenty positions were marked on the thermocline at 10 centimeter intervals, which corresponded to a measured ambient temperature as determined by sliding a thermocouple through the gradient and recording the temperature that was displayed on the Sensortek (Model BAT-12; Clifton, NJ). Gas of the desired oxygen concentration flowed through the thermocline at a constant flow rate of 1.412

L/min for the normoxic gas mixture (room air, 21%) and 1.490 L/min for the hypoxic gas mixture (10% oxygen in balance nitrogen) as determined by a Stead-wells Spirometer (Braintree, Mass.).

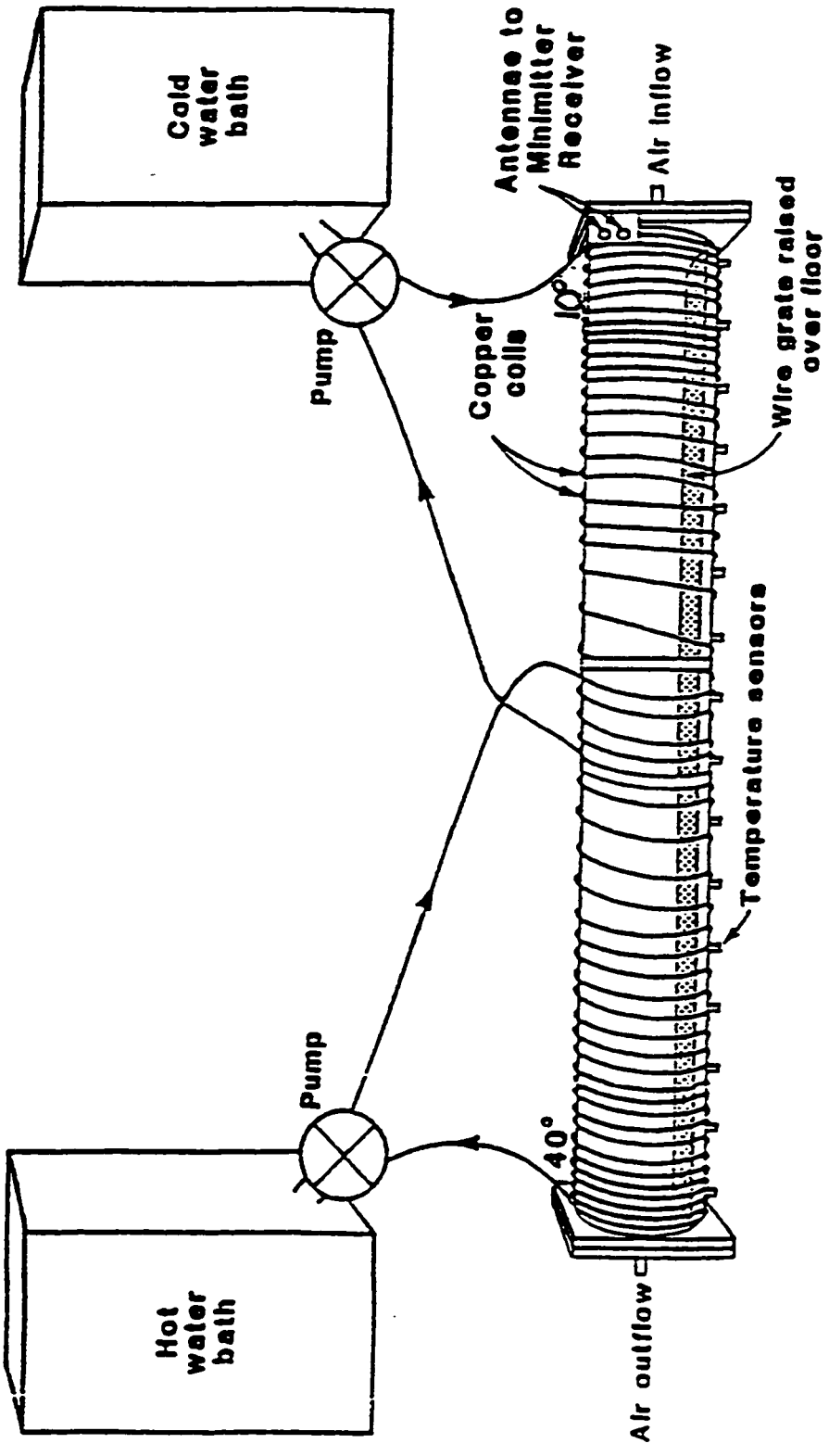

Figure 2.1. A schematic drawing of a thermocline

2.4. EXPERIMENTAL PROTOCOLS

2.4.1. Endogenous Opioids Experiments

At least 2 to 3 days were allowed to lapse between surgery and an experiment. For an experiment, each pup was removed from its mother and siblings, weighed and placed in the thermocline into which flowed room air (21% oxygen) for a period of one to two hours and allowed to select its preferred ambient temperature. At the end of this stabilization period measurements were made during a control period. A period of five consecutive measurements at two minute intervals in which core temperature did not vary more than ±0.2 °C was considered to be a suitable control period.

Following control measurements, the guinea pig was removed from the thermocline and given an intraperitoneal (i.p.) injection of naloxone hydrochloride (Narcan, Dupont Pharma; Mississauga, Ontario) at doses of 1, 2 or 4 mg per kilogram of body weight or an equal volume of vehicle (physiological saline) (Anpharm Inc.; Toronto, Ontario). The animal was then promptly returned to the thermocline and monitored for an additional 30 minutes.

Each guinea pig then underwent a 60 minute experimental period of exposure to either normoxemia (21% O_2) or to acute moderate hypoxemia (10% O_2). The experimental period was preceded by a 10 minute flush of the thermocline with the appropriate gas mixture at approximately 6 times the normal

flow rate (ie. 8.7 L/min for the normoxic gas mixture [21% O_2] and 8.4 L/min for the hypoxic gas mixture [10% O_2]) to ensure equilibration of the gas mixture throughout the thermocline. Following the experimental period each animal underwent a 60 minute recovery period of normoxemia (21% O_2). Similarly the recovery period was also preceded by a 10 minute flush period with the normoxic gas as described previously to ensure that normoxemic conditions were reinstated. Dependent variables (ie. core temperature, selected ambient temperature, oxygen consumption, respiratory rate, and heart rate) were recorded at six minute intervals during both the experimental and recovery periods.

One hundred and twenty Hartley strain guinea pigs were studied between both 5 to 10 days of age and 25 to 30 days of age to test the hypothesis that the endogenous opioids play an age specific role in mediating the "regulated" decrease in core temperature that occurs during acute moderate hypoxemia in guinea pigs. A newborn group of 59 guinea pigs was studied between 5 to 10 days of age, and an older group of 61 guinea pigs was studied between 25 to 30 days of age. The animals in each age group were randomly allocated to one of eight experimental groups. Experimental group I received an intraperitoneal (i.p.) injection of vehicle after the control period and experienced normoxemia during the experimental period (newborn n=5; older n=8); experimental group II received an i.p. injection of vehicle after the control period and experienced hypoxemia during the experimental period (newborn n=8; older n=8);

experimental group III received an i.p. injection of naloxone (1 mg/kg) after the control period and experienced normoxemia during the experimental period (newborn n=5; older n=8); experimental group IV received an i.p. injection of naloxone (1 mg/kg) after the control period and experienced hypoxemia during the experimental period (newborn n=9; older n= 8); experimental group V received an i.p. injection of naloxone (2 mg/kg) after the control period and experienced normoxemia during the experimental period (newborn n=8; older n=8); experimental group VI received an i.p. injection of naloxone (2 mg/kg) after the control period and experienced hypoxemia during the experimental period (newborn n=8; older n=8); experimental group VII received an i.p. injection of naloxone (4 mg/kg) after the control period and experienced normoxemia during the experimental period (newborn n=8; older n=7); and experimental group VIII received an i.p. injection of naloxone (4 mg/kg) after the control period and experienced hypoxemia during the experimental period (newborn n=8; older n=6).

Following an experimental procedure each guinea pig was euthanized by inhalation of carbon dioxide and the biotelemetry device was retrieved.

All experimental procedures were carried out in accordance with the "Guide to the Care and Use of Experimental Animals" provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

2.4.2. Adenosine Experiments

2.4.2.1. Aminophylline Dosing Experiments

In order to determine the appropriate dose of the adenosine antagonist aminophylline that was to be used in the experiments that are described in section 2.4.2.2., preliminary experiments were carried out to determine the plasma concentrations of theophylline in newborn and older guinea pigs following an intraperitoneal (i.p.) injection of 5, 10 or 15 mg/kg of aminophylline at 30, 60 and 90 minute intervals.

For an experiment each guinea pig was given an intraperitoneal (i.p.) injection of aminophylline at a dose of 5, 10, or 15 mg per kilogram of body weight. At 30, 60, or 90 minutes following the injection of aminophylline each guinea pig underwent a cardiac puncture to obtain a sufficient blood sample. Following the cardiac puncture procedure each guinea pig was euthanized by inhalation of carbon dioxide.

Thirty six Hartley strain guinea pigs were studied between both 5 to 10 days of age and 25 to 30 days of age. A newborn group of 18 guinea pigs was utilized between 6 to 9 days of age, and an older group of 18 guinea pigs was utilized between 25 to 30 days of age. The animals in each age group were randomly assigned to one of nine experimental groups. Experimental group I received an intraperitoneal (i.p.) injection of aminophylline (5 mg/kg) and

underwent a cardiac puncture 30 minutes after the injection (newborn n=2; older n=2); experimental group II received an i.p. injection of aminophylline (5 mg/kg) and underwent a cardiac puncture 60 minutes after the injection (newborn n=2; older n=2); experimental group III received an i.p. injection of aminophylline (5 mg/kg) and underwent a cardiac puncture 90 minutes after the injection (newborn n=2; older n=2); experimental group IV received an i.p. injection of aminophylline (10 mg/kg) and underwent a cardiac puncture 30 minutes after the injection (newborn n=2; older n=2); experimental group V received an i.p. injection of aminophylline (10 mg/kg) and underwent a cardiac puncture 60 minutes after the injection (newborn n=2; older n=2); experimental group VI received an i.p. injection of aminophylline (10 mg/kg) and underwent a cardiac puncture 90 minutes after the injection (newborn n=2; older n=2); experimental group VII received an i.p. injection of aminophylline (15 mg/kg) and underwent a cardiac puncture 30 minutes after the injection (newborn n=2; older n=2); experimental group VIII received an i.p. injection of aminophylline (15 mg/kg) and underwent a cardiac puncture 60 minutes after the injection (newborn n=2; older n=2); and experimental group IX received an i.p. injection of aminophylline (15 mg/kg) and underwent a cardiac puncture 90 minutes after the injection (newborn n=2; older n=2).

2.4.2.1.2. Cardiac Puncture Procedure

For a cardiac puncture each guinea pig was anesthetized by inhalation of halothane (2% for induction and maintenance) in oxygen. Once the animal no longer demonstrated a paw withdrawal reflex it was considered suitable for a cardiac puncture. A 10 mL heparinized syringe with a 23 gauge; 3/4" needle attatched to it was used for blood removal. Once blood was obtained it was transferred to a conical centrifuge tube (Falcon, Becton Dickson, Franklin Lakes NJ, USA) and centrifuged to obtain plasma samples. The plasma was removed and stored at -70 °C until theophylline plasma concentrations were determined.

All experimental procedures were carried out in accordance with the "Guide to the Care and Use of Experimental Animals" provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

2.4.2.1.3. Determination of Theophylline Plasma Concentrations

Theophylline levels were determined by the Applied Chemistry Laboratory at the Calgary Foothills Hospital by the COBAS FARA II chemistry system (Roche) using the principle of fluorescence polarization. This technique measures the binding of fluorescein - labeled drug (tracer) to specific antibodies, as first outlined by Dandliker and Feigen (1961).

2.4.2.2. Adenosine Thermocline Experiments

At least 2 to 3 days were allowed to lapse between surgery and an experiment. For an experiment, each pup was removed from its mother and siblings, weighed and placed in a thermocline into which flowed room air (21% oxygen) for a period of one to two hours and allowed to select its preferred ambient temperature. At the end of this stabilization period measurements were made during a control period. A period of five consecutive measurements at two minute intervals in which core temperature did not vary more than ± 0.2 °C was considered to be a suitable control period.

Following control measurements, the guinea pig was removed from the thermocline and given an intraperitoneal (i.p.) injection of Aminophylline Anhydrous (85% theophylline: 15% ethylenediamine) (U.S.P., Sel-win Chemicals Ltd., Vancouver, BC) in saline at a dose of 10 mg per kilogram of body weight. Control groups of guinea pigs which would have received an i.p. injection of an equal volume of vehicle (physiological saline) were not repeated as they were identical to the control group of animals which were described and utilized in section 2.4.1. Hence, it was thought beneficial to reduce animal number as the animals that received an i.p. injection of vehicle were suitable controls in both experimental protocols. The animal which was given an i.p. injection of aminophylline was then promptly returned to the thermocline and monitored for an additional 30 minutes.

Each animal then underwent a 60 minute experimental period of exposure to either normoxemia (21% O_2) or to acute moderate hypoxemia (10% O_2). The experimental period was preceded by a 10 minute flush of the thermocline with the appropriate gas mixture at approximately 6 times the normal flow rate (ie. 8.7 L/min for the normoxic gas mixture [21% O_2] and 8.4 L/min for the hypoxic gas mixture [10% O_2]) to ensure equilibration of the gas mixture throughout the thermocline. Following the experimental period each animal underwent a 60 minute recovery period of normoxemia (21% O_2). Similarly the recovery period was also preceded by a 10 minute flush period with the normoxic gas mixture as described previously to ensure that normoxemic conditions were reinstated. Dependent variables (ie. core temperature, selected ambient temperature, oxygen consumption, respiratory rate, and heart rate) were recorded at six minute intervals during both the experimental and recovery periods.

Twenty nine Hartley strain guinea pigs were studied between both 5 to 10 days of age and between 25 to 30 days of age to test the hypothesis that adenosine plays an age specific role in mediating the "regulated" decrease in core temperature that occurs during acute moderate hypoxemia in guinea pigs. As was mentioned previously the control group utilized in this experimental protocol was completed in section 2.4.1. and consisted of an additional 29 animals within the same age groups. A newborn group of 16 guinea pigs was studied between 5 and 10 days of age, and an older group of 13 guinea pigs was studied between 25 to 30 days of age. The animals in each age group were

randomly assigned to one of two experimental groups. Experimental group III received an i.p. injection of aminophylline (10 mg/kg) after the control period and experienced normoxemia during the experimental period (newborn n=8; older n=7); and experimental group IV received an i.p. injection of aminophylline (10 mg/kg) after the control period and experienced hypoxemia during the experimental period (newborn n=8; older n=6).

Following an experimental procedure each guinea pig was euthanized by inhalation of carbon dioxide and the biotelemetry device was retrieved.

All experimental procedures were carried out in accordance with the "Guide to the Care and Use of Experimental Animals" provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

2.5. DETERMINATION OF DEPENDENT VARIABLES

2.5.1. Core Temperature

For measurement of core temperature platform antennae (PhysioTel CTR 86; Data Sciences International; St. Paul, MN) which received output frequency (Hz) from the previously implanted biotelemetry device were placed underneath the thermocline. The received output was then fed into a peripheral processor (Dataquest III, Data Sciences International; St. Paul, MN) which was connected to an IBM computer.

2.5.2. Selected Ambient Temperature

For measurement of selected ambient temperature the position of the guinea pig in the thermocline was determined. The position was recorded and following the experiment it was correlated to an ambient temperature between 10 °C and 40 °C.

2.5.3. Oxygen Consumption

Oxygen consumption was calculated by the difference between the inflow and outflow (dry) oxygen concentration (as a decimal) (Ametek - Applied Electrochemistry S - 3A/IO2 Analyzer; Pittsburgh, PA), the flow rate (mL/min) and the mass of the guinea pig in grams. The following calculation was utilized to achieve measurements of oxygen consumption in the units (mL/min/kg):

$$(\text{inflow } O_2 - \text{outflow } O_2) \times \text{Flow rate (mL/min)} \times \frac{1}{\text{mass}} \times \frac{1000\ g}{1\ kg}$$

2.5.4. Respiratory Rate

For measurement of respiratory rate, respiratory frequency was visually counted by watching the thorax of the guinea pig for a 15 second interval. The value obtained for the 15 second interval was then multiplied by four to determine the respiratory rate in breaths per minute.

2.5.5. Heart Rate

For measurement of heart rate the platform antennae which were placed underneath the thermocline were interfaced with a Grass recorder (Model 7E Polygraph, Grass Instruments Co., Quincy, Mass.), and an electrocardiogram (ECG) signal was recorded on paper at a speed of 25 mm/sec. Following the experiment the ECG tracing was divided into 15 second increments and the ECG peaks were counted. This value was subsequently multiplied by four to determine the heart rate in beats per minute.

2.6. STATISTICAL ANALYSIS

Statistical analysis was conducted in a series of steps to determine: 1) the effect of hypoxemia on core temperature, selected ambient temperature, oxygen consumption, respiratory rate, and heart rate in both newborn and older guinea pigs and, 2) whether the prior administration of naloxone or aminophylline significantly altered the response of any of the dependent variables in compared to that which was observed following the administration of vehicle.

A four - factor analysis of variance (ANOVA) for repeated measures was carried out on raw data to determine if age, gas, drug or time significantly affected core temperature, selected ambient temperature, oxygen consumption, respiratory rate, or heart rate. A p value of <0.05 was taken to be of statistical significance and where statistical analyses revealed significant differences a Newman Keul's multiple comparison post hoc analysis test was performed to determine where the differences from the control values occurred.

Following the analysis on the raw data, statistical analyses was carried out on mean changes from control values. Once again a four - factor analysis of variance (ANOVA) for repeated measures was completed to determine if age, gas, drug or time significantly influenced core temperature, selected ambient temperature, oxygen consumption, respiratory rate or heart rate. Where statistical analyses revealed a significant difference (ie. $p<0.05$) a Newman Keul's multiple comparison post hoc analysis test was performed to determine if

the response observed with vehicle was significantly different than that observed following an i.p. injection of either 1, 2, or 4 mg/kg of naloxone or 10 mg/kg of aminophylline.

The final series of statistical analyses involved the summary statistic of core temperature indexes. Core temperature index is a numerical description of the area under the mean change from control in core temperature curve. This was determined by summing the change in core temperature over the experimental period and then dividing this value by the product of the length of the experimental period (ie. 1 hour) and the number of the measurements taken during this period (ie. 10 measurements). A schematic representation of determination of core temperature index is provided in Appendix 1.

Two separate sets of statistical analyses were conducted on the core temperature indexes. The first series was carried out using a two way analysis of variance (ANOVA) to determine if age and drug had a significant effect on core temperature indexes during the experimental period of both normoxemia and hypoxemia. The second and final statistical analysis was carried out using a three - factor analysis of variance (ANOVOA) to determine if age, drug, or gas had a significant effect on core temperature index. Where statistical analysis revealed significant differences (ie. $p<0.05$) a Newman Keul's multiple comparison post hoc analysis test was conducted to determine where the differences occurred.

All results are reported as means ± 1 standard deviation and as indicated previously a p value of < 0.05 was taken to be of statistical significance throughout.

RESULTS

3.1. THE EFFECT OF HYPOXEMIA

3.1.1. Thermoregulatory Variables

With vehicle, core temperature decreased significantly during acute hypoxemia in both newborn and older guinea pigs (Figure 3.1.1.). Contrary to previous findings in our laboratory (Clark and Fewell, 1996) there was no significant decrease in selected ambient temperature during acute moderate hypoxemia in either newborn or older animals following an intraperitoneal injection of vehicle (Figure 3.1.2.). Oxygen consumption did not change significantly with vehicle during acute moderate hypoxemia in either age group of animals (Figure 3.1.3.).

3.1.2. Cardiorespiratory Variables

Respiratory rate increased significantly in both the newborn and older guinea pigs during acute moderate hypoxemia that quickly returned toward control levels following an intraperitoneal injection of vehicle (Figure 3.1.4.). With vehicle, heart rate decreased significantly during acute moderate hypoxemia in the newborn but not in the older guinea pigs (Figure 3.1.5.).

3.2. ENDOGENOUS OPIOIDS

3.2.1. Thermoregulatory Variables

The core temperature response to acute moderate hypoxemia was not significantly altered by the prior administration of either 1, 2, or 4 mg/kg of naloxone hydrochloride as compared to the core temperature response observed following the administration of vehicle (Figures 3.2.1. & 3.2.2.). An intraperitoneal (i.p.) injection of either vehicle or 1, 2 or 4 mg/kg naloxone hydrochloride had no effect on baseline core temperature during normoxemia in both newborn and older animals (Table 3.2.1). The summary statistic of core temperature index did not differ significantly in either age group when the animals received either vehicle or 1, 2, or 4 mg/kg of naloxone hydrochloride and experienced normoxemia during the experimental period. Similarly the core temperature indexes did not differ significantly in either age group when the guinea pigs received either vehicle or 1, 2, or 4 mg/kg of naloxone hydrochloride and experienced hypoxemia during the experimental period (Figure 3.2.3). In all instances the core temperature indexes obtained during hypoxemia were significantly different from those which were obtained during normoxemia.

The selected ambient temperature response to acute moderate hypoxemia was not significantly altered by the prior administration of either 1, 2,

or 4 mg/kg of naloxone hydrochloride as compared to the response which was observed following the administration of vehicle (Figures 3.2.4. & 3.2.5.). Administration of either vehicle or any dose of naloxone hydrochloride had no significant effect on selected ambient temperature during normoxemia in both age groups of animals (Table 3.2.2.).

In newborn guinea pigs oxygen consumption decreased significantly during acute hypoxemia following the administration of both 2 and 4 mg/kg naloxone hydrochloride as compared to the response observed following the administration of vehicle (Figure 3.2.7.). Alternatively in older guinea pigs administration of any dose of naloxone did not significantly alter the response to acute moderate hypoxemia observed following the administration of vehicle (Figures 3.2.6. & 3.2.7.). In older guinea pigs neither vehicle or 1, 2, or 4 mg/kg of naloxone hydrochloride had a significant effect on oxygen consumption during normoxemia (Table 3.2.3.). Similarly, in newborn guinea pigs administration of vehicle, 1 or 2 mg/kg of naloxone hydrochloride had no significant effect on oxygen consumption during normoxemia. However prior administration of 4 mg/kg of naloxone hydrochloride caused a significant decrease in oxygen consumption in newborn animals which experienced normoxemia during the experimental period (Table 3.2.3.).

3.2.2. Cardiorespiratory Variables

The respiratory rate response to acute moderate hypoxemia was not significantly altered by prior administration of either 1, 2, or 4 mg/kg of naloxone hydrochloride as compared to the respiratory rate response that was observed following the administration of vehicle with the exception of the third epoch during the experimental period following 4 mg/kg of naloxone in the newborn guinea pigs (Figures 3.2.8. & 3.2.9.). Baseline respiratory rate decreased significantly over time in both newborn and older guinea pigs during normoxemia (Table 3.2.4.). In the newborn animals the respiration rate response was significantly altered by prior administration of either 1 or 2 mg/kg of naloxone hydrochloride. In contrast, the respiratory rate response during normoxemia in the older guinea pigs was not significantly altered by the prior administration of any dose of naloxone hydrochloride (Table 3.2.4.).

The heart rate response to acute moderate hypoxemia was not significantly altered by the prior administration of either 1, 2, or 4 mg/kg of naloxone hydrochloride as compared to the heart rate response observed following the administration of vehicle (Figures 3.2.10. & 3.2.11.). Neither vehicle nor 1, 2, or 4 mg/kg of naloxone hydrochloride had an effect on baseline heart rate in either age group of guinea pigs (Table 3.2.5.).

3.3. ADENOSINE

3.3.1. Aminophylline Dosing Experiments

In newborn guinea pigs the plasma concentrations of theophylline ranged from approximately 40 umol/L to 125 umol/L following an intraperitoneal injection of either 5, 10 or 15 mg/kg of aminophylline respectively, and these levels remained relatively constant over 30, 60 and 90 minute intervals (Figure 3.3.1.). In older guinea pigs the plasma concentrations of theophylline ranged from approximately 30 umol/L to 100 umol/L, and once again these levels remained relatively constant over 30, 60 and 90 minute intervals.

3.3.2. Thermoregulatory Variables

The decrease in core temperature during acute moderate hypoxemia was significantly attenuated by the prior administration of 10 mg/kg of aminophylline as compared to the core temperature response observed following the administration of vehicle in both newborn and older guinea pigs (Figure 3.3.2.). The significant decrease in core temperature that occurred during acute moderate hypoxemia when compared to the core temperature response during normoxemia following an i.p. injection of vehicle (Figure 3.3.3.) was eliminated by the prior administration of 10 mg/kg of aminophylline in both newborn and

older guinea pigs (Figure 3.3.4.). Neither vehicle nor aminophylline had a significant effect on baseline core temperature during normoxemia in either age group of animals (Table 3.3.1.). The summary statistic of core temperature index did not differ significantly in either age group when the animals received an intraperitoneal injection of either vehicle or 10 mg/kg of aminophylline and experienced normoxemia during the experimental period. In contrast, in both newborn and older animals the significant decrease in core temperature index during acute hypoxemia was eliminated following the administration of 10 mg/kg of aminophylline. In addition, there was a significant difference between the core temperature index during normoxemia and hypoxemia following the administration of vehicle in both age groups of animals, and this response was significantly altered by the prior administration of 10 mg/kg of aminophylline (Figure 3.3.5.).

The selected ambient temperature response that occurred during acute moderate hypoxemia following an i.p. injection of vehicle was not significantly altered by the prior administration of 10 mg/kg of aminophylline in either age group of animals (Figure 3.3.6). Neither vehicle nor aminophylline had a significant effect on selected ambient temperature during normoxemia in both newborn and older guinea pigs (Table 3.3.2.).

Oxygen consumption did not change significantly during acute hypoxemia in either newborn or older guinea pigs following an intraperitoneal injection of vehicle (Figure 3.1.3.), and this response was not altered by the prior

administration of 10 mg/kg of aminophylline (Figure 3.3.7.). Vehicle had no significant effect on oxygen consumption in both newborn and older animals. However, in newborn guinea pigs the administration of 10 mg/kg aminophylline significantly increased baseline oxygen consumption as compared to the response observed following the administration of vehicle (Table 3.3.3.).

3.3.3. Cardiorespiratory Variables

The increase in respiratory rate during acute moderate hypoxemia in both newborn and older guinea pigs was not significantly altered by prior administration of 10 mg/kg aminophylline as compared to the response that was observed following the administration of vehicle (Figure 3.3.8.). As previously outlined in section 3.2.2. baseline respiratory rate decreased significantly over time in both newborn and older guinea pigs during normoxemia. In the newborn animals this respiratory rate response was significantly altered by administration of 10 mg/kg aminophylline. Alternatively, in the older animals the respiratory rate response was not altered by aminophylline administration (Table 3.3.4.).

In the newborn but not the older animals the heart rate response to acute hypoxemia (Figure 3.1.5.) was significantly altered by the prior administration of 10 mg/kg of aminophylline as compared to the heart rate response observed following the administration of vehicle (Figure 3.3.9.). Similarly, in the newborn but not the older guinea pigs, 10 mg/kg of aminophylline had a significant effect

on baseline heart rate during normoxemia. Vehicle had no effect on heart rate in either age group of animals (Table 3.3.5.).

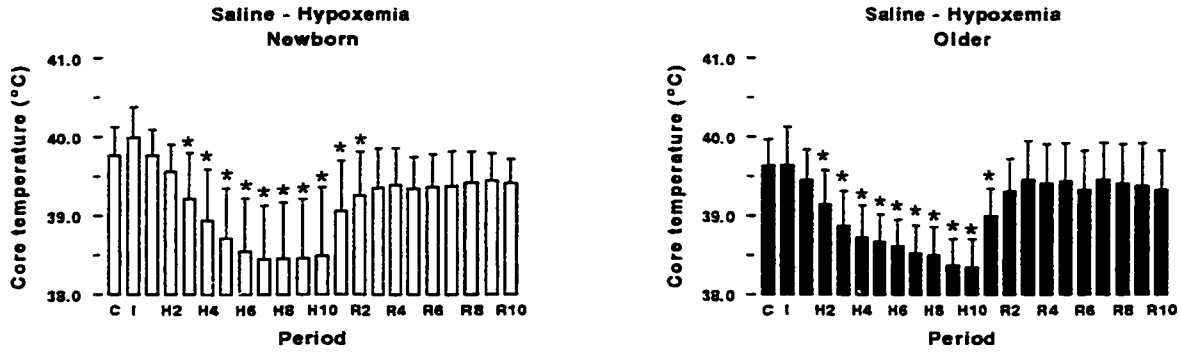

Figure 3.1.1. The effect of hypoxemia on absolute core temperature in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle. *=$p<0.05$ vs. control; C=control, I=injection, H=experimental period (hypoxemia), R=recovery period normoxemia.

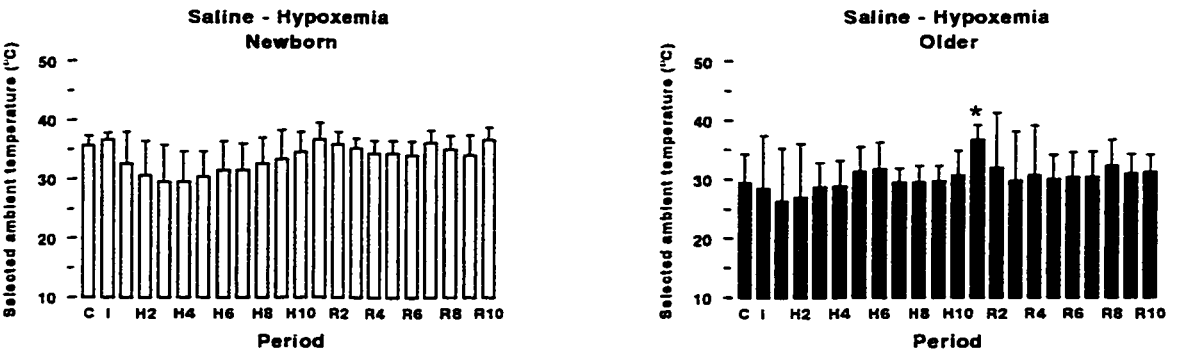

Figure 3.1.2. The effect of hypoxemia on absolute selected ambient temperature in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle. *=$p<0.05$ vs. control; C=control, I=injection, H=experimental period (hypoxemia), R=recovery period (normoxemia).

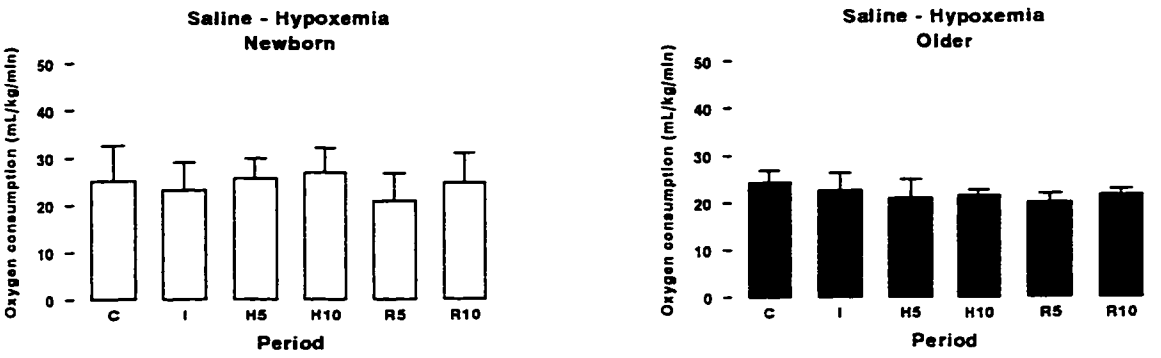

Figure 3.1.3. The effect of hypoxemia on absolute oxygen consumption in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle. C=control, I=injection, H=experimental period (hypoxemia), R=recovery period (normoxemia).

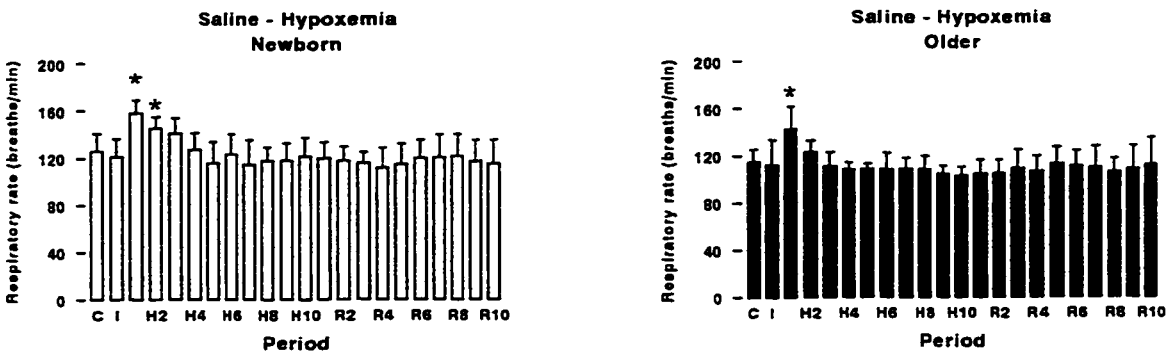

Figure 3.1.4. The effect of hypoxemia on absolute respiratory rate in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle. *=$p<0.05$ vs. control; C=control, I=injection, H=experimental period (hypoxemia), R=recovery period (normoxemia).

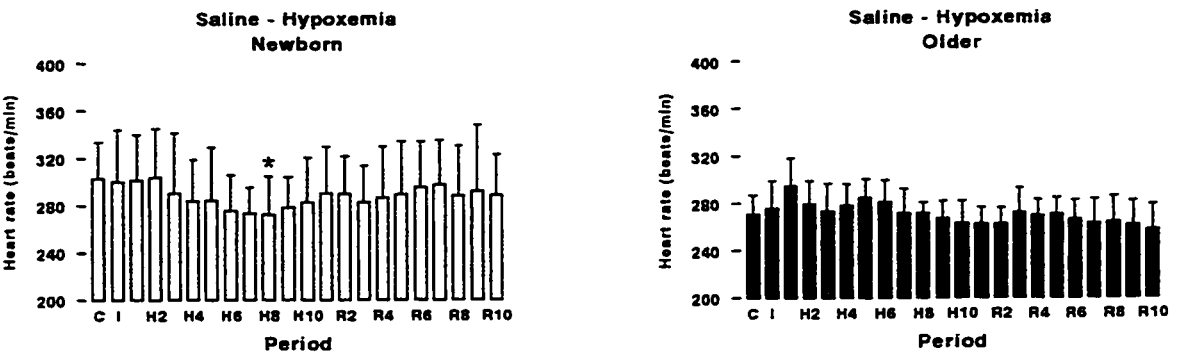

Figure 3.1.5. The effect of hypoxemia on absolute heart rate in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle. *=$p<0.05$ vs. control; C=control, I=injection, H=experimental period (hypoxemia), R=recovery period (normoxemia).

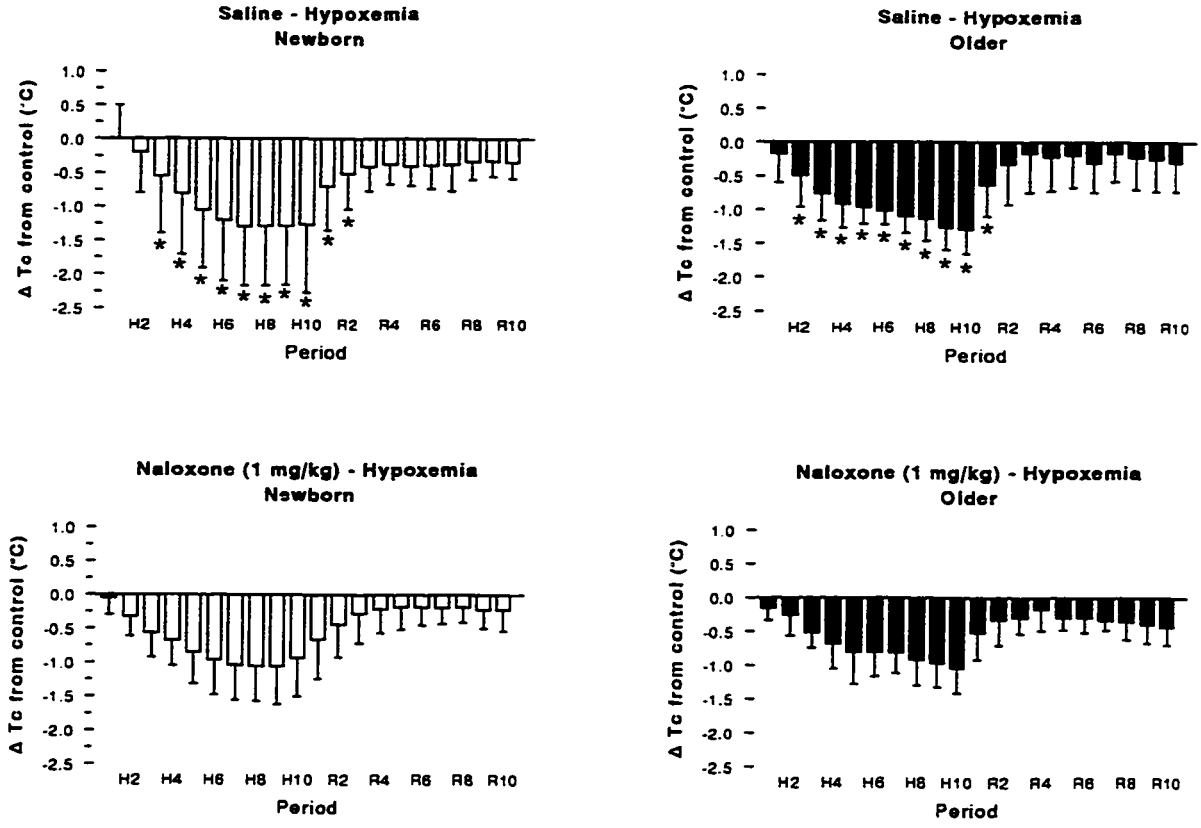

Figure 3.2.1. The effect of hypoxemia on mean changes in core temperature from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle or 1 mg/kg of naloxone. *=$p<0.05$ vs. control (Figure 3.1.1.); H=experimental period (hypoxemia), R=recovery period (normoxemia).

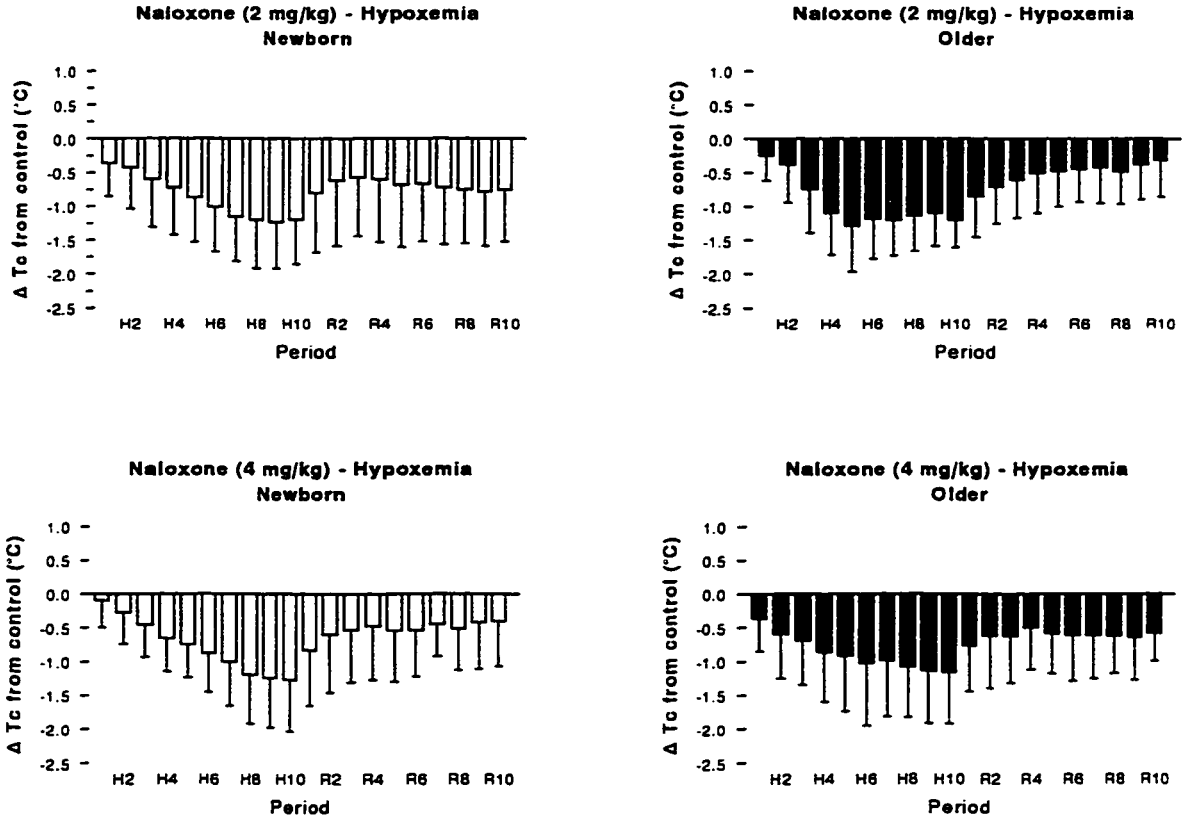

Figure 3.2.2. The effect of hypoxemia on mean changes in core temperature from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of 2 or 4 mg/kg of naloxone. H=experimental period (hypoxemia), R=recovery period (normoxemia).

Table 3.2.1. Core temperature (°C) -- change from control -- during normoxemia in newborn and older guinea pigs.

	Experimental Periods		Recovery Periods	
Drug	N5	N10	R5	R10
Vehicle				
Newborn	-0.17 ± 0.17	-0.29 ± 0.31	-0.26 ± 0.34	-0.37 ± 0.23
Older	-0.34 ± 0.25	-0.24 ± 0.33	-0.26 ± 0.34	-0.21 ± 0.49
1 mg/kg Naloxone				
Newborn	-0.37 ± 0.73	-0.44 ± 0.59	-0.19 ± 0.42	-0.26 ± 0.69
Older	-0.59 ± 0.62	-0.53 ± 0.92	-0.56 ± 0.62	-0.29 ± 0.68
2 mg/kg Naloxone				
Newborn	-0.40 ± 0.26	-0.73 ± 0.25	-0.65 ± 0.35	-0.74 ± 0.45
Older	-0.26 ± 0.31	-0.24 ± 0.38	-0.19 ± 0.30	-0.27 ± 0.50
4 mg/kg Naloxone				
Newborn	-0.36 ± 0.25	-0.47 ± 0.22	-0.61 ± 0.47	-0.65 ± 0.48
Older	-0.45 ± 0.68	-0.59 ± 0.40	-0.66 ± 0.80	-0.49 ± 0.69

Data are means ± 1 SD.

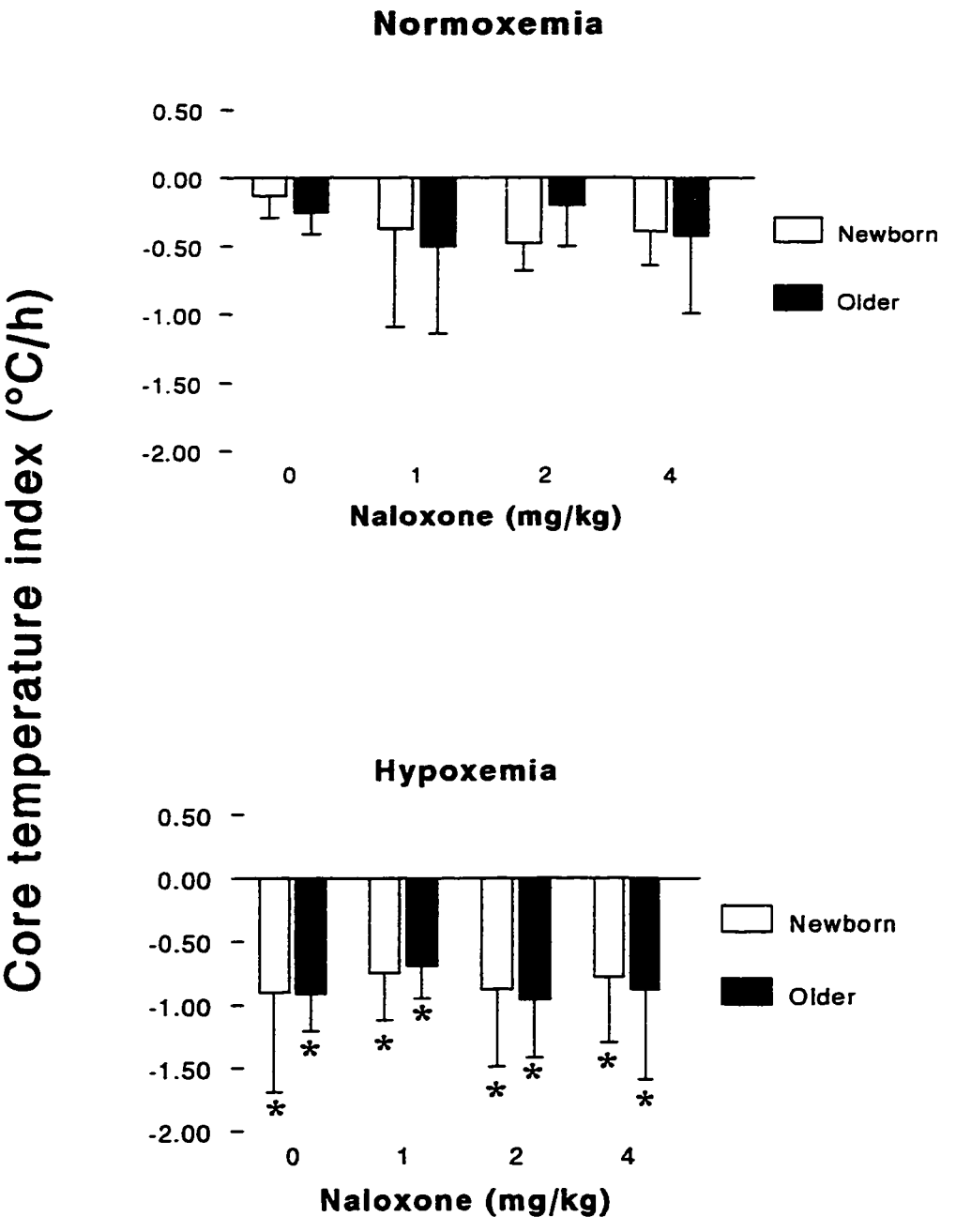

Figure 3.2.3. The effect of vehicle or 1, 2 or 4 mg/kg of naloxone on core temperature index during normoxemia and hypoxemia in both newborn (open bars) and older (closed bars) guinea pigs. *=$p<0.05$ vs. normoxemia.

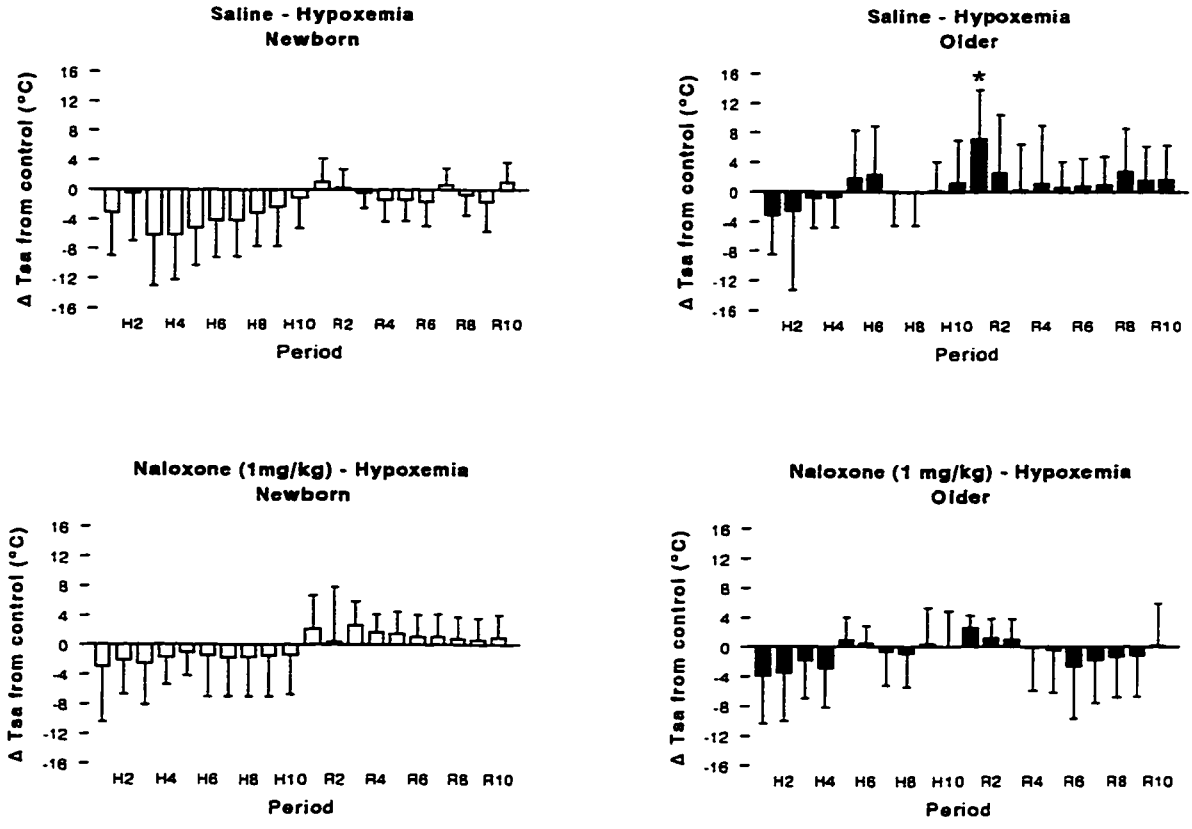

Figure 3.2.4. The effect of hypoxemia on mean changes in selected ambient temperature from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle or 1 mg/kg of naloxone. *=$p<0.05$ vs. control (Figure 3.1.2.); H=experimental period (hypoxemia), R=recovery period (normoxemia).

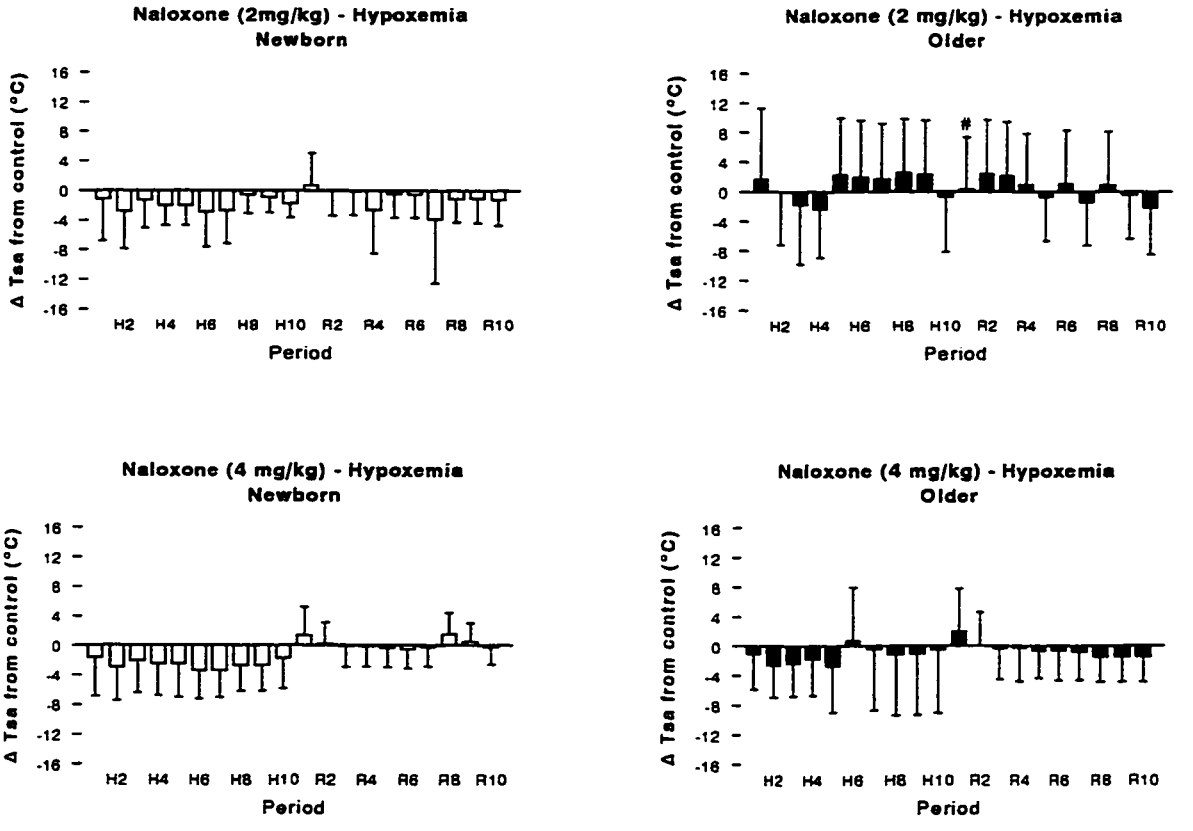

Figure 3.2.5. The effect of hypoxemia on mean changes in selected ambient temperature from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of 2 or 4 mg/kg of naloxone. $^{\#}p<0.05$ vs. response observed with vehicle. H=experimental period (hypoxemia), R=recovery period (normoxemia).

Table 3.2.2. Selected ambient temperature (°C) -- change from control -- during normoxemia in newborn and older guinea pigs.

	Experimental Periods		Recovery Periods	
Drug	N5	N10	R5	R10
Vehicle				
Newborn	-2 ± 3	0 ± 1	0 ± 2	-2 ± 4
Older	-1 ± 6	-1 ± 6	4 ± 3	-1 ± 7
1 mg/kg Naloxone				
Newborn	-2 ± 7	-2 ± 8	1 ± 4	-1 ± 2
Older	-6 ± 5	-2 ± 6	-3 ± 7	-5 ± 8
2 mg/kg Naloxone				
Newborn	0 ± 1	1 ± 2	0 ± 2	-1 ± 4
Older	2 ± 8	3 ± 2	2 ± 4	2 ± 3
4 mg/kg Naloxone				
Newborn	0 ± 2	0 ± 2	-2 ± 5	-2 ± 5
Older	3 ± 7	1 ± 5	1 ± 4	2 ± 5

Data are means ± 1 SD.

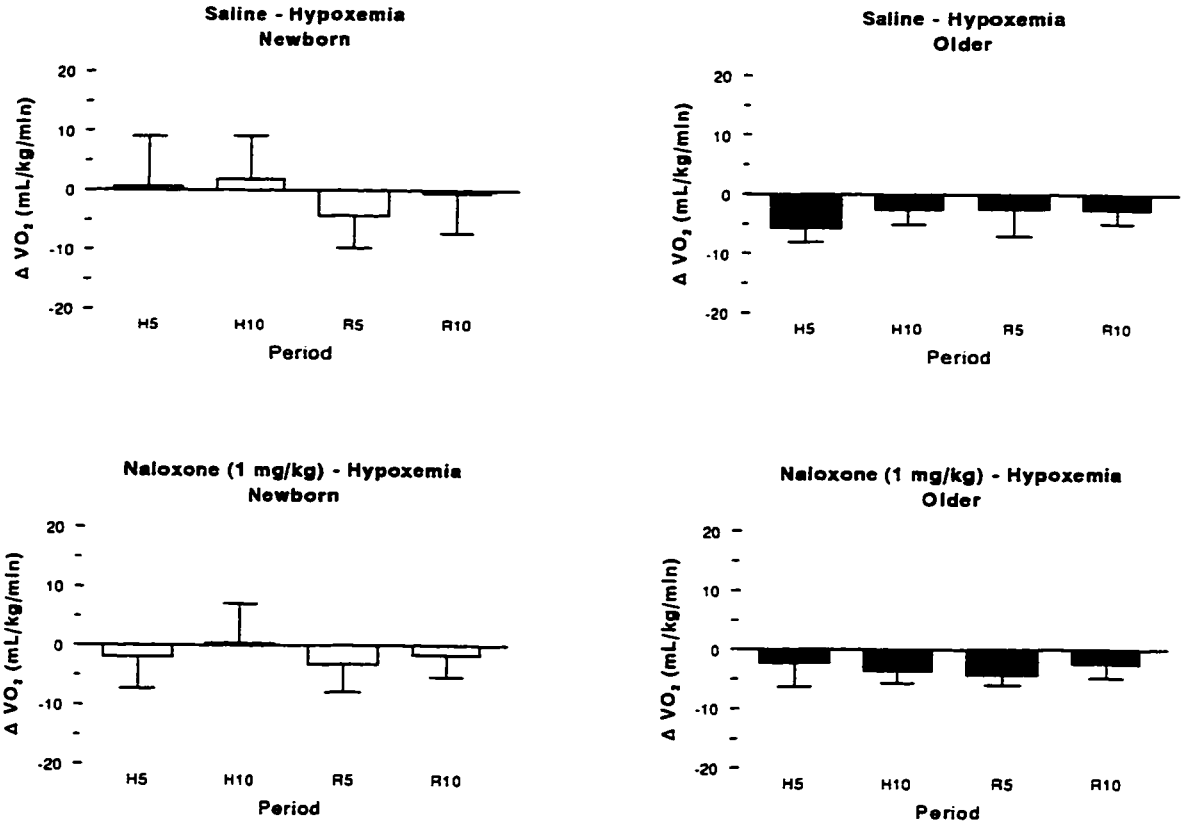

Figure 3.2.6. The effect of hypoxemia on mean changes in oxygen consumption from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle or 1 mg/kg of naloxone. H=experimental period (hypoxemia), R=recovery period (normoxemia).

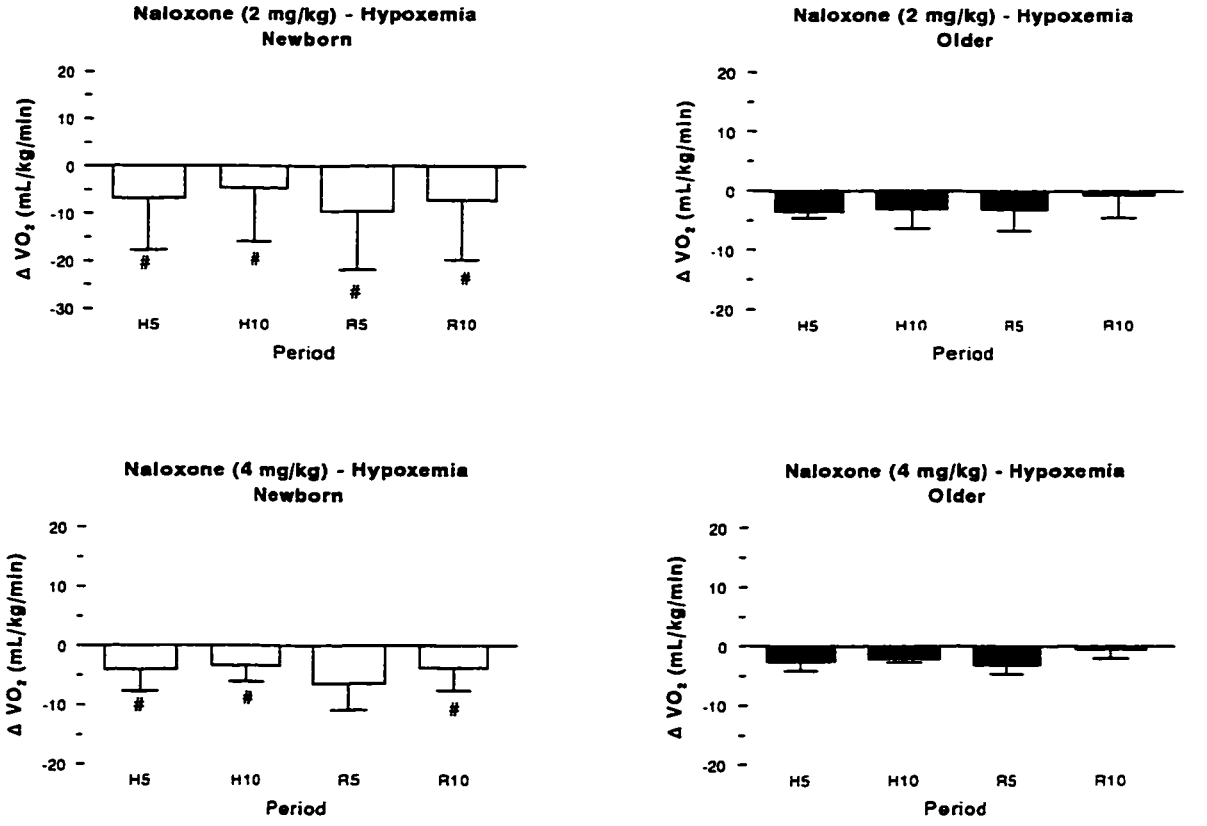

Figure 3.2.7. The effect of hypoxemia on mean changes in oxygen consumption from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of 2 or 4 mg/kg of naloxone. $^{\#}$=$p<0.05$ vs. response observed with vehicle; H=experimental period (hypoxemia), R=recovery period (normoxemia).

Table 3.2.3. Oxygen consumption (mL/min/kg) -- change from control -- during normoxemia in newborn and older guinea pigs.

	Experimental Periods		Recovery Periods	
Drug	N5	N10	R5	R10
Vehicle				
Newborn	-4 ± 2	1 ± 4	-4 ± 2	0 ± 4
Older	-4 ± 4	-4 ± 4	-4 ± 4	-3 ± 5
1 mg/kg Naloxone				
Newborn	-5 ± 6	-1 ± 6	-2 ± 10	-1 ± 8
Older	-6 ± 7	-2 ± 8	-4 ± 5	-3 ± 5
2 mg/kg Naloxone				
Newborn	-5 ± 4	-4 ± 3	-5 ± 2	-2 ± 4
Older	-5 ± 2	-2 ± 2	-4 ± 2	-2 ± 2
4 mg/kg Naloxone				
Newborn	-7 ± 11	-7 ± 11[#]	-7 ± 10	-4 ± 9
Older	-5 ± 4	-4 ± 5	-3 ± 5	-2 ± 5

Data are means ± 1 SD. [#]=$p<0.05$ vs. response observed with vehicle.

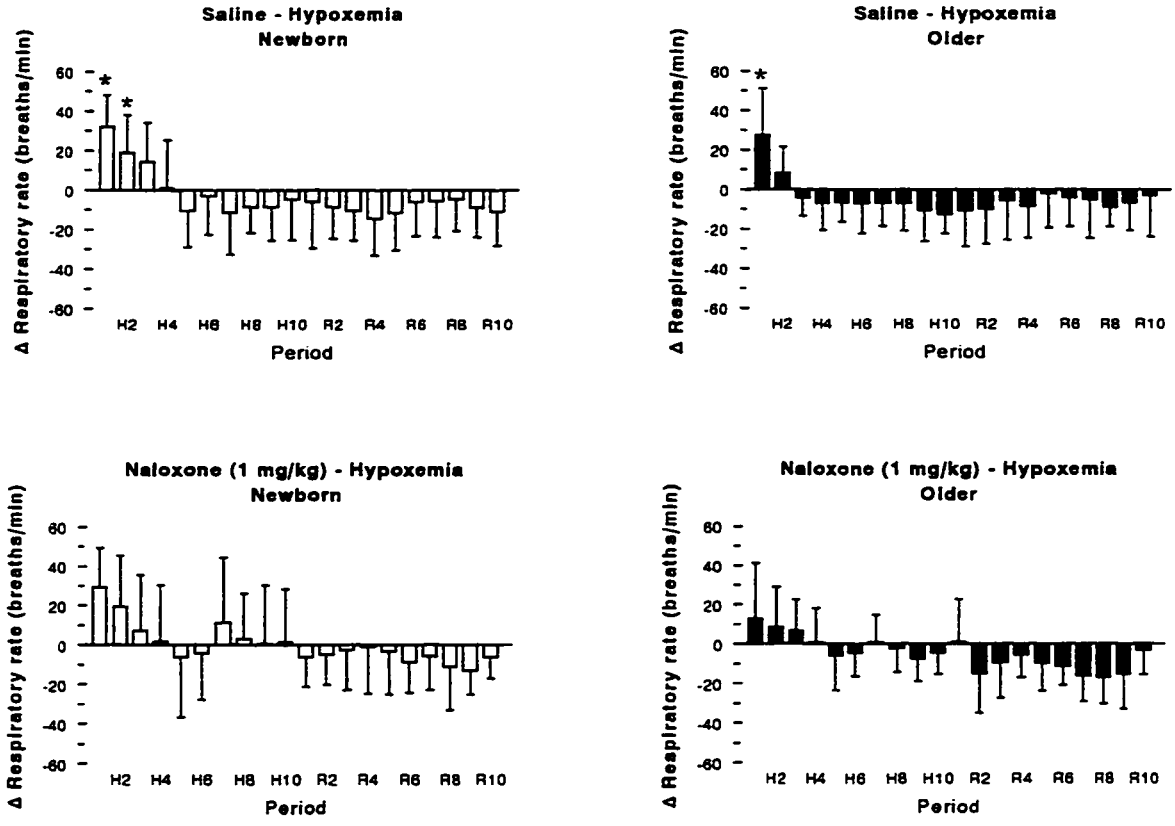

Figure 3.2.8. The effect of hypoxemia on mean changes in respiratory rate from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle or 1 mg/kg of naloxone. *=$p<0.05$ vs. control (Figure 3.1.4.); H=experimental period (hypoxemia), R=recovery period (normoxemia).

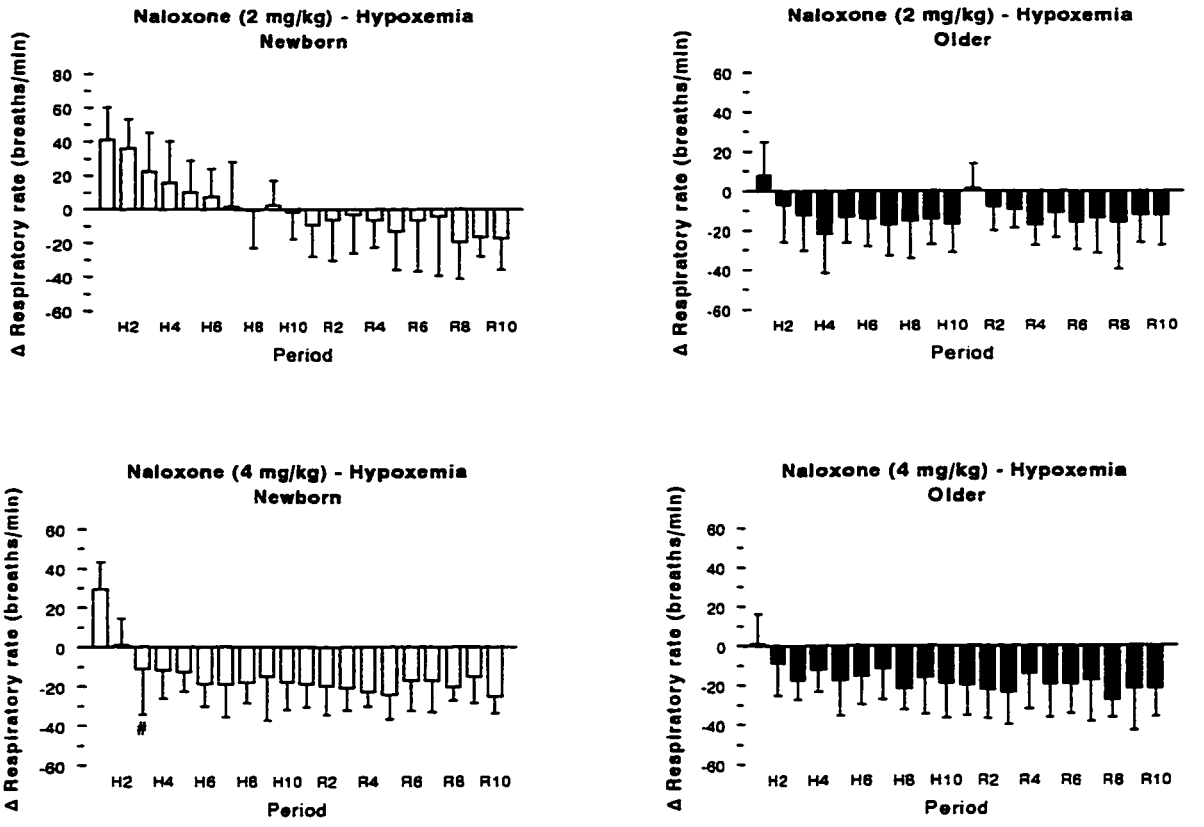

Figure 3.2.9. The effect of hypoxemia on mean changes in respiratory rate from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of 2 or 4 mg/kg of naloxone. $^{\#}$=$p<0.05$ vs. response observed with vehicle; H=experimental period (hypoxemia), R=recovery period (normoxemia).

Table 3.2.4. Respiratory rate (breaths/min) -- change from control -- during normoxemia in newborn and older guinea pigs.

	Experimental Periods		Recovery Periods	
Drug	N5	N10	R5	R10
Vehicle				
Newborn	-17 ± 26	-32 ± 27*	-27 ± 19	1 ± 34
Older	-25 ± 20*	-24 ± 25*	-16 ± 21	-13 ± 14
1 mg/kg Naloxone				
Newborn	-19 ± 19	3 ± 28#	-18 ± 22	-1 ± 26
Older	-31 ± 25	-28 ± 28	-30 ± 18	-30 ± 19
2 mg/kg Naloxone				
Newborn	-7 ± 13	-2 ± 14#	3 ± 21#	-10 ± 15
Older	-21 ± 24	-26 ± 19	-16 ± 19	-21 ± 17
4 mg/kg Naloxone				
Newborn	-14 ± 13	-13 ± 13	-17 ± 12	-9 ± 15
Older	-7 ± 20	-8 ± 14	-5 ± 10	-15 ± 15

Data are means ± 1 SD. *=$p<0.05$ vs. control; #=$p<0.05$ vs. response observed with vehicle.

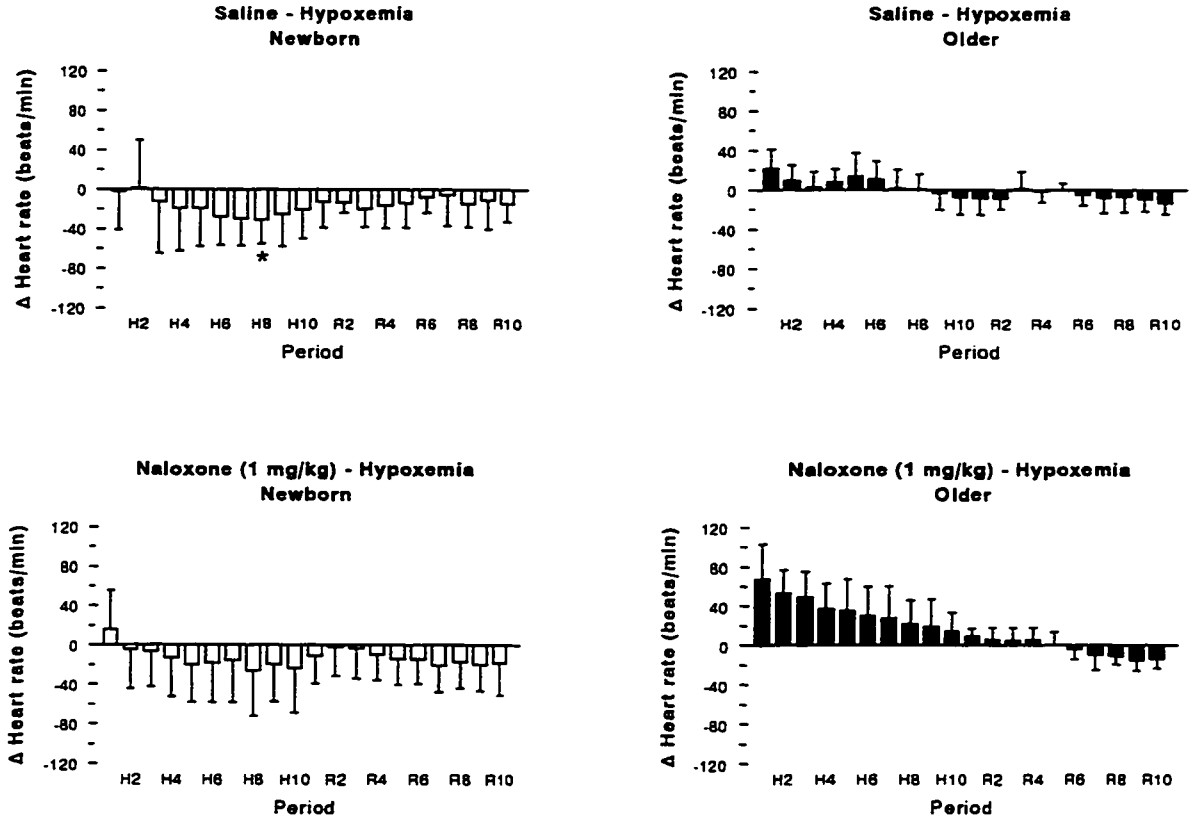

Figure 3.2.10. The effect of hypoxemia on mean changes in heart rate from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle or 1 mg/kg of naloxone. *=$p<0.05$ vs. control (Figure 3.1.5.); H=experimental period (hypoxemia), R=recovery period (normoxemia).

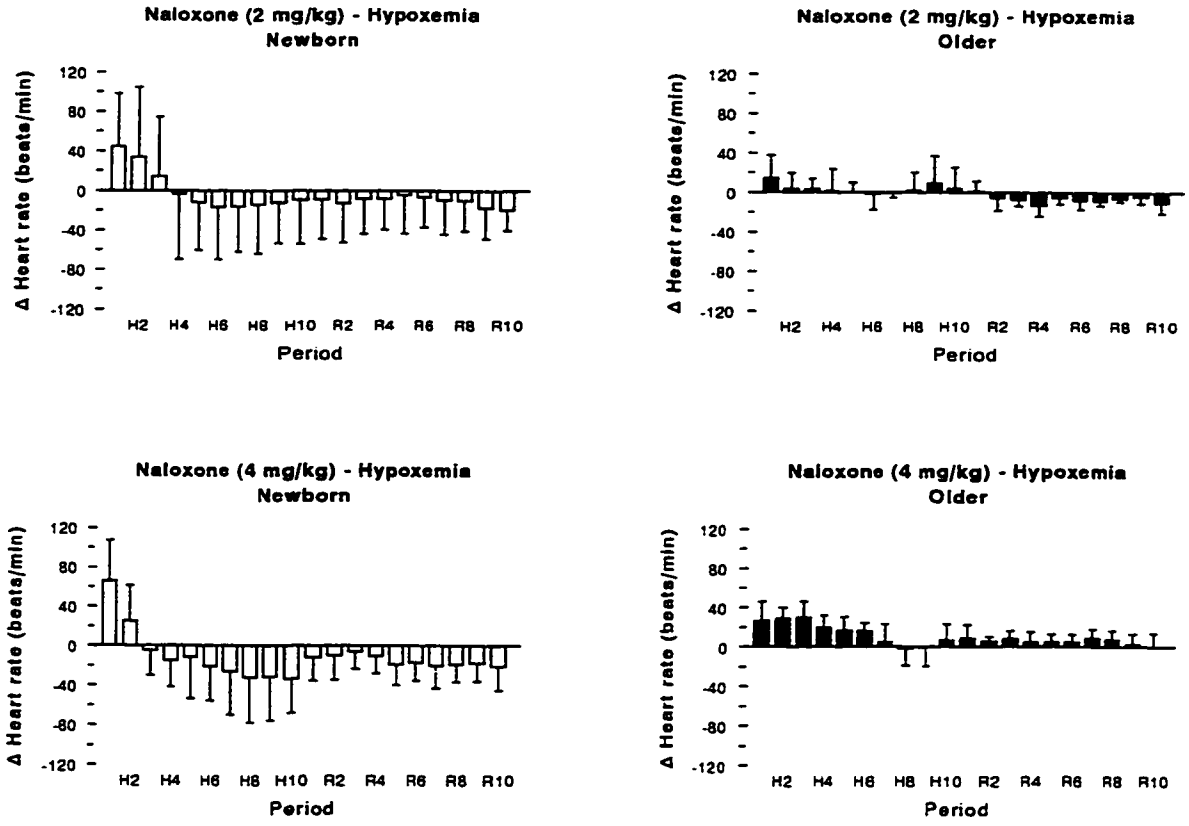

Figure 3.2.11. The effect of hypoxemia on mean changes in heart rate from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of 2 or 4 mg/kg of naloxone. H=experimental period (hypoxemia), R=recovery period (normoxemia).

Table 3.2.5. Heart rate (beats/min) -- change from control -- during normoxemia in newborn and older guinea pigs.

	Experimental Periods		Recovery Periods	
Drug	N5	N10	R5	R10
Vehicle				
Newborn	-20 ± 33	-18 ± 26	-31 ± 24	-1 ± 26
Older	-3 ± 7	-7 ± 15	-8 ± 9	-11 ± 14
1 mg/kg Naloxone				
Newborn	-2 ± 20	-1 ± 27	1 ± 32	-1 ± 32
Older	-14 ± 10	-12 ± 15	-14 ± 10	-10 ± 11
2 mg/kg Naloxone				
Newborn	-2 ± 10	2 ± 35	3 ± 26	-16 ± 10
Older	7 ± 7	3 ± 8	-4 ± 17	-6 ± 18
4 mg/kg Naloxone				
Newborn	-11 ± 20	-6 ± 18	-1 ± 24	-12 ± 16
Older	-14 ± 22	-13 ± 15	-1 ± 18	0 ± 13

Data are means ± 1 SD.

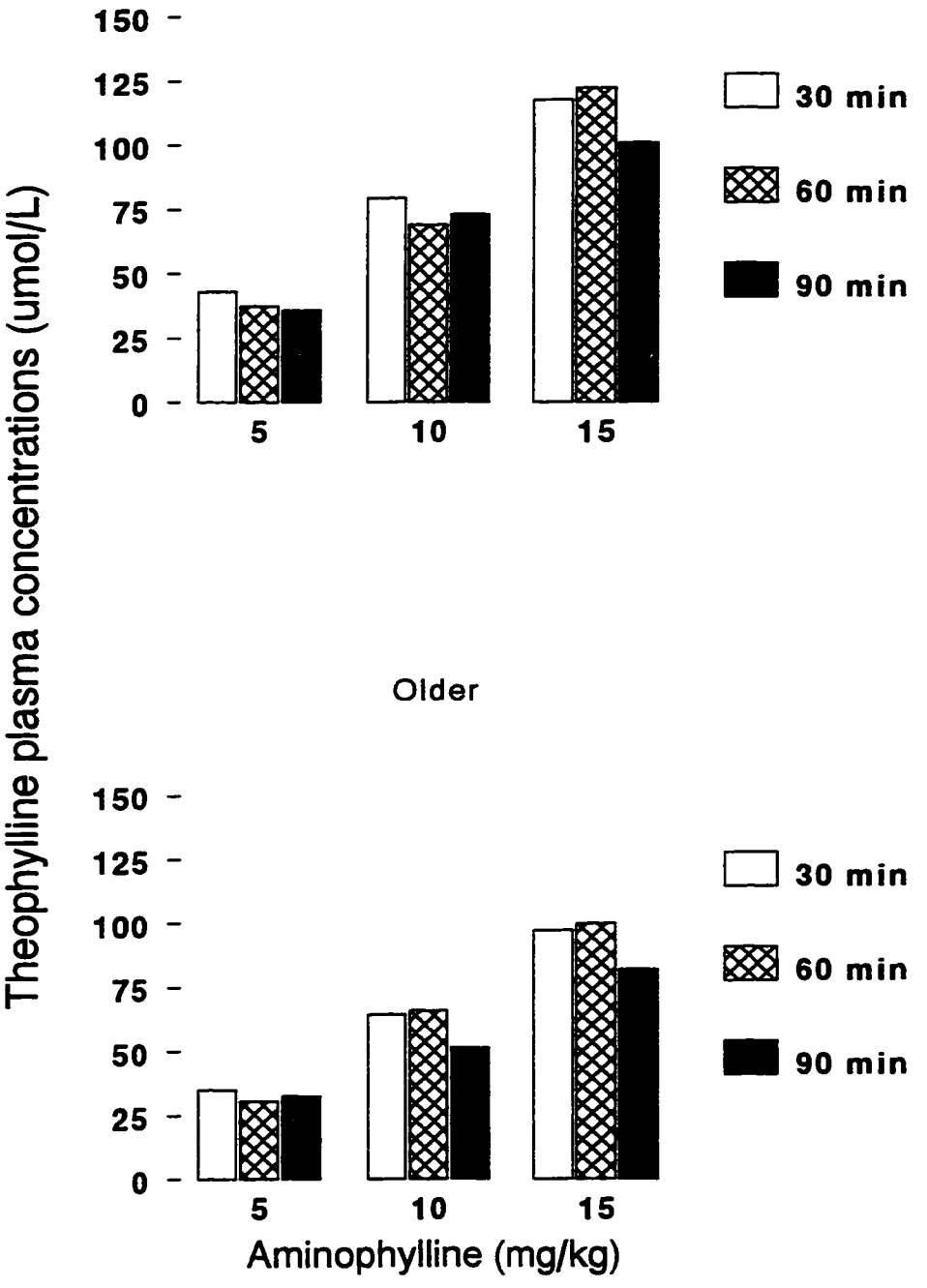

Figure 3.3.1. The effect of an i.p. injection of 5, 10 or 15 mg/kg of aminophylline on theophylline plasma concentrations at 30 (open bars), 60 (hatched bars) and 90 (closed bars) minute intervals in both newborn and older guinea pigs.

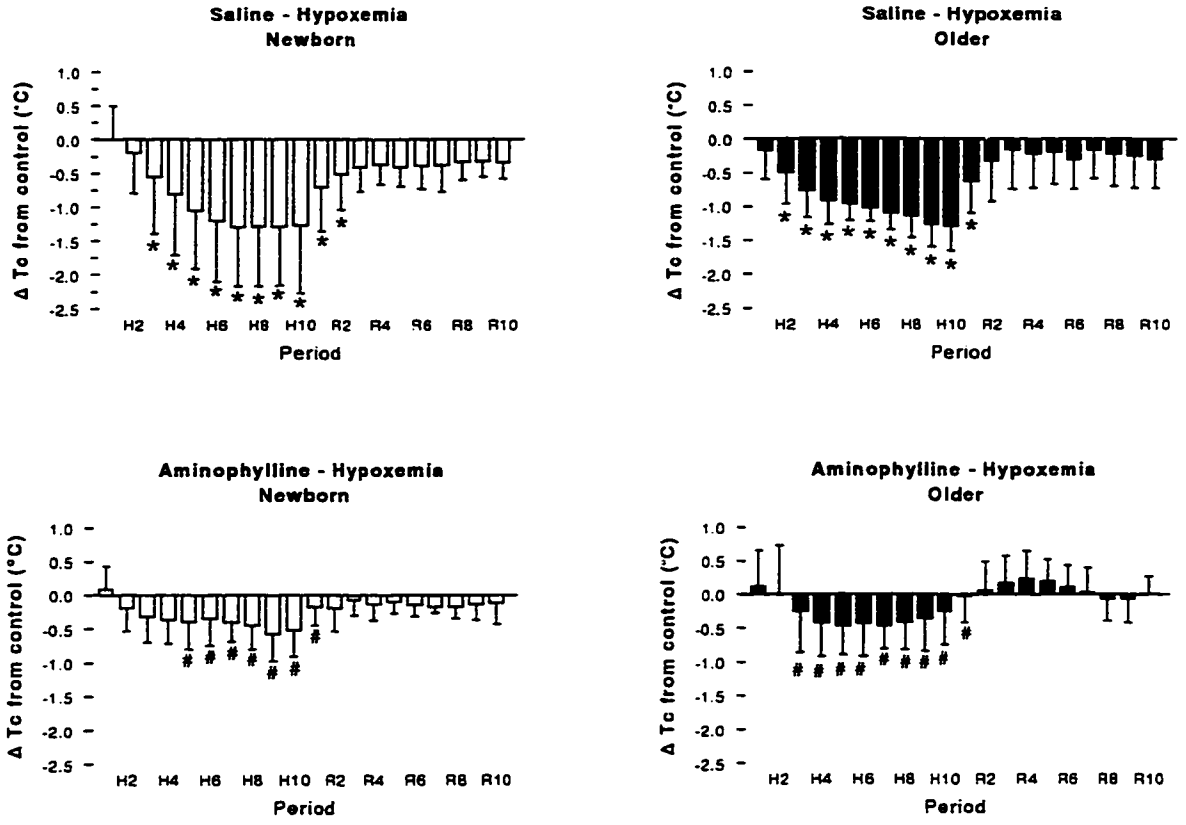

Figure 3.3.2. The effect of hypoxemia on mean changes in core temperature from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle or 10 mg/kg of aminophylline. *=$p<0.05$ vs. control (Figure 3.1.1.); #=$p<0.05$ vs. response observed with vehicle. H=experimental period (hypoxemia), R=recovery period (normoxemia).

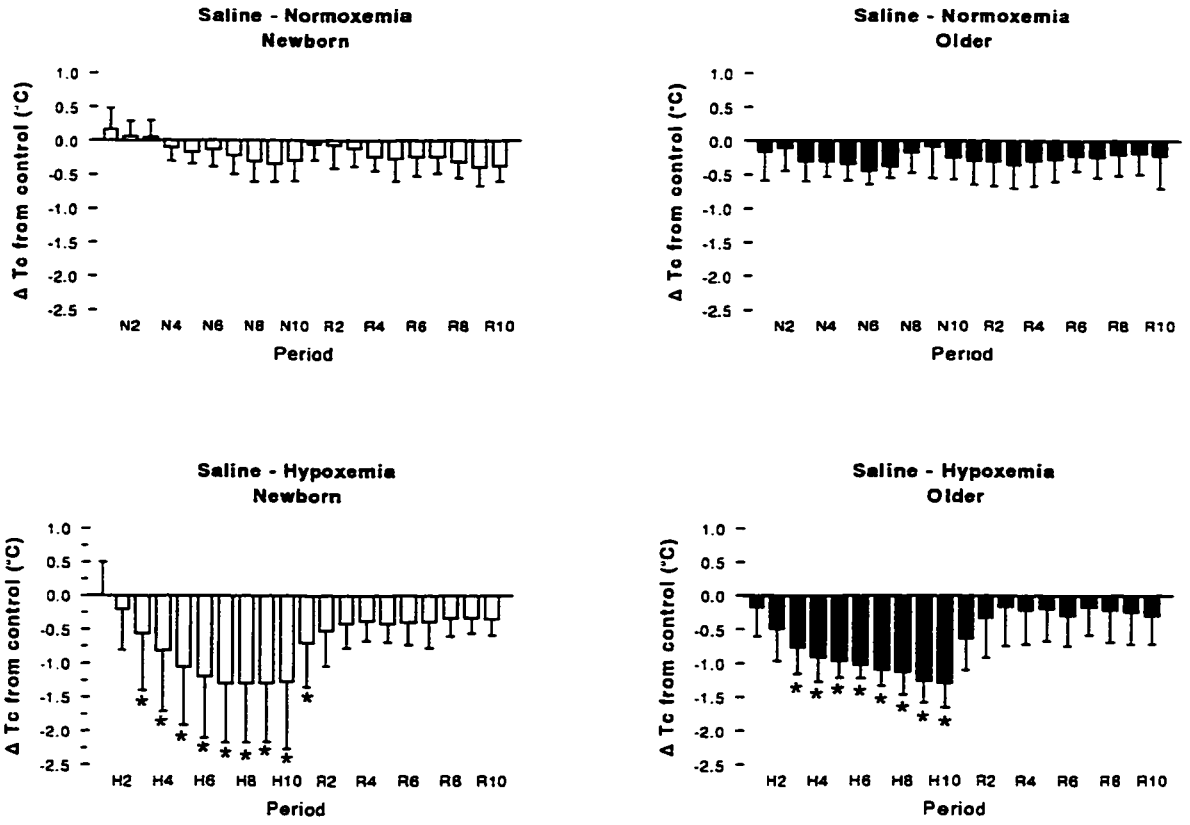

Figure 3.3.3. The effect of normoxemia and hypoxemia on mean changes in core temperature from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle. *=$p<0.05$ vs. normoxemia; N(H)=experimental period (normoxemia/ hypoxemia), R=recovery period (normoxemia)

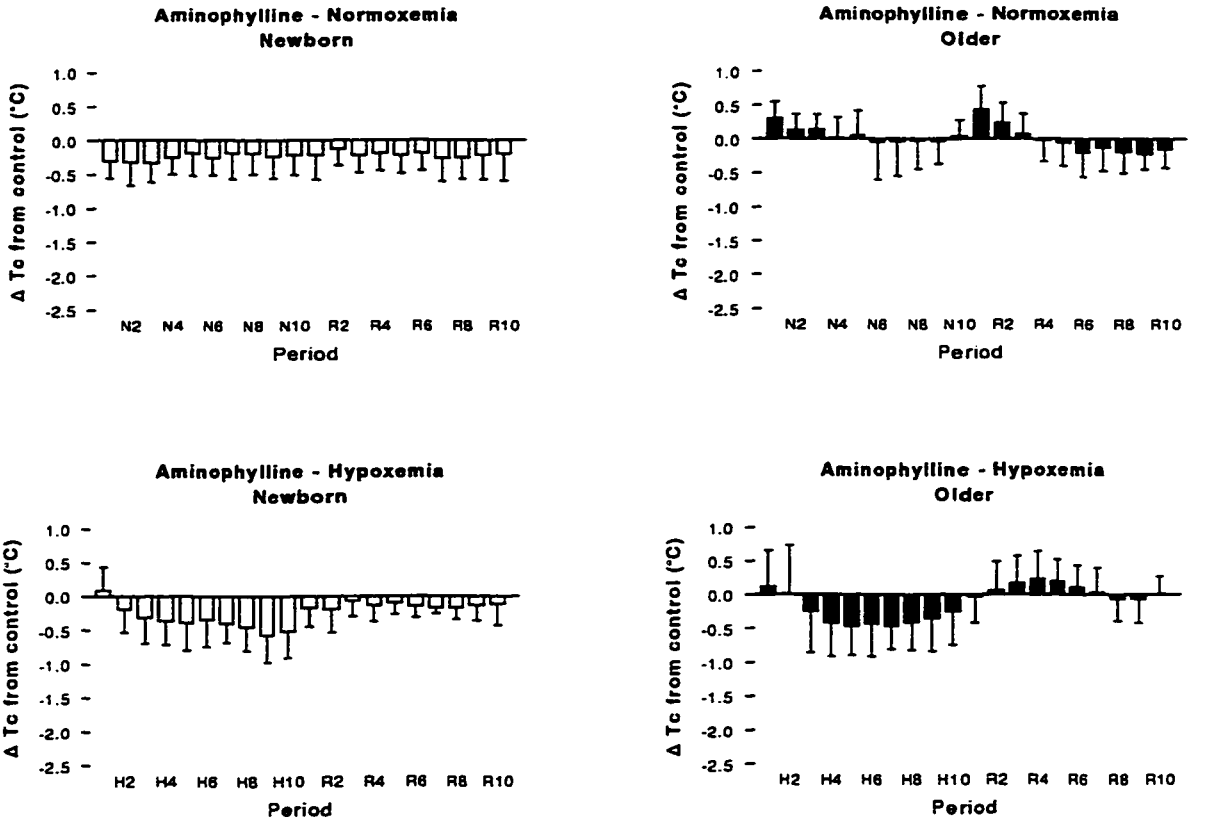

Figure 3.3.4. The effect of normoxemia and hypoxemia on mean changes in core temperature from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of 10 mg/kg of aminophylline. N(H)=experimental period (normoxemia / hypoxemia), R=recovery period (normoxemia).

Table 3.3.1. Core temperature (°C) -- change from control -- during normoxemia in newborn and older guinea pigs.

	Experimental Periods		Recovery Periods	
Drug	N5	N10	R5	R10
Vehicle				
Newborn	-0.17 ± 0.17	-0.29 ± 0.31	-0.26 ± 0.34	-0.37 ± 0.23
Older	-0.34 ± 0.25	-0.24 ± 0.33	-0.26 ± 0.34	-0.21 ± 0.49
10 mg/kg Aminophylline				
Newborn	-0.19 ± 0.33	-0.21 ± 0.30	-0.22 ± 0.26	-0.20 ± 0.40
Older	0.05 ± 0.37	0.04 ± 0.24	-0.05 ± 0.35	-0.15 ± 0.28

Data are means ± 1 SD.

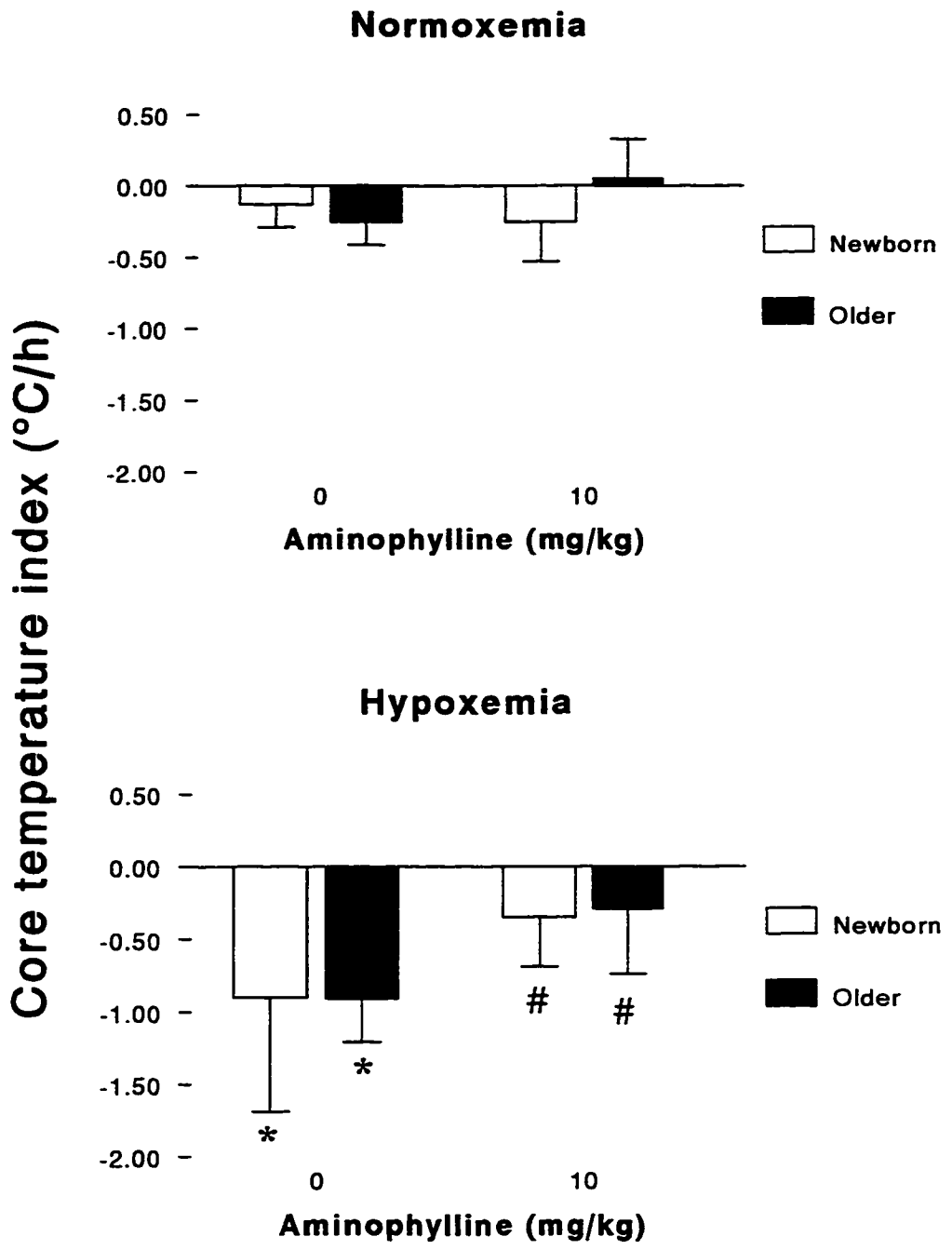

Figure 3.3.5. The effect of vehicle or 10 mg/kg of aminophylline on core temperature index during both normoxemia and hypoxemia in newborn (open bars) and older (closed bars) guinea pigs. #=$p<0.05$ vs. response observed with vehicle; *=$p<0.05$ vs. response observed during normoxemia.

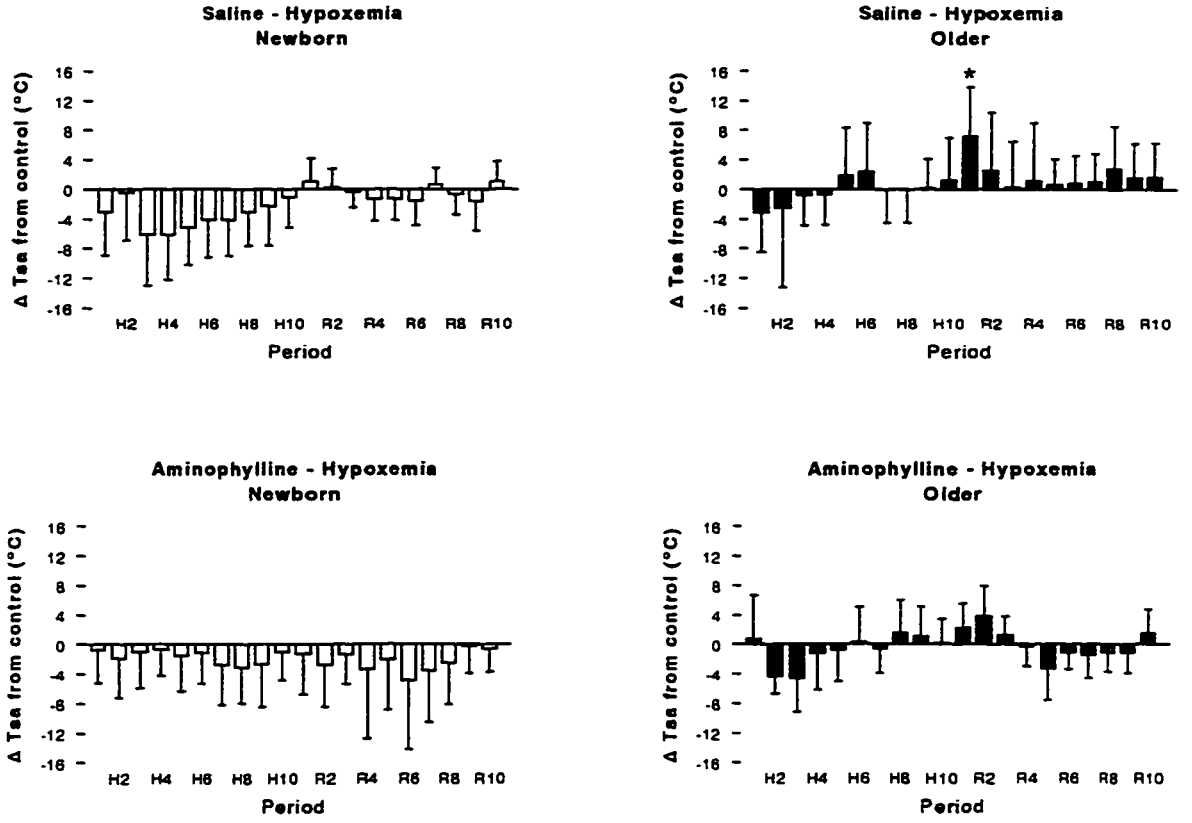

Figure 3.3.6. The effect of hypoxemia on mean changes in selected ambient temperature from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle or 10 mg/kg of aminophylline. *=$p<0.05$ vs. control (Figure 3.1.2.); H=experimental period (hypoxemia), R=recovery period (normoxemia).

Table 3.2.2. Selected ambient temperature (°C) -- change from control -- during normoxemia in newborn and older guinea pigs.

	Experimental Periods		Recovery Periods	
Drug	N5	N10	R5	R10
Vehicle				
Newborn	-2 ± 3	0 ± 1	0 ± 2	-2 ± 4
Older	-1 ± 6	-1 ± 6	4 ± 3	-1 ± 7
10 mg/kg Aminophylline				
Newborn	-3 ± 9	-6 ± 7	-1 ± 4	1 ± 3
Older	-1 ± 7	0 ± 5	-3 ± 5	-3 ± 9

Data are means ± 1 SD.

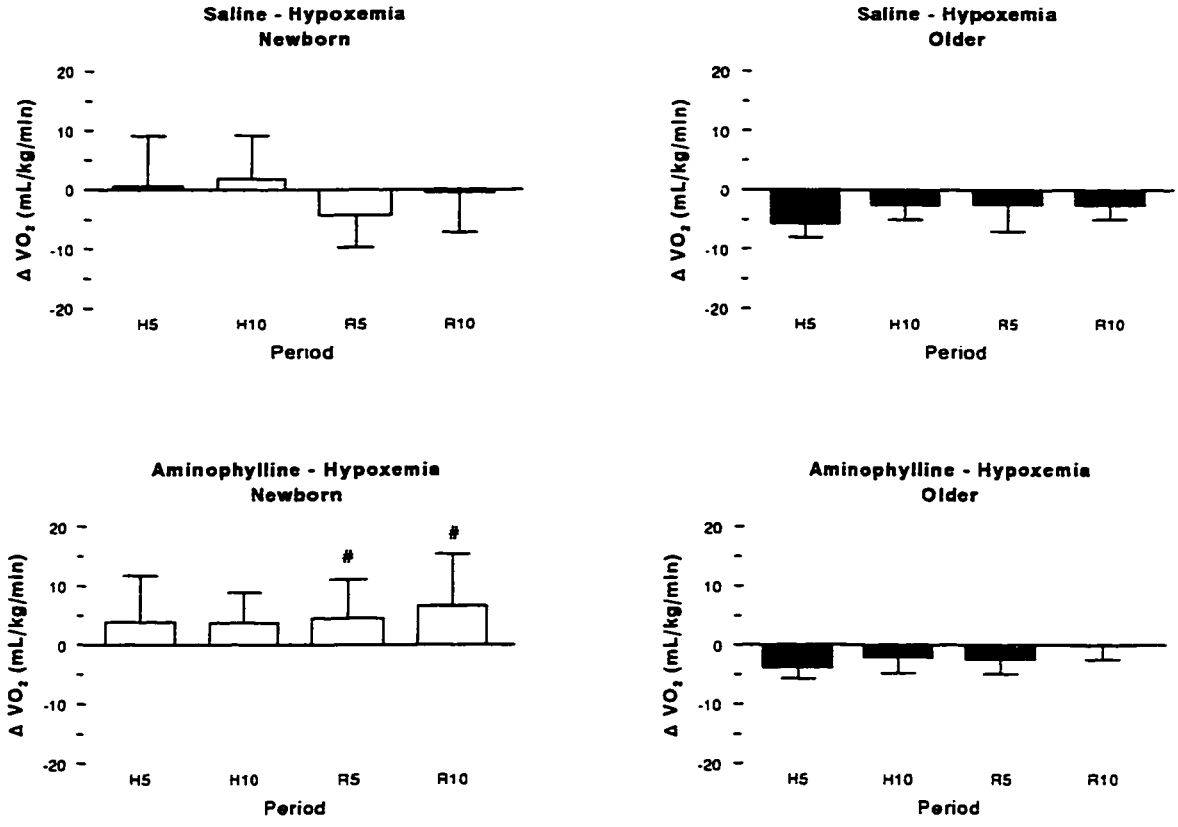

Figure 3.3.7. The effect of hypoxemia on mean changes in oxygen consumption from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle or 10 mg/kg of aminophylline. #=$p<0.05$ vs. response observed with vehicle; H=experimental period (hypoxemia), R=recovery period (normoxemia).

Table 3.3.3. Oxygen consumption (mL/min/kg) -- change from control -- during normoxemia in newborn and older guinea pigs.

	Experimental Periods		Recovery Periods	
Drug	N5	N10	R5	R10
Vehicle				
Newborn	-4 ± 2	1 ± 4	-4 ± 2	0 ± 4
Older	-4 ± 4	-4 ± 4	-4 ± 4	-3 ± 5
10 mg/kg Aminophylline				
Newborn	7 ± 8#	7 ± 5#	2 ± 5#	6 ± 5#
Older	-3 ± 2	0 ± 2	-3 ± 2	0 ± 3

Data are means ± 1 SD. #=$p<0.05$ vs. response observed with vehicle.

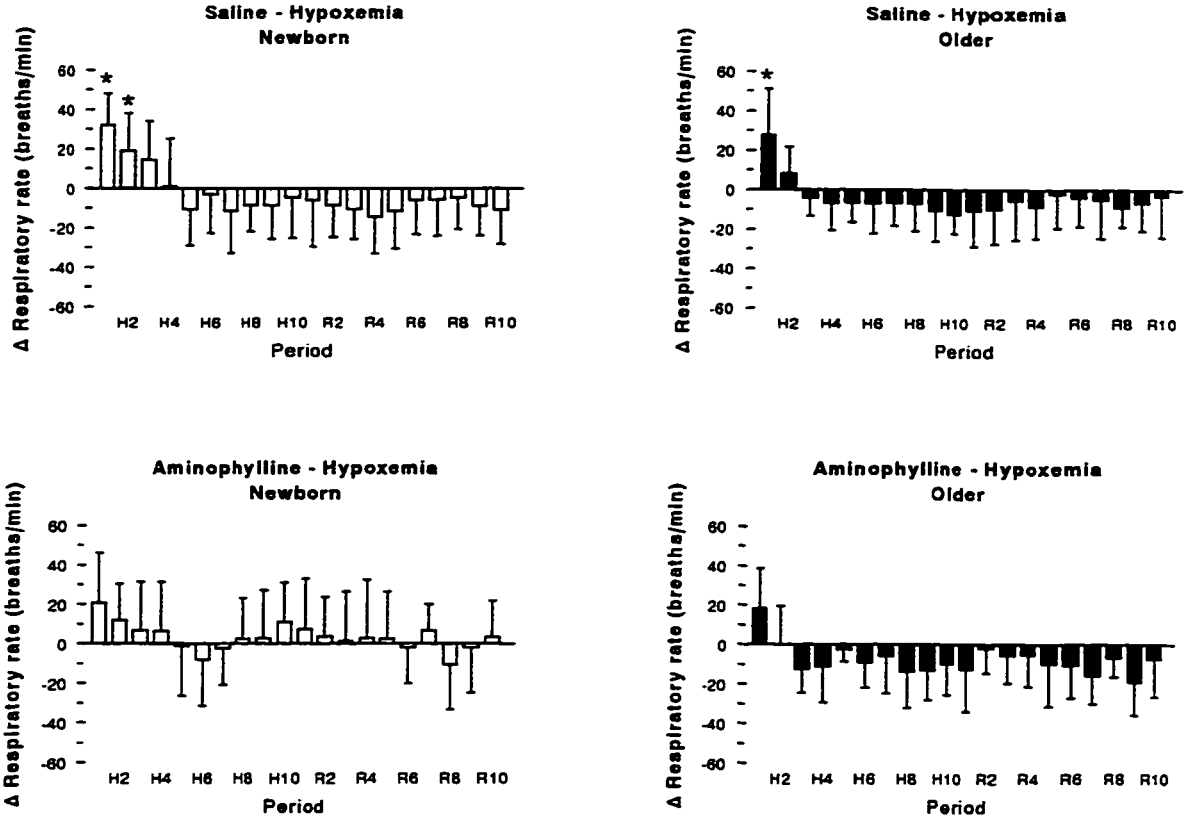

Figure 3.3.8. The effect of hypoxemia on mean changes in respiratory rate from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle or 10 mg/kg of aminophylline. *=$p<0.05$ vs. control (Figure 3.1.4.); H=experimental period (hypoxemia), R=recovery period (normoxemia).

Table 3.3.4. Respiratory rate (breaths/min) -- change from control -- during normoxemia in newborn and older guinea pigs.

	Experimental Periods		Recovery Periods	
Drug	N5	N10	R5	R10
Vehicle				
Newborn	-17 ± 26	-32 ± 27*	-27 ± 19	1 ± 34
Older	-25 ± 20*	-24 ± 25*	-16 ± 21	-13 ± 14
10 mg/kg Aminophylline				
Newborn	-3 ± 23	-2 ± 10#	-8 ± 32	-11 ± 21
Older	-3 ± 23	0 ± 16	-1 ± 9	-9 ± 19

Data are means ± 1 SD. *=$p<0.05$ vs. control; #=$p<0.05$ vs. response observed with vehicle.

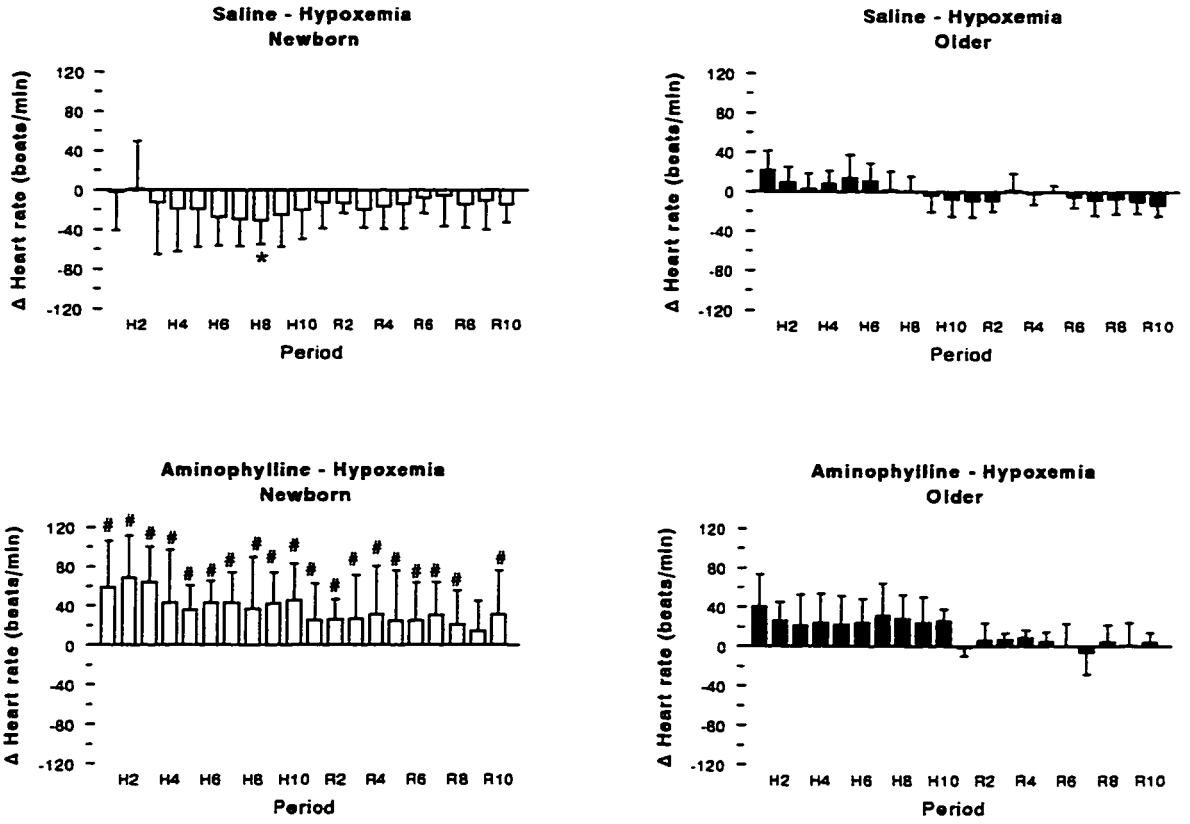

Figure 3.3.9. The effect of hypoxemia on mean changes in heart rate from control in newborn (open bars) and older (closed bars) guinea pigs following an i.p. injection of vehicle or 10 mg/kg of aminophylline. *=$p<0.05$ vs. control (Figure 3.1.5.); #=$p<0.05$ vs. response observed with vehicleH=experimental period (hypoxemia), R=recovery period (normoxemia).

Table 3.3.5. Heart rate (beats/min) -- change from control -- during normoxemia in newborn and older guinea pigs.

	Experimental Periods		Recovery Periods	
Drug	N5	N10	R5	R10
Vehicle				
Newborn	-20 ± 33	-18 ± 26	-31 ± 24	-1 ± 26
Older	-3 ± 7	-7 ± 15	-8 ± 9	-11 ± 14
10 mg/kg Aminophylline				
Newborn	24 ± 40#	15 ± 24	-3 ± 43	15 ± 69
Older	4 ± 22	6 ± 18	9 ± 13	-3 ± 16

Data are means ± 1 SD. #=$p<0.05$ vs. response observed with vehicle.

DISCUSSION

Our data provide insight into the mechanisms responsible for the "regulated" decrease in core temperature that occurs during acute moderate hypoxemia in newborn and older guinea pigs.

4.1. THE EFFECT OF HYPOXEMIA

4.1.1. Thermoregulatory variables

With vehicle, core temperature decreased during acute moderate hypoxemia in both newborn and older guinea pigs, as has been observed previously in our laboratory by Clark and Fewell (1996) and by numerous other investigators in several different animal species such as kittens, rats, mice, dogs, cats, humans and lizards (Kottke et. al., 1948; Hill, 1959; Hicks and Wood, 1985; Gautier et. al., 1987; Gordon and Fogelson, 1991). However, contrary to previous studies by Bonora and Gautier (1987) in kittens and Clark and Fewell (1996) in guinea pigs we did not observe an age dependent influence on the decrease in core temperature during hypoxemia. Clark and Fewell (1996) have shown an accentuation of the "regulated" decrease in core temperature in older versus newborn guinea pigs. The discrepancy between this and the present study may be explained in part by the duration of the

experimental period (ie. two hours versus one hour in the present study), and the method in which the data were reported (ie. 30 versus 6 minute intervals respectively). Given that the accentuation of the decrease in core temperature in the older guinea pigs was observed in the second hour of hypoxemia, it is not surprising that we did not observe this with an experimental period of only one hour. It is quite possible that if the experimental period was extended to two hours an age dependent response would have occurred. This is supported by the observation that during the one hour experimental period of hypoxemia in the present study the newborn guinea pigs seem to reach a nadir, whereas the older guinea pigs were still decreasing their core temperature near the end of the experimental period. A one hour experimental period was chosen for this series of experiments due to the half life of the antagonists that were utilized to test the hypotheses and this will be addressed further in limitations of this study.

Following an intraperitoneal injection of vehicle selected ambient temperature did not change significantly during acute moderate hypoxemia in either newborn or older guinea pigs. This is in contrast to the findings of other investigators who have reported a decrease in selected ambient temperature during acute moderate hypoxemia in several animal species from invertebrates to mammals (Hicks and Wood, 1985; Gordon and Fogelson, 1991; Wood, 1991). As well, it is also different from the results obtained by Clark and Fewell (1996) who showed that newborn but not older guinea pigs moved to a significantly lower selected ambient temperature during acute moderate hypoxemia to

facilitate the decrease in core temperature. Although there was no facilitation of the decrease in core temperature during acute moderate hypoxemia by selecting a cooler ambient temperature, perhaps more important was the observation that there was no attempt made by the animals to increase their core temperature during the hypoxemic episode by moving to a warmer selected ambient temperature. Therefore, according to the definitions of a "forced" or "regulated" thermoregulatory response outlined in section 1.2.2.2. [i.e. Selected ambient temperature (pg. 9)] the decrease in core temperature that occurred in both the newborn and older guinea pigs during acute moderate hypoxemia in the present study was indeed a "regulated" hypothermia.

In the older, but not the newborn guinea pigs the first selected ambient temperature epoch during the recovery period, in which normoxemic conditions were reintroduced, was significantly greater than control. This is interesting in light of the observation that the older guinea pigs return their core temperature towards control values quicker than the newborn animals do. Therefore, it appears that the older but not the newborn guinea pigs use behavioural means to return their core temperature to control values during the recovery period. This is in agreement with the previous investigation by Clark and Fewell (1996) in which it was also noted that the older but not the newborn guinea pigs moved to a significantly warmer selected ambient temperature during the recovery period of normoxemia. This difference between newborn and older guinea pigs in the use of behavioural effector mechanisms to return their core temperature to

control values raises the possibility of post-natal maturation influencing thermoregulatory effector mechanisms and the way in which guinea pigs recover from an episode of hypoxemia. This age-dependent response was not examined further in this series of experiments, and warrants further investigation.

Oxygen consumption did not vary significantly during acute moderate hypoxemia in either newborn or older guinea pigs. Given that the animals were studied within their thermoneutral zone (Fewell et. al., 1997), control measurements of oxygen consumption were at basal levels and hence no further decreases in oxygen consumption values during acute moderate hypoxemia were expected. Several studies have indicated that the degree of the decrease on oxygen consumption during hypoxemia in both newborn and older animals is equivalent to the control (or resting) oxygen consumption level, which is dependent upon the ambient temperature at which the animals were studied (Hill, 1959; Frappell et. al., 1992). In agreement with our observation of no change in oxygen consumption during hypoxemia, Blatteis (1964) has shown that in newborn rabbits studied within their thermoneutral zone, the decrease in core temperature during acute moderate hypoxemia was not accompanied by a concurrent decrease in oxygen consumption. However, in contrast to this, other investigators have shown that a decrease in oxygen consumption may still occur during acute moderate hypoxemia even if the animal is studied within a thermoneutral environment (Gautier et. al., 1989; Olson and Dempsey, 1978; Pappenheimer, 1977).

Selected ambient temperature and oxygen consumption did not decrease during acute moderate hypoxemia. Considering that selected ambient temperature may be taken as a measure of behavioural thermoregulatory effector mechanisms and oxygen consumption may be taken as a measure of an autonomic thermoregulatory mechanism, a question may arise as to how core temperature could decrease without causing a change in one of these effector mechanisms? In fact there are other thermoregulatory effector mechanisms which were not examined in the course of this series of experiments such as vasodilatation or respiratory evaporative heat loss which may have been enlisted by the animals to facilitate a decrease in core temperature. Since hypoxemia has been shown to induce vasodilatation (Chalmers and Korner, 1966; Iriki et. al., 1971; Iriki and Simon, 1973) which would enhance heat loss, it can be hypothesized that a thermoregulatory effector mechanism such as this, could in part, account for the decrease in core temperature that occurred during acute moderate hypoxemia in both age groups of animals.

4.1.2. Cardiorespiratory variables

With vehicle, exposure to acute moderate hypoxemia resulted in a significant increase in respiratory rate that quickly returned toward control levels in both the newborn and older guinea pigs. This has been observed previously in both newborns and adults of a number of species including humans (Cross and Warner, 1951; Cross and Oppe, 1952; Rigatto et. al., 1975; Grunstein et. al., 1981; Moss et. al., 1987; Easton et. al., 1986; Vizek et. al., 1987). Hence, both age groups of guinea pigs demonstrated the typical biphasic respiratory response to hypoxemia that has commonly been referred to as "hypoxic ventilatory decline" (Bisgard and Neubauer, 1995).

Heart rate decreased significantly during acute moderate hypoxemia following an intraperitoneal injection of vehicle in newborn but not in older guinea pigs. Interestingly, the decrease in heart rate was not preceded by an initial increase in heart rate during hypoxemia in neither newborn nor older animals as has been observed in both dogs (Resnik 1925a; 1925b; 1925c) and humans (Rahn and Otis, 1947). The statistically significant decrease in heart rate that occurred in the newborn guinea pigs during hypoxemia correlates with studies on isolated perfused guinea pigs hearts which have shown that during hypoxic conditions there was a significant slowing in ventricular rate that was primarily caused by a slowing of atrioventricular conduction time (Froldi and Belardinelli, 1990).

This same study also demonstrated that there were two different patterns in the response of guinea pig hearts to hypoxia; a response which involved atrial slowing and a second response which involved no significant atrial slowing. Froldi and Belardinelli (1990) postulated that the two different patterns observed in the course of their experiments could have been the result of varying starting heart rates. The idea of different starting heart rates determining a heart rate response to hypoxemia may, in part, explain the different heart rate response to acute moderate hypoxemia we observed between newborn and older guinea pigs. Newborn guinea pigs exhibited a decrease in heart rate, whereas older guinea pigs did not. Given that the newborn guinea pigs had a higher control heart rate, albeit not significantly different from the older guinea pigs, it may have been great enough to account for the different responses that we observed. However, it is also possible that there may be a post-natal maturation influence in determining the heart rate response to hypoxemia, and further investigation into this area should be considered.

4.2. THE ROLE OF ENDOGENOUS OPIOIDS

4.2.1. Thermoregulatory variables

Prior administration of 1, 2 or 4 mg/kg of naloxone hydrochloride - a nonspecific opioid antagonist (Reisine and Pasternak, 1996) which readily crosses the blood brain barrier (Cohen et. al., 1983) and is thought to have no intrinsic pharmacological properties (Harvey et. al., 1992) - did not significantly alter the decrease in core temperature that occurred during acute moderate hypoxemia in comparison to the response that occurred following the administration of vehicle in either newborn or older guinea pigs. The inability of any dose of naloxone to alter the core temperature response to acute moderate hypoxemia in both newborn and older guinea pigs was illustrated further by the lack of a significantly altered core temperature index between the animals that received naloxone and those that received vehicle. These results differ from our preliminary results (Crisanti and Fewell, 1996) which provided evidence that the endogenous opioids are involved at least in part in mediating the regulated decrease in core temperature during hypoxic hypoxia in newborn guinea pigs, and that low dose naloxone attenuated the decrease in core temperature during acute moderate hypoxemia in both newborn and older guinea pigs (Crisanti and Fewell, 1997). However, preliminary results are just that, and a discrepancy between preliminary results and final results are not that uncommon. There are

several possibilities as to why this difference may have occurred. In particular, we must consider the addition of the older age group and the different doses of naloxone into the statistical analysis. As well, upon completion of the study we thought it was more appropriate to compare back to control values rather than back to the values that were obtained 30 minutes after the intraperitoneal injection of either vehicle or drug, as was done for the preliminary results. And finally, the post hoc tests on the preliminary data were conducted using a more robust multiple comparisons test which may have incorrectly influenced our conclusions.

The absence of a role for the endogenous opioids in the decrease in core temperature that occurs during acute moderate hypoxemia in newborn and older guinea pigs is in contrast to the evidence provided by Mayfield and D'alecy (1992) and Mayfield et. al. (1994) which has indicated that the endogenous opioids play a role in mediating the decrease in core temperature following acute severe hypoxemia in hypoxic conditioned adult mice. As well, it also contradicts the preliminary results of Young and Malvin (1996) that imply a role for the endogenous opioids in mediating the decrease in core temperature during acute moderate hypoxemia in adult rats. Furthermore, the negative results obtained in this study do not lend support to the investigations of Grunstein et. al. (1981) in which they demonstrated that an injection of naloxone to newborn rabbits during severe hypoxemia returned decreasing rectal temperatures toward control levels.

There are several possibilities as to why our results are different from the earlier positive results which have implicated the endogenous opioids as a mediator of the "regulated" decrease in core temperature that occurs during acute moderate hypoxemia. We studied guinea pigs that were between 5 and 10 days of age, and a second group that were between 25 to 30 days of age, whereas Mayfield and D'alecy (1992), Mayfield et. al. (1994), and Young and Malvin (1996) studied adult mice and rats, respectively. Therefore, it is entirely possible that the age of the guinea pigs may have played a role in producing the negative results. However, this seems unlikely given the evidence provided by Grunstein et. al. (1981) that the endogenous opioids are involved in the decrease in core temperature during severe hypoxemia in newborn rabbits and that a guinea pig has been shown to have a full complement of brain opioid receptors - as determined by stereospecific binding of [^{3}H] naltrexone in brain homogenates - in late fetal life as compared to the levels that are observed in adult guinea pigs (Clendeninn et. al., 1976). In addition, Zhang and Moss (1995) have demonstrated that younger piglets have a greater concentration of β-endorphin, Met-enkephalin, Dynorphin-A, and Dynorphin-B in several brain regions than do older piglets. Couple these findings to those of Moss and Inman (1989) who have found that there is a greater release of β-endorphin into the plasma and cerebrospinal fluid of younger versus older piglets under both normoxemic and hypoxemic conditions, we may conclude that the absence of a role for the endogenous opioids in mediating the "regulated" decrease in core

temperature during acute moderate hypoxemia in guinea pigs is most likely not due to the age of the animals that were utilized in the experiments.

It is also possible that the different results obtained regarding the involvement of the endogenous opioids in mediating the decrease in core temperature during acute moderate hypoxemia for mice, rats, rabbits, and guinea pigs is related to a species difference in the role of opioids in thermoregulation. Studies carried out by Spencer et. al., (1990) on adult rats in a thermocline have provided evidence that intracerebroventricular opioid administration in the form of mu, kappa, or delta specific agonists results in either a "regulated" increase or decrease in core temperature, respectively. Whereas, Kandasamy and Williams (1983) have found that an intracerebroventricular injection of either mu, kappa, or delta agonists into adult male guinea pigs results in hyperthermia. This variation is interesting when one considers the evidence of a species dependent concentration and distribution of opioid binding sites within the rodent brain (Robson et. al., 1985). Therefore, it may be possible that the "regulated" decrease in core temperature during acute moderate hypoxemia occurs via different mechanisms in different rodent species.

It may be argued that the doses of naloxone employed within the course of this series of experiments (ie. 1, 2, or 4 mg/kg) were insufficient to block the effect of the released endogenous opiods in guinea pigs during acute moderate hypoxemia. However, it has been demonstrated that in adult male guinea pigs

pretreatment with an intraperitoneal injection of either 1, 3, or 5 mg/kg of naloxone was sufficient to significantly attenuate the hyperthermia due to morphine or β-endorphin injected intraventricularly (Kandasamy and Williams, 1983). Similarly, Rosow et. al. (1982) have shown that in adult mice doses of naloxone as low as 0.1 mg/kg were sufficient enough to antagonize the observed temperature effects of morphine. Therefore, we cannot attribute the negative role of endogenous opioids in mediating the "regulated' decrease in core temperature during acute moderate hypoxemia in both newborn and older guinea pigs to an insufficient dose of the general opioid antagonist -- naloxone.

Despite 1) the age differences in comparison to other investigations in this area, 2) the species differences that exist in regards to opioid concentration and receptor distribution, and 3) the possibility that the doses of naloxone (ie. 1, 2, or 4 mg/kg) used in these experiments were insufficient to completely antagonize the endogenous opioids circulating in the animals, we cannot dispute the clear evidence that the endogenous opioids do not play an age-dependent role in mediating the "regulated' decrease in core temperature that occurs during acute moderate hypoxemia in guinea pigs.

Administration of either 1, 2 or 4 mg/kg of naloxone did not significantly alter baseline core temperature in comparison to the response observed following an intraperitoneal injection of vehicle during normoxemia. This is in agreement with the data of Kandasamy and Williams (1983) who showed that an intraperitoneal injection of either 1, 3, or 5 mg/kg of naloxone did not significantly

alter core temperature in chronically instrumented adult male guinea pigs. Alternatively Blatteis et. al. (1991) have demonstrated that higher doses of naloxone (ie. 10 mg/kg) produced small changes in core temperature in adult guinea pigs, which has also been shown to occur in adult rats (Goldstein and Lowery, 1975). However, it is generally accepted that the core temperature response to opioid antagonists, such as naloxone, is too variable, small, and insensitive to conclude that the endogenous opioids play a tonic role in maintaining core temperature (Clark, 1979).

Neither newborn nor older guinea pigs chose a significantly lower selected ambient temperature during acute moderate hypoxemia following an intraperitoneal injection of vehicle, and this response was not significantly altered by the prior administration of either 1, 2, or 4 mg/kg of naloxone. Therefore, the prior administration of any dose of naloxone did not alter the behavioural thermoregulatory response in either age group of animals. In contrast to this, in the study by Mayfield et. al. (1994) administration of the opioid antagonist – naloxone, eliminated the decrease in selected ambient temperature that accompanied the decrease in core temperature following severe hypoxemia in adult mice. Therefore, the results obtained in the present study do not support the evidence that the endogenous opioids play a role in the "regulated" aspect of the decrease in core temperature that occurs during acute hypoxemia. As was discussed previously in reference to the discrepancy about the involvement of the endogenous opioids in the decrease in core temperature

that occurs during acute moderate hypoxemia, we cannot overlook the possibility of a species difference in the concentration and distribution of opioid binding sites between mice and guinea pigs which may account for the inconsistent results.

Vehicle did not have a significant effect on selected ambient temperature during normoxemia and there was no significant difference with the administration of either 1, 2, or 4 mg/kg of naloxone when compared to the response observed with saline. The lack of an alteration of this behavioural thermoregulatory effector mechanism under basal conditions is in agreement with previous findings by Spencer et. al. (1990) on adult rats studied in a thermocline, in which it was demonstrated that pretreatment with either 3 or 10 mg/kg of naloxone failed to have a significant effect on the animal's behavioural thermoregulation.

In newborn, but not in older guinea pigs oxygen consumption decreased significantly during acute moderate hypoxemia following the administration of both 2 and 4 mg/kg of naloxone as compared to the response observed following the administration of vehicle. However, this decrease in oxygen consumption was not accompanied by either an accentuation of the decrease in core temperature or an increase or decrease in selected ambient temperature that would have occurred if the decrease in oxygen consumption was in fact due to a physiological thermoregulatory response. Given that there appeared to be no attempt on behalf of the newborn guinea pigs to coordinate their autonomic and

behavioural effector mechanisms to compensate for, or facilitate, the decrease in oxygen consumption, it can be argued that this response may be a statistical artifact rather than a physiological event.

In addition to the changes in oxygen consumption that occurred during acute moderate hypoxemia, newborn but not older guinea pigs demonstrated a decrease in oxygen consumption during normoxemia with the prior administration of 4 mg/kg of naloxone in compared to the response that was observed following an intraperitoneal injection of vehicle. Therefore, we cannot overlook the possibility that the decrease in oxygen consumption that occurred during hypoxemia in the newborn animals was not due to the hypoxemic episode, but rather it was an artifact of the injection of naloxone.

Recently Xin et. al. (1997) have confirmed that both mu and kappa opioid receptor subtypes play a role in thermoregulation in the adult rat. Numerous investigators have indicated that the role of opioids and their receptors in thermoregulation is via an alteration of set point that in mice and rats influences core temperature by either increasing or decreasing oxygen consumption (Estler, 1961; Lotti et. al., 1966; Lynch et. al., 1987; Zwil et. al., 1988; Handler et. al., 1992). Hence, we must consider that naloxone which binds more preferentially to the mu receptor subtype than the kappa receptor subtype (Geller et. al., 1983) may disrupt the existing balance between the two receptors which have been shown to be involved in thermoregulation, resulting in the observed decrease in oxygen consumption. Furthermore, Malin et. al. (1985)

have shown that a subcutaneous injection of naloxone resulted in a significant decrease in oxygen consumption in adult rats. Hence, given that oxygen consumption has been shown to be altered by opioids, it is most likely that the decrease in oxygen consumption that occurred in the newborn guinea pigs during acute moderate hypoxemia was in fact a result of the injection of the higher doses of naloxone rather than the hypoxemic exposure.

It is also possible that the endogenous opioids may play a role in maintaining baseline activity levels, and therefore blocking their action via an intraperitoneal injection of 4 mg/kg of naloxone may result in the observed decrease in oxygen consumption in the newborn guinea pigs. In fact, the endogenous opioids have been implicated in maintaining baseline activity in rats and rabbits as both subcutaneous and intravenous administration of various doses of the general opioid antagonist - naloxone - have been shown to produce a decrease in general locomotor activity (Walker et. al., 1981; DeRossett and Holtzman, 1982; Schindler et. al., 1990). Alternatively, DeRossett and Holtzman (1982) and Schnur and Barela (1984) have demonstrated that the endogenous opioids are not involved in maintaining baseline activity level, as naloxone administration failed to significantly alter activity in mice and hamsters respectively. Similarly, a lack of a role for the endogenous opioids in maintaining baseline activity in guinea pigs has been illustrated by Andrews and Holtzman (1987) and Chahl and Thornton (1987).

Therefore, it is improbable that an intraperitoneal injection of naloxone caused a significant reduction in baseline activity in the newborn guinea pigs in this series of experiments which would have accounted for the observed decrease in oxygen consumption. It is true, that the opioids have been found to influence such activity in other species as previously mentioned, but given the species dependent distribution of opioid receptors within the rodent brain (Robson et. al., 1985) it is not surprising that such variation exists.

An interesting question that has emerged, is why the decreases in oxygen consumption were limited to the newborn guinea pigs? We did not observe any alterations in oxygen consumption either during normoxemia or hypoxemia in the older guinea pigs. It is possible that the different oxygen consumption responses observed may be related to the role of nonshivering thermogenesis in the newborn versus the older guinea pigs. Nonshivering thermogenesis, which utilizes brown adipose tissue, is the prevailing mechanism of heat production in the newborn guinea pig (Bruck and Wunnenberg, 1966). In contrast, by 3 weeks of age the contribution of nonshivering thermogenesis to heat production in the guinea pig is greatly reduced (Bruck and Wunnenberg, 1966). Our experiments were carried out on two age groups of guinea pigs, a newborn group that were between 5 to 10 days of age, and an older group which were between 25 to 30 days of age. Considering this, and studies by Egawa et. al. (1993) and Schlenker and Inamdar (1995) that have demonstrated a role for the opioid, β-endorphin in nonshivering thermogenesis in adult rats and hamsters

respectively, it is possible that the age dependent oxygen consumption response to naloxone during both normoxemia and hypoxemia is related to the role of nonshivering thermogenesis in newborn but not older guinea pigs. Regardless of the mechanism causing the decrease in oxygen consumption in the newborn guinea pigs, the question remains as to why the response is age-dependent and this merits further investigation.

4.2.2. Cardiorespiratory variables

The biphasic respiratory rate response that occurred during acute moderate hypoxemia following an intraperitoneal injection of vehicle was not altered by the prior administration of either 1, 2, or 4 mg/kg of naloxone in both the newborn and older guinea pigs. The third epoch during the hypoxemic experimental period in the newborn animals that received an intraperitoneal injection of 4 mg/kg of naloxone was significantly different when compared to the same point in the animals that were injected with vehicle. Although the response was different, the biphasic respiratory response characteristic of hypoxemia was not significantly altered, and therefore it was concluded that the endogenous opioids are not involved in the biphasic respiratory response to acute moderate hypoxemia in both newborn and older guinea pigs.

The apparent absence of a role of endogenous opioids in the respiratory rate response to acute moderate hypoxemia in both age groups of guinea pigs is in contrast to the findings of other investigators who have shown that naloxone is effective in reversing the hypoxemic induced depression of respiratory rate in both newborn rabbits (Grunstein et. al., 1981) and humans (DeBoeck et. al., 1984). Similar to our findings however, Steinbrook et. al. (1984) did not observe an effect of naloxone on the ventilatory responses to hypoxemia in adult rats. Therefore, as was eluded to earlier, the effect of the endogenous opioids to modulate the responses to hypoxemia seem to be species dependent and given

the differences in opioid concentration and receptor distribution in rodent brains outlined by Robson et. al. (1985) such differences are not surprising.

Respiratory rate decreased significantly during normoxemia following an intraperitoneal injection of vehicle in both newborn and older guinea pigs. The respiratory rate response was significantly altered by the prior administration of either 1 or 2 mg/kg of naloxone in newborn guinea pigs, but no dose of naloxone altered the respiratory rate response in the older guinea pigs. Considering that it has been shown that direct application of the opioids Met-enkephalin or β-endorphin on the brain stem of intact mammals leads to respiratory depression (Denavit-Saubie et. al., 1978; Florez and Mediavilla, 1977; Moss and Friedman, 1978), it is possible that the endogenous opioids may be involved in mediating the decrease in respiratory rate that was observed in both newborn and older guinea pigs that received an intraperitoneal injection of vehicle that experienced normoxemia during the experimental period.

The possible involvement of the endogenous opioids in influencing respiratory rate is supported by the observation that in newborn guinea pigs the respiratory rate response with 1 and 2 mg/kg of naloxone was significantly altered. As well, it has been shown that in both newborns and adults of several species administration of naloxone or other general opioid antagonists results in an increase in respiratory rate (Isom and Elshowihy, 1980; Grunstein et. al., 1981; Hazinski et. al., 1981; Farber and Maltby, 1983; Moss et. al., 1987). An increase in respiratory rate following the administration of an opioid antagonist

such as naloxone would implicate a tonic role for the endogenous opioids in newborn guinea pigs. Interestingly, we did not see an alteration of the respiratory rate response with any dose of naloxone in the older guinea pigs. It may be possible that we did not observe any response because the endogenous opioids may play a greater role in modulating the respiratory rate in newborn versus older guinea pigs as has been illustrated in piglets by Moss, Sugarman and Goode (1987).

However, as with other possible roles for the endogenous opioids, once again species seems to influence the response. In contrast to the evidence propagating a role for the opioids in maintaining baseline respiratory rate, DeBoeck et. al. (1984) found that in human infants no change in respiratory rate was induced by administration of 1 mg/kg of naloxone during normoxemia. Therefore, not only species, but as mentioned previously age, must be considered before a blanket statement can be made regarding the tonic role of endogenous opioids in maintaining respiratory rate. The apparent influence of the age of the guinea pig to influence the modulatory role of endogenous opioids on respiratory rate was an interesting observation made during the course of these experiments and is one that merits further investigation.

With vehicle, heart rate decreased during acute moderate hypoxemia in newborn guinea pigs and this response was not significantly altered by the prior administration of either 1, 2, or 4 mg/kg of naloxone. Similarly, administration of any dose of naloxone did not significantly alter the heart rate response observed during acute moderate hypoxemia following an intraperitoneal injection of vehicle in older guinea pigs. The role of endogenous opioids on heart rate control has been summarized by Holaday (1983) in an attempt to bring together overwhelming information that varies according to the species being studied, whether the animal is anesthetized or not, and the route of administration of the agonists or antagonists that have been utilized to elucidate the cardiovascular effects of opioids. However, it is generally well accepted that the opioids can modulate cardiovascular variables (Holaday, 1983).

There are similarities between hypoxemia and the opioids. In fact, Laubie et. al. (1977) have shown that an injection of β-endorphin into the interstitial fluid of anesthetized dogs produced a transient increase in heart rate followed by a delayed bradycardia. This response has also been observed during acute moderate hypoxemia in dogs, humans, and rats (Resnik, 1925a; 1925b; 1925c; Rahn and Otis, 1947; Thomas and Marshall, 1994). Hence, it was interesting that prior administration of any dose of naloxone did not alter the decrease in heart rate in the newborn guinea pigs during hypoxemia.

Since the decrease in heart rate was not altered by the opioid antagonist naloxone, we must consider other possibilities for the heart rate response. The

purine nucleoside, adenosine has been implicated as a modulator of the decrease in heart rate during hypoxemia in adult rats (Thomas and Marshall, 1994) and in isolated perfused rat, rabbit, and guinea pig hearts (Froldi and Belardinelli, 1990), and will be examined further in section 4.3.3. (pg. 123) of the discussion.

Prior administration of any dose of naloxone did not have a significant affect on baseline heart rate when compared to the response observed following the administration of vehicle in either newborn or older guinea pigs. The endogenous opioid system is usually quiet until there is a perturbation of the circulatory system (Holaday, 1983), given this, an intraperitoneal injection of the opioid antagonist -naloxone- into the guinea pigs should not have had an effect on heart rate. The previous statement could be made, because during an experimental period of normoxemia we would hypothesize that there would be minimal fluctuation from homeostasis. In a review prepared by Holaday and Loh (1981) they summarized that injections of naloxone into both anesthetized and unanesthetized control animals produced no significant alterations of cardiovascular variables.

4.3. THE ROLE OF ADENOSINE

4.3.1. Aminophylline dosing experiments

An intraperitoneal injection of either 5, 10 or 15 mg/kg of aminophylline-86% theophylline by weight with 14% ethylenediamine [which has been shown to be therapeutically inert (Ritchie, 1975)] added to increase solubility (Katzung, 1995; Serafin, 1996) yielded plasma concentrations of theophylline that were quite similar in both newborn and older guinea pigs, and that remained relatively constant over the 90 minute experimental period. The dose of 10 mg/kg of aminophylline that was utilized in this series of experiments, was chosen based upon the observation that the plasma levels of theophylline that were obtained following an intraperitoneal injection of this dose of aminophylline were around 70 umol/L or approximately 13 mg/L in both newborn and older guinea pigs. In a similar dosing study, a dose of 10 mg/kg of theophylline given via an orogastric tube produced similar theophylline plasma concentrations of 10 to 12 mg/L in both newborn and older rabbits (Denenberg et. al., 1982). Theophylline concentrations of around 70 umol/L have been shown by Muttitt et. al. (1988) to be within the most effective plasma concentrations of theophylline in reducing the apnea of prematurity in newborn humans. Furthermore, 10 mg/kg of aminophylline produced theophylline plasma concentrations in both the newborn and older guinea pigs that were near the upper end of the therapeutic levels of

theophylline used clinically at 15 mg/L, but not within toxic levels of greater than 20 mg/L (Katzung, 1995; Serafin, 1996). Given that theophylline has a dual effect of both adenosine receptor antagonism as well as being an inhibitor of phosphodiesterase activity (Rall, 1985), it should be noted that the theophylline plasma concentrations of around 70 umol/L which were obtained via the dose of aminophylline utilized in the course of the present experiments were well below the plasma level of theophylline concentrations of around 200 uM or within millimolar range that are thought to be required for phosphodiesterase inhibition (Rall, 1981; Wang et. al., 1989). Therefore, it is likely that the antagonistic actions of theophylline (via aminophylline) observed in this series of experiments were due to the antagonism of either A_1 or A_2 adenosine receptors rather than inhibition of phosphodiesterase activity.

4.3.2. Thermoregulatory variables

With vehicle, core temperature decreased during acute moderate hypoxemia and prior administration of 10 mg/kg of aminophylline – an adenosine antagonist – which is slightly more selective for the A_1 adenosine receptor subtype (Wang et. al., 1990) and which readily crosses the blood brain barrier (Laudignon et. al., 1991) significantly attenuated this response in both newborn and older guinea pigs. When we compared the core temperature response that occurred during hypoxemia to the core temperature response that occurred during normoxemia in newborn and older guinea pigs that received an intraperitoneal injection of vehicle, there were several points that were significantly different. However, if we carried out the same comparison on the newborn and older animals that received an intraperitoneal injection of aminophylline there were no time points that were significantly different. Therefore, it can be concluded that in guinea pigs, the decrease in core temperature that occurs during acute moderate hypoxemia was eliminated by the prior administration of the general adenosine antagonist, aminophylline and this response was not age dependent.

This conclusion was supported further by the observation that the core temperature indexes obtained for the newborn and older guinea pigs that received aminophylline and experienced hypoxemia were significantly different from the newborn and older guinea pigs that received vehicle and experienced

hypoxemia. As well, there was a significant difference between the core temperature indexes of the animals that experienced normoxemia and the animals that experienced hypoxemia following vehicle administration, which was eliminated following the administration of aminophylline.

The purine nucleoside, adenosine, was first shown to influence core temperature when Bennet and Drury (1931) intravenously administered adenosine to dogs and noticed a decrease in core temperature. Since that time adenosine has been shown to influence thermoregulation both centrally via receptors in the preoptic anterior hypothalamus (Yarbrough and McGuffin-Clineschmidt, 1981; Anderson et. al., 1994) and peripherally (Jonzon et. al., 1986; Wager-Srdar et. al., 1983) by most likely inhibiting nonshivering thermogenesis in brown adipose tissue (Bruns, 1990; Vernon et. al., 1991).

Intracerebroventricular administration of adenosine and/or adenosine analogues produced a dose dependent hypothermia in conscious adult mice, which was antagonized by an intraperitoneal injection of either an A_1 specific or a general adenosine antagonist that have been demonstrated to readily cross the blood brain barrier (Yarbrough and McGuffin-Clineschmidt, 1981; Anderson et. al., 1994). Given these results, Anderson et. al. (1994) have concluded that the hypothermia produced in adult mice by centrally administered adenosine analogues is the result of activation of the adenosine A_1 receptor subtype located within the preoptic anterior hypothalamus. Ticho and Radulovacki (1991) demonstrated that there are indeed adenosine A_1 receptor subtypes

present in the preoptic area of the anterior hypothalamus of rats. Given that the preoptic anterior hypothalamus is the major site for thermoregulatory control in mammals (Bleigh, 1973), the fact that adenosine analogues influenced thermoregulation by producing hypothermia when injected into this brain area lends further support to a central role for adenosine in thermoregulation.

In addition to a central thermoregulatory role for adenosine, a peripheral role has also been postulated given its inhibitory influence on brown adipose tissue (Bruns, 1990; Vernon et. al., 1991). Several investigators, including Nedergaard and Lindberg (1982) and Schimmel et. al. (1985;1987) have demonstrated an inhibitory role for adenosine on brown adipose tissue via a modulatory inhibition on β3 - adrenergic stimulated adenylate cyclase activity. Furthermore, experiments carried out on aged adult rats, which have an elevated level of adenosine, have shown that administration of the adenosine antagonist aminophylline can reverse the inhibitory effects of adenosine on brown adipose tissue allowing the animals to mount a metabolic response to a cold environment thereby improving cold tolerance (Wang et. al., 1990; Wang and Lee, 1990; Lee et. al., 1990).

Adenosine levels in brain interstitial fluid have been shown to increase during periods of acute moderate hypoxemia in adult rats (Winn et. al., 1981; Phillis et. al., 1987; Barraco et. al., 1991) and in newborn piglets (Park et. al., 1987). Considering the elevated levels of adenosine during acute moderate hypoxemia we should note the similarities between the thermoregulatory effects

of adenosine and hypoxemia. In both newborns and adults of a number of species including humans, core temperature decreases during acute moderate hypoxemia (Behauge et. al., 1927; Gellhorn and Janus, 1936; Kottke et. al., 1948; Cross et. al., 1955; Hill, 1959; Gautier et. al., 1987; Mortola, 1993). In addition, hypoxemia has also been shown to inhibit nonshivering thermogenesis in newborn mammals (Moore and Underwood, 1962; Szekely et. al., 1971). As was discussed previously adenosine decreases core temperature both centrally via actions on the A_1 receptors within the preoptic anterior hypothalamus (Anderson et. al., 1994) and peripherally via actions on nonshivering thermogenesis (Bruns, 1990). The overlap between the effects of hypoxemia and adenosine is intriguing, and the elimination of the decrease in core temperature during acute moderate hypoxemia in both newborn and older guinea pigs that was observed in the present series of experiments provides evidence that adenosine is the mediator of the "regulated" decrease in core temperature during acute moderate hypoxemia in both newborn and older guinea pigs.

Contrary to our hypothesis we did not observe an age - dependent role for adenosine, as administration of the adenosine antagonist aminophylline eliminated the decrease in core temperature during acute moderate hypoxemia in both newborn and older guinea pigs. The lack of an age - dependent response was not expected given that the brain of a newborn rat has been shown to have a lower concentration of adenosine and a higher concentration of

adenosine metabolites (ie. inosine and hypoxanthine) than adult rats (Aranda et. al., 1989) and that Laudignon et. al. (1991) have shown that there is nearly a 3 fold greater increase in cerebrospinal fluid adenosine concentration during hypoxemia in 1 month old versus 1 to 3 day old piglets.

However, unlike previous findings in our laboratory, we did not observe an accentuation of the decrease in core temperature during acute moderate hypoxemia in the older versus the newborn guinea pigs following an intraperitoneal injection of vehicle. Therefore, it is possible that the apparent lack of an age-dependent role for adenosine in mediating the decrease in core temperature that occurs during acute moderate hypoxemia in guinea pigs was an artifact of the absence of an age-dependent core temperature response to hypoxemia itself in this series of experiments. In addition, we cannot overlook the obvious difference that these experiments were conducted on guinea pigs, which may have different adenosine concentrations and responses to hypoxemia than have been observed in piglets or rats, and species differences must be taken into account.

Vehicle did not have an effect on baseline core temperature and this response was not significantly altered by the prior administration of 10 mg/kg of aminophylline in neither newborn nor older guinea pigs. This is in agreement with previous work by Kandasamy and Williams (1983) who have shown that there were no significant changes in core temperature following an intracerebroventricular injection of either 10 or 30 ug of theophylline in adult

male guinea pigs. Furthermore, an intrahypothalamic injection of 50 ug of theophylline failed to cause a significant change in core temperature of adult rabbits (Woolf et. al., 1975). These findings are contradictory to the increase in core temperature that was shown to occur in adult rats following either an intracerebroventricular injection of 200 ug or intraperitoneal injections of 5 to 30 mg/kg of theophylline at both room temperature and at 8°C (Lin et. al., 1980). Similarly Lee et. al. (1990) have demonstrated that an intraperitoneal injection of aminophylline increases core temperature in cold exposed rats via an enhancement of thermogenesis. However, given that adenosine has been shown to inhibit presynaptic neurotransmitter release within the central nervous system (Fredholm and Dunwiddie, 1988; Phillis and Wu, 1987; Newby, 1984), it has been argued that the increase in core temperature and heat production following an injection of the general adenosine antagonist, aminophylline was due to a general behavioural excitation (Lin et. al., 1984) rather than an antagonism of adenosine receptors within the preoptic anterior hypothalamus or removal of the inhibitory effects of adenosine on brown adipose tissue thermogenesis.

Neither newborn nor older guinea pigs chose a significantly lower selected ambient temperature during acute moderate hypoxemia following an intraperitoneal injection of vehicle, and this response was not altered by the prior administration of 10 mg/kg of aminophylline. The insignificant alteration of the selected ambient temperature response during acute moderate hypoxemia with

the prior administration of 10 mg/kg of aminophylline is interesting considering that pretreatment with aminophylline eliminates the decrease in core temperature during acute moderate hypoxemia in both newborn and older guinea pigs. Of particular interest is the observation that the animals did not choose a cooler ambient temperature during acute moderate hypoxemia with the prior administration of aminophylline.

Based on the ideas of "forced" and "regulated" thermoregulatory responses outlined previously [Section 1.2.2.2. (pg. 9)] if the maintenance of core temperature during acute moderate hypoxemia with aminophylline pretreatment was a "forced" thermoregulatory response, then the animals would have chosen a cooler ambient temperature to compensate for the increase in core temperature above the "set point". However, if the maintenance of core temperature during acute moderate hypoxemia with aminophylline pretreatment was a "regulated" thermoregulatory response, then the animals would have chosen the same or warmer selected ambient temperature to facilitate the maintenance of the warmer core temperature. Since there was no statistically significant difference in selected ambient temperature between the guinea pigs treated with vehicle and those treated with aminophylline, we may conclude that the elimination of the decrease in core temperature during acute moderate hypoxemia with the prior administration of the general adenosine antagonist was not the result of a forced increase in core temperature due to the drug, but rather

it was the result of an inhibition of the influence of adenosine in mediating the "regulated" decrease in core temperature.

In both newborn and older guinea pigs vehicle had no significant effect on selected ambient temperature and prior administration of aminophylline did not alter this response. These findings are in contrast to a study conducted by Kavaliers (1980) in which it was shown that theophylline administration caused a dose dependent reduction in the water bath temperatures selected by white sucker fish. These results indicate that theophylline affects the positional changes encompassing behavioural thermoregulation which is employed by poikilothermic animals, such as the white sucker fish to control their core temperature. Selection of a cooler ambient temperature may be the result of administration of theophylline causing either a "forced" increase in core temperature or a "regulated" decrease in core temperature in the white sucker fish. However, core temperature was not reported in the study conducted by Kavaliers (1980).

As previously mentioned, Lin et. al. (1980) and Lee et. al. (1990) have demonstrated that an intraperitoneal injection of aminophylline results in an increase in core temperature in adult rats. Hence, we may speculate that the increase in core temperature that occurs following aminophylline administration in rats may be a "forced" thermoregulatory response. It is possible that in the present series of experiments we did not observe a significant alteration in selected ambient temperature following aminophylline administration when

compared to the response observed following the administration of vehicle because there was no significant change in core temperature in either newborn or older guinea pigs that received an intraperitoneal injection of aminophylline and experienced normoxemia during the experimental period. Therefore, since there was no elevation in core temperature, it was not necessary that the animals employ behavioural thermoregulatory effector mechanisms to account for "forced" alterations in core temperature.

Oxygen consumption did not change significantly during acute moderate hypoxemia in either the newborn or older guinea pigs that had received an intraperitoneal injection of vehicle and prior administration of 10 mg/kg of aminophylline did not alter this response. As was previously mentioned, since the animals were allowed to choose their preferred selected ambient temperature during the control period, oxygen consumption values were at basal levels and no further decreases in oxygen consumption during acute moderate hypoxemia were expected. Despite there being no difference in oxygen consumption during acute moderate hypoxemia, there was a significant increase in oxygen consumption during the recovery period in the newborn guinea pigs that received an intraperitoneal injection of aminophylline, in comparison to the newborn guinea pigs that received an intraperitoneal injection of vehicle. This increase in oxygen consumption was most likely an artifact of the increase in oxygen consumption that was observed to occur in the newborn animals after administration of 10 mg/kg of aminophylline.

Vehicle had no effect on oxygen consumption in neither the newborn nor the older guinea pigs. In newborn, but not older animals an intraperitoneal injection of 10 mg/kg of aminophylline caused a significant increase in oxygen consumption during normoxemia when compared to that which occurred following an intraperitoneal injection of vehicle. An increase in oxygen consumption following aminophylline administration has been shown to occur in various other spices including rats (Lin et. al., 1980; Lundberg et. al., 1981; Vonlanthen et. al., 1989), dogs (Olsen and Schlitt, 1981), human infants (Milsap et. al., 1980; Gerhardt et. al., 1979), and adults (Lakshminarayan et. al., 1978). As well, administration of adenosine and/or adenosine analogues have been shown to result in a decrease in oxygen consumption in rabbits (Matuszek and Gagalo, 1996) and rats (Green and Stoner, 1950; Wang et. al., 1992). Considering this, and the fact that in this series of experiments we observed an increase in oxygen consumption following the administration of the adenosine antagonist aminophylline, it would be logical to conclude that adenosine plays a role in maintaining basal levels of oxygen consumption in the newborn guinea pig.

An interesting question emerges as to why we observed an increase in oxygen consumption with aminophylline in the newborn but not the older guinea pigs. As has been mentioned previously there is a different role for nonshivering thermogenesis in newborn versus older guinea pigs. Nonshivering thermogenesis, which utilizes brown adipose tissue, is the prevailing mechanism

of heat production in the newborn guinea pig (Bruck and Wunnenberg, 1966). In contrast, by three weeks of age the contribution of nonshivering thermogenesis to heat production in the guinea pig is greatly reduced (Bruck and Wunnenberg, 1966). Our experiments were carried out on two groups of guinea pigs, a newborn group that were between 5 and 10 days of age, and an older group which were between 25 to 30 days of age.

It is known that adenosine inhibits brown adipose tissue thermogenesis (Bruns, 1990; Vernon et. al., 1991) by modulating β3 - adrenergic adenylate cyclase activity (Nedergaard and Lindberg, 1982; Schimmel et. al., 1985;1987), and that administration of the adenosine antagonist aminophylline can reverse the inhibitory effect of adenosine on nonshivering thermogenesis in adult rats (Wang et. al., 1990; Wang and Lee, 1990; Lee et. al., 1990). Given the action of adenosine and consequently aminophylline on brown adipose tissue, it is most likely that the increase in oxygen consumption that occurred in the newborn guinea pigs with an intraperitoneal injection of aminophylline was due to an increase in brown adipose tissue thermogenesis, and the differential use of brown adipose tissue in newborn and older guinea pigs can explain the lack of an oxygen consumption response in the older animals.

However, it is also possible that the increase in oxygen consumption in the newborn guinea pigs with aminophylline administration may be due to an increase in activity. Considering that adenosine has been shown to be an inhibitor of presynaptic neurotransmitter release within the central nervous

system (Newby, 1984; Phillis and Wu, 1987; Fredholm and Dunwiddie, 1988) and thereby causing marked sedative effects and decreased motor activity (Haulica et. al., 1973; Snyder et. al., 1981), we cannot overlook the possibility that administration of aminophylline may cause a general behavioural excitation. Lin et. al. (1984) have argued that the increase in oxygen consumption they observed in rats following aminophylline administration was due to a general behavioural excitation.

In the present series of experiments behavioural observations were noted, however, no numerical activity index was used as in previous experiments in our laboratory. Therefore, we cannot draw concise conclusions on any behavioural differences that occurred in the animals that received aminophylline in comparison to the animals that received an intraperitoneal injection of vehicle. If indeed the increase in oxygen consumption in the newborn guinea pigs following aminophylline administration was due to an increase in general behavioural activity, the question as to why it only occurred in the newborn and not the older guinea pigs remains to be answered and would warrant further investigation.

4.3.3. Cardiorespiratory variables

The biphasic respiratory rate response that occurred during acute moderate hypoxemia following an intraperitoneal injection of vehicle was not significantly altered by the prior administration of 10 mg/kg of aminophylline in either the newborn or older guinea pigs. The apparent inability of aminophylline to maintain the elevated respiratory rate during acute moderate hypoxemia in both newborn and older guinea pigs is consistent with the previous findings of Milerad (1987) in which it was demonstrated that administration of theophylline did not enhance the respiratory drive during an hypoxemic insult in human infants. Similarly, Thomas and Marshall (1994) have demonstrated that although administration of the adenosine antagonist 8-PT significantly altered the tidal volume response, it did not alter the respiratory rate response to hypoxemia in adult rats. It is possible that an intraperitoneal injection of aminophylline may have affected the tidal volume response to hypoxemia in the guinea pigs studied in the present series of experiments, but given the untethered nature of the experimental design, tidal volume was not assessed and hence cannot be commented upon.

In contrast to the present findings, Darnall (1985) and Darnall and Bruce (1987) have clearly demonstrated a role for adenosine in mediating the biphasic respiratory rate response to hypoxemia in newborn piglets, as pretreatment with aminophylline decreased the amount of ventilatory depression during hypoxemia

by preventing a decrease in respiratory frequency. A similar alteration of the biphasic respiratory response to hypoxemia with aminophylline has been illustrated by experiments conducted on glomectomized cats (Millhorn et. al., 1984) and adult humans (Easton and Anthonisen, 1988). The involvement of adenosine in the respiratory rate response to hypoxemia in these species lends support to the hypothesis put forth by Barraco et. al. (1991) that adenosine serves as a regulatory metabolite in the cerebral spinal fluid that influences the brainstem cardiorespiratory control mechanisms that would respond to insults such as hypoxemia.

Respiratory rate decreased significantly over time during normoxemia following an intraperitoneal injection of vehicle in both newborn and older guinea pigs. The respiratory rate response was significantly altered by the prior administration of 10 mg/kg of aminophylline in the newborn but not the older animals. Aminophylline administration did have an effect on respiratory rate in the older guinea pigs, but the effect did not reach statistical significance. Considering that application of adenosine and/or adenosine analogues systemically, to the fourth cerebral ventricle, to the cerebral cisternal system, or directly to respiratory related nuclei results in a decrease in respiratory rate in several newborn and adult animal species (Runold et. al., 1986; Yamamota et. al., 1985; Lagercrantz et. al., 1984; Eldridge et. al., 1984; Hedner et. al., 1985), it is possible that adenosine may be involved in mediating the decrease in respiratory rate that was observed in both the newborn and older guinea pigs

that experienced normoxemia during the experimental period in the present series of experiments.

A possible role for adenosine in mediating baseline respiratory rate is supported by the observation that both newborn and older guinea pigs had an altered respiratory rate response following aminophylline administration, however, only the newborn response reached a level of statistical significance. These observations are consistent with reports that administration of an adenosine antagonist stimulates baseline respiratory rate in cats (Eldridge et. al., 1983; 1985), newborn rabbits (Lagercrantz et. al., 1984), preterm rabbits (Hedner et. al., 1985), newborn humans (Gerhardt et. al., 1979) and adult rats (Neylon and Marshall, 1991). However, other investigators have shown that an adenosine antagonist, aminophylline, has no influence on baseline respiratory rate in both newborn piglets (Darnall, 1985) and adult rats (Thomas and Marshall, 1994). Since aminophylline is composed of theophylline as well as the therapeutically inert ethylenediamine, Eldridge et. al. (1983) carried out experiments to determine if the stimulatory role of aminophylline was due to the adenosine antagonistic actions of theophylline or whether it was the result of ethylenediamine. As was anticipated, ethylenediamine was found to have neither a stimulatory nor a inhibitory effect on baseline respiratory rate. Therefore, despite the contradictory evidence surrounding the involvement of adenosine and its antagonists on respiratory rate, Eldridge et. al. (1985) have concluded that adenosine acts as a tonic modulator of respiratory rate and that

stimulation of respiratory rate following aminophylline is due to its antagonist actions of centrally located adenosine receptors.

The question remains as to why we observed a statistically significant influence on respiratory rate following an intraperitoneal injection of aminophylline in the newborn and not the older guinea pigs. Lagercrantz et. al. (1984) have found that both an intraperitoneal injection or direct application to the fourth ventricle of an adenosine analogue produced a decrease in respiratory rate, that could be reversed by an intraperitoneal injection of aminophylline and which was age dependent, being more pronounced in the newborn versus the older rabbit kits. Similarly, Runold et. al. (1986) have also demonstrated an age dependent respiratory rate response to an intraperitoneal injection of an adenosine analogue, whereby the newborn rabbits had a much more pronounced decrease in respiratory rate than the older rabbits.

In an attempt to explain the discrepancy between the responses in the newborn and older rabbits, Runold et. al. (1986) conducted an analysis of receptor number and binding affinity for adenosine analogues in the rabbit brain. In support of the age dependent responses reported by Lagercrantz et. al. (1984) and Runold et. al. (1986) it was found that there were only minor differences between the number of adenosine binding sites in the brain of newborn versus older rabbits, however, the binding affinity was much higher in the newborn versus the older and adult rabbits (Runold et. al., 1986). Therefore, the more pronounced respiratory rate response to adenosine in the newborn

rabbits could be partially explained by the higher affinity of the adenosine receptor for the exogenously administered analogue. Given that there is a greater affinity for adenosine in the newborn brain, we may expect that there may be an equal affinity for a competitive antagonist, such as aminophylline. This possibility would provide an explanation for the age dependent respiratory rate response to aminophylline observed in the present series of experiments, in which the decrease in respiratory rate which occurred during normoxemia in both newborn and older guinea pigs was significantly altered by the prior administration of aminophylline in the newborn but not the older animals.

Although the binding affinity for adenosine and/or its analogues has been shown to be greater in newborn versus older animals we must combine this information with that provided by Aranda et. al., (1989) and Laudignon et. al. (1991) which illustrated that there is a lower concentration of adenosine in the brain and cerebrospinal fluid of newborn versus older rats and piglets, respectively. Hence, the binding affinity of the adenosine receptors in the newborn brain may need to be greater due to the decreased amount of adenosine that may be present at any time. Regardless of whether the difference in the respiratory rate response to aminophylline between newborn and older guinea pigs is due to circulating levels of adenosine or whether it is due to the binding affinity of receptors within the brain, this age dependent response is an interesting observation that has emerged and one that would be of interest to examine more fully.

With vehicle, heart rate decreased during acute moderate hypoxemia in newborn guinea pigs and this response was significantly altered by the prior administration of 10 mg/kg of aminophylline. Alternatively, administration of 10 mg/kg of aminophylline did not significantly alter the heart rate response observed during acute moderate hypoxemia following an intraperitoneal injection of vehicle in the older animals. A decrease in heart rate during hypoxemia has been demonstrated in several species including dogs (Resnik 1925a; 1025b; 1925c) humans (Rahn and Otis, 1947) rats (Neylon and Marshall, 1991) and in the isolated perfused hearts of rats, rabbits, and guinea pigs (Froldi and Belardinelli, 1990). Since adenosine levels have been shown to increase during periods of hypoxemia in both newborn and older animals (Winn et. al., 1981; Phillis et. al., 1987; Park et. al., 1987), the decrease in heart rate that occurs during hypoxemia has been attributed to an increase in adenosine levels (Berne et. al., 1983; Froldi and Belardinelli, 1990; Neylon and Marshall, 1991; Thomas and Marshall, 1994). It is known that adenosine and/or adenosine analogues can influence heart rate (Drury and Szent-Gyorgyi, 1929; Bruns, 1990; Berne, 1986). As well, administration of adenosine has been shown to decrease heart rate in both whole animal preparations and in isolated perfused hearts (Jonzon et. al., 1986; Froldi and Belardinelli, 1990).

In a review article, Ohisalo (1987) stated that evidence is accumulating that the decrease in heart rate (via a decrease in sinus-atrial node firing and a decrease in atrioventricular conduction) that occurs during hypoxemia is due to

adenosine. Ohisalo (1987) referred to studies carried out on several newborn and adult species which have demonstrated a clear role for adenosine in mediating the heart rate response to hypoxemia (Darnall, 1985; Darnall and Bruce, 1987; Muttitt et. al., 1988; Froldi and Belardinelli, 1990; Neylon and Marshall, 1991; Thomas and Marshall, 1994; Thomas et. al., 1994). In these experiments administration of an adenosine antagonist such as theophylline attenuated or eliminated the decrease in heart rate that has been observed to occur under hypoxemic conditions.

The prior administration of 10 mg/kg of aminophylline caused a significant alteration of the heart rate response observed following an intraperitoneal injection of vehicle in the newborn but not the older guinea pigs. An intraperitoneal injection of aminophylline caused a significant increase in heart rate in comparison to that which occurred following vehicle. The ability of aminophylline to influence baseline heart rate is in agreement with the findings of Darnall (1985), Verlato and Borgdorff (1990) and Neylon and Marshall (1994) which demonstrated an increase in heart rate following aminophylline administration in newborn piglets, rabbits, and rats, respectively. Couple these findings to those which have shown an inhibitory modulatory role for adenosine on heart rate under basal conditions in rats (Jonzon et. al., 1986) and we can infer a tonic role for adenosine in maintaining baseline heart rate levels in newborn guinea pigs.

As with the respiratory rate response, we observed an age dependent response on heart rate with an intraperitoneal injection of aminophylline under both normoxemic and hypoxemic conditions. Administration of aminophylline resulted in an increase in heart rate in the newborn but not the older guinea pigs during both hypoxemia and normoxemia. This age dependent response may, in part, be explained by the higher binding affinity of adenosine receptors in the brain of a newborn versus an older animal as determined by Runold et. al. (1986). It is also possible that the theophylline plasma concentrations attained were insufficient in the older animals to block the adenosine receptors responsible for modulating heart rate. But since the plasma concentrations of theophylline in the present series of experiments were similar in both newborn and older guinea pigs and have been sufficient enough to eliminate the decrease in core temperature that occurs during acute moderate hypoxemia, an insufficient level of antagonist in the older animals is an improbable explanation for the lack of a heart rate response following aminophylline administration. Whether there is a differential binding affinity of aminophylline for adenosine receptors or an inability of adenosine antagonists to modulate the heart rate response in an older guinea pig, the question as to why age influences the heart rate response to aminophylline in guinea pigs merits further investigation.

4.4. CONCLUSIONS

The present experiments were carried out to test the hypotheses that 1) the endogenous opioids play an age-dependent role in mediating the "regulated" decrease in core temperature that occurs in guinea pigs during acute moderate hypoxemia, and 2) that adenosine plays an age-dependent role in mediating the "regulated" decrease in core temperature that occurs in guinea pigs during acute moderate hypoxemia.

Our data do not support the first hypothesis. The endogenous opioids do not play an age-dependent role in mediating the "regulated" decrease in core temperature that occurs in guinea pigs during acute moderate hypoxemia as an intraperitoneal injection of either 1, 2, or 4 mg/kg of naloxone, a general opioid antagonist, failed to significantly alter the core temperature response to hypoxemia in comparison to that which occurred following an intraperitoneal injection of vehicle. In addition, blocking the endogenous opioids had no effect on the selected ambient temperature response to hypoxemia in either the newborn or the older guinea pigs. Oxygen consumption did not change significantly during hypoxemia, however, our data provide evidence that the endogenous opioids may play a role in maintaining oxygen consumption in the newborn guinea pig where nonshivering thermogenesis is the principle mechanism of heat production.

The endogenous opioids do not appear to be playing a significant role in the biphasic respiratory rate response to acute moderate hypoxemia in either newborn or older guinea pigs. However, we have found a possible role for the endogenous opioids in maintaining the baseline respiratory rate in newborn but not older guinea pigs. The heart rate response to acute moderate hypoxemia in both the newborn and older guinea pigs was not significantly altered by the prior administration of any dose of naloxone. Similarly, naloxone had no effect on baseline heart rate in either age group of animals.

With respect to the second series of experiments, our data provide evidence to support the hypothesis that adenosine mediates the "regulated" decrease in core temperature that occurs in guinea pigs during acute moderate hypoxemia, however, this response was not age-dependent. This conclusion is based upon the observation that prior administration of 10 mg/kg of aminophylline, an adenosine antagonist, eliminated the decrease in core temperature that occurs during acute moderate hypoxemia in both newborn and older guinea pigs as there was no statistical difference between core temperature in those animals that received aminophylline and experienced hypoxemia and those that experienced normoxemia during the experimental period. It is possible that we never observed an age-dependent role for adenosine in mediating this response, because there was no difference in the core temperature response to hypoxemia in the newborn and older animals in the present series of experiments.

The "regulated" aspect of the core temperature response to acute moderate hypoxemia was not influenced by inhibiting adenosine action as there was no statistical difference between the selected ambient temperature response observed following an intraperitoneal injection of vehicle and the selected ambient temperature response that occurred following an intraperitoneal injection of aminophylline in either the newborn or older animals during hypoxemia. Similarly, aminophylline had no effect on baseline selected ambient temperature in either age group of animals.

Oxygen consumption did not vary significantly during hypoxemia in the newborn and older guinea pigs and the prior administration of aminophylline did not alter this response. However, as with the endogenous opioids, our data provide evidence that adenosine may play a tonic role in modulating basal levels of oxygen consumption in the newborn guinea pigs as administration of the adenosine antagonist - aminophylline - resulted in a significant increase in oxygen consumption values during normoxemia.

Adenosine does not seem to be playing a role in modulating the biphasic respiratory rate response to hypoxemia in either newborn or older guinea pigs. However, our data provide evidence that adenosine may play a role in maintaining baseline respiratory frequency in the guinea pig in an age-dependent manner. The heart rate response to acute moderate hypoxemia was significantly altered by the prior administration of aminophylline in the newborn but not the older guinea pigs. Therefore, we may conclude that adenosine is

involved in mediating the bradycardia that occurred during hypoxemia in the newborn animals. In addition to the modulatory role of adenosine during hypoxemia, our data provide evidence to imply an age-dependent role for adenosine in maintaining baseline heart rate levels in the guinea pig.

The data obtained during the course of the present experiments were vast and in many instances the study raised more questions than it was designed to answer. However, despite the ambiguity surrounding several of the examined variables, the present data undoubtedly provide evidence that adenosine, not the endogenous opioids, mediates the "regulated" decrease in core temperature that occurs in guinea pigs during acute moderate hypoxemia and this response is not age-dependent.

4.5. LIMITATIONS OF THE EXPERIMENTAL DESIGN

Although the experiments outiined in the course of this thesis were conducted in the manner we believed most appropriate a number of limitations have been identified

One limitation was that the experimental period of hypoxemia in the present experiments was 1 hour, this is in contrast to previous experiments conducted in our laboratory in which the experimental period was 2 hours. The 1 hour experimental period was chosen in this study due to the half life of the endogenous opioid antagonist - naloxone - which is around 90 minutes (Goodman and Gilman, 1996). The 1 hour experimental period may have influenced the response to hypoxemia such that we did not observe an accentuation of the decrease in core temperature during acute moderate hypoxemia in the older guinea pigs.

Another limitation of the study was the design of the experimental apparatus. Because of the length of the thermocline and the way in which hypoxemic conditions were introduced during the experimental period we could not be sure of proper oxygen consumption values being obtained until approximately 30 minutes into the experimental and recovery periods. This time point was based upon calculations involving the volume of the thermocline, the flow of the gas into the thermocline, and the animals position in the thermocline

once the flow rate of the desired inflow gas was returned to normal flow rate values.

In terms of the respiratory response to hypoxemia, due to the untethered nature of the experiments we were only able to record the respiratory rate response. Therefore, if tidal volume or other respiratory related variables were altered during the course of the experiments we were unaware of these changes. In a similar manner we were limited to counting heart rate via the QRS complex which was transferred onto the Grass Polygraph recorder and therefore could not comment on whether changes that occurred during the course of the experiments were due to alterations in sinoatrial node firing rate or whether they were due to atrioventricular conduction.

Another limitation to study design that has emerged is that we did not have any measure of other thermoregulatory effector mechanisms, such as vasodilatation or respiratory evaporative heat loss that may have helped to explain exactly how the decrease in core temperature occurred during acute moderate hypoxemia, since there was no decrease in oxygen consumption or selected ambient temperature in either age group of animals. We did attempt to look at values of thermal conductance, but since the animals were in a thermocline and allowed to change their selected ambient temperature, rather than being at a fixed ambient temperature, values of thermal conductance were extremely variable and uninformative and hence were not reported.

A further limitation to the study was that there was no numerical activity index utilized to determine if administration of the antagonists altered baseline activity or whether the behavioural response to hypoxemia was different in the drug treated versus the vehicle treated animals. Given that both naloxone and aminophylline have been implicated in altering activity levels, a numerical activity index may have been useful in interpreting data and drawing conclusions.

In the present experiments we used general antagonists of both the endogenous opioids and adenosine despite there being specific antagonists for the various opioid and adenosine receptor subtypes. Given the general nature of the antagonists we cannot be sure that all receptor subtypes were blocked equally and this must be taken into consideration. However, these experiments were not designed to determine the exact receptor subtypes that are involved in mediating the "regulated" decrease in core temperature during acute moderate hypoxemia in guinea pigs, they were merely designed to elucidate whether the endogenous opioids or adenosine play a role in mediating this response.

Despite these limitations, the experiments that were conducted produced valuable data that provide clear evidence that adenosine, not the endogenous opioids, are involved in mediating the "regulated" decrease in core temperature that occurs in guinea pigs during acute moderate hypoxemia and this response is not age-dependent.

4.6. FUTURE DIRECTIONS

As with any series of experiments more questions than answers emerge as the scientific process continues, and this thesis was no exception. Throughout the course of the discussion whenever points for future experiments arose they were addressed, therefore this section will provide a summary of these points of further investigation as well as other ideas that would be interesting to examine more fully.

Considering that the present experiments were conducted using a general adenosine antagonist, a future series of experiments should examine whether there is a specific adenosine receptor involved in mediating the "regulated" decrease in core temperature during acute moderate hypoxemia in both newborn and older guinea pigs, and if the role of the receptors is influenced by the age of the animal. In keeping with the idea that the maturity of the animal may influence the role of adenosine in mediating the "regulated" decrease in core temperature during acute moderate hypoxemia, another series of experiments could determine if maturity at birth alters the role of adenosine in this response. At the present time experiments are being conducted in our laboratory to determine if maturity at birth (ie. precocial vs. altricial species) influences the "regulated" thermoregulatory response to hypoxemia. Therefore, once the thermoregulatory profile to hypoxemia is determined in an altricial species, such as the rabbit, then a series of experiments comparable to the ones

conducted for this thesis should be carried out to determine the mediator of the core temperature response to hypoxemia.

Throughout the discussion it became apparent that the age of the guinea pigs were instrumental in determining the response of various variables to either hypoxemia, drug administration, or a combination of drug administration and hypoxemia. For example, oxygen consumption was affected by the administration of naloxone in the newborn but not the older guinea pigs. Similarly, aminophylline had an effect on oxygen consumption in the newborn but not the older animals. This age dependent effect of the endogenous opioids and adenosine on oxygen consumption in the guinea pig should be examined more closely to determine if it is indeed due to a differential use of brown adipose tissue, or whether there are different mechanisms controlling oxygen consumption in newborn and older guinea pigs.

In addition to oxygen consumption, respiratory rate and heart rate were also influenced by the age of the guinea pigs in the present series of experiments. Therefore, future investigations should be conducted which would examine the influence of age on the possible role of the endogenous opioids and adenosine in mediating both tonic respiratory rate and heart rate control as well as the cardiorespiratory responses to hypoxemia in a much more in-depth manner than the these experiments permitted.

Another developmental question that emerged was the possibility that newborn and older guinea pigs employ different thermoregulatory mechanisms

to respond to an hypoxemic episode and consequently to recover from a decrease in core temperature that occurs during acute moderate hypoxemia. Hence, a series of experiments, which would measure the various effector mechanisms and their response to hypoxemia in both newborn and older guinea pigs, may provide some novel information about a postnatal maturation effect on the thermoregulatory responses to acute moderate hypoxemia.

In the present series of experiments the animals were studied in a thermoneutral environment. It would be interesting to determine if the mediator of the "regulated" decrease in core temperature in both newborn and older guinea pigs would differ depending on whether the animals were studied within a thermoneutral environment or whether they were clamped at a specific environmental temperature.

Several areas that warrant further investigation have emerged from the present set of experiments. Hopefully these experiments will be conducted so that we may gain a more complete understanding of the physiological role of both the endogenous opioids and adenosine as mediators of homeostasis and the role they play during episodes of hypoxemia in both newborn and older guinea pigs.

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APPENDIX

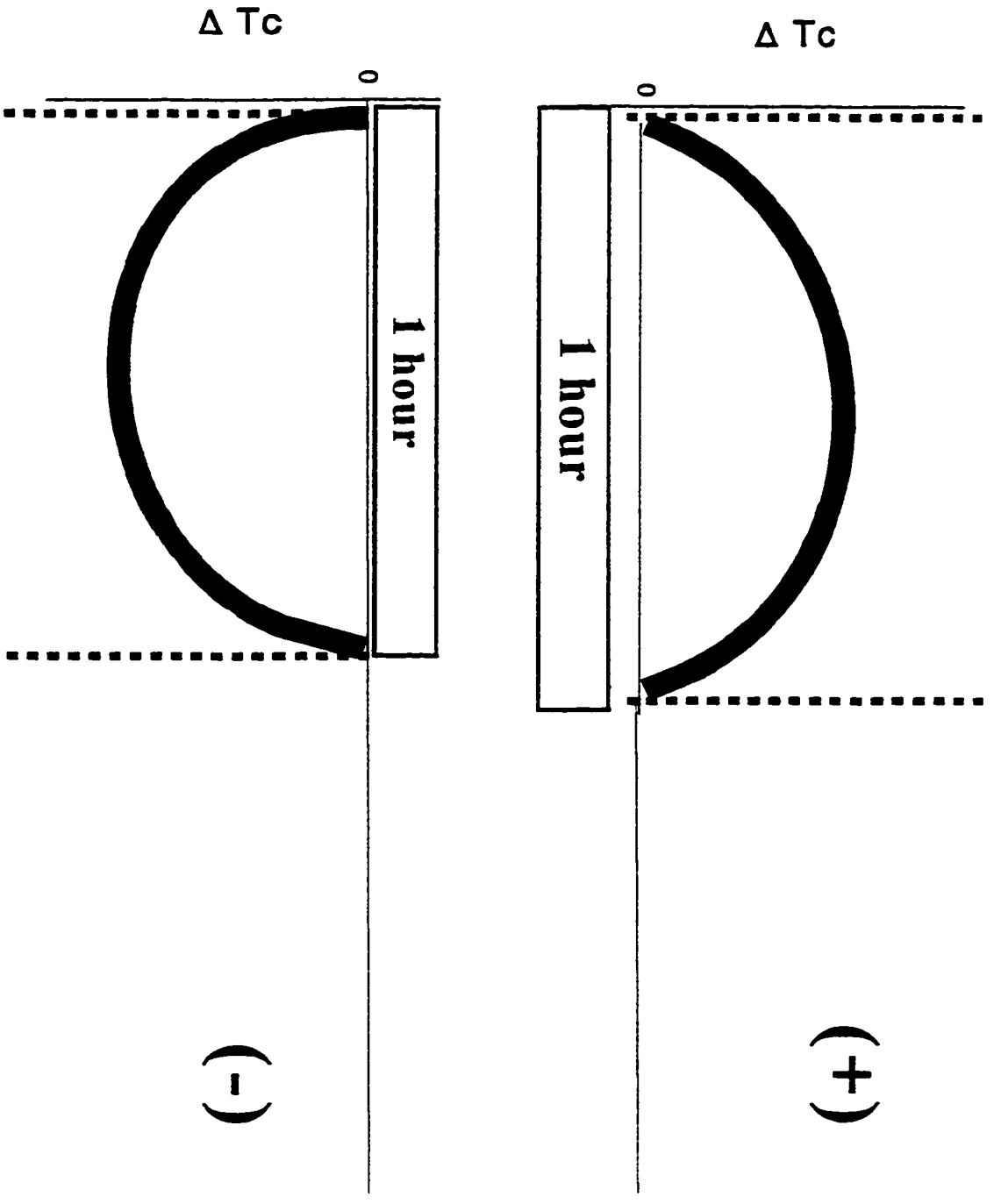
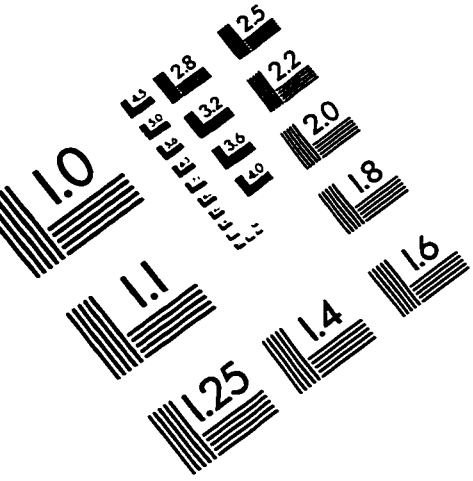
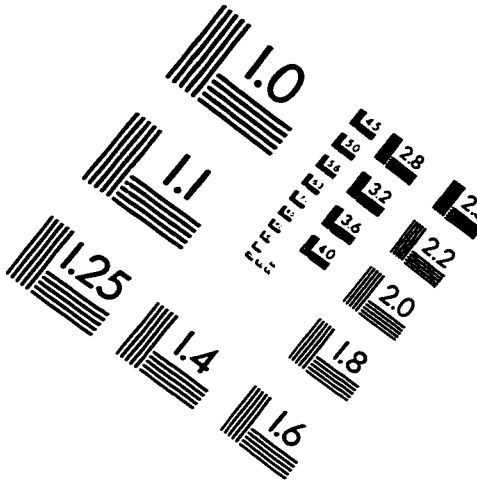
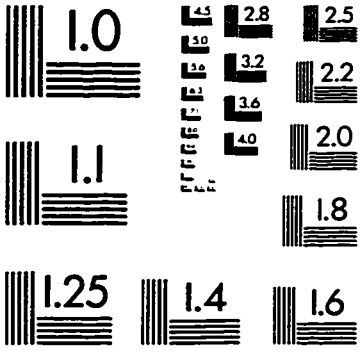


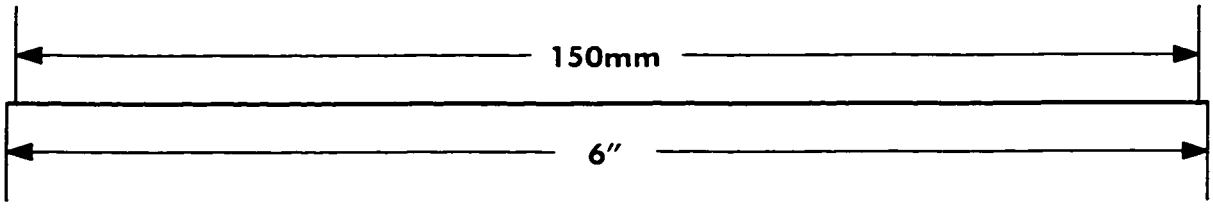
Figure A.1. Schematic representation of the determination of core temperature indexes

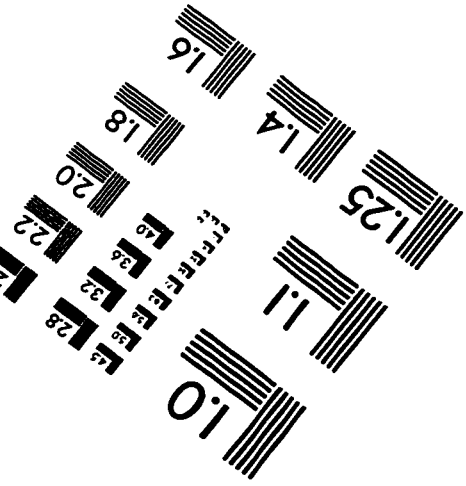
IMAGE EVALUATION TEST TARGET (QA-3)

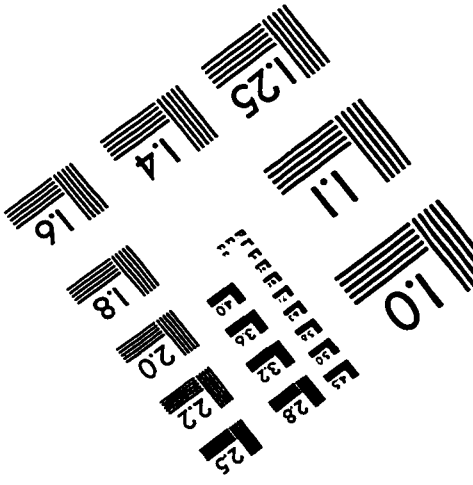












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1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989