The Blood Pressure and Thermal Effects of Clonidine Microinjected into the Paraventricular Hypothalamic Nucleus In Conscious Normotensive and Goldblatt Hypertensive Rats

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BY

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Abstract

The paraventricular nucleus of the hypothalamus (PVN) innervates sympathetic preganglionic cell bodies in the spinal cord that control both cardiovascular and thermoregulatory systems effector systems. Electrical stimulation of this nucleus increases blood pressure (BP) and heart rate, while its lesioning attenuates the pressor and tachycardic responses that occur following aortic baroreceptor deafferentiation. High concentrations of alpha-2 adrenoceptors have been also found in the PVN, and stimulation of these receptors in other central regions suppresses both BP and thermogenesis. However, the BP and thermal effects of the selective stimulation of the PVN alpha-2 receptors are unknown. The one-kidney Goldblatt (1K-GB) model of hypertension is characterized by altered (a) sympathetic activity purportedly mediated by central catecholaminergic mechanisms, including those in the PVN, and (b) sensitivity to pharmacologically or environmentally induced thermal challenges. Accordingly, the present study examined the cardiovascular and thermoregulatory effects of the selective stimulation of the PVN alpha-2 receptors with clonidine, a selective alpha-2 agonist, in conscious 1K-GB hypertensive and normotensive rats. Accordingly, 26 male rats underwent the induction of 1K-GB hypertension, while another 27 rats composed the normotensive group. All animals were progressively adapted to mild, physical

restraint, and implanted with cannulae in the PVN or in areas adjacent to the PVN, and with chronically indwelling aortic and jugular catheters. Following recovery from catheterization, all animals underwent a 3 day metabolic and BP testing procedure. Half of the hypertensive and normotensive animals received an intravenous injection of saline followed 30 minutes later by either 0 nmol, 1 nmol or 20 nmol of clonidine (0.5 ul), into the PVN or into adjacent areas over the 3 day testing period. The remaining half of the animals pretreated with an intravenous dose of rauwolscine, a selective alpha-2 antagonist, 30 minutes prior to each of the 3 microinjections of clonidine. Dependant measures included mean arterial BP, rectal temperature, and oxygen consumption. The results showed that clonidine microinjections into the PVN or non-PVN areas produce produced very small, and probably physiologically non- relevant effects on BP in either normotensive or 1K-GB hypertensive rats. Clonidine produced dose-dependant decreases in rectal temperature and oxygen consumption when injected into the PVN or non-PVN areas of normotensive animals. Rauwolscine was able to antagonize the hypothermic effects produced by the 20 nmol dose of clonidine in normotensive animals. However, when clonidine was injected into the PVN of 1K-GB animals, a larger hypothermic response was found in response to the 20 nmol dose than in normotensive animals, but not when injected into non-PVN

areas. Furthermore, rauwolscine actually potentiated the hypothermic effects of the 20 nmol dose of clonidine. The lack of BP effects following PVN administration of clonidine may be due to stimulation of parvocellular descending autonomic fibers. This stimulation produces selective vasoconstriction in various vascular beds without causing an overall change in BP. Such stimulation may shunt blood away from the core and hindquarter region, resulting also in decreases in rectal temperature. The increased responsiveness of 1K-GB animals to clonidine injected into the PVN, and to pretreatment with rauwolscine, suggests an altered central alpha-2 receptor population, particularly in the PVN.

The Blood Pressure and Thermal Effects of Clonidine Microinjected into the Paraventricular Hypothalamic Nucleus in Conscious Normotensive and Goldblatt Hypertensive Rats

The hypothalamus is a phylogenetically old component of the vertebrate brain that functions primarily to maintain internal homeostasis. One of its principle functions is to coordinate autonomic processes, including both oxygen and heat transport. The paraventricular nucleus of the hypothalamus (PVN) is a neuroanatomically complex nucleus. Its contribution to both these processes is suggested by evidence that (a) it contains a subpopulation of alpha receptors that, in other brain regions, have been linked to both blood pressure and temperature regulation, and (b) it controls both parasympathetic and sympathetic mechanisms by descending pathways to the brainstem and to the intermediolateral cell column of the spinal cord. Dysfunction of the autonomic nervous system has been implicated in the pathogenesis of arterial hypertension. The one-kidney Goldblatt model of hypertension is associated with alterations in central and peripheral catecholamine activity. Two premises emerge from these general observations: First, the PVN contributes to both cardiovascular and thermoregulatory functioning in the normotensive endotherm. Thus, selective neuropharmacological stimulation of the PVN alpha receptors

may evoke a coordinated cardiovascular and thermogenic response. Second, perturbations of the PVN's alpha adrenergic control of one autonomic process would alter the neuropharmacological sensitivity of those receptors for the remaining process. Thus, a model of arterial hypertension linked pathogenically to sympathetic excitation, such as the one-kidney Goldblatt model, may be accompanied by an altered profile of both cardiovascular and thermoregulatory responses. The purpose of this study was, therefore, to examine the role of the PVN in cardiovascular and thermoregulatory functioning by selective stimulation of its alpha receptors in both normotensive and one-kidney Goldblatt hypertensive rats.

Basic Mechanisms in BP Regulation

Blood pressure (BP) reflects both myocardial and vascular components. The myocardial component, cardiac output (CO), refers to the volume of blood pumped by each ventricle per unit time (Levine, 1976). It is determined by a multiplicative interaction of stroke volume, the volume of blood ejected by each ventricle per contraction, and heart rate (HR), the number of contractions per unit time. The vascular component, total peripheral resistance (TPR), refers to the BP consequences of changes in smooth muscle contraction of arteries and arterioles. Vasoconstriction, a

decrease in the diameter of blood vessels, elevates BP, while vasodilation, an increase in diameter of blood vessels, lowers BP (Levine, 1976).

Interaction of Cardiovascular and Thermoregulatory Systems

The cardiovascular and thermoregulatory systems are often considered to function independently. However, the evolution of complex behavior patterns among vertebrates required an increased aerobic capacity or endothermy. This increased oxygen processing ability required the co-evolution of several physio-chemical changes associated with oxygen uptake and transport, including an elevation in blood flow and pressure (Bennett & Ruben, 1979). Consequently, the vascular system not only serves to transport nutrients, such as oxygen, but also to control the thermal gradient between the body's core and its shell, and between the shell and the external environment. Exposure to a cold environmental temperature results in vaso-constriction, in an attempt by the body to conserve heat and maintain an adequate body temperature. Conversely, an adequate body temperature is maintained in a warm environment by vasodilation (Richards, 1973). Exposure to either cold or warm temperatures results in increases in oxygen consumption and carbon dioxide elimination and, therefore, metabolic rate (Christensen & Galbo, 1983).

The finding that both cardiovascular and thermoregulatory processes may be interdependant suggests that alterations in either system influence the functional integrity of the remaining system. Morishima and Gale (1972) proposed that the preoptic/anterior (POAH) region of the hypothalamus may serve as a site of regulation for both these systems. This hypothesis was based on their findings that cooling the POAH produced elevations in BP, HR and midbrain temperatures. This study suggests, therefore, an interdependance between the cardiovascular and thermoregulatory systems that may be centrally mediated. This central mediation was also proposed by Wasserstrum & Herd (1977). They showed that exposure to an ambient temperature of 10°C increased BP, HR, and oxygen consumption, and decreased rectal temperature in unanesthetized squirrel monkeys. Thus, the cardiovascular responses to low ambient temperatures involve more than vasoconstriction of skin blood vessels and, as the authors suggest, are centrally mediated.

Classical Notion of Cardiovascular Regulation

The baroreceptor reflex arc is involved in homeostatic control of BP and is composed of the nucleus tractus solitariius (NTS) and its afferent and efferent connections. The NTS is a medullary relay station in this arc and receives afferent input from baroreceptors (pressure receptors) located in the aortic arch and carotid sinus (Panneton & Loewy, 1980; Wallach & Loewy, 1980). In turn, efferent fibers from the NTS project to the spinal cord monosynaptically or polysynaptically via the dorsal motor nucleus of the vagus (DMV) and nucleus ambiguus, or indirectly via a hypothetical area in the rostral medulla termed the vasomotor center (Howe, 1985). The vasomotor center is generally located in the ventrolateral medulla (Howe, 1985) since electrical or chemical stimulation of this area results in vasoconstriction and hypertension (Dampney, Goodchild, Robertson, & Montgomery, 1982; McAllen, Neill, & Loewy, 1982); and lesioning reduces vasomotor tone and BP (Kumada, Dampney, & Reis, 1979; McAllen et al., 1982). If BP increases, the baroreceptors activate the NTS which, in turn, inhibits the vasomotor center and excites the DMV. The inhibition of the vasomotor center decreases TPR, while the excitation of the DMV decreases HR and, therefore, CO (Struyker-Boudier, Evenwell, Smits, & Van Essen, 1982). Decreases in both TPR and CO result in a fall in BP.

The ventrolateral medulla contains the classic "vasomotor center" and can be partitioned into the rostral C1 adrenergic group and the caudal A1 noradrenergic group (Reis, Granata, Joh, Ross, Ruggiero, & Park, 1984). The C1 group is considered a pressor area since its electrical stimulation increases BP (Dampney & Moon, 1980) and provides tonic vasomotor tone (Ross, Ruggeiro, Joh, Park, & Reis, 1983). It has, therefore, been equated with the vasomotor center (Reis et. al., 1984). It is an integral part of the baroreflex arc (Loewy & Burton, 1978), and projects to the intermediolateral cell column (Reis et. al., 1984). The A1 noradrenergic group projects to the hypothalamus but apparently does not participate in the baroreceptor reflex arc (Sawchenko & Swanson, 1982).

Although the functions previously attributed to the vasomotor center are now thought to be due to the actions of the C1 area, the notion of the vasomotor center persists. It is thought to receive descending projections from the hypothalamus. The anterior hypothalamus has been considered a depressor area since its electrical stimulation presumably inhibits the vasomotor center (Isaac, 1980) and decreases BP and HR (Hilton & Spyer, 1971). Conversely, the posterior hypothalamus has been labelled a pressor area since its electrical stimulation excites the vasomotor area (Isaac, 1980) and increases BP and HR (Przuntek, Guimaraes, & Philippu, 1971).

This classical notion of BP regulation involving the baroreflex arc, vasomotor center, and anterior and posterior hypothalamic nuclei is inadequate to account for the myriad of factors involved in BP control (Isaac, 1980). It is now known that other hypothalamic nuclei are involved in cardiovascular control as a result of their projections to the intermediolateral (IML) cell column of the spinal cord.

Sympathetic Control of Blood Pressure

Sympathetic preganglionic cell bodies are primarily located in the IML cell column of the thoraco-lumbar divisions of the spinal cord (Chung, Chung & Wurster, 1975). The IML cells relay central and peripheral input to autonomic effector organs such as the heart and blood vessels, via postganglionic neurons and, therefore, constitute the final common pathway controlling BP regulation. It is also through the IML cells that descending supramedullary input, including that from the brainstem and hypothalamus, controls BP (Petras & Cummings, 1972).

The most important brainstem input to the IML cell column is from the C1 adrenergic and A2 noradrenergic cell groups. Both these groups are located in the vicinity of the NTS, are involved in the baroreceptor reflex arc (Reis et al., 1984), and project directly to the IML cells (Lipski &

Trzebski, 1975; Reis et al., 1984). Additionally, the A5 (ventrolateral pons) (Loewy, McKellar & Saper, 1979), A6 (locus coeruleus) (McKellar & Loewy, 1979), and A7 (lateral pontine reticular formation) (Satoh, Tohyama, Yamamoto, Sakumoto, & Shimizu, 1977) noradrenergic cell groups also project to the IML cells.

Paraventricular Input to the IML Cell Column

Another major source of input to the preganglionic cell bodies in the IML cell column is the PVN. The existence of a paraventriculo-spinal pathway possessing reciprocal connections between the PVN and NTS and DMV, and between the PVN and IML cells has been established (Hosoya & Matsushita, 1979; Saper, Loewy, Swanson & Cowan, 1976; Sawchenko & Swanson, 1982; Swanson & Kuypers, 1980). In fact, Hosoya and Matsushita (1979) have found that nearly one-third of all hypothalamic fibers that project to the spinal cord originate in the PVN. The PVN consists of at least eight distinct cell groups cytoarchitectonically classified as either magnocellular or parvocellular (Swanson & Sawchenko, 1980). The magnocellular groups project primarily to the neurohypophysis, and to a lesser extent to the NTS and DMV and IML, while the parvocellular groups project primarily to the spinal cord, and NTS and DMV (Swanson & Kuypers, 1980).

The PVN and BP Regulation

Given the reciprocal connections between the PVN and brainstem nuclei involved in cardiovascular functioning, and between the PVN and sympathetic preganglionic fibers, relatively few studies have examined the PVN's role in cardiovascular regulation. Ciriello and Calaresu (1980a) electrically stimulated PVN parvocellular sites that give rise to the paraventriculo-spinal tract in anesthetized cats. Following sympathetic denervation of the heart by C2 transection, stimulation produced only tachycardia, whereas both tachycardia and pressor responses were found in bilaterally vagotomized animals. These results suggested that stimulation of the PVN (1) produced cardioacceleration by sympathoexcitation because tachycardia was found in the absence of parasympathetic innervation, (2) produced tachycardia also by vagal inhibition because this response was found in the absence of sympathetic innervation, and (3) produced a pressor response in vagotomized animals that was vasoconstrictor mediated because it could not be blocked by the beta-blocker, propranolol.

Zhang and Ciriello (1985) examined the effects of PVN lesions on the pressor and tachycardic responses produced by aortic baroreceptor denervation. They found that these responses were attenuated regardless of whether the lesions

were made before or after denervation. These results suggest that the PVN is involved in both the development and maintenance of the pressor and tachycardic responses that result from baroreceptor denervation.

These electrophysiological observations suggest that the PVN participates in BP regulation through elevations in HR and TPR. The cardiovascular consequences of chemical stimulation of the PVN are unknown. However, the finding that reciprocal connections exist between the PVN and catecholaminergic cell bodies in the NTS (A2) and the caudal ventrolateral medulla (A1) suggests a role for catecholaminergic receptors.

Alpha-2 Adrenoceptors and Anti-hypertensive Drugs

Two subclassifications of alpha-adrenoceptors based on their synaptic location and drug affinity have been proposed. A presynaptic location is inferred from observations that (a) norepinephrine (NE) release is increased by alpha blockade in <u>in vitro</u> preparations lacking postsynaptic effector cells (Vogel, Silberstein, Berv, & Kopin, 1972) in both alpha and beta receptor mediated effector organs (Dubocovich & Langer, 1974; Farah & Langer, 1974), and that (b) 6-hydroxydopamine (6-OHDA) induced degeneration of NE nerve endings in the rat heart decreases specific binding of tritiated-dihydroergocryptine, an

alpha-adrenoceptor ligand (Story, Briley, & Langer, 1979). A distinction between alpha-1 and alpha-2 receptors based on receptor affinities overlaps with the subclassification based on synaptic location. Accordingly, alpha-1 receptors are regarded to be largely postsynaptic but exhibit a high affinity for phenylephrine and prazosin, an alpha agonist and antagonist respectively (Starke & Langer, 1979). By comparison, alpha-2 receptors are predominantly pre-synaptically located but exhibit a selective affinity for the agonists clonidine and guanabenz, and the antagonists rauwolscine and yohimbine (Starke & Langer, 1979). When activated, these alpha-2 presynaptic receptors control a negative feedback system that inhibits additional vesicular release of NE either by decreasing the availability of calcium, or by preventing the propagation of the action potential (Langer, 1981). These alpha-2 receptors, however, can be both pre- or post-synaptically located (U'Prichard & Snyder, 1979). Thus, the criteria for distinguishing between alpha-1 and alpha-2 receptors provided by their synaptic location and drug affinities are not necessarily redundant.

Central alpha-1 receptors purportedly contribute little to cardiovascular regulation (Van Zwieten, 1985). By comparison, alpha-2 receptors are distributed in many central regions linked to cardiovascular functioning,

including the NTS, locus coeruleus, portions of the medial hypothalamus that circumvent the parvocellular region of the PVN (Young & Kuhar, 1980), with a high concentration within the PVN itself (Leibowitz, Jhanwar-Uniyal, Dvorkin, & Makman, 1982; Unnerstall, Fernandez, & Orensanz, 1985). These receptors participate in the depressor actions of many centrally acting anti-hypertensive agents (Weber & Drayer, 1984).

Clonidine is an imidazoline derivative, and a centrally acting, therapeutically effective anti-hypertensive drug. Its binding affinity for alpha-2 receptors is 1000 times greater than for alpha-1 adrenergic sites (Kellar, Quest, Spera, Buler, Conforti, Dias Souza, & Gillis, 1984) although at high concentrations, its proportional alpha-2 receptor selectivity decreases (Jarrott, Lewis, Conway, Summers, & Louis, 1984). Intravenous administration of clonidine (5 ug/kg) in chloralose-urethane anesthetized rats produces an initial pressor response that peaks at 10 to 20 min after injection, recovers to pre-injection levels by 60 min, and is followed by long-lasting hypotension, with bradycardia accompanying both phases (Bolme & Fuxe, 1971). The initial pressor phase is thought to reflect a sympathomimetic vasoconstricting action on peripheral alpha-adrenergic receptors, since it can be inhibited by alpha-adrenergic blockers such as phenoxybenzamine and phentolamine (Constantine & McShane, 1968; Rand & Wilson, 1968).

The long-lasting hypotension is thought to be centrally mediated for several reasons. First, decreases in CO or HR seldom occur when clonidine is applied to isolated, perfused hearts of rats and other animals in concentrations comparable to hypotensive doses in vivo (Constantine & McShane, 1968). Second, vasodilation does not occur after infusion of isolated perfused vascular sections with clonidine (Boissier, Giudicelli, Fichelle, Schmitt, & Schmitt, 1968; Constant & McShane, 1968). Third, the hypotension is prevented by spinal transection (Constantine & McShane, 1968; Rand & Wilson, 1968) or ganglionic blockade (Boissier, et al., 1968; Nayler, Rosenbaum, McInness, & Lowe, 1966) yet, clonidine itself has no significant ganglionic-blocking properties (Rand & Wilson, 1968). Fourth, the systemic administration of clonidine decreases spontaneous activity in pre- and post-ganglionic sympathetic nerve fibers such as those in the sympathetic trunk and splachnic nerve of rats, cats and dogs (Schmitt, Schmitt, Boissier, & Giudicelli, 1967; Schmitt, Schmitt, Boissier, Giudicelli, & Fichelle, 1968). Klupp, Knappen, Otsuka, Streller and Teichmann (1970) found a dose-dependent decrease (5-30 ug/kg clonidine, in spontaneous discharge of the splanchnic nerve and the cervical sympathetic trunk within 10 to 20 minutes after the injection, when the inital pressor response had disappeared. More importantly, Guyenet

and Cabot (1981) directly applied clonidine to the sympathetic preganglionic fibers located in thoracic spinal cord of pigeons and reported that clonidine had an inhibitory action on these fibers. Fifth, when administered intracisternally, clonidine produces an even greater decrease in BP, HR, and CO than the same doses given intravenously (Boissier, et. al., 1968; Kobinger & Walland, 1967; Onesti, Schwartz, Kim, PazMartinez, & Swartz, 1971); while transections rostral to the medulla fail to prevent the hypotensive and bradycardic effects of clonidine (Schmitt & Schmitt, 1969; Shaw, Hunyor, & Korner, 1971). These observations suggested that the medulla might be an important site of action of clonidine

Clonidine presumably inhibits excitatory medullary cardiovascular neurons. Svensson, Bunney and Aghajanian (1975) found that intravenous (iv) and micro-iontophoretically applied clonidine inhibits NE neurons in the locus coeruleus, and that intravenous but not microiontophoretic application of clonidine inhibits serotonin neurons in the dorsal raphe nucleus. Sharma, Sandrew, and Wang (1978) iontophoretically applied clonidine to neurons in the vasomotor area that were identified as excitatory based on their responses to intravenously injected NE. Clonidine decreased the spontaneous firing of these excitatory neurons and inhibited their firing

following NE microiontophoretic application. These results suggest that clonidine has an antagonistic action at this level of the brain.

Bousquet, Feldman, and their colleagues showed that small doses of clonidine (75 ng/kg) microinjected into the nucleus reticularis lateralis (NRL) in the ventrolateral medulla cause a hypotensive response (Bousquet, Feldman, Velly, & Bloch, 1975), while lesions of this nucleus prevent the hypotensive response of peripherally injected clonidine (Bousquet, Feldman, Bloch, & Schwartz, 1981). These results indicated that the NRL is a main site for the hypotensive action of clonidine. They recently found that the alpha-2 blocker, yohimbine, caused a hypotensive response while the alpha-1 blocker, prazosin, caused a hypertensive response (Bousquet & Feldman, 1986). The investigators suggested that the effects of clonidine and these two alpha-adrenergic antagonists are independent of the particular receptor type in the NRL and, therefore, receptors in the NRL might be specific to the imidazoline compound.

Intracerebroventricular (icv) injections of clonidine (30-ug in 50 ul) decrease BP and HR in anesthetized cats (Kellar et al, 1984) but in conscious rats (30 ug/kg icv) actually produces a transient hypertensive and bradycardic response that was usually replaced within 25 minutes by

hypotension and tachycardia (Imai, Nolan & Johnston, 1986). Conversely, Kawaki and Takasaki (1986) found that clonidine (2-50 ug icv) produced a dose-dependent, long-lasting hypertensive response (lasting over 60 min) and bradycardia in conscious, unrestrained rats. Since these icv clonidine effects are blocked by central yohimbine and, to a smaller extent, by central prazosin, they were thought to be mediated by central alpha-2 receptors. The additional observation that central yohimbine still abolished clonidine-induced hypertension in animals pretreated with 6-OHDA suggested that these effects were post-synaptically mediated.

The Cardiovascular Effects of Clonidine in Hypothalamic Areas.

The classical notion of BP regulation identifies two areas of importance in the hypothalamus, the anterior depressor and the posterior pressor areas. Philippu, Demmeler, and Roensberg (1974) found that depending on the dose, clonidine infused over a period of one hr, either decreased or increased the pressor response to electrical stimulation of the posterior hypothalamus. Lower doses of clonidine (1 to 5 x 10^{-5} M) increased the pressor response while higher doses (1 to 10 x 10^{-3} M) decreased it. These non-linear effects were thought to reflect a dual action for

clonidine: Low doses activate post-synaptic receptors, thereby potentiating NE release resulting in a pressor response, whereas high concentrations of clonidine activate presynaptic receptors, inhibit NE release and, therefore, the expression of the pressor response. Injections of clonidine and NE into the anterior hypothalamus produced hypotension and bradycardia and both these responses could be blocked by phentolamine (Struyker-Boudier, Smeets, Brouwer, & van Rossum, 1974).

The cardiovascular effects of clonidine micro-injected into the PVN are unknown, although such administrations elicit another autonomically-mediated response, namely feeding. Goldman, Marino and Leibowitz (1985) showed that PVN clonidine (20 nmol) elicited a feeding response in satiated rats within two hours after injection that approximates the response induced by PVN NE (40 nmol). Furthermore, this clonidine-induced feeding was selectively inhibited by the alpha-2 antagonists yohimbine and rauwolscine, but not by either the alpha-1 antagonists, prazosin or corynanthine, or the catecholamine(CA)-synthesis inhibitor alpha-methyl-para-tyrosine. These observations suggest that the appetitive consequences of PVN clonidine are mediated by post-synaptic alpha-2 receptors.

Hypothermic Actions of Clonidine

The thermoregulatory effects of clonidine have received little attention. Available evidence suggests that clonidine may exert a hypothermic effect (Livingston, Low & Morris, 1984; Ozawa, Chen, Watenable & Vematsu, 1977) that is blocked by the general alpha-antagonists phentolamine and phenoxybenzamine (Reid, Lewis & Myers, 1975; Tsoucaris-Kupfer & Schmitt, 1972) but not by the alpha-2 antagonist yohimbine (Livingston et. al., 1984). However, clonidine produced a hyperthermic effect in high ambient temperature that was blocked by yohimbine (Mogilnicka, Klimek, Nowak, & Czyrak, 1985). Additionally, the depletion of brain CA fails to alter the hypothermic effects of clonidine (Reid et. al., 1975) and the peripheral administration of imidazolines related to clonidine, that do not cross the blood brain barrier also produce hypothermic effects (Tsoucaris-Kupfer & Schmitt, 1972). Therefore, these results can best be described as revealing a non-descript thermolability that is not specific to an alpha-adrenoceptor subtype. Nevertheless, if regionally microinjected, the thermal response characteristics of clonidine may not only reflect its depressor response, but unveil a specific central mode of action. The preoptic-anterior hypothalamus (POAH) is a possible site for the thermoregulatory actions of clonidine because its role

in temperature regulation is well established. In conscious rats, local warming of the POAH decreased the core and peripheral temperatures, suppressed behavioral responding to radiant heat in cool ambient temperature, and promoted vasodilation and salivation in neutral ambient temperatures. Conversely, cooling the POAH produced shivering and increased body temperature in both neutral (25° C) and cool (5° C) environments (Carlisle & Laudenslager, 1979) and increased behavioral responding for heat when the POAH was cooled (Satinoff, 1964). Neurons in this region are cold-sensitive and respond to local cooling by increasing their firing rate (Boulant & Hardy, 1974), while others are warm-sensitive, and increase their firing rates with local warming (Boulant & Hardy, 1974). Furthermore, these thermosensitive neurons receive a considerable amount of thermal afferent input from peripheral thermoreceptors as well as deep-body thermosensitive neurons (Guieu & Hardy, 1970). These studies suggest that the POAH may serve to integrate hypothalamic thermal information with that from peripheral and deep-body structures (Boulant, 1981).

The posterior hypothalamus has also been implicated in thermoregulation. Although this region has traditionally been viewed as a center for heat conservation and production, it also contributes to behavioral thermoregulation. For example, Refinetti and Carlisle

(1986) found that alterations in the temperature of the posterior hypothalamus did not influence metabolic heat production in an ambient temperature of 15° C, but did influence behavioral responding for warm air in a cold environment (5° C).

A thermoregulatory role for the PVN has been postulated. The PVN is reported to be connected to the POAH via tubero-infundibular fibers (Renaud, Blume, Kearney, MacKenzie, & Pittman, 1978) and the discharge rates of certain PVN neurons that project to the posterior pituitary are reportedly altered following POAH warming (Matsumura, Nakayama, & Ishikawa, 1983). These responses of PVN neurons to thermal stimulation may occur through synaptic contacts with the POAH or may reflect an inherent thermosensitivity. The latter explanation has been postulated by Inenaga, Osaka & Yamashita (1987) who have demonstrated that both magnocellular and parvocellular neurons alter their discharge rates in response to local warming. An involvement of the PVN in thermoregulation via its autonomic connections has also been postulated. Lefeuvre, Rothwell & Stock, (1987) administered corticotropin-releasing factor into the PVN and observed a rise in the temperature of brown adipose tissue, a sympathetically controlled effector organ for some forms of thermogenesis. However, Holt, Wheal & York (1988) demonstrated that electrical stimulation of the

PVN had no effect on brown adipose tissue temperature. Therefore, although these observations suggest a thermoregulatory role for the PVN, the specific neuroanatomical and neurochemical substrates for this role remain unexplained.

The Role of Central Alpha-Adrenoceptors in Thermoregulation

Catecholamines have been traditionally implicated in thermoregulation (Myers, 1970). Cantor and Satinoff (1976) showed that icv injections of NE (0.5-5 ug) produced hypothermia that is blocked by the alpha-adrenergic antagonist phentolamine, but not the beta-adrenergic blocker, LB-46. Later, Mora, Lee, and Myers (1983) showed that the hypothermia, bradycardia, and lowered metabolism induced by icv injections of NE were attenuated by pretreatment with phentolamine and, to a lesser extent, by the beta-adrenergic antagonist propranolol.

Research on the specific central sites for the thermoregulatory actions of NE have concentrated on the POAH. However, microinjections of NE in the POAH have yielded conflicting results, with both hypothermia (e.g., Myers, 1970) and hyperthermia (e.g, Beckman, 1970) being found. Nevertheless, the actions of NE injected into the POAH seem to be mediated by both alpha-1 and alpha-2 adrenoceptors. Myers, Beleslin and Rezvani (1987) found a

dose dependent hypothermia induced by injections of clonidine (5-50 ug), NE (5-50 ug) and phenylephrine (5-50 ug) into the POAH of cats. The clonidine-induced hypothermia was antagonized by yohimbine, while the hypothermia induced by NE and phenylephrine was antagonized by phentolamine. They also found that microinjections of alpha-methyl-para-tyrosine into the POAH do not prevent the clonidine-induced hypothermia, suggesting that post-synaptic mechanisms are involved. However, the findings that (a) lesions of the POAH fail to alter the hypothermia produced by icv NE, and (b) the majority of studies reporting hypothermia with NE injections into the POAH used nonphysiological doses suggest that extra-POAH hypothalamic regions may contribute to NE-induced hypothermia (Cantor & Satinoff, 1976).

Renovascular Hypertension

Due to the importance of the kidney in human hypertension (Buggy & Fink, 1982), various animal models of renal hypertension have been proposed. The one-kidney Goldblatt (1K-GB) model is derived from the work of Goldblatt and his colleagues (Goldblatt, Lynch, Hanzel, & Summerville, 1934). In this model, chronic hypertension is produced by unilateral nephrectomy and partial constriction or "clipping" of the renal artery to the remaining kidney by

the application of a solid silver clip. The level of hypertension produced is proportional to the degree of constriction of the renal artery (Leenen & deJong, 1971).

Ledingham and Cohen (1964) reported that, in 1K-GB rats, extracellular fluid volume increased for up to 7 days after renal artery constriction, but by Day 14 it had returned to values comparable to those of the normotensive control group. The CO also increased within 10 days after clipping due to the increased atrial filling pressure and myocardial contractility accompanying the increased extracellular fluid volume. By Day 15, however, CO had returned to normotensive levels. This increased CO is thought to support the development of hypertension in 1K-GB animals before Day 4. Ferrario (1974) has found in 1K-GB dogs that this increased CO reflects an increased HR and SV. He also showed that by two weeks after clipping, CO, HR and SV decreased but a compensatory increase in TPR developed to maintain the hypertension. By 5 weeks, BP had reached an asymptote, the myocardial variables had returned to normal levels, while TPR was still markedly elevated.

Various mechanisms have been linked to the pathogenesis of 1K-GB hypertension. For example, sodium retention is thought to increase fluid volume and therefore CO. Swales, Thurston, Queiroz, and Medina (1972) have found that 1K-GB

rats had a progressively higher sodium balance (sodium intake minus sodium excretion) for up to 14 days after clipping. However, Seymour et al. (1981) have found that 1K-GB rats on a sodium restricted diet still developed hypertension comparable to that of 1K-GB rats on a normal sodium diet.

The renin- angiotensin system has also been implicated in 1K-GB hypertension. Plasma renin activity is transiently elevated for up to 1 week in 1K-GB rats (Bengis & Coleman, 1964) and up to 3 days in 1K-GB dogs (Brown, Davis, Olichney & Johnston, 1966). However, Watkins, Davis, Freeman, DeForrest and Stephens (1978) found that continuous angiotensin blockade by either Saralasin or the converting enzyme inhibitor SQ 20881, for 7 days following renal artery constriction, failed to prevent the development of hypertension in 1K-GB dogs. It was Seymour et al (1981) who showed that simultaneous suppression of the renin-angiotensin system by SQ 14225, and of fluid volume expansion by dietary sodium restriction, for 12 days after clipping prevented the development of 1K-GB hypertension in rats. If, however, administration of SQ 14225 was discontinued at Day 12 after clipping, BP increased so that by Day 19 after clipping, these animals were hypertensive as compared to controls. These results suggest that the development of 1K-GB hypertension is largely

volume-dependent but, if volume factors are minimized by sodium restriction, the renin-angiotensin system dominates to promote hypertension. The mechanisms involved in the increased TPR seen in the maintenance of 1K-GB hypertension are unclear. Sodium volume repletion or depletion does not seem to influence this maintenance (Stephens, Davis, Freeman, DeForrest, & Early, 1979) while the vasoconstrictor or sympathoexcitatory role of the renin-angiotensin system during this phase is unclear.

The Role of the Sympathetic Nervous System in 1K-GB Hypertension.

The sympathetic nervous system (SNS) is involved in the pathogenesis of many models of hypertension including the 1K-GB renovascular model. Henning (1969) examined NE turnover in various organs of 1K-GB hypertensive and normal rats. Four hours after injection of H44/68, a tyrosine hydroxylase (TH) inhibitor, NE decreased faster in the heart and femoral muscle of 1K-GB hypertensive rats than in control rats, suggesting that these tissues are subject to greater basal sympathetic tone. Reid, Dargie, Franklin and Fraser (1976) examined changes in BP and plasma NE levels in 1K-GB and control rats at 24 hours, 7, 14 and 28 days after renal artery clipping. They found that at each sampling interval, 1K-GB animals exhibited elevated BP's. Plasma NE

levels were elevated for the hypertensive group at each interval except 24 hours. These observations were supported later by Dargie, Franklin, and Reid (1977). Similarly, DeQuattro, Eide, Eide, Myers, Eide, Kolloch and Wigham (1978) observed that at 21 days after renal artery clipping, BP and plasma NE were elevated in 1K-GB rats as compared to controls. These findings of apparent sympathetic activation between days 7 and 28 after renal artery constriction suggest that the SNS contributes to the etiology of 1K-GB hypertension.

Vlachakis, Ransom, Kogosov, Woodcock, Alexander, and Maronde (1984) studied plasma and cardiac NE levels and density of alpha- and beta-aderenergic receptors in the heart at 3 days and 4 weeks after renal artery constriction in 1K-GB hypertensive and normotensive rats. They found that, in contradiction to other studies, plasma NE levels of 1K-GB rats were decreased at 3 days and 4 weeks whereas cardiac NE content was similar at 3 days and lower at 4 weeks. There was also a significant decrease in density of cardiac alpha and beta receptors at 4 weeks in the 1K-GB rats. These decreases may be a result of chronic exposure of the receptors to increased quantities of CA and do not necessarily contradict the enhanced sympathetic activity hypothesis of 1K-GB hypertension.

The mechanism by which peripheral SNS activity is altered in 1K-GB rats is unclear. Katholi and his colleagues focussed on the role of renal nerve activity in this model. They have found that renal denervation performed two weeks after clipping attenuates 1K-GB hypertension although not to pre-clipping levels (Katholi, Winternitz, & Oparil, 1981) and results in plasma NE levels comparable to those of normotensive animals (Katholi, Winternitz, & Oparil, 1982a). Furthermore, denervated animals showed smaller absolute BP decreases to ganglionic blockade by hexamethonium bromide than did hypertensive animals (Katholi et.al., 1982a). These findings suggest that clipping of the renal artery produces renal afferent nerve signals which, through an unknown process, is expressed as an alteration in peripheral sympathetic activity.

Central Dysfunction of 1K-GB Hypertension.

Since the sympathetic preganglionic fibers are largely regulated by supraspinal input, neurochemical alterations of this input may characterize 1K-GB hypertension. Dargie et. al. (1977) examined the effect of intracisternal injections of 6-OHDA, made two weeks before nephrectomy and renal artery constriction, on BP and plasma NE in 1K-GB rats. They found that 6-OHDA administration prevented the hypertension and elevation of plasma NE levels at seven days after

nephrectomy and clipping. This observation suggested that increasec sympathetic activity in 1K-GB rats is at least partly mediated by central CA fibers. Petty and Reid (1977, 1979) examined NE concentrations and synthesis in several brainstem and hypothalamic areas at 72 hours, 7 and 28 days after renal artery clipping in 1K-GB and normotensive animals. Results showed that 72 hours after clipping, the elevation of mean aortic BP was evident and NE concentrations were reduced in the NTS, NRL of the brainstem, and the anterior, posterior and paraventricular hypothlamic nuclei. These changes in the posterior and paraventricular nuclei coincided with reduced levels of TH, the rate limiting factor in NE synthesis. This apparent reduction in regional NE levels and synthesis were not detected at 7 or 28 days even though large group differences in BP persisted. However, more prolonged changes in regional CA activity have been observed by other investigators. DeQuattro et al. (1978) examined NE content and the activities of TH and Dopamine-beta-hydroxylase in the hypothalamus, brainstem and forebrain 21 days after clipping in 1K-GB rats, suggesting that the hypothalamus may contribute to the increased sympathetic activity found in 1K-GB animals. Similar results were obtained by Eide, Myers, DeQuattro, Kolloch, Eide and Wigham (1980) and Katholi, Winternitz and Oparil (1982b). Furthermore,

Katholi et al. (1982b) reported that renal denervation reinstated the increased hypothalamic NE levels in 1K-GB rats to normal. Some of the discrepancies in these studies may be attributed to methodological differences. For instance, Petty and Reid measured NE content of specific hypothalamic nuclei and perhaps did not include the specific nuclei contributing to the elevated hypothalamic NE levels found by DeQuattro and his colleagues.

A number of hypothalamic areas have been specifically implicated in renal hypertension. Bilateral lesions of the anteroventral third ventricle region prevent the development of 1K-GB hypertension (Brody, Fink, Buggy, Haywood, Gordon & Johnson, 1978). Similarly, lesioning of the posterior hypothalamus (Bunag & Eferakeya, 1978) or the ventromedial nucleus-medial eminence region (Johnson, Buggy, Fink, & Brody, 1981) prevents the development of 1-kidney Grollman hypertension produced by uninephrectomy and figure eight ligature of the remaining kidney.

Given the postulated role of the PVN in the development of 1K-GB hypertension and given its anatomical connections to the sympathetic preganglionic fibers, it is possible that this region is also involved in 1K-GB hypertension.

Thermoregulatory Consequences of Hypertension

Since heat exchange is often accomplished by a redistribution of blood between the body core and the available surface area, any changes in peripheral circulation may have thermoregulatory consequences. The chronic elevation in TPR found in a number of models of arterial hypertension approximate the condition of altered peripheral circulation. Yet, few studies have assessed the thermoregulatory consequences of the induction of hypertension. In 1972, it became evident that the Spontaneously Hypertensive Rat tended to have a basal body temperature nearly 0.5^{0}C higher than did the normotensive control (Tanaka, Takaori & Okamoto, 1972). Wilson, Wilson & DiCara (1976) confirmed this observation, but also reported that this genetic model of hypertension exhibited a lethal sensitivity to warm ambient temperatures that were tolerated by both the normotensive inbred control and the standard Sprague- Dawley rat. Wilson, Fyda & Berczi (1983) found that the induction of a mineralocorticoid, or DOCA-salt, hypertension emerged thermobehaviorally. Specifically, DOCA-salt hypertension was accompanied by an increased tendency to escape relatively mild elevations (39^{0} C) in the ambient temperature, but exhibiting longer tolerance to mild reductions (17^{0} C) in the ambient temperature. Similar evidence of altered tolerance to thermal challenges have

been obtained in renovascular hypertension. Fregly (1954) reported that inducing renovascular hypertension with latex encapsultion of both kidneys reduced the rat's tolerance to warm environments. More recently, Wilson & Fyda (in press, 1989) observed in sialoadenectomized, shaved 1K-GB hypertensive rats, that this reduced tolerance to warm environments enhanced heat escape responding, without altering behavioral thermoregulation in a cool environment. Fyda (1987) assessed the influence of systemically administered adrenoceptor stimulation on both thermophysiological and pressor reactivity in conscious 1K-GB hypertensive rats. She found that 1K-GB hypertension is accompanied by an increased pressor reactivity to alpha stimulation, but a decreased thermogenic reactivity to beta stimulation.

Statement of the Problem

Several lines of evidence suggest that the PVN is involved in both cardiovascular and thermal functioning. First, its electrical stimulation produces pressor and tachycardic responses, whereas its lesions attenuate these responses following aortic baroreceptor denervation. Secondly, the PVN possesses reciprocal projections with brainstem nuclei involved in cardiovascular functioning such as the NTS and DMV. Thirdly, the PVN projects directly to

the IML cell column of the spinal cord that is the site of origin of sympathetic projections that subserve both cardiovascular and temperature regulation. Fourthly, recent work has identified thermosensitive neurons within the PVN and has suggested a role for this nucleus in the regulation of BAT, a primary organ in thermogenesis. The specific neurochemical basis for the PVNs role in both the cardiovascular and thermoregulatory systems is unknown, although the large number of alpha-2 receptors, and their modulation of functioning of both these systems in a number of other hypothalamic nuclei suggests their involvement. Clonidine is a centrally-acting anti-hypertensive agent that selectively stimulates alpha-2 receptors. One question that emerges from these and related observations is whether the selective stimulation of the alpha-2 receptors in the PVN, with clonidine, will alter indices of BP and temperature in conscious, normotensive rats.

The 1K-GB model of hypertension is characterized by altered peripheral sympathetic and central catecholaminergic activity, including activity in the PVN. Although the specific receptors mediating this catecholaminergic activity are unknown, alpha-2 involvement is possible since the 1K-GB model is also characterized by increased pressor reactivity to alpha stimulation. Existing evidence has also shown that this model may exhibit thermal maladaptiveness.

Accordingly, the second question that emerges is whether the onset of 1K-GB hypertension would alter the cardiovascular and thermal response profile obtained with selective stimulation of PVN alpha-2 receptors in normotensive rats.

Thus, the present study was designed to assess the dose-dependant influence of PVN microinjections of clonidine alone, or following pretreatment with rauwolscine, a selective alpha-2 antagonist, on blood pressure, rectal temperature, and oxygen consumption.

Method

Subjects

Fifty-three male, Sprague-Dawley rats served as subjects and weighed 80-100 g upon arrival in the lab. The animals were initially housed in individual wire-mesh hanging cages with free access to standard rat chow and water. The colony room was maintained on a 12/12 hr light/dark cycle (lights on 0800-2000) at 23°C

Apparatus for Determination of Expired Gases

Testing was conducted in a metabolic chamber (Figure 1) consisting of a plexiglass cylinder, 37 cm long with an internal diameter of 13.8 cm located inside a temperature-controlled cabinet. The cylinder contained

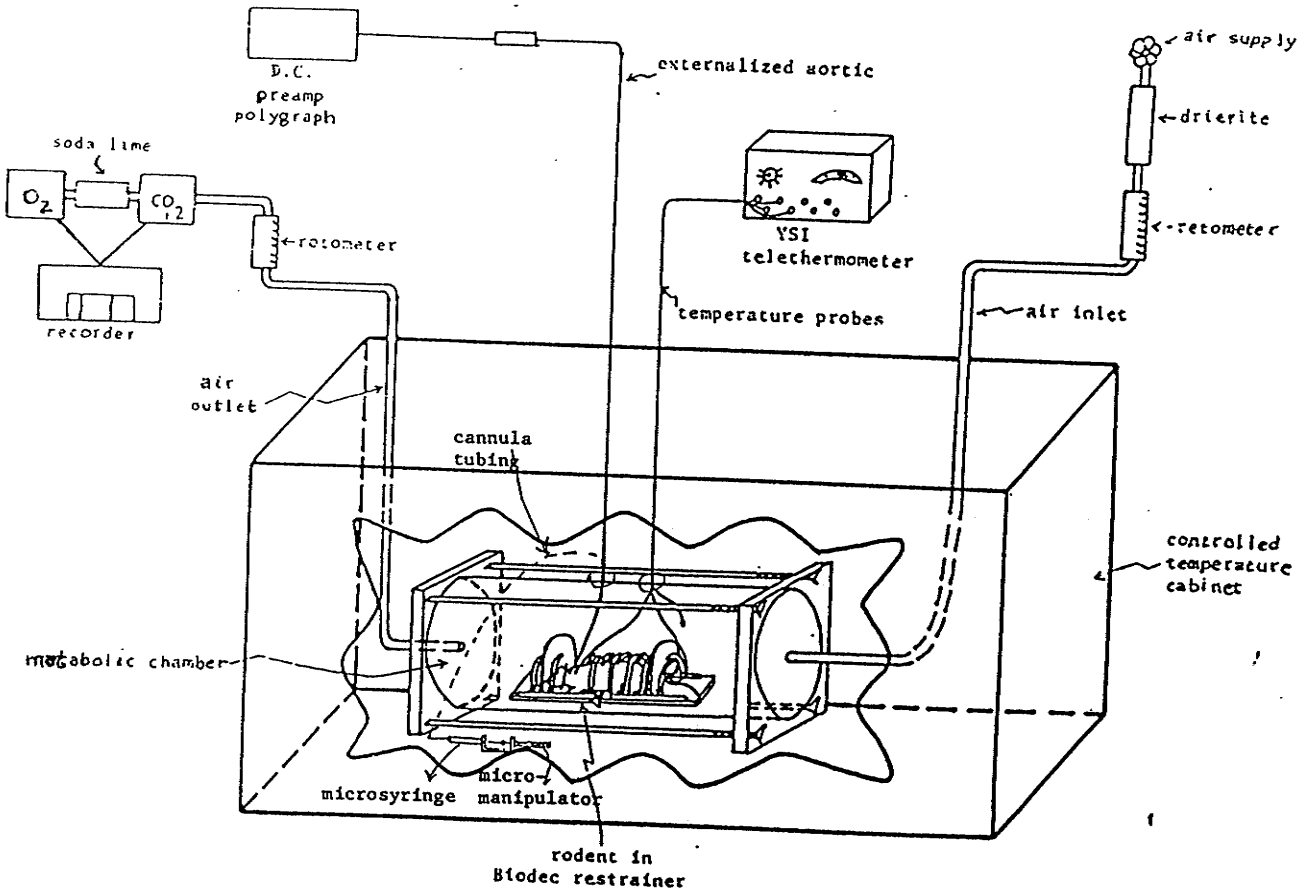

Figure 1. Schematic representation of the testing apparatus.

access ports for the temperature probe, the aortic and jugular catheters, and the cannula injector system. Two plexiglass end plates provided an air-tight seal in the chamber and contained air inlet and outlet ports. Both the temperature controlled cabinet and metabolic chamber were maintained at 26° C. Dry air was circulated through the chamber at a rate of 760 ml/min. Expired gases were dried by passage through Drierite and analyzed for oxygen content using a Beckman OM-11 Oxygen Analyzer. The formula used for oxygen consumption is presented in Appendix A. Prior to oxygen consumption analysis expired gases were passed through a Soda Lime (6 to 12 mesh) tube to remove carbon dioxide. Oxygen content of the metabolic chamber air was then measured.

Surgical Procedures

Prior to all surgical manipulations the animals were food deprived for approximately 12 hr, then anesthetized with sodium pentobarbital (60 mg/kg ip, Allen and Hanburys) and given an intramuscular injection of atropine sulfate to suppress mucosal secretions. Under aseptic conditions the site of the incision was shaved. All incisions were sutured with 00 silk surgical suture, and a topical anti-bacterial cream applied to the wound (Furacin, 0.2%, Austin). The animals were given an injection of a local analgesic (2%

Xylocaine HCl, Astra), an intramuscular injection of a broad spectrum antibiotic (Ethacilin, Rogar STB, 1.5 mg/kg).

Induction of 1K-GB hypertension or sham-operation. The surgical induction of hypertension was conducted in two phases: a nephrectomy phase and a renal artery clipping phase. For the nephrectomy phase, a right laporotomy was made, the kidney cleared of surrounding fascia, the vasculature sealed with a double ligation of 00 silk surgical suture, and the kidney removed. For the clipping phase, 1K-GB animals underwent a left laporotomy following which the left kidney was lifted and the renal vasculature cleared of surrounding tissue. A solid silver clip (4 x 2 x 1.6 mm) with a slit of .2 mm diameter was applied to the renal artery, proximal to the aorta. This technique has been demonstrated by Leenen & DeJong (1971) to produce a 60% increase in BP in 78% of their rats. The sham-operated animals also underwent a left laparotomy, but the renal clip was not applied.

PVN cannulations. Anesthetized rats were placed in a Kopf stereotaxic instrument. Stereotaxic measurements for the PVN were made from bregma, midline, and the surface of the skull with the incisor bar positioned 0.0 mm dorsal relative to the horizontal interaural plane. Coordinates used to locate the PVN were : 0.9-1.0 mm posterior to

bregma, 1.7 mm lateral to midline, and 8.4 mm ventral to the surface of the skull (Brown, personal communication). A guide cannula consisting of a 3-cm piece of 24-ga. stainless steel tubing epoxied inside a threaded 1 cc tuberculin syringe tip was implanted with these coordinates, but lowered only 7.4 mm ventrally through an aperture drilled in the skull. This guide cannula was affixed to the skull with acrylic cement. The guide cannula was plugged with a 31-ga. stainless steel dummy stylette fixed with epoxy cement inside a threaded 1-cc tuberculin syringe cap. All guide cannulae were sterilized in 95% ethanol, flushed with saline, and dried overnight before being reused. For testing, the dummy stylette was replaced by a 31-ga. injector cannula which was threaded into the guide cannula to the level of the PVN at a depth of 8.4 mm below the skull surface.

Aortic catheterization. Chronically indwelling catheters were implanted in the subdiaphragmatic descending aorta. The catheters had a Teflon tip (Small Parts Inc., OD-.034 in., ID-.022 in.) with a blunt, tapered end, attached to a 45 cm length of Tygon tubing (OD-0.75 cm, ID-0.15 cm). The connection was made by soaking 1 to 2 cm of the Tygon tubing in 1,2-dichloroethane (Fisher Scientific Co. Ltd.) for 4 to 5 min and inserting the Teflon tip approximately 1cm into the Tygon tubing. The completed

catheter was flushed with distilled water and left to dry overnight. One day before catheterization, the catheter was filled with TDMAC heparin complex (2%, Polysciences Inc.), soaked for 30 min, and then dried by infusing air through it.

A 2 to 3 cm incision was made through the midabdominal region. The intestines were gently retracted, and the descending aorta, approximately 2 cm caudal to the left renal artery, exposed and isolated. The catheter was led subcutaneously to the nape of the neck and externalized. The aorta was gently lifted with a pair of forceps to briefly occlude blood flow, and a small puncture made with a 27-ga. hypodermic needle. The Teflon tip of the catheter was inserted 1.2 cm into the aorta, and the blood flow reestablished. The catheter was anchored to the surrounding muscle by two sutures. The catheter was filled with heparinized isotonic saline (100 U/ml, Sigma Chemical Co.) and closed off with a stainless steel obturator.

Jugular catheterization. While the animals were still under sodium pentobarbital anesthesia from the aortic catheterization, a 0.17 ml jugular catheter (PE50, Intramedic, OD-0.038 in., ID-0.023 in.) was implanted. The right jugular vein was exposed by making a 2 cm incision anterior to the clavicle. The vein was isolated from the

surrounding tissues and punctured with a 22-ga. stainless steel hypodermic needle. The catheter was then inserted 3 cm into the vein, ligated to the vein rostral and caudal to the point of insertion, anchored to surrounding muscle, led subcutaneously and externalized at the nape of the neck. After it was filled with heparinized isotonic saline (100 U/ml), the catheter was closed off with a stainless steel obturator. Both the aortic and jugular catheters were taped to an Elizabethan collar that was placed around the neck of the animal and the animal was returned to its home cage.

Drugs

Clonidine HCl (Sigma) was dissolved in artificial cerebrospinal fluid. The ph of the artificial cerebrospinal fluid was adjusted to 7.4. A 0.5 ul volume of the clonidine solution was infused into the PVN at the following three doses: 0 or artificial cerebrospinal fluid, 1 nmol and 20 nmol. The 20 nmol dose has been shown to be nontoxic when injected into the PVN (Leibowitz, 1985). Rauwolscine (Roth, West Germany) was dissolved in physiological saline and injected iv at a dose of 1.0 mg/kg determined through pilot observations. Both clonidine and rauwolscine solutions were made fresh daily. If the same dose of clonidine was to be administered to more than one animal in one day, the solution was wrapped in aluminum foil, stored in the fridge,

and and brought to room temperature before being placed in the injector cannula system of the next animal. A new rauwolscine solution was made for each animal.

Pretest Procedure

All animals were allowed 2 days to acclimate to the housing conditions. Half of the animals (n = 26) were assigned to the 1K-GB hypertensive group (HT), while the remaining half served as the normotensive control group (NT,n = 27). On Day 3, all subjects underwent a right nephrectomy. On Day 7, HT animals had a silver clip placed on the left renal artery, while the NT animals underwent only a left laporotomy. On Day 10, a 7-day progressive restraint adaptation procedure began. Accordingly, the animals were placed in a Biodec restrainer for only one hr. On the following day (Day 11), the duration of restraint increased to 2 hr. On Day 12, they were restrained for 3 hr, and on Days 13 and 14 for 4 hr each. Finally, on Days 15 and 16, animals were restrained for 5 hr each. At the end of the restraint period on Day 16, animals were anesthetized and unilaterally implanted with a cannula into the PVN. They were allowed to recover for 48 hr. HT and NT animals were then assigned to one of two drug conditions: (a) administration of saline iv followed 30 min later by one of three doses of clonidine (the Sal group, n = 13

HTanimals, n = 15 NT animals) or (b) administration of rauwolscine iv followed thirty minutes later by one of three doses of clonidine (the Rauwol group, n = 13 HT animals, n = 12 NT animals). On Day 18, all animals were implanted with chronically indwelling aortic and jugular catheters and allowed to recover for 24 hours. On Day 19, a 3 day metabolic and cardiovascular testing procedure began. Upon completion of testing, placement of cannulas in all animals was histologically confirmed. Based on this placement, the HT (n = 26) and NT (n = 27) animals were assigned either to a PVN group (n = 27) or a non-PVN (NPVN) group (n = 26). The design for this study is illustrated in Figure 2. The numbers in parentheses refer to the cell sizes used in the analyses of all BP data.

Metabolic and Cardiovascular Testing Procedure

On Day 19, a cannula injector system consisting of the 31-ga. injector cannula attached to PE10 (Intramedic) tubing and a lul Hamilton microsyringe, was filled with a clonidine solution of predetermined concentration. Then the injector cannula was inserted into the 26-ga. guide cannula and positioned in the PVN. The aortic and jugular catheters were unwound from the Elizabethan collar; the animal guided into a Biodec restrainer, and the restrainer was placed inside the metabolic chamber.

DESIGN

PVN CANNULAE

	Sal + 0 nmol	Sal + 1 nmol	Sal + 20 nmol	Rau + 0 nmol	Rau + 1 nmol	Rau+ 20 nmol
1K-GB Hypertensive			n=6(6)			n=7 (5)
Normotensive			n=8(6)			n=6(5)

N=27

NON-PVN CANNULAE

	Sal + 0 nmol	Sal + 1 nmol	Sal + 20 nmol	Rau + 0 nmol	Rau + 1 nmol	Rau + 20nmmol
1K-GB Hypertensive			n=7(5)			n=6(5)
Normotensive			n=7(5)			n=6(5)

N=26

Figure 2. The design for the study.

A YSI 402 probe was inserted 4cm into the rat's rectum for rectal temperature measurement, and the wire was externalized through an access port. The ambient temperature in the metabolic chamber and rectal temperatures were measured with a YSI telethermometer (Model 46 TUC). The aortic and jugular catheters were be externalized through an access port as was the injector cannula tubing attached to the Hamilton microsyringe. The aortic catheter was connected to a Gould Statham pressure transducer (Model P23Gb) with Intramedic polyethylene tubing (PE100), and mean arterial BP (MABP) recorded on a Grass Model 5B polygraph. The metabolic chamber was then completely sealed. The jugular catheter was attached to a 1-ml syringe and filled with 0.17 ml of saline or rauwolscine depending on whether animal was assigned to the Sal group or Rau group. A 90-min stabilization period then began, of which the last 30-min constituted the predrug baseline. Measurements of oxygen consumption, rectal temperature, MABP and HR were taken at 10-min intervals during baseline and all testing sessions. At the end of the 30-min baseline period, saline or rauwolscine was infused iv over a 5-min period. Thirty min later, 0.5 ul of one of three doses of clonidine was administered into the PVN over a 1-min period by depression of the plunger of the microsyringe. Readings were taken for the next 2 hr. At the end of the 2 hr testing period, the

rat was removed from the restrainer and replaced in its home cage. This procedure was repeated over the next 2 days, such that by the end of the 3 days of testing, all animals received vehicle or rauwolscine followed 30-min later by each of the three doses of clonidine injected into the PVN. The order of drug administration was counterbalanced throughout the experiment.

Histological Verification

Upon completion of testing, all animals were euthanized with sodium pentobarbital and perfused successively with saline and 10% formalin. Brains were removed and soaked in 10% formalin until sectioning. All brains were coronally sectioned at 100 u in a freezing microtome and the sections mounted onto gelatin-coated slides and dried. All sections were stained with cresyl violet and subsequently verified for cannula placement by light microscopy.

Statistical Analyses

Retrospective assignment of all animals to either the PVN or NPVN groups led to unequal cell sizes. Independent variables were Placement (PVN or NPVN), BP Status (HT or NT), Rauwol/Sal group (Sal or Rau), Clonidine Dose (0, 1nol, 20nmol) and Sampling Intervals (10-min intervals).

Data for each dependant variable, MABP, rectal temperature and oxygen consumption were collected in three consecutive phases: the predrug baseline, the rauwol/sal baseline and a clonidine administration phase. The predrug baseline consisted of the last three 10-min intervals of the 90 min stabilization period and preceded the administratio of Rau or Sal. This data was analyzed in a 2 x 2 x 3 (Placement x BPStatus x Time analysis of variance (ANOVA) with repeated measures on the last factor. The rauwol/sal baseline consisted of the next three 10-min intervals and preceded the administration of clonidine. These values were transformed for analysis to percentage change from the value obtained during the last 10-min sampling interval of the predrug baseline. This strategy was adopted to ameliorate the bias contributed by group differences that could exist prior to pharmacological manipulation. The rauwol/sal baseline data were analyzed in a 2 x 2 x 2 x 3 (Placement x BPStatus x Rauwol/Sal administration x Time) ANOVA with repeated measures on the last factor. The clonidine administration data consisted of the next twelve 10-min sampling intervals. These values were also transformed for analysis to percentage change from the value obtained during the last 10-min interval of the rauwol/sal baseline. This data was analyzed in a 2 x 2 x 2 x 3 x 12 (Placement x BPStatus x Rau/Sal administration x Clondose x Time) ANOVA

with repeated measures on the last two factors. Post-hoc analyses using the Tukey- Kramer modification for unequal n were performed for all ANOVAs.

Results

Histological Analysis

On the basis of reconstructions of the cannula placements, illustrated in Figure 3, HT and NT animals were retrospectively sorted into PVN or NPVN groups. The PVN group consisted of placements throughout the anterior posterior extent of the PVN and were immediately dorsal to, or in the dorsal portion of the PVN. Photomicrographs of two PVN placements are given in Figure 4. The majority of NPVN placements were posterior to the PVN in the dorsal and ventromedial regions of the hypothalamus. A number of NPVN placements were anterior to the PVN or dorsally located in the zona incerta or thalamic nuclei.

Predrug baseline

Mean arterial blood pressure. A difference in MABP was found between the NT and HT animals, $F(1,38=227.14$, $p<.001$, collapsed across placements and across the three sampling intervals. The MABPs of the NT and HT animals were 130 mm Hg and 185 mm Hg, respectively. No significant differences were found between PVN and NPVN animals or across the three sampling intervals during this baseline.

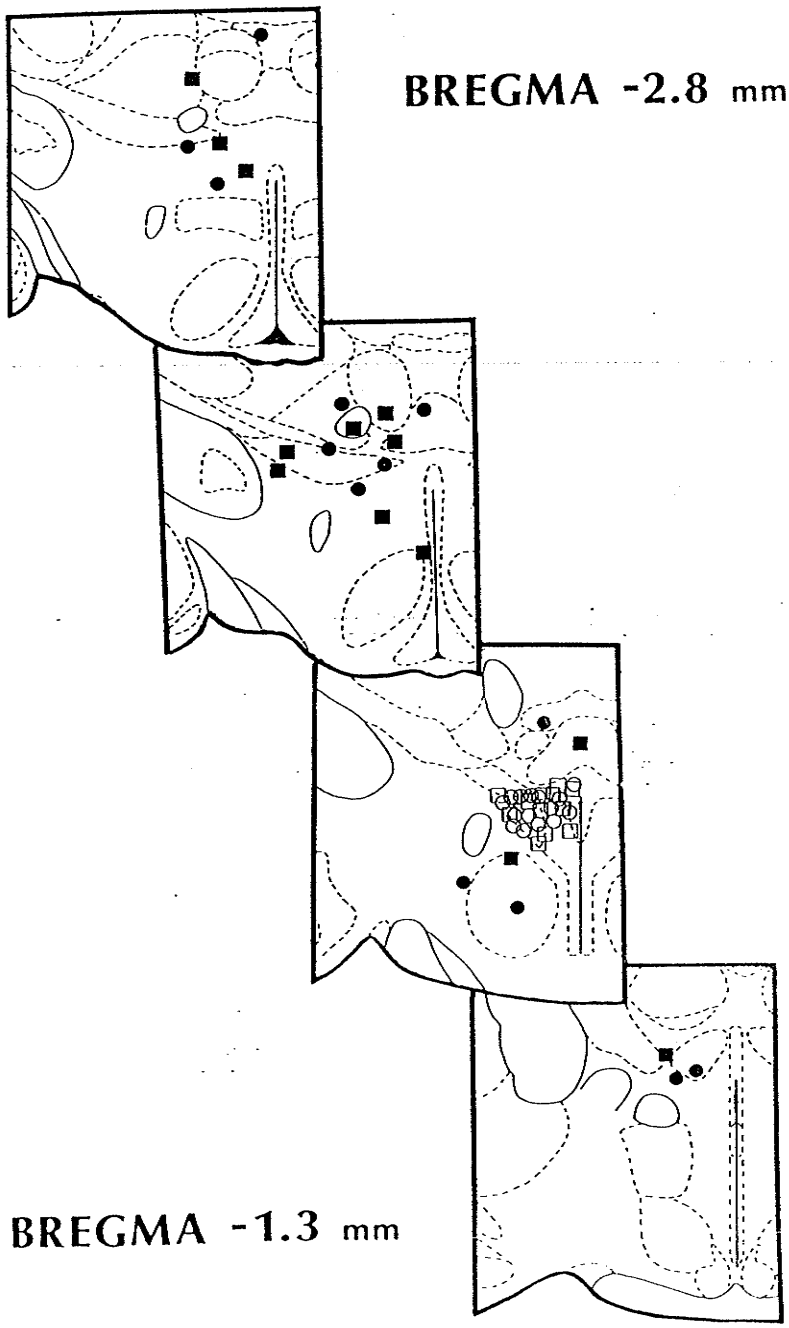

Figure 3. Histological reconstructions for cannula placements in NPVN-NT (●), NPVN-HT (■), PVN-NT (○) and PVN-HT (□) rats. Taken from Paxinos & Watson,(1982).

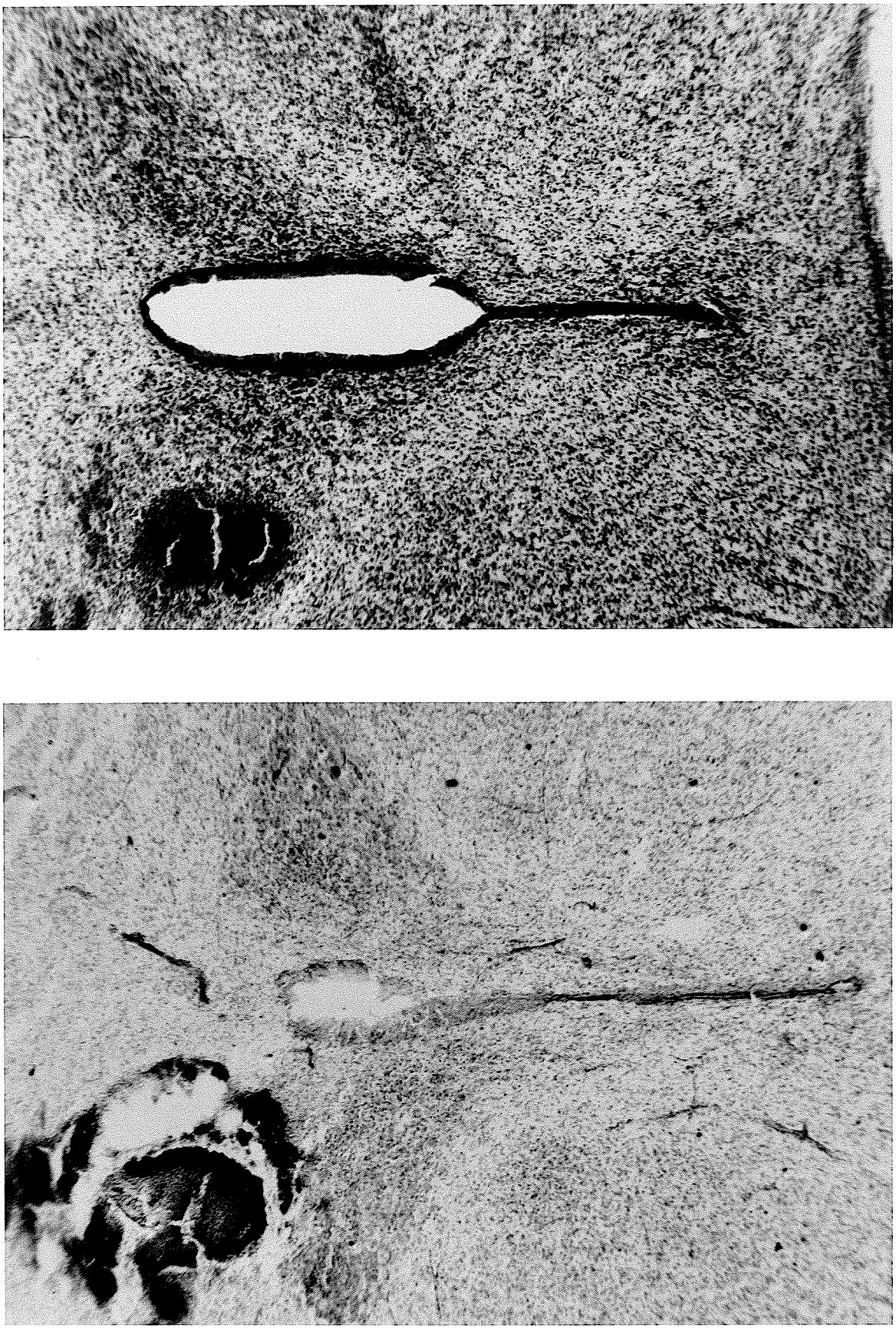

Figure 4. Photomicrographs of two PVN placements.

Rectal temperature. A Sampling Interval effect, $F(2,98)=8.07$, $p<.001$, revealed that Tr values generally decreased over the three sampling intervals from 37.7°C to 37.6°C collapsed across placements and across BP status. No significant differences were found between PVN and NPVN animals or between NT and HT animals.

Oxygen consumption. A three-way Placement x BP Status x Sampling Interval effect, $F(2,96)=5.75$, $p<.004$ was obtained, and is illustrated in Figure 5. Post-hoc analyses revealed that the oxygen consumption or VO^2 values for the PVN-NT group were significantly higher at all three intervals than either the PVN-HT, NPVN-NT, or NPVN-HT groups. No significant differences were found between PVN and NPVN between NT and HT groups, or over the three sampling intervals across all groups.

Rauwol/Sal baseline

Mean arterial blood pressure. No significant differences were found between PVN and NPVN animals or across the three sampling intervals. However, an overall difference was found between NT and HT animals, $F(1,34)=7.83$, $p<.008$, when collapsed across placements, across the three sampling intervals, and across Rauwol or Sal pretreatment. The MABPs of NT animals increased by 1.62% from 130 mm Hg at predrug baseline to 131 mm Hg and the MABPs of HT animals decreased

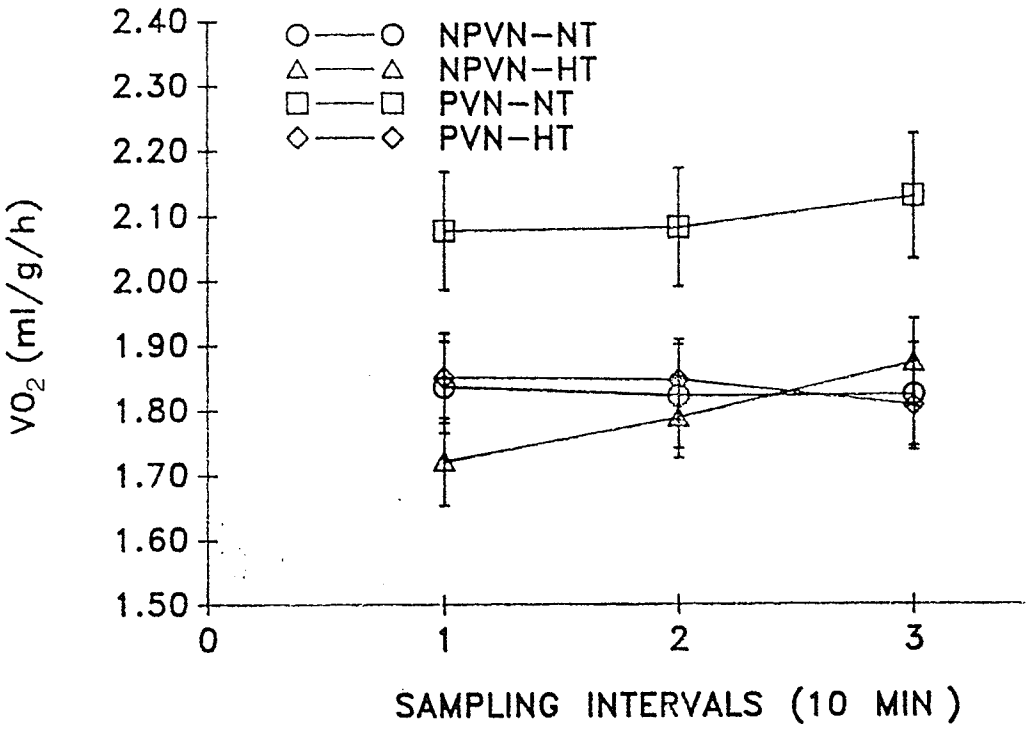

Figure 5. Mean (+SEM) in oxygen consumption (VO_2) during the three 10-min intervals of the predrug baseline for normotensive (NT) and 1K-GB hypertensive (HT) animals tht have cannulae implanted in areas outside the PVN (NPVN) or in the PVN.

by 1.70% from 184 mm Hg at predrug baseline to 181 mm Hg. Furthermore, a Placement x BP Status interaction, $F(1,34)=11.02$, $p<.002$, indicated that, regardless of whether Rauwol or Sal was administered, MABP increased in PVN-NT animals, while having no change or decreasing in the PVN-HT, NPVN-NT and NPVN-HT groups. Finally, a three-way Placement x BP Status x Rauwol/Sal interaction, $F(1,34)=4.58$, $p<.04$, was observed, and is illustrated in Figure 6. Post-hoc analyses revealed that, regardless of whether Rauwol or Sal was administered, the increases in the MABPs of the PVN-NT group were greater than for the PVN-HT group. Following Rauwol administration, the change in MABPs for the PVN-NT group was greater than the change for the NPVN-NT group.

Rectal temperature. A Sampling interval effect, $F(2,90)=20.24$, $p<.001$, was found with Tr values decreasing by 0.17%, to 0.49% from the first to the third interval after Sal or Rauwol administration, collapsed across PVN and NPVN placements, across NT and HT animals, and across Rauwol/Sal pretreatment. These percentage changes represented decreases from 37.6°C at predrug baseline to 37.6° C to 37.5° C following Rauwol or Sal administration. No significant differences were found between PVN and NPVN animals or between NT and HT animals. Also, no differences between the administration of Sal or Rauwol across all groups were found.

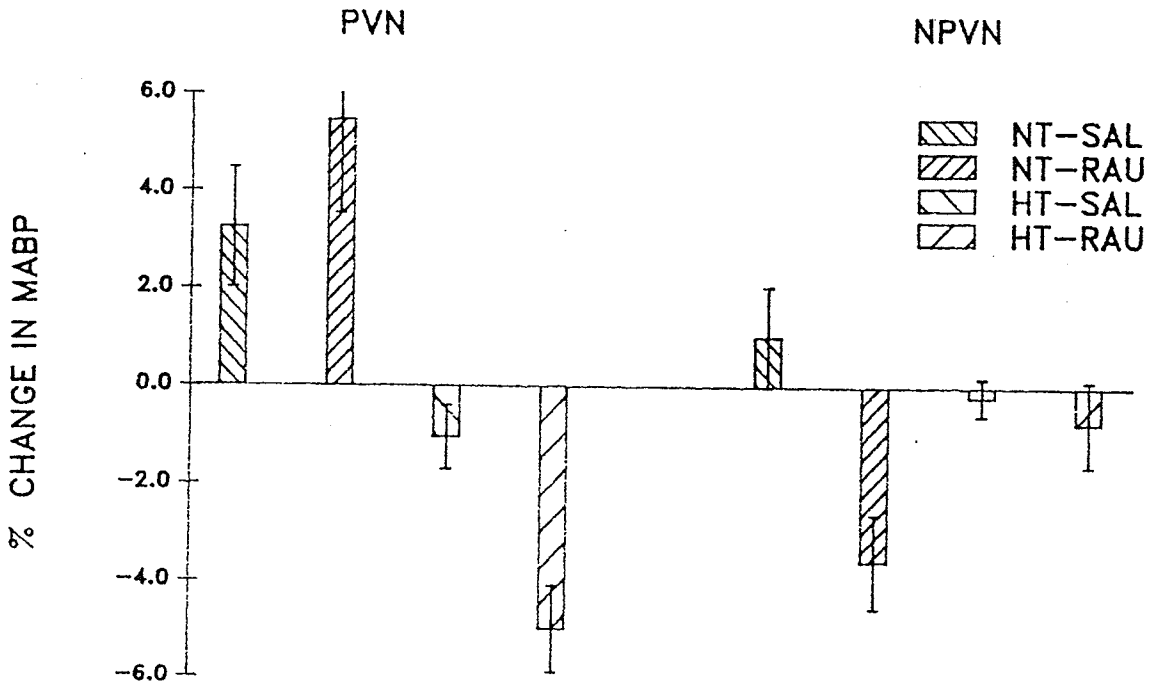

Figure 6. Mean (+SEM) percentage change in mean arterial blood pressure (MABP) from the previous baseline in normotensive (NT) and 1K-GB hypertensive (HT) animals that have received saline (SAL) or rauwolscine (RAU), following SAL or RAU administration.

Oxygen Consumption. A Sampling Interval effect, $F(2,88)=6.03$, $p<.003$, was found with VO_2 increasing from 1.26%, to 4.12% from the first to the third interval following administration of either Sal or Rauwol, collapsed across placements, across BP status, and across Rauwol/Sal pretreatment. These percentage changes represented increases from 1.91 ml O_2/g/h at predrug baseline to 1.93 ml O_2/g/h to 1.97 ml O_2 /g/h. No differences were found between PVN and NPVN groups or between NT and HT groups.

Clonidine administration

Mean arterial blood pressure. A Placement effect, $F(1,34)=6.03$, $p<.019$, indicated that clonidine a 0.36% increase in MABP for the the PVN animals (from 156 mm Hg at Rauwol/Sal baseline to 157 mm Hg) that differed from the 1.89% decrease observed for the NPVN animals (from 156 mm Hg at Rauwol/ Sal baseline to 152 mm Hg) when collapsed across BP Status, the three doses of clonidine, the twelve sampling intervals, and across Rauwol/Sal pretreatment. Furthermore, a BP Status effect, $F(1,34)=5.74$, $p<.022$, revealed that clonidine's overall effect on MABP depended on whether it was NT or HT in status. Specifically, the MABPs of the NT animals decreased by 1.81%, from 131 mm Hg at Rauwol/Sal baseline to 128 mm Hg, and that of HT animals increased by only 0.38%, to 181 mm Hg. Finally, a Rauwol/Sal effect,

$F(1,34)=12.71$, $p<.001$, and a Rauwol/Sal x Sampling Interval effect, $F(1,34)=12.71$, $p<.001$, suggested that Rauwol buffered, and perhaps even reversed, the overall depressor effect obtained with clonidine administration (Figure 7). Pretreatment with Rauwol increased MABP from 153 mm Hg at the Rauwol/Sal baseline to 154 mm Hg, wheras pretreatment with Sal decreased MABP from 159 mm Hg at the Rauwol/Sal baseline to 155 mm Hg. Post-hoc analyses revealed that animals pretreated with Rauwol generally had higher MABPs than animals that did not receive Rauwol. These differences between Rauwol and Sal pretreatment occurred primarily between 50 and 100 min after clonidine administration.

Rectal temperature. A significant overall Clonidine Dose effect, $F(2,90)=14.92$, $p<.001$, was obtained when Tr values were collapsed across placement, BP status, across Rauwol/Sal pretreatment, and across the twelve sampling intervals. Post-hoc analyses revealed that clonidine administration resulted in a dose-dependant decrease in Tr, with the 20 nmol dose producing a decrease of up to 1.29%, from 37.5° C at Rauwol/ Sal baseline to 37.4° C. An overall Sampling Interval effect, $F(11,495)=12.5$, $p<.001$, indicated that Tr decreased to a maximum of 0.95%, from 37.5°C at Rauwol/Sal baseline to 37.1°C 70 min after administration of clonidine. Post-hoc analyses revealed, however, that this decrease occurred largely by 30 min after the clonidine

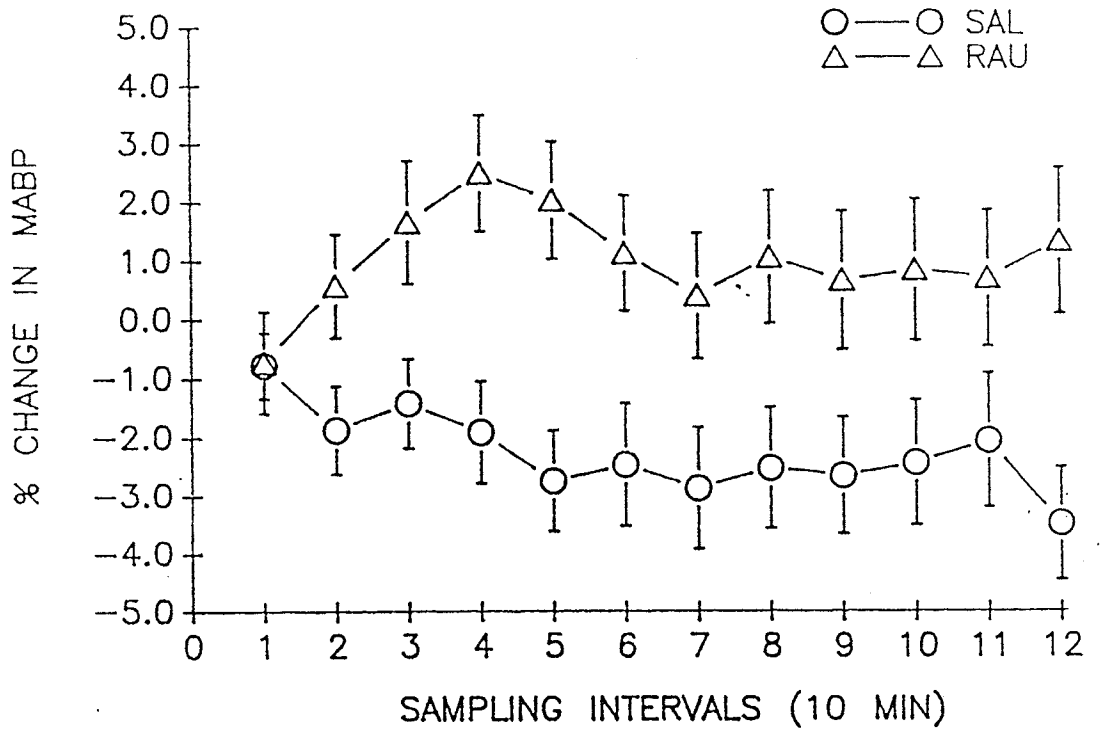

Figure 7. Mean (+SEM) percentage change in mean arterial blood pressure (MABP) from previous baseline in rauwolscine pretreated (RAU) or saline (SAL) pretreated rats during the twelve 10-min intervals following central administration of clonidine.

injection, since there were no differences between the sampling intervals after the first 30 min. Similarly, an overall Clonidine Dose x Sampling Interval interaction effect, $F(22,990)=3.56$, $p<.001$, was also found (Figure 8) indicating that the 0 nmol dose decreased Tr by a maximum of 0.36%, from 37.5°C at Rauwol/Sal baseline to 37.3° C, at 70 min after clondine administration. The 1 nmol dose decreased Tr by a maximum of 0.93%, from 37.5°C at Rauwol/Sal baseline to 37.2° C, at the last sampling interval suggesting that Tr was still decreasing at 2 h after administration of clonidine. The 20 nmol dose produced decreases in Tr of up to 1.66%, from 37.5°C to 36.8° C, but these decreases reached a maximum at the end of the first hour after clonidine administration. At the end of testing, there was no significant differences between the decreases produced by the lo dose and those produced by the hi dose, suggesting that the effects of the hi dose had reached asymptote.

Significant Placement x BPStatus x Clonidine Dose, $F(2,90)=4.58$, $p<.013$, and Placement x BPStatus x Clonidine Dose x Sampling Interval, $F(22,990)=2.15$, $p<.002$, interactions revealed that, although a dose dependant hypothermic effect was found in NPVN-NT animals, no such effect was found in NPVN-HT animals (Figure 9). Both the 1nmol and 20 nmol doses of clonidine produced hypothermic

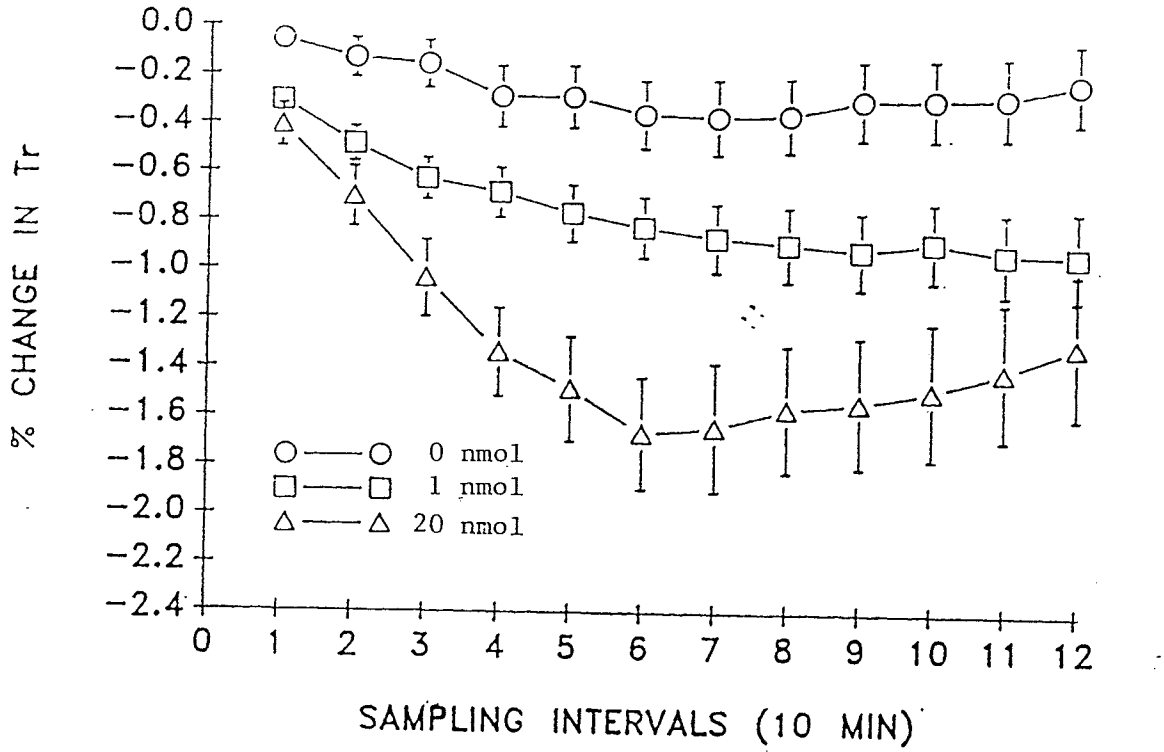

Figure 8. Mean (+SEM) percentage change in rectal temperature (tTr) from previous baseline during the twelve 10-min intervals following central administration of each of the three doses of clonidine, 0 nmol, 1 nmol and 20 nmol.

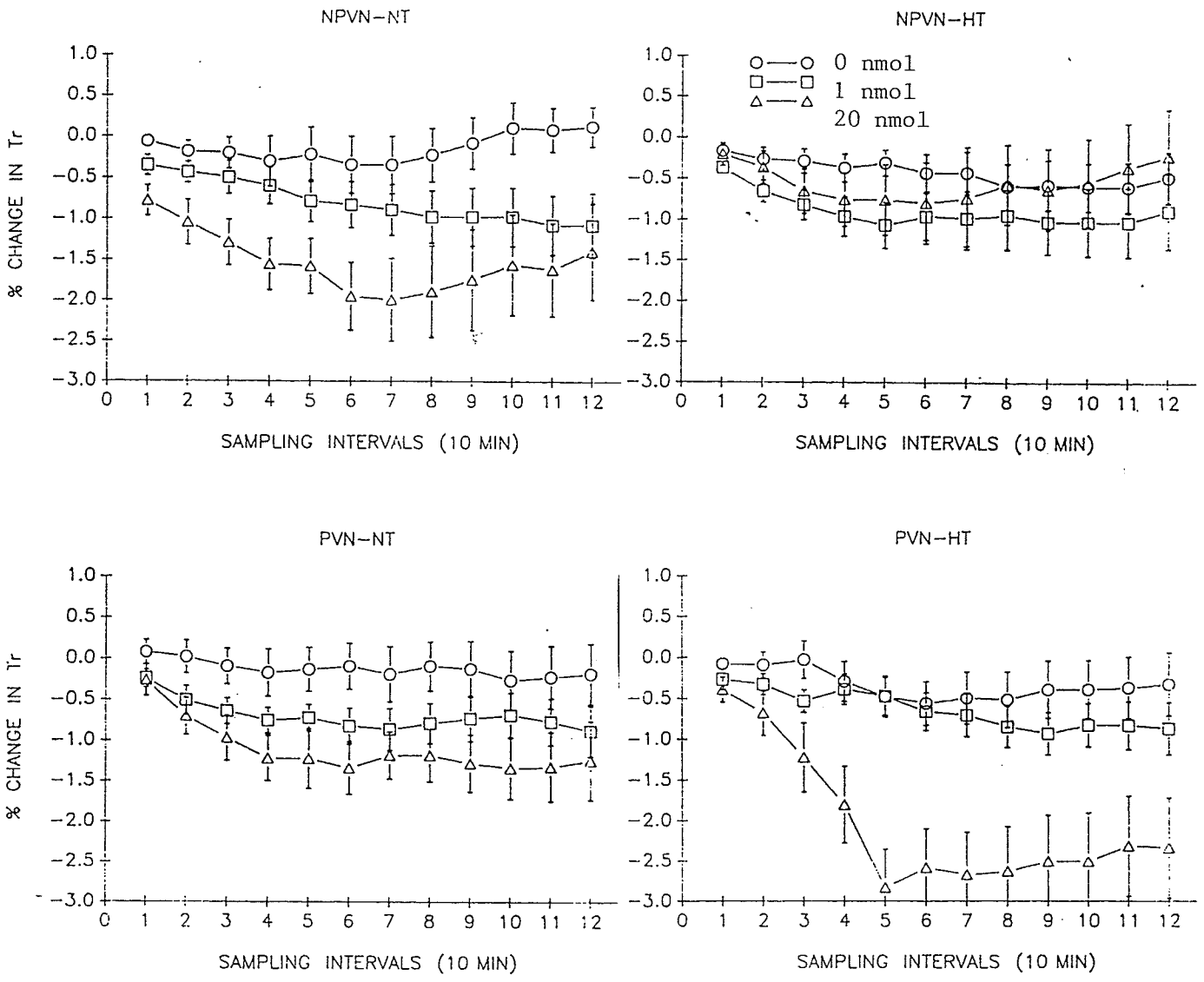

Figure 9. Mean (+SEM) percentage change in rectal temperature (Tr) from previous baseline in normotensive (NT) and 1K-GB hypertensive (HT) animals implanted with cannulae in the PVN or in areas outside the PVN (NPVN) during the twelve 10-min intervals following central administration of each of the three doses of clonidine, 0 nmol, 1 nmol, and 20 nmol.

effects in PVN-NT animals in comparison to the 0 nmol dose. The 20 nmol dose produced a large hypothermic effect in PVN-HT animals but the effects of the 1 nmol dose were not different from those for the 0 nmol dose. A significant BPStatus X Rauwol/Sal x Clonidine Dose x Sampling Interval effect, $F(22,990)=1.83$, $p<.012$, was found and is illustrated in Figure 10. This effect revealed that, when collapsed across placements, decreases in Tr attribuable to the 20 nmol dose were attenuted by Rauwol in NT animals, but were apparently potentiated in HT animals.

Oxygen consumption. A Sampling Interval effect, $F(11, 484)=29.11$, $p<.001$, indicated a progressive increase in VO_2, such that by the end of the testing session, VO_2 had increased by 13.18%, from 1.97 ml O_2/g/h to 2.21 ml O_2/g/h from baseline. A Placement x BP Status effect, $F(1,44)=5.94$, $p<.019$, was found when collapsed across Rauwol/Sal pretreatment, the three doses of clonidine, and the twelve sampling intervals. This effect indicated the VO_2 values for the NPVN-NT, PVN-NT, and PVN-HT groups were suppressed relative to the NPVN-HT group (Figure 11). A similar suppression was also apparent over time in a Placement x BP Status x Sampling Interval effect, $F(11,484)=3.3$, $p<.001$, when collapsed across all other groups. Finally, a Placement x Rauwol/Sal x Clonidine Dose x Sampling Interval effect, $F(22,968)=2.14$, $p<.002$, when

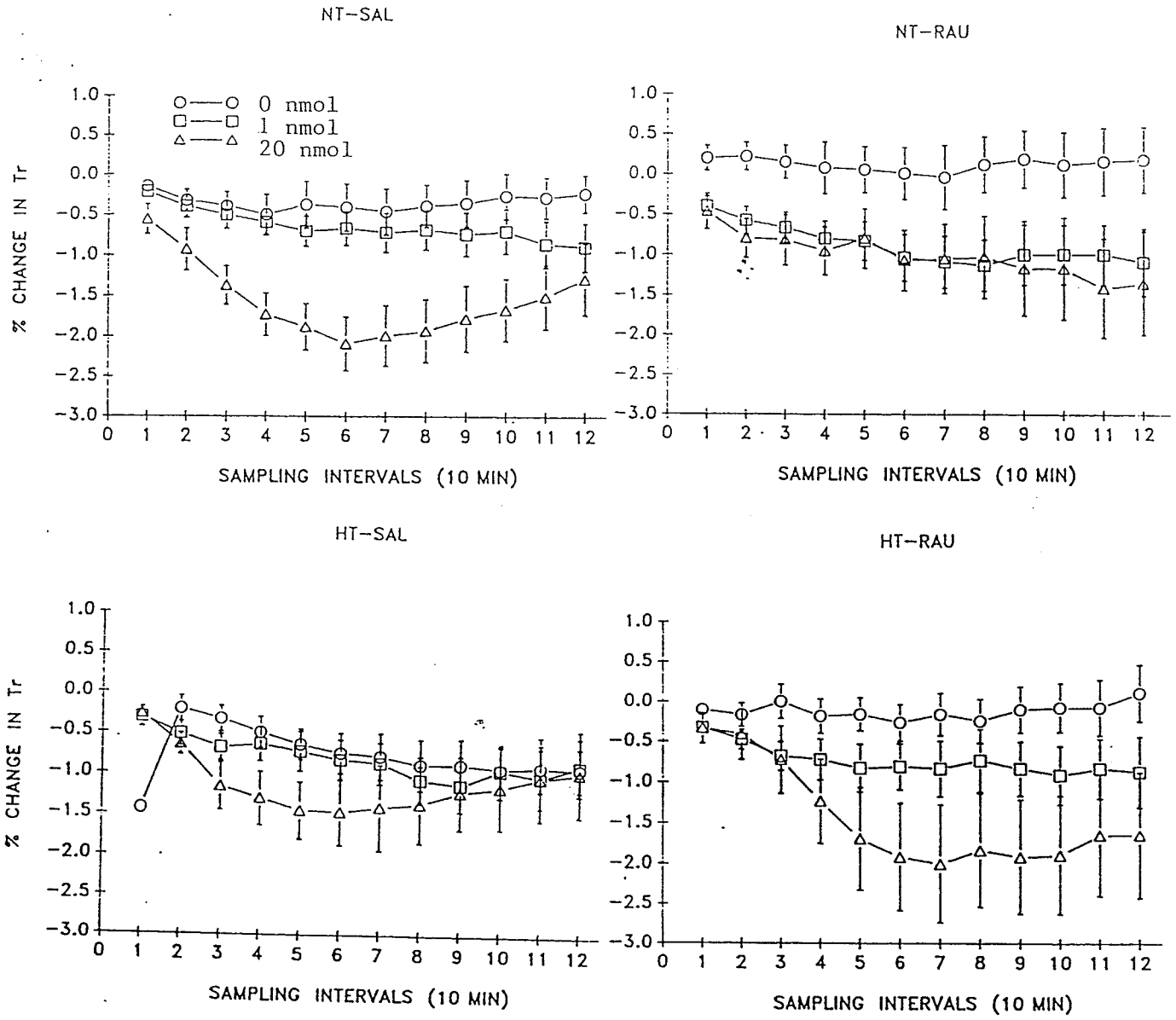

Figure 10. Mean (+SEM) percentage change in rectal temperatures (Tr) from the previous baseline in normotensive (NT) and 1K-GB (HT) animals that have been pretreated with either saline (SAL) or rauwolscine (RAU) during the twelve 10-min intervals following central administration of each of the three doses of clonidine, 0 nmol, 1 nmol, and 20 nmol.

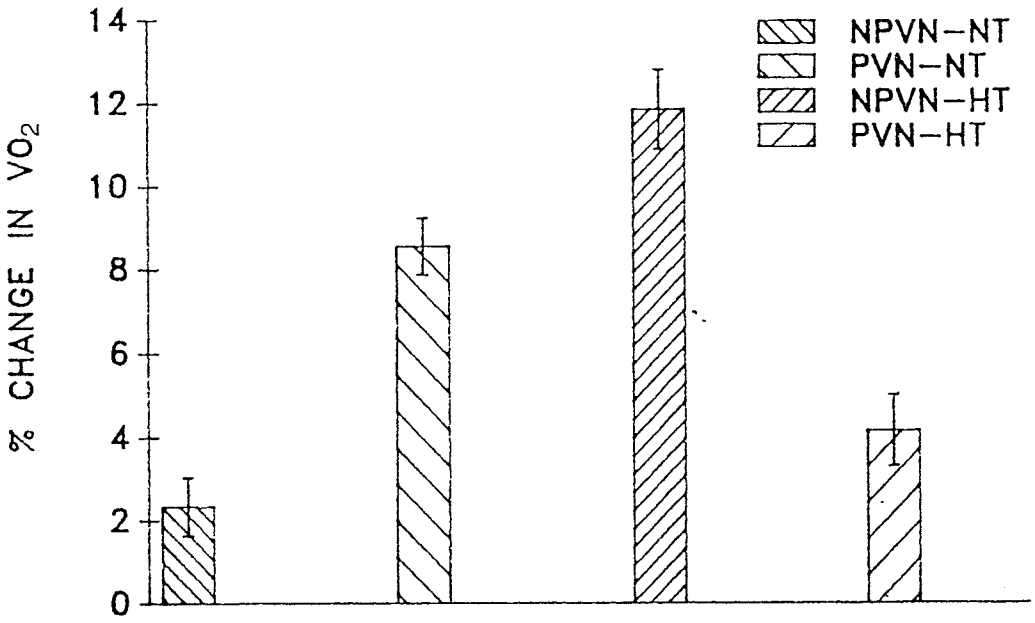

Figure 11. Mean (+SEM) percentage change in oxygen consumption (VO_2) from prevous baseline in normotensive (NT) and 1K-GB hypertensive (HT) animals with cannulae implanted in the PVN or in areas outside the PVN (NPVN) following central injection of clonidine.

collapsed across BP Status. This effect showed that the 1 nmol and 20 nmol doses of clonidine tended to suppress VO_2 in PVN animals that had not been pretreated with Rauwol, although this suppression was only exhibited in the second hour after administration of clonidine. No such suppression was obtained in PVN animals that were pretreated with Rauwol, or in NPVN animals.

Discussion

A number of observations are apparent upon examination of the results for the predrug baseline data. The first observation is that the application of a clip to the renal artery was successful in inducing hypertension, whereas sham-operated animals were indeed normotensive. The second observation is the finding that there were no thermal or MABP differences between PVN and NPVN animals suggesting that placement of cannulae in the PVN or areas adjacent to the PVN did not produce abnormal changes in Tr or MABP. The third observation is that there were no differences between the three days of testing suggesting that pharmacological manipulations administered on one day did not cause any changes that could influence subsequent testing. All these observations suggest that the subjects in this study were healthy, viable animals. The results from the Rau/Sal Baseline analyses suggest that the dose of Rau used (1 mg/kg) in this study did not have any effects on its own

that were different from those produced by saline in the 30-min period between its administration and the administration of clonidine.

The administration of clonidine into the PVN produced a negligible increase in MABP, whereas there was a somewhat larger decrease in MABP for the NPVN animals. Similarly, there was a small increase in MABP in HT animals following clonidine administration and a larger decrease in NT animals. Rauwol pretreatment resulted in a general increase in MABP, whereas animals pretreated with Sal exhibited lower MABPs. The physiological relevance of these effects is questionable, however, since they represent changes of 1 to 4 mmHg. Thus, overall, clonidine injected into PVN or NPVN areas, in NT or HT animals, did not produce relevant effects with the present methodology. A numer of possible mechanisms can account for the lack of BP effects found in this study. The first one involves parvocellular descending autonomic fibers that terminate at the IML in the spinal cord. These descending projections may be modulated by NE since Decavel et al (1987) observed NE immunoreactive bodies in parvocellular areas. Porter and Brody (1986) have shown that electrical stimulation of the parvocellular neurons in the PVN results in selective vasoconstriction in the mesentery and renal regions while there was an increased flow to hindquarter regions (Porter & Brody, 1986). The

investigators observed that sino-aortic baroreceptor deafferentation resulted in an enhancement of the selective vasoconstrictor responses found in baroreflex-intact rats suggesting that this reflex buffers effects of parvocellular stimulation. It is possible, therefore, that the administration of clonidine could result in stimulation of descending fibers to the IML and cause selective vasoconstriction in some vascular beds. BP, under these conditions, may change very little since the baroreflex arc will attempt to buffer any pressor effects that may result from increasing sympathetic activity. Secondly, a number of studies that have examined the effects of clonidine on specific hypothalamic nuclei have infused clonidine, instead of microinjecting it, resulting in longer-lasting responses. Therefore, the results of this study do not exclude the possibility that clonidine produced short latency responses within ten minutes after administration. Thirdly, it is possible that the doses used in this study are not effective in eliciting relevant cardiovascular responses, but higher doses may be able to elicit such responses.

Clonidine microinjections produced hypothermic effects in both the PVN and NPVN areas. In NT animals, PVN or NPVN injections of clonidine produced dose-dependant hypothermic effects were found. However, in HT animals, clonidine only produced a hypothermic effect when injected into the PVN but

not in NPVN areas. Furthermore, this hypothermic effect only occurred with the high, 20 nmol dose of clonidine. Interestingly, the three groups in which clonidine produced hypothermic effects, the NPVN-NT, PVN-NT, and PVN-HT groups, also exhibited a suppression in metabolism.

Clonidine-induced hypothermic effects in NPVN areas (though only for NT animals) are not unexpected since such responses have been found following clonidine administration into a number of hypothalamic areas. Injection of clonidine into the anterior hypothalamic nucleu has been shown to result in hypothermic effects (Myers, Beleslin, & Rezvani, 1987). Both the posterior and ventromedial nuclei have been implicated in thermoregulation and posterior nucleus has further been implicated in the cardiovascular effects of clonidine (Philipu, Demmeler, & Roensberg, 1974). The finding in this study that the hypothermic effect in NPVN animals was accompanied by a suppresssion of metabolism suggests that clonidine was able to exert coordinated thermal responses in a number of areas outside the PVN.

PVN injections of clonidine produced hypothermic effects and suppression of metabolism. These results support the hypothesis that alpha-2 receptors in the PVN may mediate thermoregulatory and metabolic variables. A possible mechanism that may account for clonidine's hypothermic

effect and suppression of metabolism when it is administered into the PVN involves the Porter and Brody study described earlier. Clonidine may be able to stimulate descending autonomic fibers from the parvocellular regions of the PVN, and elicit a selective vasoconstriction, resulting in the shunting of blood away from the core or hindquarter region to more peripheral vascular beds and a corresponding decrease in Tr.

Clonidine produced differential hypothermic responses in NT animals than in HT animals. When clonidine was injected into NPVN areas, a dose-dependant hypothermic effect occurred in NT, but not in HT animals. However, when injected into the PVN, the hypothermic effect of the 20 nmol dose of clonidine was potentiated in HT animals in comparison to NT animals. This increased thermal reactivity to PVN alpha-2 receptors has never before been demonstrated in the 1K-GB model, although this model doses exhibit an increased pressor reactivity to general alpha stimulation (Fyda, 1987). The results of the present study suggest that the alpha-2 receptor population in the PVN of 1K-GB animals is somehow altered. This alteration may involve an up-regulation of alpha-2 receptors leading to greater responsiveness following stimulation.

A further difference between NT and 1K-GB animals occurs in the response to the 20 nmol dose of clonidine following pretreatment with Rauwol. Rauwol blocked the decrease in Tr produced by the 20 nmol dose in NT animals pretreated with Sal. However, in HT animals, Rauwol potentiated the effects of the 20 nmol dose of clonidine in comparison to HT animals pretreated with Sal. These results suggest a generally altered alpha-2 receptor population in 1K-GB animals compared to the NT control animals.

To summarize, the results of the present study suggest that clonidine injected into the PVN on in NPVN areas of HT or NT animals did not produce physiologically relevant changes in BP. These results suggest that the doses of clonidine, the microinjection paradigm, or the temporal parameters of the measurement of BP utilized in this study may be responsible. An alternate explanation for the lack of effects in PVN animals involves parvocellular descending autonomic fibers. Stimulation of these fibers by clonidine may have produced a selective vasoconstriction in various vascular beds without causing an overall change in BP. Clonidine injected into the PVN or NPVN areas of NT animals produced dose-dependant hypothermic effects. Pretreatment with Rauwol blocked the hypothermic effects of the 20 nmol dose in NT animals. This same dose injected into the PVN of 1K-GB animals produced a strong hypothermic effect that was

not present when this dose of clonidine was administered into NPVN areas. Furthermore, the hypothermic effect observed in HT animals was potentiated when animals were pretreated with Rauwol. Hypothermic effects following clonidine administration into NPVN areas are not unexpected, since such effects have already been demonstrated. The hypothermic effects following clonidine administration into the PVN may again involve parvocellular autonomic fiber stimulation, causing a selective vasoconstriction such that blood is shunted away from the core and hindquarter region, resulting in a decrease in Tr. The greater hypothermic effect in HT animals following clonidine administration alone or with Rauwol pretreatment suggests an altered alpha-2 receptor population in the 1K-GB model of hypertension.

Future studies should examine the specific mechanisms underlying the effects of PVN alpha-2 stimulation found in this study. Additionally, the altered alpha-2 receptor population in 1K-GB hypertensive animals, particularly the PVN, requires further examination, as does the presence of such altered receptor populations in other models of hypertension.

References

Beckman, A.I. (1970). Effect of intrahypothalamic norepinephrine on thermoregulatory responses in the rat. American Journal of Physiology, 218, 1596-1604.

Bengis, R.G., & Coleman, T.G. (1979). Antihypertensive effect of prolonged blockade of angiotensin formation in benign and malignant one- and two-kidney Goldblatt hypertensive rats. Clinical Science, 57, 53-62.

Benetos, A., Gavras, I., & Gavras, H. (1986). Norepinephrine Applied in the Paraventricular Hypothalamic Nucleus Stimulates Vasopressin Release. Brain Research, 381, 322-326.

Bennett, A.F., & Ruben, J.A. (1979). Endothermy and activity in vertebrates. Science, 206, 649-654.

Boissier, J.R., & Giudicelli, J.F., Fichelle, J., Schmitt, H., & Schmitt, H. (1968). Cardiovascular effects of 2-(2,6-dichlorophenylamino)-2-imidazoline-hydrochloride (St 155) I. Peripheral sympathetic system. European Journal of Pharmacology, 2, 333-339.

Bolme, P., & Fuxe, K. (1971). Pharmacological studies in the hypotensive effects of clonidine. European Journal of Pharmacology, 13, 168-174.

Boulant, J.A. (1981). Hypothalamic mechanisms in thermoregulation. Federation Proceedings, 40, 2843-2850.

Boulant, J.A., & Hardy, J.D. (1974). The effect of spinal and skin temperatures on the firing rate and thermosensitivity of preoptic neurons. Journal of Physiology, 240, 639-660.

Bousquet, P., & Feldman, J. (1986). The blood pressure effects of alpha-adrenoceptor antagonists injected in the medullary site of action of clonidine: the nucleus reticularis lateralis. Life Science, 40, 1045-1052.

Bousquet, P., Feldman, J., Bloch, R., & Schwartz, J. (1981). The nucleus reticularis lateralis: a region highly sensitive to clonidine. European Journal of Pharmacology, 69, 389-392.

Bousquet, P., Feldman, J., Velly, J., & Bloch, R. (1975). Role of the ventral surface of the brain stem in the hypotensive action of clonidine. European Journal of Pharmacology, 34, 151-156.

Brody, M.J., Fink, G.D., Buggy, J., Haywood, J.R., Gordon, F.J., & Johnson, A.K. (1978). The role of the anteroventral third ventricle (AV3V) region in experimental hypertension. Circulation Research, 43, I2-I13.

Brown, T.C., Davis, J.O., Olichney, J.J., & Johnston, C.I. (1966). Relation of plasma renin to sodium balance and arterial pressure in experimental renal hypertension. Circulation Research, 18, 475-483.

Buggy, J., & Fink, G.D. (1982). Experimental renal hypertension. In: F.M. Radzialowski (Ed.), Hypertension Research. Methods and Models (pp. 344-391).

Bunag, R.D., & Eferakeya, A.E. (1976). Immediate hypotensive after-effects of posterior hypothalamic lesions in awake rats with spontaneous, renal or DOCA-hypertension. Cardiovascular Research, 10, 663-671.

Calaresu, F.R., & Ciriello, J. (1981a). Renal afferent nerves affect discharge rate of medullary and hypothalamic single units in the cat. Journal of the Autonomic Nervous System, 3, 311-320.

Calaresu, F.R., & Ciriello, J. (1981b). Altered concentration of catecholamines in the hypothalamus of the rat after renal denervation. Canadian Journal of Physiology and Pharmacology, 59, 1274-1277.

Calaresu, F.R., & Ciriello, J. (1980). Projections to the hypothalamus from buffer nerves and NTS in cats. American Journal of Physiology, 239, R130-136.

Carlisle, H.J., & Laudenslager, M.L. (1979). Observations on the thermoregulatory effects of preoptic warming in rats. Physiology and Behavior, 23, 723-732.

Cantor, A., & Satinoff, E. (1976). Thermoregulatory responses to intraventricular norepinephrine in normal and hypothalamic damaged rats. Brain Research, 108, 125-141.

Christensen, N.J., & Galbo, SH. (1983). Sympathetic nervous activity during exercise. Annual Review of Physiology, 45, 139-153.

Ciriello, J., & Calaresu, F.R. (1980a). Role of paraventricular and supraoptic nuclei in central cardiovascular regulation in the cat. American Journal of Physiology, 239, R137-142.

Ciriello, J., & Calaresu, F.R. (1980b). Hypothalamic projections of renal afferent nerves in the cat. Canadian Journal of Physiology and Pharmacology, 58, 574-476.

Chung, J.M., Chung, K., & Wurster, R.D. (1975). Sympathetic preganglionic neurons of the cat spinal cord: horseradish peroxidase study. Brain Research, 91, 126-131.

Constantine, J.W., & McShane, W.K. (1968). Analysis of the cardiovascular effects of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (Catapres). European Journal of Pharmacology, 4, 109-123.

Coote, J.H., & MacLeod, V.H. (1974). The influence of bulbospinal monoaminergic pathways on sympathetic nerve activity. Journal of Physiology, 241, 453-475.

Cowley, A.W., & Liard, J.F. (1988). Vasopressin and arterial blood pressure regulation. Hypertension 11 (Suppl 1): I-25 - I-32.

Dahlstrom, A., & Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurons in the CNS. I. Demonstration of monoamines in the cell bodies in brain stem neurons. Acta Physiologica Scandinavia, 62, 1-55.

Dampney, R.A.L., & Moon, E.A. (1980). Role of ventrolateral medulla in vasomotor responses to cerebral ischemia. American Journal of Physiology, 239, H349-358.

Dampney, R.A.L., Goodchild, A.K., Robertson, L.G., & Montgomery, W. (1982). Role of ventrolateral medulla in vasomotor regulation: a correlative anatomical and physiological study. Brain Research, 249, 223-235.

Dargie, H.J., Franklin, S.S., & Reid, J.L. (1977). Plasma noradrenaline concentrations in experimental renovascular hypertension in the rat. Clinical Science and Molecular Medicine, 52, 477-483.

Decavel, C., Geffard, M., & Calas, A. (1987). Comparative study of dopamine-and noradrenaline-immunoreactive terminals in the paraventricular and supraoptic nuclei of the rat. Neuroscience Letters, 77, 149-154.

DeQuattro, V., Eide, I., Myers, M.R., Eide, K., Kollach, R., & Whigham, H. (1978). Enhanced hypothalamic noradrenaline biosynthesis in Goldblatt I renovascular hypertension. Clinical Science and Molecular Medicine, 55, 109s-111s.

Dubocovich, M.L., & Langer, S.Z. (1973). Effects of flow-stop on the metabolism of 3H-noradrenaline released by nerve stimulation in the perfused cat's spleen. Naunyn-Schmiedeberg's Archives of Pharmacology, 278, 179-194.

Eide, I., Myers, M.R., DeQuattro, V., Kolloch, R., Eide, K., & Whigham, M. (1980). Increased hypothalamic noradrenergic activity in one-kidney, one-clip renovascular hypertensive rats. Journal of Cardiovascular Pharmacology, 2, 833-841.

Farah, M.B., Langer, S.Z. (1974). Protection by phentolamine against the effects of phenoxybenzamine on transmitter release elicited by nerve stimulaiton in the perfused cat heart. British Journal of Pharmacology, 52, 549-557.

Ferrario, C.M. (1974). Contribution of cardiac output and total peripheral resistance to experimental renal hypertension. American Journal of Physiology, 226, 711-717.

Fregly, M.J. (1954). Effects of extremes of temperatures on hypertensive rats. American Journal of Physiology, 176, 275-281.

Fyda, D.M. (1987). The influence of One-Kidney Goldblatt hypertension and cold acclimation on adrenergically-induced cardiovascular and

thermoregulatory adjustments in rats. Doctoral Dissertation, University of Manitoba, Winnipeg, Manitoba, 1987.

Goldblatt, H., Lynch, J., Hanzel, R.F., & Summerville, W. (1934). Studies on experimental hypertension. I. The production of persistent elevation of systolic blood pressure by means of renal ischemia. Journal of Experimental Medicine, 59, 347-379.

Goldman, C.K., Marino, L., & Leibowitz, S.F. (1985). Postsynaptic alpha-2-noradrenergic receptors mediate feeding induced by paraventricular nucleus injection of norepinephrine and clonidine. European Journal of Pharmacology, 115, 11-19.

Guieu, J.D., & Hardy, J.D. (1970). Effects of heating and cooling of the spinal cord on preoptic unit activity. Journal of Applied Physiology, 29, 675-683.

Guyenet, P.T., & Cabot, J.B. (1981). Inhibition of sympathetic preganglionic neurons by catecholamines and clonidine: mediation by an alpha-adrenergic receptor. The Journal of Neuroscience, 1(8), 908-917.

Henning, M. (1969). Noradrenaline turnover in renal hypertensive rats. Journal of Pharmacology and Pharmacy, 21, 61-63. Hilton, S.M., & Spyer, K.M. (1971). Participation of the anterior hypothalamus in the baroreflex arc. Journal of Physiology, 218, 271-293.

Hokfelt, T., Fuxe, K., Goldstein, M., & Johansson, O. (1974). Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. Brain Research, 66, 235-251.

Hosoya, Y., & Matsushita, M. (1979). Identification and distribution of the spinal and hypophysial projection neurons in in paraventricular nucleus of the rat. Experimental Brain Research, 35, 315-331.

Howe, P.R.C. (1985). Blood pressure control by neurotransmitters in the medulla oblongata and spinal cord. Journal of the Autonomic Nervous System, 12, 95-115.

Imai, Y., Nolan, P., & Johnston, C. (1986). Tachycardic and hypertensive effects of centrally administered clonidine in conscious rats. Clinical and Experimental Hypertension- Theory & Practice, 44, 239-258.

Isaac, L. (1980). Clonidine in the central nervous system: site and mechanism of hypotensive action. Journal of Cardiovascular Pharmacology, 2, S5-S19.

Itoh, S. (1968). Effect of vasopressin on lipid metabolism. In: Integrative Mechanisms of Neuroendocrine System, Hokkado Univ. Sch. Med., Sapporo, 175-197.

Johnson, A.K., Buggy, J., Fink, G.D., & Brody, M.J. (1981). Prevention of renal hypertension and of the central pressor effect of angiotensin by ventromedial hypothalamic ablation. Brain Research, 205, 255-264.

Jarrott, B., Lewis, S., Conway, E.L., Summers, R., & Louis, W.J. (1984). The involvement of central alpha adrenoceptors in the anti-hypertnsive action of methyldopa and clonidine in the rat. Clinical and Exprimental Hypertension- Theory and Practice, A6(1&2), 387-400.

Kannan, H., Niijima, A., & Yamashita, H. (1987). Inhibition of renal sympathetic nerve activity by electrical stimulation of the hypothalamic paraventricular nucleus in anesthetized rats. Journal of the Autonomic Nervous System, 21, 73-80.

Katholi, R.E., Winternitz, S.R., & Oparil, S. (1981). Decrease in symapthetic nervous system activity and attenuation in response to stress following renal denervation in the one-kidney, one-clip Goldblatt hypertensive rat. Clinical and Exprimental Hypertension, A4(4&5), 707-716.

Katholi, R.E., Winternitz, S.R., & Oparil, S. (1982). Decrease in peripheral sympathetic nervous system activity following renal denervation or unclipping in the one-kidney, one-clip Goldblatt hypertensive rat. Journal of Clinical Investigations, 69, 55-62.

Kawasaki, H., & Takasaki, K. (1986). Central alpha-2 adrenoceptor-mediated hypertensive response to clonidine in conscious, normotensive rats. Journal of Pharmacology and Experimental Therapeutics, 236, 810-818.

Kellar, K.J., Quest, J.A., Sper, A.C., Buller, A., Conforti, A., Dias Souza, J.D., & Gillis, R.A. (1985). Comparative effects of urapidil, prazosin, and clonidine on ligand binding to central nervous system receptors, arterial pressure, and heart rate in experimental animals. American Journal of Medicine, Oct. 5, 87-95.

Kiss, J.Z. (1988). Dynamism of Chemoarchitecture in the Hypothalamic Paraventricular Nucleus. Brain Research Bulletin, 20, 699-708.

Klupp, H., Knappen, K., Otsuka, Y., Streller, Il, & Teichmann, H. (1970). Effects of clonidine on central sympathetic tone. European Journal of Pharmacology, 10, 225-229.

Kobinger, W., & Walland, A. (1967). Investigations into the mechanism of the hypotensive effect of 2-(2,6 dichlorophenylamino)-2-imidazoline hydrochloride. European Journal of Pharmacology, 2, 155-162.

Kumada, M., Dampney, R.A.L., & Reis, D.J. (1979). Profound hypotension and abolition of the vasomotor component of the cerebral ischemic response produced by restricted lesions of the medulla oblongata: relationship to the so-called tonic vasomotor center. Circulation Research, 45, 63-70.

Langer, S.Z. (1981). Presynaptic regulation of the release of catecholamines. Pharmacological Reviews, 32, 337-362.

Ledingham, J.M., & Cohen, M.B. (1964). Changes in extracellular volume and cardiac output during the development of experimental renal hypertension. Canadian Medical Association Journal, 90, 292-294.

Leenen, F.H.H., & deJong, W. (1975). Plasma renin and sodium balance during development of moderate and severe renal hypertension in rats. Circulation Research, 36, 179-186.

Leibowitz, S.F., Jhanwar-Uniyal, M., Dvorkin, B., & Makman, M.H. (1982). Distribution of alpha-adrenergic, beta-adrenergic and dopaminergic receptors in discrete hypothalamic areas of the rat. Brain Research, 233, 97-114.

Levine, H.J. (Ed.). (1976). Clinical cardiovascular physiology. New York: Green & Shatton Publ.

Lipski, J., & Trzebski, A. (1975). Bulbo-spinal neurons activated by baroreceptor afferents and their possible role in inhibition of preganglionic sympathetic neurons. Pflugers Archives, 356, 181-192.

Livingston, A., Low, J., & Morris, B. (1984). Effects of clonidine and xylazine on body temperature. British Journal of Pharmacy, 81, 189-193.

Loewy, A.D., & Burton, H. (1978). Nuclei of the solitary tract: efferent projections to the lower brain stem of the cat. Journal of Comparative Neurology, 181, 421-450.

Loewy, A.D., McKellar, S., & Saper, C.B. (1979). Direct projections from the A5 catecholamine cell group to the intermediolateral cell column. Brain Research, 174, 309-314.

Matsumura, K., Nakayama, T., & Ishikawa, Y. (1983). Effects of preoptic thermal stimulation on electrical activities of neurosecretory cells in paraventricular and periventricular nuclei of the hypothalamus. Brain Research, 289, 330-333.

Mogilnicka, E., Klimek, V., Nowak, G., & Czyrak, A. (1985). Clonidine and beta-agonists induce hyperthermia in rats at high ambient temperature. Journal of Neural Transmission, 63, 223-235.

McAllen, R.M., Neil, J.J., & Loewy, A.D. (1982). Effects of kaninic acid applied to the ventral surface of the medulla oblongata on vasomotor tone, the baroreceptor reflex and hypothalamic autonomic responses. Brain Research, 238, 65-76.

McKellar, S., & Loewy, A.S. (1979). Spinal projections of noradrenaline-containing neurons in the rat. Neuroscience Abstracts, 5, 344-350.

Mora, F., Lee, T.F., & Myers, R.D. (1983). Involvement of alpha- and beta-adrenoceptors in the central action of norepinephrine on temperature, metabolish, heart and respiratory rates of the conscious primate. Brain Research Bulletin, 11, 613-616.

Morishima, M.S., & Gale, C.C. (1972). Relationship of blood pressure and heart rate to body temperature in baboons. American Journal of Physiology, 223, 387-395.

Myers, R.D. (1970). The role of hypothalamic transmitter factors in the control of body temperature. In J.D. Hardy, A.P. Gagge, & A.J. Stolwijk (Eds.), Physiological and behavioral thermoregulation (pp. 648-666). Springfield, Ill.: C.E. Thomas Publ.

Myers, R.D., Beleslin, D.B, & Rezvani, A.H. (1987). Hypothermia: role of alpha-1 and alpha-2 noradrenergic receptors in the hypothalamus of the cat. Pharmacology, Biochemistry and Behavior, 26, 373-379.

Nayler, W.G., Price, J.M., Stone, J., & Lowe, T.E. (1969). Further observations on the cardiovascular effects of St155 (Catapres). Journal of Pharmacology and Experimental Therapeutics, 166, 364-373.

Nayler, W.G., Rosenbaum, M., McInness, J., & Lowe, T.E. (1966). Effect of a new hypotensive drug, St155, on the systemic circulation. American Heart Journal, 72, 764-770.

Onesti, G., Schwartz, A.B., Kim, K.E., Paz-Martinez, V., & Swartz, G.H. (1971). Anti-hypertensive effects of clonidine. Circulation Research, 28(Suppl. 2), 53-67.

Ozawa, H., Chen, C.-S., Watenable, M., & Vematsu, T. (1977). Effect of clonidine on blood pressure, heart

rate and body temperature in conscious rats. Japanese Journal of Pharmacy, 27, 47-54.

Panneton, W.M., & Loewy, A.D. (1980). Projections of the carotid sinus nerve to the nucleus of the solitary tract in the cat. Brain Research, 191, 239-244.

Paxinos, G., & Watson, C. (1982). The rat Brain in stereotaxic coordinates. Toronto: Academic Press.

Petras, J.M., & Cummings, J.F. (1972). Autonomic neurons in the spinal cord of the rhesus monkey: A correlation of the findings of cytoarchitectonics and sympathectomy with fiber degeneration following dorsal rhizotomy. Journal of Comparative Neurology, 146, 189-218.

Petty, M., & Reid, J. (1977). Changes in noradrenaline concentration in brainstem and hypothalamic nuclei during the develoment of renovascular hypertension. Brain Research, 136, 376-380.

Petty, M.A., & Reid, J.L. (1979). Catecholamine synthesizing enzymes in brainstem and hypothalamus during the development of renovascular hypertension. Brain Research, 163, 277-284.

Philippu, A., Demmeler, R., & Roensberg, G. (1974). Effects of centrally applied drugs on pressor responses to hypothalamic stimulation. Naunyn-Schmideberg's Archives of Pharmacology, 282, 389-400.

Porter, J. R., & Brody, M.J. (1986). A Comparison of the Hemodynamic Effects produced by electrical stimulation of subnuclei of the paraventricular nucleus. Brain Research, 375, 20-29.

Przuntek, Yl, Guimaraes, S., & Philippu, A. (1971). Importance of adrenergic neurons of the brain for the rise of blood pressure evoked by hypothalamic stimulation. Naunyn-Schmideberg's Archives of Pharmacology, 271, 311-319.

Rand, M.J., & Wilson, J. (1968). Mechanisms of the pressor and depressor actions of St155 (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, catapresan). European Journal of Pharmacology, 3, 27-33.

Refinetti, & Carlisle (1986). Effects of anterior and posterior hypothalamic temperature changes on thermoregulation in the rat. Physiology and Behavior, 36, 1099-1103.

Reid, J.L., Dargie, H.J., Franklin, S.S., & Fraser, B. (1976). Plasma noradrenaline and renovascular hypertension in the rat. Clinical Science and Molecular Medicine, 51(Suppl. 11), 4395-4425.

Reid, J.L., Lewis, P.J., & Myers, A.G. (1975). Role of central dopaminergic mechanisms in piribedil and clonidine induced hypothermia in the rat. Neuropharmacology, 14, 215-220.

Reis, D.J., Granata, A.R., Joh, T.H., Ross, C.A., Ruggiero, D.A., & Park, D.H. (1984). Brain stem catecholamine mechanisms in tonic and reflex control of blood pressure. Hypertension, 6(Suppl. 11), II-7-II-15.

Renaud, L.P., Blume, H.W., Kearney, R.E., MacKenzie, B.W., & Pittman, Q.J. (1978). Physiological Anatomy of the Hypothalamus. Current Studies of Hypothalamic Function, 2, 1-16.

Richards, S.A. (1973). Temperature regulation. London: Wykeham.

Ross, C.A., Ruggiero, D.A., Joh, T.H., Park, D. H., & Reis, D.J. (1983). Adrenaline synthesizing neurons of the rostral ventrolateral Brain Research, 273, 356-361.

Saper, C.B., Loewy, A.D., Swanson, L.W., & Cowan, W.M. (1976). Direct hypothalamo-autonomic connections. Brain Research, 117, 305-312.

Satinoff, E. (1964). Behavioral thermoregulation in response to local cooling of the rat brain. American Journal of Physiology, 206, 1389-1394.

Satoh, K., Tohyama, M., Yamamoto, K., Sakumoto, T., & Shimizu, N. (1977). Noradrenaline innervation of the spinal cord studied by horse-radish peroxidase method combined with monoamine oxidase staining. Experimental Brain Research, 30, 175-186.

Sawchenko, P.E., & Swanson, L.W. (1982). The organization of noradrenergic pathways from the brainstem to the paraventricular and Brain Research Reviews, 4, 275-325.

Schmitt, H.H., & Schmitt, H. (1969). Localization of the hypotensive effect of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (St155, Catapesan). European Journal of Pharmacology, 6, 8-12.

Schmitt, H., Schmitt, H., Boissier, J.R., & Giudicelli, J.F. (1967). Centrally mediated decrease in sympathetic tone induced by 2-(2,6-dichlorophenylamino)-2-imidazoline (St155, Catapresan). European Journal of Pharmacology, 2, 147-148.

Schmitt, H., Schmitt, H., Boissir, J.R., Giudicelli, J.F., Fichelle, J. (1968). Cardiovascular effects of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (St155). II. Central sympathetic tone. European Journal of Pharmacology, 2, 340-344.

Seymour, A.A., Davis, J.O., Freeman, R.H., DeForrest, J.M., Rowe, B.P., Stephens, G.A., & Williams, G.M. (1981). Sodium and angiotensin in the pathogenesis of experimental renal hypertension. American Journal of Physiology, 240, H788-H792.

Sharma, J.N., Sandrew, B.B., & Wang, S.C. (1978). Central nervous system site of clonidine-induced hypotension: A

microiontophoretic study of bulbar cardiovascular neurons. Brain Research, 151, 127-133.

Shaw, J., Hunyor, S.N., & Korner, P.J. (1971). Sites of central nervous system action of clonidine on reflex autonomic function in the unanesthetized rabbit. European Journal of Pharmacology, 15, 66-71.

Shido, O., Kifune, A., & Nagasaka, T. (1984). Japanese Journal of Physiology, 34, 397-406.

Starke, K. (1981). Alpha-adrenoceptors subclassification. Reviews in Physiology, Biochemistry and Pharmacology, 88, 199-236.

Starke, K., & Langer, S.Z. (1979). A note on terminology for presynaptic receptors. In S.Z. Langer, K. Starke, & M.L. Dubocovich (Eds.), Presynaptic receptors (pp. 1-3). Oxford: Pergamon Press.

Stephens, G.A., Davis, J.O., Freeman, R.SH., DeForrest, J.M., & Early, D.M. (1979). Hemodynamic, fluid, and electrolyte changes in sodium-depleted, one-kidney, renal hypertensive dogs. Circulation Research, 44, 316-321.

Story, D.F., Briley, M.S., & Langer, S.Z. (1979). The effects of chemical sympathectomy with 6-hydroxydopamine on alpha-adrenoceptor and muscarinic cholinoceptor binding in rat heart ventricle. European Journal of Pharmacology, 57, 423-426.

Struyker-Boudier, H.A.J., Evenwell, R.T., Smits, J.F.M., & vanEssen, H. (1982). Baroreflex sensitivity during the development of spontaneous hypertension in rats. Clinical Science, 62, 589-594.

Struyker-Boudier, H.A.J., Smeets, G.W.M., Brouwer, G.M., & vanRossum, J.M. (1974). Hypothalamic alpha adrenergic receptors in cardiovascular regulation. Neuropharmacology, 13, 837-946.

Svensson, T.H., Bunney, B.S., & Aghajanian, G.K. (1975). Inhibitionof both noradrenergic and serotonergic neurons in brain by Brain Research, 92, 291-306.

Swales, J.D., Thurston, H., Queitoz, F.P., & Medina, A. (1972). Sodium balance during the development of experimental hypertension. 539-547.

Swanson, L.W., & Kuypers, H.G.J.M. (1980). The paraventricular of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labelling methods. The Journal of Comparative Neurology, 194, 555-570.

Tsoucaris-Kupfer, D., & Schmitt, H. (1972). Hypothermic effect of alpha-sympathomimetic agents and their antagonism by adrenergic and cholinergic blocking agents. Neuropharmacology, 11, 625-635.

Unnerstall, J.R., Fernandez, I., & Orensanz, L.M. (1985). The alpha-adrenergic receptor: radiohistochemical analysis of functional characteristics and biochemical differences. Pharmacology, Biochemistry and Behavior, 22, 859-874.

U'Prichard, D.C., & Snyder, S.H. (1979). Distinct alpha-noradrenergic receptors differentiated by binding and physiological relationships. Life Sciences, 24, 79-88.

Vatner, S.F., & Boettchner, D.H. (1978). Regulation of cardiac output by stroke volume and heart rate in conscious dogs. Circulation Research, 42, 557-561.

Vlachakis, N.D., Ranson, F., Kogosov, E., Woodcock, E., Alexander, N., & Maronde, R.F. (1984). Sympathetic activity and cardiac adrenergic receptors in one kidney, one clip hypertension in rats. Hypertension, 6, 654-659.

Vogel, S.A., Silberstein, S.D., Berv, K.R., & Kopin, I.J. (1972). Stimulation-induced release of norepinephrine from rat superior cervical ganglion in vitro. European Journal of Pharmacology, 20, 308-311.

Wallach, H., & Loewy, A.D. (1980). Projections of the aortic nerve to the nucleus tractus solitariius in the rabbit. Brain Research, 188, 247-251.

Wasserstrum, N., & Herd, J.A. (1977). Elevation of arterial blood pressure in the squirrel monkey at 10oC. American Journal of Physiology, 1, H459-H463.

Watkins, B.E., Davis, J.O., Freeman, R.H., DeForrest, J.M., & Stephens, G.A. (1978). Continuous angiotensin II blockade throughout the acute phase of one-kidney hypertension in the rat. Circulation Research, 42, 813-821.

Weber, M.A., & Drayer, J.I. (1984). Centrally acting anti-hypertensive agents: a brief overview. Journal of Cardiovascular Pharmacology, 6(Suppl. 5), S803-807.

Wilson, J.R., Berczi, A., & Fyda, D.M. (1981). Metacorticoid hypertension facilitates operant thermoregulation during heat stress in desalivated/depilitated rats. Proceedings of the 6th Annual Federation of American Societies of Experimental Biology|, Atlanta, Ga., 118.

Wilson, J.R. & Fyda, D.M. (1989). Goldblatt Hypertension and operant thermoregulation in shaved, sialoadenectomized rats. Physiology and Behavior, in press.

Winternitz, S.R., Katholi, R.E., & Oparil, S. (1982). Decrease in hypothalamic norepinephrine content following renal denervation in the one-kidney, one-clip Goldblatt hypertensive rats. Hypertension, 4, 369-373.

Young, W.S. III, & Kuhar, M.J. (1980). Noradrenergic alpha-1 and alpha-2 receptors: Light microscopic autoradiographic localization. Proceedings of the National Academy of Sciences USA, 77, 1696-1700.

Zerihun, L., & Harris, M. (1981). Electrophysiological identification of neurones of paraventricular nucleus sending axons to both the neurohypophysis and the medulla in the rat. Neuroscience Letters, 23, 157-160.

Zhang, T.-X., & Ciriello, J. (1985). Effect of paraventricular nucleus lesions on arterial pressure and heart rate after aortic baroreceptor denervation in th rat. Brain Research, 341, 101-109.

Appendix A

Formulae Used in the Determination of Oxygen Consumption (VO_2)

$VO_2 = (V_1) \times (F_{IO} - F_{EO}/1 - F_{EO}) / \text{Body Weight (g)}$

Where:

V_1 = volume of dry air through metabolic chamber/unit time @ STP

= 760 ml/min x 60 x Pb/760 x 273/273 + ta ^{o}C

F_{IO} = fractional concentration of O_2 of inspired air

= 0.2094

F_{EO} = fractional concentration of O_2 of expired air

= OM-11/100

Hill, R. W. (1972). Determination of oxygen consumption by use of paramagnetic oxygen analyzer. Journal of Applied Physiology, 33(2), 261-263.