The Effects of Central I_1-Imidazoline and α_2-Adrenergic Receptors on Body Temperature Regulation in Conscious Rats

BY

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Thomas R. Harrigan

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of Doctor of Philosophy

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The amount of energy, fortitude, and devotion necessary to deliver a doctoral dissertation is staggering. Thus, without the help, guidance, and encouragement of my academic advisors, family, and friends, the completion of this work would not have been possible. I'm dedicating the present work to my mom, Evelyn C. Harrigan, and the memory of my dad, Albert G. Harrigan, who encouraged and supported me unconditionally. I know they're smiling now.

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Abstract

Clonidine, an α_2-adrenergic receptor agonist, is a classic pharmacological tool used to study the sympathetic control of cardiovascular and thermoregulatory processes. Clonidine's ability to inhibit sympathetic output may be linked more to its affinity for non-adrenergic I_1-imidazoline receptors than for α_2-adrenoceptors. Previous research has focused on the role of medullary α_2-adrenergic and I_1-imidazoline receptors in regulating blood pressure; yet, structures rostral to the brainstem also influence sympathetic output. What role I_1-imidazoline receptors located in the medulla, or those located in the region of the third ventricle, exert towards the regulation of body temperature is largely unexplored. The present study assessed the relative contributions of diencephalic and medullary α_2-adrenergic and I_1-imidazoline receptors on core body temperature in conscious rats. In Experiment 1, 24 rats received chronically indwelling thermistors, for recording body temperature, and intracerebroventricular (ICV) cannulae targeted to the third ventricle, an area near the hypothalamus, for drug administration. In a repeated measures design, 12 rats were pretreated with central administration of 4 µl of saline or efaroxan, an I_1-antagonist; 20-min later moxonidine, an I_1 agonist, was centrally administered in 1of 3 doses (0, 1, 10 nmol) delivered in a 4 µl saline over 45-60 s. The other 12 rats were similarly pretreated with saline or SK&F 86466, an α_2-adrenergic receptor antagonist, followed 20 min later with 1 of 3 doses (0, 1, 10 nmol) of UK 14304, an α_2-adrenergic receptor agonist. Body temperature was monitored at 30-min intervals for 4 hr. The result were contrary to what might have been predicted from reports of moxonidine-induced reductions in blood pressure in that 10-nmol of moxonidine increased core body temperature (>1.5 °C, $p<.02$). However, the increase in body

temperature was reversed with efaroxan. UK 14304 did not alter body temperature. In Experiment 2, 24 rats underwent the same procedure as described above, except the drugs were delivered to the fourth ventricle, an area near the medulla. In this case, neither moxonidine nor UK 14304 had any significant effect on body temperature. These findings support the notion that I_1-imidazoline and α_2-adrenergic receptors in the diencephalon and medulla are functionally distinct, and that the thermoregulatory contribution of diencephalic I_1-imidazoline receptors is different from what would be predicted from their sympathoinhibitory action exhibited in the medulla.

Preface

A high mean resting metabolism is a hallmark of warm-blooded terrestrial vertebrates, or endotherms. Though expending much energy, it sustains a high and constant body temperature and promotes cold tolerance with minimal reliance on behaviour. Despite a lower resting metabolism, cold-blooded terrestrial vertebrates, or ectotherms, can reach similar body temperatures, but only for brief periods with bursts of nonsustainable locomotion at great metabolic expense, or for prolonged periods by selecting a thermoneutral microenvironment at little metabolic expense. Endotherms also have a high mean arterial blood pressure. Evolutionary biologists (e.g., Bennett, 1978; Bennett & Reuben, 1979; Else & Hulbert, 1981, 1983; Ruben, 1995, 1996) note that elevated blood pressure is the functional consequence of a pervasive evolutionary restructuring of the cardiopulmonary and skeletomotor systems that developed to support the high metabolism by maximizing the capacity for aerobic metabolism. This restructuring also granted behavioural advantages. It improved behavioural stamina, enabling the endotherm to better pursue food, escape from danger, or acquire and defend large territories for the purpose of reproduction, and it better equipped the endotherm to metabolize energy when coping with stress (Sapolsky, 1992). In contrast, modest levels of activity outstrip an ectotherm's aerobic capacity, and any increase in activity beyond a slow walk entails anaerobic metabolism that exhausts quickly and recovers slowly.

Accordingly, an endotherm is a striking, though energetically costly, adaptation. Its evolutionary success stems not only from improvements in cold tolerance, which could have been accomplished more economically by simply increasing thermal

insulation. Instead, the success evidently stems from a restructuring that maximized aerobic capacity and elevated arterial blood pressure. To the extent that endothermy is functionally related through evolution to these developments, then the central and psychological processes regulating normal and stress-induced elevations in aerobic capacity and blood pressure may well be inextricably linked to those processes traditionally associated with the regulation of body temperature.

Introduction

Clonidine

Clonidine, chemically known as 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, is a first generation therapeutically effective antihypertensive medication. Although originally it was synthesized as an α-adrenergic receptor stimulating agent and nasal decongestant (Stahle, 1966), its sedative, bradycardic and hypotensive effects soon became the major focus of attention (Kobinger, 1978). Within 5 min of an intravenous delivery of clonidine, a sympathoinhibitory profile emerges in anesthetized or conscious laboratory animals that involves tonic reductions in blood pressure, heart rate, plasma noradrenergic levels, and spontaneous discharges from pre- and postganglionic sympathetic fibers (Robson & Kaplan, 1969; Sattler & van Zwieten, 1967; Schmitt, Schmitt, Boissier, & Giudicelli, 1967). In humans, intravenous clonidine produces a similar sympathoinhibitory profile with reductions in blood pressure (Zaimis & Hanington, 1969), heart rate, and in urinary and plasma catecholamines (Hokfelt, Hedeland, & Dymling, 1970).

In the mid-1960s and 1970s reports indicated that sympathoinhibitory profile of clonidine reflected a central site of action. For example, Kobinger (1967, cited in Kobinger, 1978) injected clonidine into the lower brainstem of vagotomized cats in sufficiently low doses to preclude most, if not all, peripheral action. He found that clonidine given via this route lowered blood pressure and heart rate more than the same dose given intravenously. Sattler and van Zwieten (1967), using cats, found that doses of clonidine too low to influence blood pressure when administered intravenously reduced blood pressure when administered centrally through the vertebral artery. Other studies observed that, while intravenously or intracerebroventricularly (ICV) administered clonidine in the intact rat reduced the spontaneous discharges of the splanchnic and renal nerves (Schmitt & Schmitt, 1969; Schmitt, Schmitt, Boissier, & Giudicelli, 1967), intravenous or intrathecal administration of clonidine in the spinal preparation failed to lower blood pressure (Schmitt & Schmitt, 1969). Sherman, Grega, Woods, and Buckley (1969) performed parabiotic experiments in dogs where the recipient animal had an intact nervous system, but the vascular supply to its head was provided by the systemic circulation from the donor. The investigators found that although systemic injection of clonidine had no discernible effect on the donor it markedly lowered the recipient's blood pressure and heart rate. This selective hypotensive action in the recipient, coupled with the absence of any such effect in the donor, suggested that clonidine acted centrally. Later, Kobinger and Pichler (1975) found that brain transections placed at the level of the caudal medulla in rats and dogs abolished clonidine-induced reductions in sympathetic

nerve activity and blood pressure; whereas, transections placed rostral to the medulla had no such effect. Finally, Reid, Wing, Mathias, and Frankel (1976) reported that clonidine had little effect on quadraplegic patients, in whom the descending bulbosopinal fibers had been severed indicating that clonidine acted centrally rather than peripherally.

The Medullary Site of Clonidine Sympathoinhibition

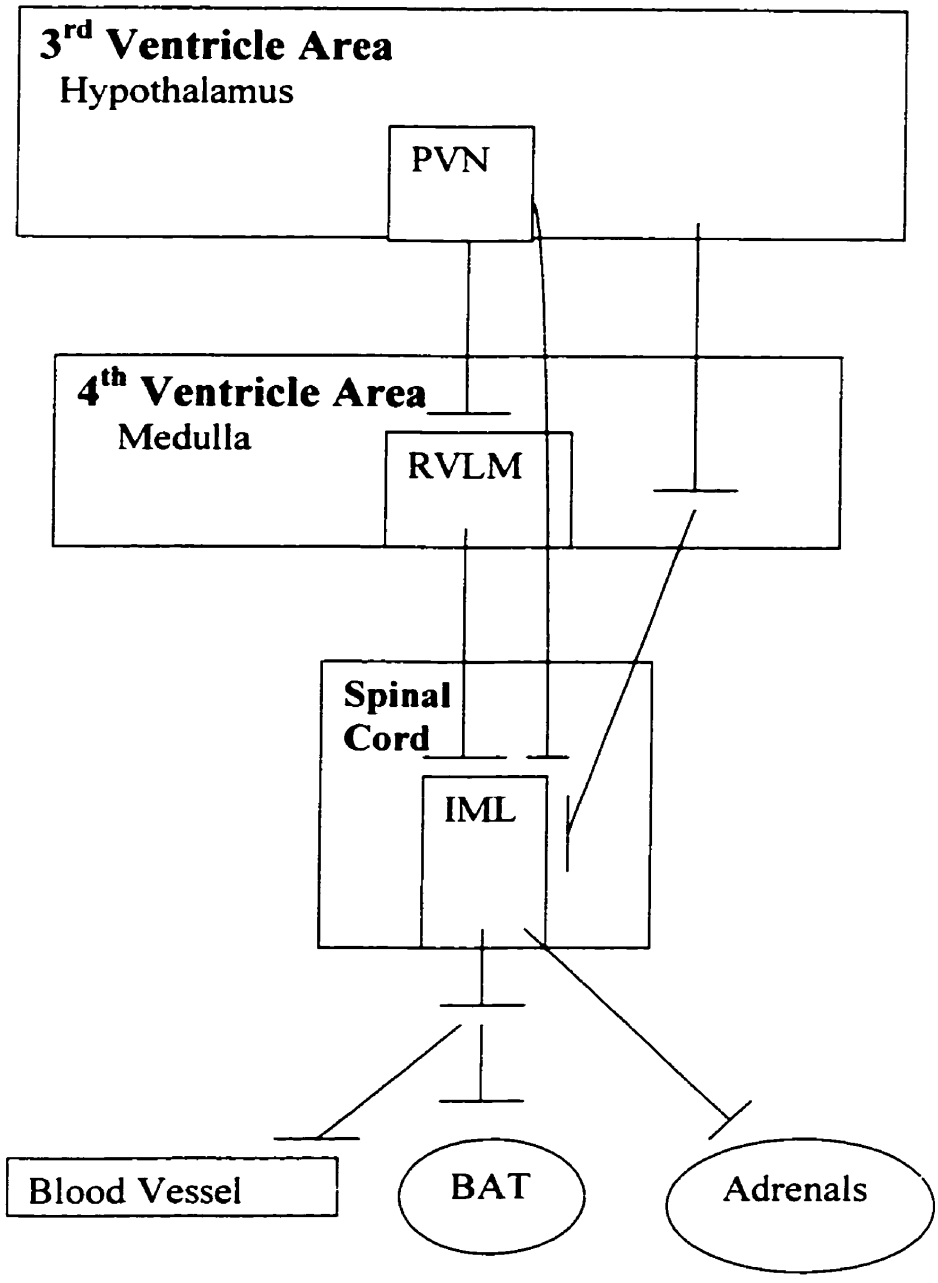
To localize clonidine's central site of action researchers soon began to administer clonidine into anatomically defined areas of the brain. For example, Schmitt and Schmitt (1969) applied clonidine topically to the floor of the fourth ventricle in brain-transected cats. The most sensitive sites for clonidine-induced bradycardia, hypotension, and reductions in sympathetic nerve activity occurred within the medulla, caudal to a coronal transection placed between the level of the pons and rostral level of the medulla. Bousquet and Guertzenstein (1973) later confirmed these findings. However, an indication developed that other areas of the medulla contributed to clonidine's sympathoinhibitory effects. For example, early electrophysiological data had demonstrated that the nucleus tractus solitarius (NTS), which resides in the medulla near the floor of the fourth ventricle, mediated baroreceptor reflexes (e.g., Crill & Reis, 1968). Kobinger and Walland (1973) hypothesized that clonidine's hypotensive action resulted from enhancing the sensitivity of the NTS-mediated baroreceptor reflex. Although the NTS was a plausible site of clonidine's sympathoinhibitory action, Antonaccio and Halley (1977) demonstrated that cats with bilateral NTS lesions responded normally to central injections of clonidine, while Bousquet, Feldman, Bloch, and Schwartz (1981)

reported that the dose of clonidine needed to lower blood pressure when microinjected into the NTS exceeded by 100-fold the dose needed when injected along the floor of the fourth ventricle. Accordingly, although sensitive to clonidine, the NTS was evidently not the sole or primary mediator of clonidine's sympathoinhibitory effects.

Bousquet and Guertzenstein (1973) identified an alternative vasodepressor site of clonidine action in cats along the floor of the fourth ventricle in cats. Specifically, this site was found in the ventrolateral region of the medulla at the level of the nucleus reticularis lateralis. The vasodepressor consequences of perfusing this area with clonidine are now well documented in rabbits (Dampney, Goodchild, Robertson, & Montgomery, 1982) and rats (Willette, Barcas, Krieger, & Sapru, 1984). In the rat, this depressor area occurs in the rostral ventrolateral medulla (RVLM) (Ross, Ruggiero, & Reis, 1985). The RVLM (see Figure 1) is a heterogeneous collection of cell bodies located caudal and adjacent to the facial nucleus in the rat. It receives direct input from several locations, including the NTS and paraventricular nucleus (PVN) of the hypothalamus, and projects monosynaptically to the intermediolateral (IML) cell column in the thoracic spinal cord (Barman & Gebber, 1985). These IML cells give rise to sympathetic preganglionic fibers that control vascular resistance (Dampney, 1994), participate in baroreceptor reflexes (Reis, Ruggiero, & Morrison, 1989), and constitute the final common pathway for the regulation of blood pressure and other sympathetically mediated autonomic processes. This neural circuit has provided the conceptual framework for investigations of the neuropharmacological and neuroanatomical

Figure Caption

Figure 1. Selected neuroanatomical pathways of the rostral ventrolateral medulla (RVLM). The RVLM recieves axons from the paraventricular nucleus of the hypothalamus (PVN) and projects axons to the intermediolateral portion of the spinal cord (IML). The IML, in turn, regulates the responses of sympathetic effector tissues, including blood vessels, brown adipose tissue (BAT), and the adrenals.


3rd Ventricle Area
Hypothalamus
PVN
4th Ventricle Area
Medulla
RVLM
Spinal Cord
IML
Blood Vessel
BAT
Adrenals

substrates of clonidine-induced sympathoinhibition.

Several lines of evidence have demonstrated that the vasodepressor action of clonidine is linked to the RVLM. Bousquet, Feldman, Velly, and Bloch (1975) have administered clonidine peripherally to cats that had received either sham lesions or lesions to the RVLM. Clonidine reduced blood pressure in the sham-operated cats, while it elevated blood pressure in RVLM-lesioned cats. Reports that clonidine's hypotensive effect is absent in rats if administered more than 2 mm away from the RVLM (Ernsberger, Giuliano, Willette, & Reis, 1990; Haxhiu, Dreshaj, Erokwu, Schafer, Christen, & Ernsberger, 1992) and most pronounced in cats when administered 2 mm caudal to the RVLM (Gatti, Hill, Da Silva, Norman, & Gillis, 1988; McAuley, Macrae, & Reid, 1989) also emphasize the anatomically sensitive nature of clonidine's hypotensive action when administered in the RVLM.

Adrenergic Receptors and Clonidine's Mechanism of Action

Until recently clonidine's hypotensive and sympathoinhibitory effects have been linked to clonidine's ability to stimulate α_2-adrenergic receptors. This notion emerged from several observations. First, many of the effects of peripherally administered clonidine resembled those obtained with peripheral administration of other purported α-adrenergic agonists, including α_2-methlydopa and norepinephrine (Kobinger, 1978; van Zwieten & Timmermans, 1983). Second, in rats and rabbits peripheral clonidine produced a variety of sympathomimetic effects in peripheral end-organs possessing α_2-adrenergic receptors, including the intestine, blood vessels, nicitating membrane, and

pupils (Autret, Schmitt, Fenard, & Peillot, 1971). Third, the effects of peripherally administered clonidine could be blocked by the well known α_2-adrenergic receptor antagonists rauwolscine, yohimbine, or piperoxan (Boissier, Giudicelli, Fichelle, Schmitt, & Schmitt, 1968; Schmitt, Schmitt, & Fenard, 1971; Timmermans, Schoop, Kwa, & van Zwieten, 1981).

α_2-Adrenergic receptors have been subclassified based on differing affinities for various agents (Langer, Enero, Adler-Graschinsky, Dubocovich, & Celuchi, 1975; Piletz & Sletten, 1993). To date, at least three subtypes of the α_2-adrenergic receptor have been identified, α_{2A}, α_{2B}, α_{2C}, and each subtype has been shown to have approximately equal binding affinity for clonidine (Piletz, Zhu, & Chikkala, 1996). α_2-Adrenergic receptors are located both pre- and postsynaptically and exhibit a selective affinity for the α_2-adrenergic agonists clonidine and guanabenz, and for the α_2-adrenergic antagonists rauwolscine, yohimbine, and SK&F 86466 (Langer, 1981; Starke, 1981). Presynaptic α_2-adrenergic receptors exert a negative feedback by inhibiting synthesis and release of the axon's endogenous neurotransmitter (Langer). Although α_2-adrenergic receptors also occur postsynaptically (Langer; Starke), an entirely different population of postsynaptic receptors exhibits a high affinity for phenylephrine and prazosin, which are α_1-adrenergic agonists and antagonists, respectively (Langer; Starke; U'Prichard & Snyder, 1979). These receptors, termed α_1-adrenergic receptors, rarely occur presynaptically and demonstrate a modest affinity for clonidine. Collectively, these observations suggest that clonidine's sympathoinhibitory profile may be linked to the action of central presynaptic

α_2-adrenergic receptors.

A variety of methodological strategies have strengthened the position that clonidine's effectiveness results from activation of central presynaptic α_2-adrenergic receptors. One strategy involved manipulating clonidine's sympathoinhibitory effect with drugs possessing varying affinities for α_2-adrenergic receptors. For example, Timmermans et al. (1981) showed that when the specific α_2-adrenergic antagonists, rauwolscine and yohimbine, were delivered systemically at high doses, much of clonidine's sympathoinhibitory profile was attenuated. Granata, Numao, Kumada, and Reis (1986) showed that the medullary administration of a spectrum of α_2-adrenergic agonists, including clonidine and α-methylnorepinephrine, reduced heart rate, blood pressure, and sympathetic nervous activity. Another strategy used radioligand binding. Bylund and U'Prichard (1983) assessed clonidine's binding characteristics from purified receptors that had been extracted from cell membranes. The receptors were then exposed to ^{3}H-clonidine, a radiolabelled form of clonidine, and rinsed. Judging from the amount of ^{3}H-clonidine that remained after rinsing, the investigators determined that clonidine had a strong affinity for the α_2-adrenergic receptor subtype. Other radioligand binding studies have reported a site for α_2-adrenergic receptors in the RVLM (Ernsberger, Meeley, Mann, & Reis, 1987; Unnerstall, Kopaitic, & Kuhar, 1984), the proposed site of clonidine's action. Furthermore, Punnen, Urbanski, Krieger, and Sapru's (1987) reported that delivery of idazoxan, an α_2-adrenergic receptor antagonist, to the RVLM inhibited the reductions of blood pressure that normally accompany the intravenous administration

of clonidine. These observations support the contention that clonidine's vasodepressor and sympathoinhibitory action is attributed to the stimulation of presynaptic α_2-adrenergic receptors in the RVLM.

The purported role of RVLM α_2-adrenergic receptors in clonidine's action is, however, difficult to reconcile with evidence that RVLM-induced sympathoinhibition involves a nonadrenergic mechanism. For example, reports that phentolamine, an α_2-adrenergic receptor antagonist, lowered blood pressure when injected into either the cerebral aqueduct of cats (Vollmer & Buckley, 1977) or the RVLM of rats (Granata et al., 1986) could not be explained by antagonism of α_2-adrenergic receptors in the RVLM. The fact that both the α_2-adrenergic agonist, clonidine, and the α_2-adrenergic antagonist, phentolamine, lowered blood pressure suggested that α_2-adrenergic-mediated excitation was not the sole contributor to the RVLM's hypotensive action. These ostensibly irreconcilable findings prompted a reevaluation of the α_2-adrenergic receptor theory, challenged clonidine's exclusive role as an α_2-adrenergic receptor agonist, and established a more receptive attitude for identifying a nonadrenergic mechanism of action for clonidine.

Imidazoline Receptors: Do They Exist?

Clonidine and phentolamine contain an imidazoline ring, which is a five-membered, heterocyclic ring containing an imino group and a tertiary nitrogen. This common structure suggested that the sympathoinhibitory consequences of clonidine and phentolamine could reflect the action of an unidentified nonadrenergic receptor binding to

the imidazoline moiety. Specifically, reports emerged that microinjection into the RVLM of (a) imidazoline agents that exhibit little affinity for α_2-adrenergic receptors, such as cirazoline and ST 587, produce clonidine-like reductions in pressure (Bousquet, Feldman, & Schwartz, 1984), (b) nonimidazoline based α_2-adrenergic blockers, such as yohimbine, fail to block clonidine-induced reductions in blood pressure when given centrally (Tibirica, Feldman, Mermet, Gonon, & Bousquet, 1991); whereas, (c) imidazoline α_2-adrenergic blockers, such as idazoxan, abolish clonidine-induced reductions in blood pressure (Gatti et al., 1988; Punnen et al., 1987). Other evidence supporting participation of a nonadrenergic receptor in clonidine's sympathoinhibition has revealed a double dissociation. For example, Bousquet and Schwartz (1983) reported that the nonimidazoline α-adrenergic agonists, α-methylnorepinephrine (α-MNE) and norepinephrine (NE), but not clonidine, produce hypotension when delivered to the nucleus of the solitary tract in anesthetized cats. In contrast, the imidazoline clonidine, but not NE or α-MNE, produces hypotension when microinjected into the RVLM. This evidence suggests that something besides clonidine's α_2-adrenergic stimulating properties contributed to clonidine's sympathoinhibitory effects when administered into the RVLM. Bousquet et al. (1984) assessed the blood pressure effects of RVLM-microinjections of agonists (α_1 and α_2) to the RVLM of anesthetized cats; α-MNE and clonidine were chosen as the α_2 agonists, while ST 587 and cirazoline were chosen as the α_1 agonists. The results showed that clonidine, cirazoline, and ST 587, drugs that acted at different adrenergic sites yet possessed a common imidazoline ring, decreased blood pressure in a

dose-dependent manner, whereas α-MNE had no effect. These results promoted the notion that clonidine and clonidine-like substances may be exerting their hypotensive effects through the action of imidazoline-preferring, rather than α_2-adrenergic, receptors in the RVLM.

The radioligand binding evidence indicating that clonidine bound with high affinity to α_2-adrenergic receptors (e.g., Bylund & U'Prichard, 1983) used membrane receptor extracts taken from brain cortical tissue or blood platelets. These membranes have high concentrations of α_2-adrenergic receptors, but may not be representative of the receptor composition within the medulla or RVLM. In this regard, Ernsberger, Meeley, and Reis (1986) conducted a radioligand binding study using brain medullary extracts and found that clonidine bound not only to α_2-adrenergic receptors, but also to another quite different and unidentified receptor site. The unidentified sites were considered "nonadrenergic" because only imidazoline-based adrenergic agents exhibited affinity for these sites. Thus, it appeared that another receptor mechanism, located in the RVLM, with high affinity for imidazoline-based compounds may contribute to the sympathoinhibitory properties of clonidine.

The presence of central nonadrenergic imidazoline receptors presupposes the existence of an endogenous ligand. In this regard, Atlas and Burstein (1984) were the first to characterize an endogenous clonidine displacing substance (CDS) from the frontal cortices of bovine brains, which displaced [^{3}H]-clonidine from bovine frontal membrane extracts in a dose-dependent manner. This displacement appeared to be selective in that

CDS failed to displace either [^{3}H]-prazosin, a α_1-adrenergic antagonist, or ^{125}I-cyanopindolol, a β-adrenergic antagonist. Meeley, Ernsberger, Granata, and Reis (1986) also conducted two experiments to characterize CDS. The first experiment estimated the radioligand binding properties of CDS to membranes extracted from the bovine ventrolateral medulla. The binding properties of CDS were estimated by determining its ability to displace radio-labelled α-adrenergic agents. The results showed that CDS, like clonidine, potently inhibits the binding of para-aminoclonidine (^{3}H-PAC), a substance that binds to the same membrane sites as clonidine, but with greater affinity. However, 25-30% of the sites to which ^{3}H-PAC bound were also displaced by phentolamine, an α_1- and α_2-adrenergic receptor antagonist, but not by norepinephrine, an α_1- and α_2-adrenergic agonist. The second experiment assessed whether microinjecting purified CDS into the rat RVLM influenced cardiovascular function and whether this influence resembled that obtained with clonidine. The results showed that RVLM administrations of both CDS and clonidine reduced blood pressure and heart rate. These effects, however, were not obtained if the injection site extended 1 mm beyond the border of the RVLM. The results of these two experiments, coupled with the fact that clonidine, ^{3}H-PAC, and phentolamine possess an imidazoline base, suggested to the investigators that (a) nearly 30% of the purported α_2-adrenergic receptors in the rostral ventrolateral medulla were imidazoline-preferring sites that constituted either a unique subclass of α_2-adrenergic receptors, or a *non*adrenergic receptor, and (b) these sites accounted for the depressor properties of CDS and clonidine. Unfortunately, CDS has not been structurally

identified.

Two other endogenous candidates for the imidazoline receptor have been structurally identified, agmatine and harmane. Agmatine exhibits a high affinity for imidazoline receptors and appears to be concentrated in the hypothalamus (Li, Regunathan, Barrow, Eshraghi, Cooper, & Reis, 1994), however, when injected into the rabbit RVLM it exhibited a poor ability to reduce blood pressure (Head, Chan, & Godwin, 1997). Harmane binds to imidazoline receptors with the same affinity has clonidine (Hudson, Price, Tyacke, Lalies, Parker, & Nutt, 1999), and effectively lowers blood pressure when delivered to the RVLM of rats (Musgrave & Badoer, 2000).

Several lines of evidence indicate that imidazoline-preferring sites occur elsewhere in the brain and in peripheral organs. For example, mapping the distribution of α_2-adrenergic receptors in the rat brain, Boyajian and her colleagues (Boyajian & Leslie, 1987; Boyajian, Loughlin, & Leslie, 1987) observed differences in the sites labelled by [^{3}H]-rauwolscine and [^{3}H]-idazoxan, two purportedly selective α_2-adrenergic antagonists. Specifically, only a portion of the sites labelled by [^{3}H]-idazoxan were labelled by [^{3}H]-rauwolscine, and each antagonist attached to unique brain regions. These findings, coupled with the fact that idazoxan possesses an imidazoline ring, suggests that the idazoxan binding site not recognized by [^{3}H]-rauwolscine, a nonimidazoline, may be an imidazoline-preferring receptor. Moreover, imidazoline-preferring receptors have been identified in the kidneys of rats (Ernsberger, Feinland, Meeley, & Reis, 1990) and rabbits (Hamilton, 1992; Hamilton, Yakubu, Jardine, & Reid, 1991), and account for nearly 30%

and 90% of the receptors in the rat and rabbit kidney, respectively. Imidazoline preferring sites have also been found in the mouse pancreas (Schulz & Hasselblatt, 1989), human and rabbit liver (Tesson, Prip-Buus, Lemoine, Pegorier, & Parini, 1991), rabbit urethra (Yablonsky, Riffaud, Lacolle, & Dausse, 1988), rabbit adipocytes (Langin & Lafontan, 1989), rabbit and rat blood vessels and platelets (Piletz et al, 1991; Gothert & Molderings, 1992), rat vas deferens (Diamant & Atlas, 1986), and in rat and cow adrenal glands (Ernsberger, Westbrooks, Christen, & Schafer, 1992; Regunathan, Meeley, & Reis, 1992).

Because nearly 30% of the clonidine-binding receptors in the RVLM are purportedly nonadrenergic (Meeley et al., 1986), an effort was made to identify drugs with a preferential affinity for these receptors. Ernsberger, Giuliano, Willette, and Reis (1990) conducted two experiments with rats. The first experiment was designed to assess, through the inhibition of ^{3}H-PAC binding, the affinity of several drugs at suspected α_2-adrenergic and nonadrenergic imidazoline-preferring receptors in the RVLM. Two classes of drugs were tested. One class was imidazoline-based, α_2-adrenergic agonist drugs, including clonidine and cimetidine, imidazole-4-acetic acid, idazoxan, naphazoline, oxymetazoline and para-aminoclonidine (PAC). The other class of drugs were nonimidazoline-based, α_2-adrenergic agonists and included epinephrine, guanabenz, α-MNE, norepinephrine, phenylephrine, and SKF-86466. The first experiment not only confirmed Meeley's (Meeley et al., 1986) finding that nearly 30% of the receptor sites in the RVLM were nonadrenergic imidazoline-preferring, but noted

three patterns of binding. The first pattern was characterized by a complete inhibition of ^{3}H-PAC binding and occurred with the imidazoline-based α_2-adrenergic receptor agonists clonidine, PAC, naphazoline and antagonist idazoxan. The second pattern was characterized by an inhibition of only 60-65% of ^{3}H-PAC binding and occurred with nonimidazoline-based α_2-adrenergic agonists epinephrine, guanabenz, α-MNE, norepinephrine and phenylephrine. The third binding pattern was characterized by an inhibition of only 30% of ^{3}H-PAC binding and occurred with cimetidine and imidazole-4-acetic acid, imidazoline agonists possessing no α_2-adrenergic properties. The second experiment determined whether drugs possessing these three binding patterns differentially influenced blood pressure and heart rate. The results showed that, when micropipetted into the rat RVLM, imidazoline-based drugs produced greater drops in blood pressure and heart rate than their nonimidazoline based counterparts. Moreover, for imidazoline-based drugs, this reduction was proportional to the drug's affinity for imidazoline-preferring sites. No such relationship was obtained with binding affinity at α_2-adrenergic sites in the rat RVLM. Accordingly, the sympathoinhibitory action of imidazoline-based clonidine-like agents was more closely related to their affinity for RVLM imidazoline-preferring receptors than to their affinity for α_2-adrenergic receptors.

The most definitive evidence for the presence of imidazoline receptors is derived from studies in molecular biology. Parini (1994), for example, cloned a unique I_2-imidazoline binding site, which was substantially different than the sites cloned for all α_2-adrenergic receptor subtypes. This I_2 binding site was found to be encoded by the gene

that encodes the enzyme monoamine oxidase (MAO), and localized to the outer mitochondrial membrane (Raddatz, Parini, & Lanier, 1997). Most recently, Piletz and his coworkers (Piletz et al., 2000) cloned a unique protein sequence with characteristics of another, I_1, subclass of imidazoline receptors.

Imidazoline Classification and Function

In their review of imidazoline pharmacology Michel and Insel (1989) concluded that three distinct receptors exist apart from the α_2-adrenergic receptor. First, there are receptors specific for [^{3}H]PAC. These receptors were noted to predominate in the bovine brain and rat kidney and display high affinity for most imidazoline-based drugs. However, these receptors fail to recognize more potent α_2-agonists, such as the guanidino compounds or amiloride. Second, there are receptors specific for [^{3}H]idazoxan. These receptors exhibit high affinity for most imidazolines, guanidino compounds and amiloride. Finally, they noted the presence of other receptors specific for [^{3}H]idazoxan found predominantly in rats, pigs, and humans. These sites have high affinity for some imidazoline compounds, guanidino and benzazepine compounds, but low affinity for amiloride and certain other imidazolines. These unique imidazoline binding characteristics gave rise to Michel and Ernsberger's (1992) classification system for imidazoline receptors, where I_1 receptors are those receptors that exhibit a high affinity for clonidine and clonidine-displacing substance (CDS) and I_2 receptors are those receptors that display an affinity for [^{3}H]idazoxan.

Moxonidine and rilmenidine, two imidazoline receptor agonists that exhibit greater affinity for I_1 receptors relative to either α_2-adrenergic or I_2 receptors (Ernsberger et al., 1992; Piletz, Chikkala, & Ernsberger, 1995), have proven useful for studying the function of central imidazoline-preferring receptors. Most research has focused on the role of RVLM I_1 receptors in cardiovascular regulation, where the evidence suggests that RVLM I_1 receptors mediate the hypotensive effects of clonidine through an inhibition of the sympathetic nervous system (Piletz, Chikkala, & Ernsberger, 1995; Piletz, Halaris, & Ernsberger, 1995). Haxhiu et al. (1992) examined the mechanism by which moxonidine reduces blood pressure and heart rate in anesthetized, paralyzed, and artificially ventilated spontaneously hypertensive rats. Their experiments initially identified what regions within the RVLM were sympathoexcitatory judging by the pressor response obtained following the regional microinjection of L-glutamate, an excitatory amino acid. Microinjection of moxonidine into these sympathoexcitatory areas revealed a dose-dependent reduction in blood pressure evidently produced by inhibition of the sympathoexcitatory neurons in the RVLM. The effects were neuroanatomically specific because delivery of moxonidine 1-1.5 mm dorsal to the RVLM, or more caudally into the caudal ventrolateral medulla, had little effect.

Tibirica and colleagues (Tibirica, Feldman, Mermet, Gonon, & Bousquet, 1989; Tibirica et al., 1991) compared the effects of systemically administered clonidine and rilmenidine on blood pressure while recording electrical activity in the rat RVLM. They showed that both drugs lowered blood pressure and inhibited the electrical activity of

RVLM neurons, in a dose-dependent, idazoxan-reversible manner. These observations suggested that reductions in sympathetic output were directly related to the firing rate of RVLM neurons. Gomez, Ernsberger, Feinland, and Reis (1991) examined the type and the site of the receptor mediating the vasodepressor action of rilmenidine. They monitored blood pressure while administering rilmenidine either systemically or directly into the RVLM, NTS, and caudal ventrolateral medulla (CVLM) of anesthetized rats. In order to determine the type of receptor to which rilmenidine bound, radioligand binding assays were performed using receptors taken from bovine membrane extracts. The results showed that (a) rilmenidine produced a dose-dependent reduction in blood pressure and heart rate when delivered only to the RVLM, and (b) reductions in blood pressure accompanying intravenously delivered rilmenidine were blocked by the RVLM pretreatment with idazoxan, a mixed I_1- and I_2-imidazoline and α_2-adrenergic receptor antagonist, but not SKF-86466, an α_2-adrenoceptor antagonist with no imidazoline properties. Moreover, rilmenidine competed with ^{3}H-PAC, a clonidine analogue, for binding sites in the bovine ventrolateral medulla. Collectively, these observations suggested that clonidine, and related imidazoline-based antihypertensive agents, inhibit sympathetic preganglionic fibers that regulate vascular resistance and fluid volume, thereby lowering blood pressure. This apparent sympathoinhibition occurs, at least in part, by stimulating imidazoline-preferring receptors in the RVLM.

Supraspinal Mechanisms: The Hypothalamus

Although the medulla provides much of the basic neuroanatomical circuitry necessary for maintaining sympathetic tone, supraspinal regions like the hypothalamus are needed to integrate this circuitry with that controlling behaviour and emotions. The hypothalamus represents the most ventral division of the diencephalon, lying between the basal telencephalon and ventral mesencephalon, located on either side of the third ventricle and directly above the pituitary to which it is attached. It constitutes a small percentage of the brain's weight and volume, yet its multiple collections of nerve cell bodies and fiber systems play a major role in controlling and integrating a spectrum of autonomic and behavioral processes that are essential for homeostasis and survival. For instance, electrical stimulation of the hypothalamus in conscious cats can produce 'fight or flight' responses, which are associated with well-documented increases in sympathetic activity (Bard, 1928).

One of the best neuroanatomically defined collections of cell bodies in the hypothalamus is the paraventricular nucleus (PVN). The PVN is composed of large (magnocellular) and smaller (parvocellular) cells, which occupy distinct regions of the PVN and are distinguished with a Nissl stain (Saper, Loewry, Swanson, & Cowan, 1976). Both the magnocellular and parvocellular portions of the PVN project to the pituitary where they regulate hormonal outflow and can consequently influence autonomic parameters (Dampney, 1994). The parvocellular portion of PVN also contains cells that project monosynaptically to the RVLM and the intermediolateral (IML) column of the

spinal cord. Both the RVLM and IML contain cell bodies that are connected to sympathetic preganglionic motor neurons (Dampney; Swanson & Sawchenko, 1983), which mediate blood pressure and other sympathetically mediated autonomic processes. Thus, neuroanatomically the hypothalamus is strategically positioned to modulate sympathetic activity.

The Hypothalamus and Cardiovascular Function

Electrical stimulation studies indicated that the anterior region of the hypothalamus decreases blood pressure and heart rate (e.g., Hilton & Spyer, 1971), whereas the posterior portion of the hypothalamus increases blood pressure and heart rate (Przuntek, Guimaraes, & Philippu, 1971). In humans, electrical stimulation of the posterior hypothalamus causes elevations in blood pressure, tachycardia, and a strong fear response (Schvarcz, 1977). From his review of the literature, Isaac (1980) proposed that the anterior and posterior hypothalamic areas inhibit and excite, respectively, the medullary vasomotor centers.

Further evidence linking the hypothalamic area to blood pressure and strengthening Isaac's (1980) proposal comes from studies that lesion or chemically stimulate parts of hypothalamus. By electrically lesioning parts of the posterior hypothalamus in baboons, Smith, Astley, DeVito, Stein, and Walsh (1980) blocked the increase in blood pressure normally associated with a conditioned emotional fear response. Zhang and Ciriello (1985) lesioned the parvocellular region of the PVN with kainic acid and attenuated the hypertension observed in sinoaortic-denervated rats. Wilbe, Luft, and DiMicco (1988)

stimulated the hypothalamic area with GABA and suppressed sympathetic outflow to the cardiovascular system. Martin, Segura, and Haywood (1991) observed elevations in blood pressure and heart rate following chemical stimulation of the PVN with bicuculline, a gamma-aminobutyric acid (GABA) antagonist, or monosodium glutamate. Glutamate stimulation of the dorsomedial hypothalamus, in conscious rats, with N-methyl-D-aspartic acid (NMDA), increased arterial blood pressure and heart rate, an effect that was reversed with NMDA blockade (Soltis & DiMicco, 1992).

The role of hypothalamic catecholamines in cardiovascular regulation has also been investigated. Kendrick and Leng (1988), in rats, and Philippu (1988), in cats, reported that the anterior and posterior hypothalamus evoked reciprocal changes in catecholamine levels by experimentally manipulating blood pressure either neuropharmacologically or with volumetric challenges. Specifically, elevations of blood pressure lowered catecholamine levels in the posterior hypothalamus and increased catecholamine levels in the anterior hypothalamus. Conversely, lowering blood pressure increased catecholamine levels in the posterior hypothalamus and decreased catecholamine levels in the anterior hypothalamus. These observations, coupled with findings that 6-hydroxydopamine depletion of catecholamines in the posterior hypothalamus lowers blood pressure (e.g., Kawasaki et al., 1991) suggested that catecholamines in the anterior hypothalamus suppress sympathetic function and blood pressure, while in the posterior hypothalamus they enhance sympathetic function and blood pressure. Moreover, Ebihara, Kawasaki, Nakamura, Takasaki, and Wada (1993) observed the development of a dose-dependent

increase in blood pressure, despite a compensatory bradycardia, following delivery of clonidine to the PVN of conscious rats. This result differs from what one might anticipate from clonidine's depressor action traditionally associated with the RVLM, given the direct link from the PVN to the RVLM (Saper et al., 1976). However, this finding suggests that α_2-adrenergic or imidazoline receptors in the PVN may tonically inhibit the RVLM, a proposal consistent with the general observation that imidazoline and α_2-adrenergic receptors are inhibitory in nature.

Thermoregulation

Mammals are endotherms, they regulate, physiologically and metabolically, their body temperature around a temperature or range of temperatures known as the thermal set point. Set point theory argues that there are four types of body temperatures: (a) normothermia, where body temperature and the set point are similar; (b) hypothermia, where body temperature is below the set point; (c) hyperthermia, where body temperature is higher than the set point; and (d) fever, where the set point is raised and body temperature may or may not rise to the new level (Kuger, O'Reilly, Shope, & Vander, 1987; Kluger, 1979; Moltz, 1993). The thermal set point is variable and its magnitude can be affected by a combination of factors, including intrinsic brain temperature, the presence of fever, exercise, or the animal's state of arousal. When inputs from sensory apparatus in the skin and core indicate that body temperature is greater than or less than the set point, compensatory or defensive adjustments are evoked through metabolic, physiological, and/or behavioural changes.

The sympathetic nervous system (SNS) participates in the mediation of the physiological thermoregulatory effectors responsible for heat distribution. For example, changes in vascular resistance and water balance can either facilitate heat loss or promote heat retention (Gordon, 1990). The SNS also mediates heat production or thermogenesis. Obligatory thermogenesis is defined as the basal or resting metabolic rate measured at thermoneutrality and in the postabsorptive state. It is generally unresponsive to changes in the environment, occurs in all bodily organs, and is controlled principally by thyroid hormones beyond what neural control is required for basal functioning. Facultative thermogenesis is defined as the increase in basal metabolic rate that develops as a consequence of increased activity, ingestion of food, or exposure to ambient temperatures below thermoneutrality (Jansky, 1973). Thus, it is facultative thermogenesis that allows the animal to regulate and adjust its body temperature in response to real or anticipated challenges in either the internal or external environment.

Facultative thermogenesis includes shivering and nonshivering thermogenesis. Shivering thermogenesis is involuntary and is manifested by random, uncoordinated, and simultaneous contractions of flexor and extensor muscles. Since no mechanical work occurs during shivering, all energies released during the muscular contractions are released as heat. Nonshivering thermogenesis does not involve muscular contraction, but occurs as a consequence of prolonged cold exposure or ingestion of food (Jansky, 1973). Carlson and his colleagues (Cottle & Carlson, 1956; Hsieh, Carlson, & Gray, 1957) characterized nonshivering thermogenesis as an increase in oxygen consumption that

occurs in endotherms upon prolonged exposure to a cold environment. Their characterization is based on a set of experiments that manipulated ambient temperature and measured whole body oxygen uptake. In one set of experiments, rats were exposed to decreases in ambient temperature from 30 ^{0}C to 10 ^{0}C. Within 40 min, the rats increased oxygen consumption by 20%. In another experiment, ambient temperature was dropped from 30 to 5 ^{0}C; yet, the rats were prevented from shivering by administration of curare, a nicotinic neuromuscular blocker. These rats still exhibited a 25% increase in oxygen consumption that peaked after 90 min of cold exposure. However, sympathetic ganglionic blockade with hexamethonium lowered oxygen consumption by 30% at 30 ^{0}C and diminished oxygen consumption during cold exposure. Finally, these researchers assessed the adaptive nature of nonshivering thermogenesis by chronically exposing rats to a low ambient temperature of 5 ^{0}C for 28 days. This cold acclimation procedure increased resting metabolic rate by 20% compared to controls at 30 ^{0}C. When the room temperature was dropped to 10 ^{0}C, the cold-acclimated animals increased oxygen consumption by 100% and consumed 64% more oxygen than controls. Thus, through activation of the sympathetic nervous system, rats attempt to maintain adequate core temperature by increasing nonshivering thermogenesis when exposed to moderate cold ambient temperatures.

The major effector in nonshivering thermogenesis is brown adipose tissue (BAT). This is a highly vascularized tissue located in the interscapular, axillary, and paraspinal regions within the thoracic and abdominal cavities (Nedergaard & Lindberg, 1982).

Foster and Frydman (1979) assessed blood flow and oxygen consumption to specific BAT locations in rats that were cold acclimated through prolonged (3-week) exposure to a cold (5 ^{0} C) environment. The results showed that, when tested at normal ambient temperatures, cold acclimation enhanced basal and norepinephrine-induced increases in oxygen consumption and blood flow to BAT. The investigators reported that about 30% of the cardiac output was channelled to BAT and that between 65 – 80% of the increased oxygen consumption accompanying cold-acclimated rats could be attributed to BAT metabolism.

In an effort to understand the neural components of BAT activation, Young, Saville, Rothwell, Stock, and Landsberg (1982) examined norepinephrine (NE) turnover in BAT following either acute or prolonged cold exposure. Acute cold exposure (4 ^{O}C for 6 h) increased brown adipose tissue NE turnover by 4- to 12-fold; whereas, chronic cold exposure, 4^{O}C for 9 days, increased NE turnover by 109% compared to control animals. Foster (1985) conducted a series of neuropharmacological experiments to elucidate BAT's receptor profile. In one study, oxygen consumption was measured in response to intravenous infusion of NE following pretreatment with selective α_1- or α_2-antagonists. The results showed that both the α_1-antagonist, prazosin, and the α_2-antagonist yohimbine, inhibited NE-induced increases in oxygen consumption; however, prazosin was twice as effective, suggesting that α_1-adrenergic receptors play a more prominent role than α_2-adrenergic receptors in BAT. To assess the contribution of β-adrenergic receptors in BAT, Foster exposed rats to chronic cold and measured oxygen consumption

to an intravenous injection of isoproterenol, a β-adrenergic receptor agonist. Isoproterenol produced a large dose-dependent increase in oxygen consumption. These findings, in conjunction with observations that BAT cells contain a high density of β-adrenergic receptors (Mohell, Connolly, & Nedergaard, 1987), suggested that BAT thermogenesis occurs mainly in response to sympathetic activation of local β-adrenergic receptors, but that simultaneous activation of α_1-adrenergic receptors can potentiate thermogenesis. More recent work suggests that (a) activation of BAT results from post-ganglionic release of norepinephrine onto specific β_3-adrenergic receptors and (b) BAT contains a unique mitochondrial protein, termed UCP or uncoupling protein (Himms-Hagen, 1990). All mitochondria, except those found in BAT, create energy by operating an electron transport system. The energy produced by electron transport in the mitochondrial system is self-limiting, since the reaction allows for the accumulation of a proton gradient outside the mitochondrion's intermembrane, which hampers the ability to further dissipate protons. However, following activation of the β_3-adrenergic receptor, the UCP in BAT mitochondria permits for efficient energy metabolism since it rapidly dissipates the proton gradient. This enhanced UCP-dependent metabolism creates high levels of heat production within BAT mitochondria. Thus, BAT is considered to be a central effector in thermogenesis and energy balance.

Central Innervation of BAT

The major neuroanatomical region implicated in controlling thermoregulation is the hypothalamus (Bruck & Zeisberger, 1987). As mentioned previously, cell bodies in the

parvocellular component of the PVN give rise to fibers that project monosynapically to the RVLM and IML of the spinal cord, sites implicated in BAT innervation (see Figure 1.; Coote, Yang, Pyner, & Deering, 1998; Landsberg, Saville, & Young, 1984; Trayhurn & Ashwell, 1987). Bamshad, Song, and Bartness (1999) recently strengthened this argument. They administered the Bartha's K strain of the pseudorabies virus (PRV) to BAT tissue in hamsters in order to determine the neuroanatomic chain of neurons connected to interscapular BAT. The animals were sacrificed 4 to 6 days postinjection and infected neurons were visualized by immunocytochemistry. Heavily infected neurons were observed in the IML of the spinal cord, the RVLM, and PVN, suggesting that these sites contribute significantly to BAT's innervation. Other evidence suggesting that the PVN may contribute to the thermoregulation include reports that electrical stimulation of the PVN in conscious rats increases both blood pressure (Kannan, Hayastuda, & Yamashita, 1989) and BAT thermogenesis (Freeman & Wellman, 1987). Moreover, PVN microinjections of corticotrophin-releasing factor (LeFeuvre, Rothwell, & Stock, 1987) or prostaglandin E_2 (Bhatnagar, Meaney, & Amir, 1993) have also increased BAT thermogenesis in anesthetized rats. Amir (1990), using anesthetized rats, reported that the increased BAT temperatures that accompanied PVN microinjections of glutamate were prevented with ganglionic blockers, again suggesting that the thermoregulatory role of the PVN was sympathetically mediated. Accordingly, this evidence indicates that the PVN and RVLM may influence sympathetically mediated control of BAT thermogenesis.

Imidazoline and Adrenergic Receptor Influence on Thermoregulation

Clonidine and clonidine-like drugs have been injected peripherally to assess the influence of α-adrenergic receptors on temperature regulation. For example, Tsoucaris-Kupfer and Schmitt (1972) assessed the peripheral and central thermal consequences of several imidazoline-based α-adrenergic agonists, including clonidine, tolazoline, phentolamine, tetrahydrozoline, naphazoline, and piperoxane, in anesthetized rats. When administered peripherally, clonidine and the imidazoline-based agents produced a dose-dependent hypothermia. Clonidine, the most potent hypothermic agent tested, was antagonized by both nonspecific imidazoline-based α-adrenergic antagonists, such as tolazoline, phentolamine, and piperoxane, and nonimidazoline-based α-adrenergic antagonists. The thermal effects following the central administration of clonidine and other imidazoline-based drugs were site and agent specific. Specifically, hypothalamic and intracisternal injections of clonidine produced a marked, rapid onset hypothermia that was antagonized by pretreatment with all nonspecific α- and β-adrenergic antagonists used. However, microinjections of clonidine into the lateral ventricle did not alter body temperature. Hypothalamic injections of tetrahydrozoline produced a biphasic response, hypothermia followed by hyperthermia. Hypothalamic injections of naphazoline produced hyperthermia.

VonVoigtlander, Triezenberg, and Losey (1978), Bugajski, Zancy, and Zdebska (1980), and Zancy (1982) have reported clonidine-induced decreases in body temperature following either peripheral or ICV administrations in mice and rats. These investigators

found that α-adrenergic antagonists attenuate but do not block clonidine's hypothermic effect, suggesting that imidazoline receptors may be contributing to the hypothermia. Romanovsky, Shido, Ungar, and Blatteis (1993) also reported a biphasic thermal response following the preoptic hypothalamic microinjection of clonidine, an initial hypothermia that lasted about 45 min followed by a delayed onset hyperthermia, which lasted for approximately 90 min. Only the initial hypothermic phase was blocked by rauwolscine, a selective α_2-adrenergic antagonist. The hyperthermic response to clonidine was blocked by indomethacin, an inhibitor of prostaglandin E_2 synthesis.

Indirect evidence for a thermal role of imidazoline receptors also has emerged from studies of experimental and genetic models of hypertension. Wilson, Bhatnagar, and Kirouac (1989) examined the influence of PVN microinjections of clonidine on body temperature in Goldblatt hypertensive rats, an experimental model of renal hypertension. In normotensive rats clonidine produces a mild, rauwolscine-reversible hypothermia when delivered to either the PVN or adjacent control areas. Yet, in hypertensive rats, delivery of clonidine into the PVN produced a marked, rauwolscine-potentiated, dose-dependent hypothermia. In addition, the spontaneously hypertensive rat (SHR) has been characterized as possessing a genetically altered thermal set-point (Travis & Boulant, 1989; Wilson, Wilson, & DiCara, 1977) and a reduced heat tolerance to both *in vivo* (Collins, Hunter, & Blatteis, 1987; Wright, Iams, & Knecht, 1977) and *in vitro* (Malo, Schlager, Tremblay, & Hamet, 1989) situations. Evidently, the SHR is hypertensive and thermally maladaptive. The SHR expresses an increased density of central α_2-adrenergic

receptors, of the total specific ^{3}H-PAC hypothalamic binding sites nearly 30% are nonadrenergic (Kamisaki, Ishikawa, Takao, Omodani, Kuno, & Itoh, 1990). Moreover, repeated exposure to the imidazolines idazoxan or cirazoline produces greater increases in the density of hypothalamic imidazoline-preferring receptors in the SHR than in inbred normotensive controls (Olmos, Miralles, Barturen, & Garcia-Sevilla, 1992). Collectively, these observations have suggested that imidazoline receptors may contribute to both the cardiovascular and thermoregulatory features of the SHR.

Szreder (1997) reported the effects of the intravenously administered α_2-adrenergic agonist, UK 14304, and antagonist rauwolscine, in rabbits, on body temperature and blood pressure. The results showed that under normal thermal conditions UK 14304 reduced *both* body temperature and blood pressure, while rauwolscine elevated *both* body temperature and blood pressure. This is one of only a few studies to monitor both blood pressure and temperature regulation and to demonstrate that the adrenergic mechanisms involved in temperature regulation may also be involved in regulating blood pressure.

Statement of the Research Problem

Much of what is understood about the central control of blood pressure stems from investigations of the cardiovascular effects of clonidine. Previous studies with clonidine have promoted the notion that (a) medullary RVLM presynaptic α_2-receptors inhibit sympathetic preganglionics that innervate a spectrum of myocardial and vascular effectors, and (b) clonidine and clonidine-like drugs are centrally-acting, therapeutically effective antihypertensive agents. At the same time, other investigators, have adopted

clonidine as a neuropharmacological tool to assess the influence of central α_2-adrenergic receptors on another sympathetically mediated autonomic process, namely, thermoregulation. The latter studies have demonstrated unequivocally that clonidine's central action reduces both blood pressure and body temperature in a dose-dependent manner: whereas clonidine withdrawal produces compensatory elevations in both blood pressure and body temperature. Although the purported role of medullary α_2-adrenergic receptors in inhibiting the sympathetic preganglionics was a formative influence in studies of the central control of blood pressure, their role in the central control of body temperature has not been verified.

The presumption that clonidine's imidazoline structure contributes only to its binding affinity to α_2-adrenergic receptors is difficult to reconcile with several observations. Despite being considered a classic α_2-adrenergic agonist, clonidine exhibits a similarly high affinity for binding to medullary nonadrenergic imidazoline-preferring receptors. Second, selective stimulation of these nonadrenergic imidazoline-preferring receptors, but not the α_2-adrenergic receptors, has been shown to mimic clonidine's ability to reduce both blood pressure and the activity of RVLM neurons controlling sympathetic preganglionics, and this effect is blocked by pretreatment with imidazoline receptor antagonists. Third, an endogenous extract has been partially purified, called clonidine displacing substance (CDS), and the action of this ligand mimics the sympathoinhibitory, including antihypertensive, effects of clonidine administered to the RVLM. Finally, imidazoline receptors have been identified in numerous organs, and

regions of the brain other than the RVLM. These and related observations have demonstrated that the sympathoinhibitory properties until recently credited solely to medullary α_2-adrenergic receptors are linked, at least in part, to the activation of an entirely distinct class of medullary *non*adrenergic I_1-imidazoline receptors.

In the rat brain I_1-imidazoline and α_2-adrenergic receptors coexist in al least two major sites, both of which have earned dominant roles in controlling sympathetic preganglionics. One site is medullary and is confined largely to the RVLM. This site contains a 70:30 ratio of α_2-adrenergic to I_1 receptors that, when activated, apparently produce a net inhibition of those sympathetic preganglionics maintaining blood pressure. The other site is the hypothalamus, an autonomically important collection of cell bodies including the paraventricular nucleus of the hypothalamus (PVN). The ratio of α_2-adrenergic to I_1 receptors for the hypothalamus is 73:27 (Kamisaki et al., 1990), and within the hypothalamus resides perhaps the largest concentrations of clonidine displacing substance (CDS). The PVN is one of several hypothalamic structures that is strategically organized to control a spectrum of sympathetic preganglionics or autonomic effectors, either directly through neural and neuroendocrine influences or indirectly through reciprocal interactions with the medullary RVLM.

The findings that central I_1-imidazoline receptors contribute to reductions in blood pressure strongly suggests that they might also contribute to reductions in body temperature. What effect stimulating I_1-imidazoline receptors exert on thermoregulation, an autonomic process that is inextricably linked to the regulation of blood pressure,

remains to be explored systematically. Moreover, despite the acknowledged role of the hypothalamus in thermoregulation, few investigators have attempted to assess the thermal contribution of hypothalamic α_2-adrenoreceptors, and no studies have examined the thermal contribution of hypothalamic I_1-imidazoline receptors. Accordingly, the following series of two experiments using conscious rats were performed to assess whether I_1-imidazoline receptors and α_2-adrenoreceptors located in the area of the RVLM and hypothalamus, two regions known to influence sympathetic preganglionics and thermal effectors, play a role in the regulation of body temperature.

In this study, central I_1-imidazoline receptors were stimulated by intracerebroventricular microinjections of moxonidine, an I_1-imidazoline receptor agonist with greater selectivity for this site than α_2-adrenergic sites (Chrisp & Faulds, 1992; Piletz et al., 1996). Intracerebroventricular pretreatment microinjections with either vehicle or efaroxan, an I_1-antagonist with greater selectively for I_1 sites over α_2-adrenergic sites (Ernsberger et al., 1995), provided additional evidence that moxonidine was acting through an I_1-imidazoline receptor. Central α_2-adrenoreceptors were stimulated by intracerebroventricular microinjections of UK 14304, an α_2-adrenoreceptor agonist with greater selectivity for the adrenergic site than for I_1-imidazoline receptors. UK 14304 microinjection followed central pretreatments with either vehicle or SK&F 86466, an α_2-adrenoreceptor, with selectively for α_2-adrenergic over I_1-imidazoline sites (Ernsberger et al., 1992). These agents were delivered to the third or fourth ventricles to gain access to sites near the hypothalamus and medulla, respectively.

The general purpose of these experiments was to clarify the understanding of the extent to which central processes regulating blood pressure and those regulating body temperature are related. It was anticipated that (a) delivery of moxonidine or UK 14304 to the third (Experiment 1) or fourth (Experiment 2) ventricle would reduce body temperature and (b) efaroxan and SK&F 86466 pretreatment delivered to either the third or fourth ventricles would antagonize the hypothermic effect of moxonidine and UK 14304, respectively.

Experiment 1

Method

Subjects

Twenty-four male Sprague-Dawley rats weighing approximately 275-325 g at the onset of the experiment served as subjects. The rats were purchased from the University of Manitoba central breeding colony and housed individually in hanging wire (16 X 48 X 24 cm) cages in a room in the Psychology Animal Holding facilities with a 12-hr light cycle (lights on 0700 hr). Room temperature was maintained within the rat's thermoneutral range of 22 $\pm$ 2°C (Poole & Stephenson, 1977) with 30-50% relative humidity. Standard rat-chow (Wayne Rat Blox) and water was available continuously. Following a 3-day facility adjustment period, each rat was gently handled and weighed daily for approximately 2 min for an additional 7 days to familiarize the animals with human interaction. All experimental procedures conformed to the guidelines outlined by the Canadian Council on Animal Care.

Surgical Procedures

Each rat was anesthetized with Nembutal (Allen & Hanburys, Toronto) (50 mg/kg ip) supplemented with 0.2 ml of 0.4 mg/ml atropine sulfate (British Drug Houses Ltd., Canada) to suppress mucosal secretions. Once the rat was unconscious, the incision site, for intraperitoneal implantation of a cold-sterilized, 1.5 g paraffin-coated telemetered AM transmitter (Models V-M, XM-FH; Mini Mitter Inc., Sunriver, OR), was shaved and the skin wiped with a 70% alcohol swab and sterile saline (.15 M sodium chloride). A 2 to 3-

cm incision was made 1 to 2-cm lateral of midline through the abdominal wall. Then the transmitter was inserted through the incision to the abdominal cavity. The body wall and epidermis were successively sutured (OO silk), and a local analgesic (2% Xylocaine HCl, Astra) and topical antibacterial (Furacin, 0.2%, Austin) cream were applied.

Once the abdominal incision was dressed, the interaural scalp hair was clipped (Hair Clipper, Oster, No. 80), and the shaved scalp wiped with 70% ethanol and sterile saline. The rat's head was then secured with nontraumatic ear bars to a Kopf stereotaxic apparatus, and each was eye coated with chloramphenicol (Upton, Quebec) ointment to prevent corneal drying. A 3-cm incision was made through the scalp in a rostral-caudal direction over the skull. The skin and underlying fascia were retracted and clamped to expose the underlying skull. The skull was continually moistened with sterile saline. Once the skull was levelled to within 0.1 mm by determining the dorsal-ventral coordinates for Bregma and Lambda, its surface was mapped to determine the position for implantation of a 22-ga. guide cannula (Plastics One Inc., Roanoke, VA, USA). The coordinates used for the 3rd ventricle site were 0.8 mm posterior to Bregma, 0.1 mm lateral to the longitudinal suture, and 6.8 mm ventral to the ventral surface of the skull (Paxinos & Watson, 1986). Then the skull was pierced with a 30-ga. drill bit mounted to a hand-held rotary drill (Dremel). Three non-piercing indentations were made around the area of the pierced site. Stainless-steel jewellers' screws (4 mm in length) were set into each of these indentations. The guide cannula was lowered to its final position within the 3rd ventricle. Dental acrylic cement (Jet Acrylic, Lang Dental Mfg. Co., Inc., Chicago,

IL) adhered the guide cannula to the three jewellers' screws imbedded in the skull. Once the cannula assembly was secure, the fascia and skin were sutured around the dental acrylic, and topical anesthetic (2% Xylocaine hydrochloride, Astra Pharmaceuticals, Canada Ltd., Mississauga, ON) and antibacterial cream (0.2% Furacin, Austin, Division of Vetoquinol Canada Inc., Joliette, Quebec) applied to both the abdominal and cranial incision sites. In addition, a broad spectrum antibiotic (Ethacilin, Rogar STB, 45000 units) was administered intramuscularly before the rats were moved to the experimental room for recovery. To prevent infection and reflux through the guide cannula, a 24-ga. inner stylet was placed in the guide cannula. The inner stylet extended 0.5 mm beyond the guide cannula.

Drugs

The α_2-adrenergic receptor agonist UK 14304 (Pfizer Ltd., Sandwich, Kent, UK) and the I_1 imidazoline receptor moxonidine (Beiersdorf, AG, Hamburg, Germany) were dissolved in 0.9% sterile physiological saline (0.15 M, vehicle) and microinjected centrally in a 4.0-µl volume. Both drugs were divided into three groups of doses: 0.0, 1.0, and 10.0 nmol. The α_2-adrenergic receptor antagonist SK&F 86466 (SmithKline Beecham, King of Prussia, PA) and the I_1 imidazoline receptor antagonist efaroxan (Research Biochemicals Incorporated, Natick, MA, U.S.A.) were dissolved in physiological saline and microinjected centrally in a 4-µl volume containing 3 nmol. All solutions were made weekly and refrigerated between injections.

Apparatus and Procedure

Prior to implantation of the AM transmitters for temperature recording, equations for converting AM pulse frequency to core body temperature (T_C) were established through calibration of each transmitter in a Lauda (Model B-1) temperature-controlled water bath.

In the experimental room the rats were housed in polypropylene cages (16 X 48 X 24 cm) with wood-shaving bedding, topped with inverted wire covers held in place by an elastic harness. Each cage was positioned on an AM receiver (DataQuest III, Model R1010, Data Sciences International, St. Paul, MN) connected to a signal amplifier, and each signal was recorded on a personal computer. Rats were handled and weighed daily over the 10-day postoperative recovery period. Handling lasted for approximately 2 min and simulated the testing procedure that involved removing each rat from its cage, petting and weighing it, removing and reinserting its cannula's inner stylet, and returning it to its homecage.

Drug injection began on Postsurgery Day 11. The rats were randomly divided into either a moxonidine (n=12) or UK 14304 (n=12) drug treatment group. Half of the rats in each drug treatment were then randomly assigned to either a vehicle (n=6) or antagonist (n=6) pretreatment condition. Efaroxan, an imidazoline antagonist with low affinity for α_2-adrenergic receptors, served as moxonidine's pretreatment condition. SK&F 86466, an α_2-adrenergic antagonist with low affinity for imidazoline receptors, served as UK 14304's pretreatment. In a repeated measures, counterbalanced design, each rat received a

designated pretreatment drug followed 30 min later by one of the three drug doses: 0 nmol, 1 nmol, or 10 nmol. The 0 nmol dose was a 4-µl injection of vehicle. Accordingly, on Day 11 postsurgery, a rat would receive an ICV pretreatment of either efaroxan (3 nmol) or physiological saline followed 30 min later by one of three doses of moxonidine. On Day 13 postsurgery, the rat would receive the same drug pretreatment followed 30 min later by one of the two remaining doses of moxonidine. On the last day of the experiment, Day 15 postsurgery, the rat would receive the same drug pretreatment followed 30 min later by the last remaining dose of moxonidine. All drugs were delivered in a 4-µl volume over 45 s.

Approximately 2.5 hr before drug injections, 0800 h, baseline core temperature (T_C) were established for each rat by averaging across four T_Cs sampled at 30-min intervals. Thirty min after the baseline T_C had been established, during which time the pretreatment and drug treatment conditions were administered, T_C was sampled every 30 min and averaged at 2-hr intervals for a period of 4 hr. For statistical analysis the T_Cs sampled during this time were converted to percentage change from baseline T_C.

Histological Verification

Upon completion of testing, all rats were euthanized with an overdose of sodium pentobarbitol, and perfused intracardially successively with saline and 10% formalin. Before each animal's brain was removed and fixed in 10% formalin, 4 µl of black India ink was microinjected into the guide cannula. After fixing for at least 2 days, the brains were sectioned coronally with a razor and inspected under light microscopy for cannula

placement. A hit occurred if particles of ink could be identified in either the third or fourth ventricle by postmortem histological examination. If the guide cannula placement was errant, the data from that animal were not subjected to statistical analyses.

Results

Surgery. Of the 27 Sprague-Dawley rats that underwent surgery for Experiment 1, 24 contributed data to the study. Of these, 1 died during surgery, 1 lost their cannula head assembly before completion of the experiment, and 1 rat was eliminated from the analysis due to incorrect cannula placement.

Core Body Temperature (T_C). Table 1 describes the average baseline T_C. Baseline T_C for each animal were calculated by sampling T_C every 30 min over 2 hr and then averaging the 4 T_Cs (see Table 1). These baseline values served as the reference point for describing T_C change over the course of the experiment. Percentage change from baseline was calculated in the following manner:

$$\% \text{ change in } T_C = ((T_C - \text{baseline } T_C) / \text{baseline } T_C) \text{ X } 100$$

During each experimental treatment, three T_Cs for use in statistical analyses were established. The first T_C, termed 0 hr, was sampled immediately following experimental treatment. The second T_C, 2 hr, was determined 2 hr after treatment. It was calculated by averaging the four 30-min T_C samples following treatment. The third T_C, 4 hr, was an average of the four 30-min T_C samples between 2- and 4-hr post-treatment. These three T_Cs were then converted to percentage change from baseline. Thus, subsequent statistical analyses consisted of 3 measures, 0 hr, 2 hr, and 4 hr,whose values represented.

Table 1

Mean and SE Preinjection Baseline Core Temperatures (T_C) For Experiment 1

Condition	T_C (^oC)
Vehicle Pretreatment	
Moxonidine	
0 nmol	37.11 ± 0.29
1 nmol	36.74 ± 0.18
10 nmol	36.75 ± 0.30
UK 14304	
0 nmol	36.80 ± 0.13
1 nmol	36.79 ± 0.10
10 nmol	36.92 ± 0.13
Efaroxan Pretreatment	
Moxonidine	
0 nmol	37.06 ± 0.11
1 nmol	37.02 ± 0.16
10 nmol	37.03 ± 0.14
SK&F 86466 Pretreatment	
UK 14304	
0 nmol	36.72 ± .26
1 nmol	36.79 ± .14
10 nmol	36.67 + .35

Note. 0 nmol is vehicle injection.

percentage change from baseline. All omnibus effects were evaluated with a Pillai-Bartlett multivariate F. Alpha level was set to .05 for all planned comparisons.

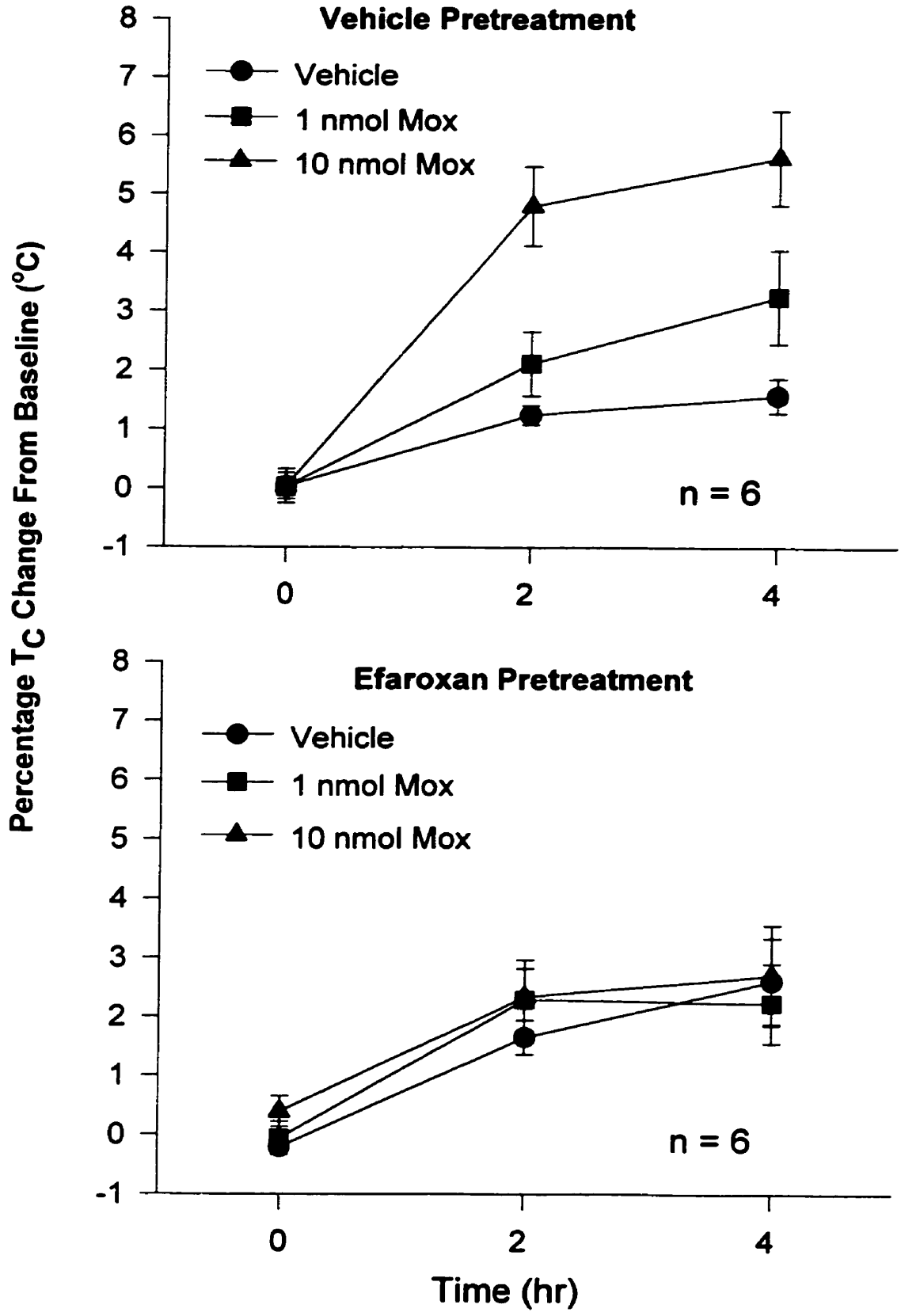
Moxonidine. Figure 2 (top panel) illustrates moxonidine's effect on T_C following vehicle pretreatment. A 3 x 3 (Dose x Time) repeated measures analysis of variance (ANOVA) with repeated measures on Dose and Time was used to determine whether moxonidine influenced, in a dose-dependent manner, T_C after vehicle pretreatment. The analyses showed that moxonidine elevated T_C in a dose-dependent fashion, $F(2, 4) = 12.67$, $p < .02$, and increased T_C over time $F(2, 4) = 16.54$, $p < .02$. No dose by time interaction was observed, $F(2, 4) = 2.12$, $p > .05$. Paired t-test contrasts across dose revealed that the percentage change of T_C in the 10-nmol moxonidine condition ($M = 3.49$, $SE = 1.17$) was elevated relative to T_C obtained for both the vehicle dose ($M = 0.95$, $SE = 0.66$), $t(18) = 3.77$, $p < .01$, and 1-nmol moxonidine dose ($M = 1.79$, $SE = 0.40$), $t(18) = 3.95$, $p < .01$. While not statistically significant, $t(18) = 1.7$, $p > .05$, the vehicle - 1 nmol moxonidine condition tended to elevate T_C compared to the vehicle - vehicle condition.

Paired t-test contrasts averaging across time revealed differences between T_C obtained at 0 hr ($M = 0.04$, $SE = 0.21$) and T_C obtained at both 2 hr ($M = 2.72$, $SE = 0.82$) and 4 hr ($M = 3.48$, $SE = 1.06$), $t(18) = 5.39$, $p < .01$, and $t(18) = 5.58$, $p < .01$, respectively. T_C at 4 hr was also significantly greater than T_C at time 2 hr, $t(18) = 2.65$, $p < .03$.

Figure 2 (lower panel) shows the effect of moxonidine on T_C following efaroxan pretreatment. Efaroxan pretreatment blocked moxonidine's dose-dependent

Figure Caption

Figure 2. Effect of third ventricle injection of moxonidine on core body temperature (T_C) following either vehicle or efaroxan pretreatment. In the top panel, the 10 nmol moxonidine group differed significantly from both the vehicle-treated control group and the 1 nmol moxonidine group. In the bottom panel, no significant group differences were observed following efaroxan pretreatment. Data points (n = 6) represent the mean percentage change in T_C from baseline over the 4-hr; vertical lines depict standard errors of the means.


Vehicle Pretreatment
Vehicle
1 nmol Mox
10 nmol Mox
n = 6
Efaroxan Pretreatment
Vehicle
1 nmol Mox
10 nmol Mox
n = 6
Percentage T_C Change From Baseline (°C)
Time (hr)
8
7
6
5
4
3
2
1
0
-1
0
2
4

hyperthermia, F (2, 4) = .56, p > .05. However, efaroxan pretreatment followed by moxonidine microinjection yielded a time effect, F (2, 4) = 6.96, p = .05. Paired t-test contrast across time revealed that T_C at 0 hr (M = .04, SE = 0.10) was lower than T_C at either 2 hr (M = 2.09, SE = 0.48), t (18) = 6.40, p < 0.01, or 4 hr (M = 2.5, SE = 0.7), t (18) = 5.37, p < 0.01. No difference was found between time 2 hr and time 4 hr. No dose by time interaction, F (2, 4) = 11.29, p > .05 was observed.

UK 14304. Figure 3 (top panel) illustrates UK 14304's effect on T_C following vehicle pretreatment. A 3 x 3 (Dose x Time) repeated measures ANOVA was used to assess whether UK 14304 would influence T_C after saline pretreatment. Figure 3 shows that following saline pretreatment, UK 14304 increased T_C across time, F (2, 4) = 8.79, p < .05. However, unlike moxonidine, no dose effect on T_C was observed, F (2, 4) = 1.31, p > 0.3. A dose by time interaction was not present, F (2, 4) = 0.74, p > 0.6.

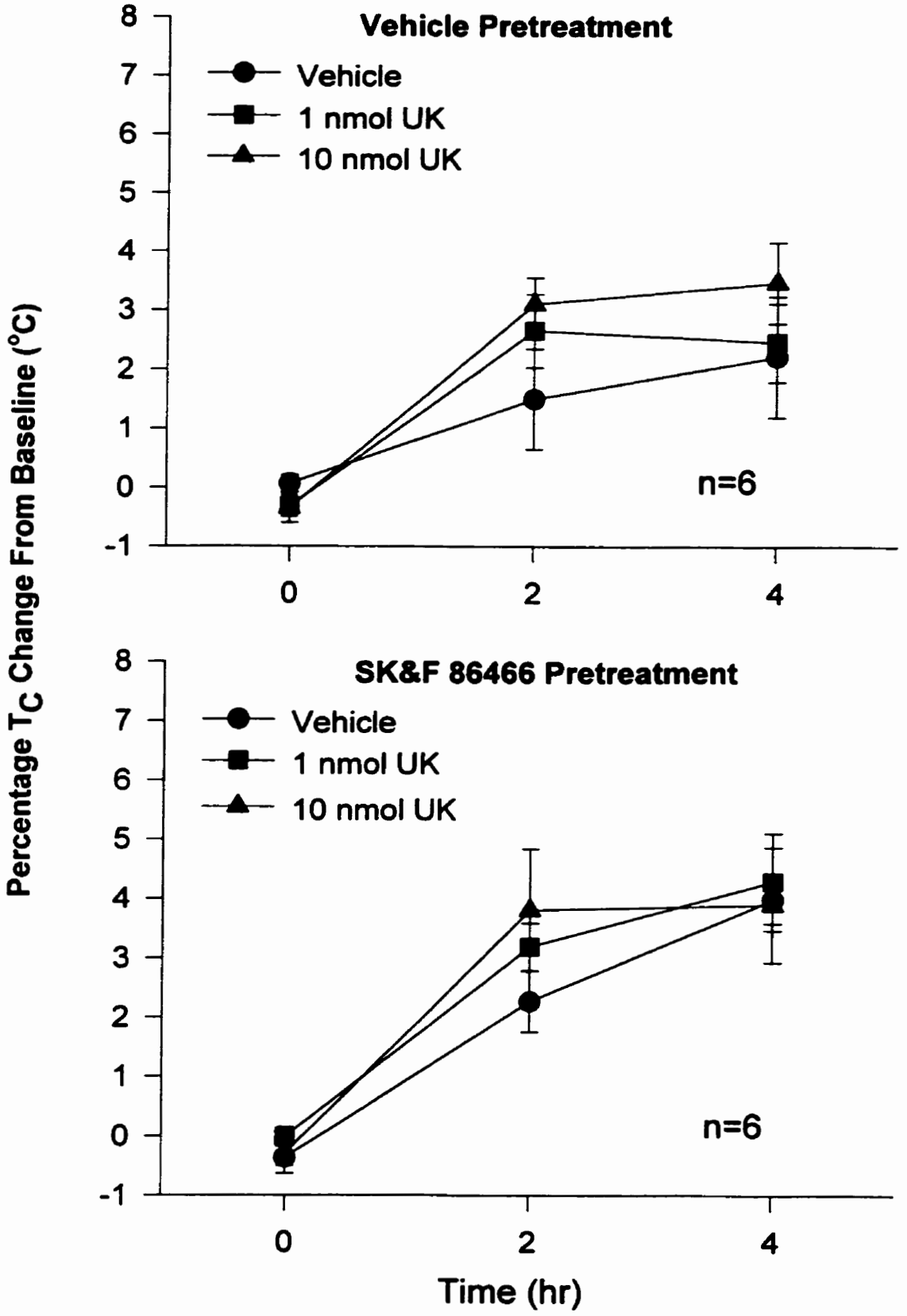
Figure 3 (lower panel) shows similar results for UK 14304 when animals were pretreated with SK&F 86466. T_C increased over time, F (2, 4) = 23.19, p < 0.01; however, no dose or dose by time effect was observed, F (2, 4) = 1.07, p > 0.4, and F (2, 4) = 1.08, p > 0.5, respectively.

Discussion

Previous reports have shown that α_2-adrenergic agonists, most notably clonidine, are injected in or adjacent to the third ventricle, changes occur in blood pressure and T_C.Though the direction of these changes depends on the injection site, those previous findings have indicated that hypothalamic α_2-adrenergic receptors may influence the

Figure Caption

Figure 3. Effect of third ventricle injection of UK 14304 on core body temperature (T_C) following either vehicle or SK&F 86466 pretreatment. The top panel illustrates a significant UK 14304 increase in T_C over time, but no dose-dependent UK 14304 effect following saline pretreatment. The bottom panel also demonstrates a significant rise in T_C over time, and no UK 14034 dose effect, following SK&F 86466 pretreatment. Data points (n = 6) represent the mean percentage change in T_C from baseline over the 4-hr; vertical lines depict standard errors of the means.


Vehicle Pretreatment
Vehicle
1 nmol UK
10 nmol UK
n=6
SK&F 86466 Pretreatment
Vehicle
1 nmol UK
10 nmol UK
n=6
Percentage T_C Change From Baseline (°C)
Time (hr)
8
7
6
5
4
3
2
1
0
-1
0
2
4

activity of sympathetic preganglionic fibers. What role, if any, I_1-imidazoline receptors play in these responses to imidazolines has remained unknown.

Experiment 1 is the first study to our knowledge to investigate whether I_1-imidazoline receptors near the third ventricle influence thermoregulation. The delivery of moxonidine, an I_1-imidazoline receptor agonist, to this area elevated T_C in a prolonged and dose-dependent fashion. This hyperthermic action, coupled with reversal by pretreatment with efaroxan, an I_1-imidazoline antagonist, suggests that I_1-imidazoline receptors near the hypothalamus influence sympathetic effectors controlling thermoregulation. There are at least four interpretations of these data. The first interpretation stems from recent characterizations of the I_1-imidazoline receptor signaling pathway. The I_1-imidazoline receptor is purportedly a membrane receptor that binds an extracellular ligand, in this case moxonidine, to initiate an intracellular response. Separovic, Kester, and Ernsberger (1996) have shown that activation of I_1-imidazoline receptors leads to the production of two secondary messengers, diacylglyceride and arachidonic acid. They observed that moxonidine not only induces the production of diacylglycerides, but that this increase is selectively abated by efaroxan, a selective I_1-imidazoline receptor antagonist, and not by SK&F 86466, a selective α_2-receptor antagonist. From these observations, Ernsberger et al. (1999) suggested that moxonidine's activation of the I_1-imidazoline receptor may, in turn, activate phosphatidylcholine-selective phospholipase C, which converts phosphatidylcholine into phosphocholine and diacylglyceride. Diacyglyceride is converted by lipases into

arachidonic acid and released extracellularly. This notion merits consideration because it is well established that arachidonic acid can be metabolized to prostaglandin E2 (PGE_2). PGE_2, an eicosanoid, may serve as a central neurotransmitter since its affinity is high at specific brain (e.g., Fleming et al., 1998). PGE_2 also contributes to sympathetic activity since injection of PGE_2 into the 3rd ventricle increases splanchnic nerve activity (e.g., Ando, Ichijo, Katafuchi, & Hori, 1995). Furthermore, PGE_2 promotes the development of fever since ICV injection produces regulated increases in body temperature and temperature of interscapular BAT (e.g., Chen, Hirasawa, Takahashi, Landgraf, & Pittman, 1999). Thus, the hyperthermic effects of may be explained by imidazoline-mediated release of PGE_2.

Romanovsky et al. (1993) reported a biphasic thermal response following delivery of clonidine to the hypothalamic area. They observed that clonidine produced a short latency, phasic hypothermia, which was blocked by rauwolscine, a selective α_2-adrenergic antagonist. A longer latency, tonic hyperthermia was then observed. The latter was blocked by indomethacin, an inhibitor of PGE_2. These observations may suggest that clonidine's late onset hyperthermia may have resulted from activation of I_1-imidazoline receptors, which led to release of PGE_2 and subsequently elevated body temperature. Romanovsky et al.'s findings, in conjunction with the present findings, suggests that the hyperthermic response to both clonidine and moxonidine may have been triggered by I_1-imidazoline receptor-mediated release of PGE_2. This interpretation may explain why both indomethacin and efaroxan blocked the hyperthermia.

A second, less direct interpretation of moxonidine's hyperthermic action stems from observations that BAT thermogenesis and increases in body temperature occur in response to psychological stress (Kluger et al., 1987; Moltz, 1993; Rothwell, 1994; Singer, Harker, Vander, & Kluger, 1986; Kluger, 1991) and overeating (Himms-Hagen, 1990). Accordingly, the hyperthermic action of moxonidine could be secondary to the stress associated with the injection procedure itself. This notion, however, would be difficult to reconcile with the fact that the animals were habituated before the injections and that an identical injection protocol was used in vehicle-treated controls. Habituation was meant to familiarize the animals with the injection protocol, and minimized any stress experienced by the animals. The use of a comparable injection protocol ensured that any thermal effects resulting from the procedure itself were similar in all experimental conditions. In addition, moxonidine's dose-dependent effect suggested that the injection procedure itself was not the main contributor to the elevation in body temperature, or that moxonidine potentiated any stress attributable to the injection procedure.

A third interpretation is that moxonidine's hyperthermic action might be linked to diet-induced thermogenesis. Diet-induced thermogenesis is dependent on activity of BAT and is caused by eating, digesting and assimilating food (Rothwell & Stock, 1982). It is influenced minimally by carbohydrate and lipid ingestion. Conversely, protein consumption can increase metabolic rate by 30% for 12 h (Himms-Hagen, 1990). Since food was continuously available during the experimental period, the observed

hyperthermia might be considered to be secondary to moxonidine's effect on food intake. In this regard, Jackson, Griffin, and Nutt (1991) and Menargues, Cedo, Artiga, Obach, and Garcia-Sevilla (1994) investigated the role of the I_2-imidazoline agonist, idazoxan, on food intake, in rats, following an intraperitoneal injection. Both studies observed that idazoxan increased food intake in a dose-dependent fashion up to 6 hr postinjection. Jackson and Nutt (1996) also reported that RX 801077, a specific I_2-imidazoline agonist, produced short-term increases in eating. Since moxonidine binds marginally to I_2-imidazoline receptors (Olmos, Alemany, Boronat, & Garcia-Sevilla, 1999), and idazoxan binds somewhat to I_1-imidazoline receptors (Ernsberger et al. 1992), Jackson and Nutt's observations may stem from activating I_1- rather than I_2- imidazoline receptors. Moreover, recent data from our lab indicate that delivery of moxonidine to the 3rd ventricle increases short-term (6 hr), but not long-term (24 hr) food consumption in a dose-dependent fashion. Thus, moxonidine may have increased body temperature secondarily through the thermogenic consequences of short-term increases in food intake.

A fourth interpretation of moxonidine's hyperthermic action relates to neural circuitry. Imidazoline research has concentrated on I_1 receptors near the 4th ventricle, most notably the RVLM. I_1-imidazoline receptors in the RVLM reduce sympathetic output by inhibiting sympathetic preganglionic neurons in the spinal cord (Haxhiu et al., 1992). However, the RVLM is, in turn, innervated by descending projections from several diencephalic structures including the paraventricular nucleus (PVN) of the hypothalamus (Coote et al., 1998; Saper et al., 1976). The PVN is located adjacent to the

3rd ventricle injection site targeted in the current protocol. The inhibitory nature of RVLM I_1-imidazoline receptors suggests that the diencephalic I_1-imidazoline receptors may also be inhibitory. If so, activation of these inhibitory I_1-imidazoline receptors in the PVN may disinhibit the RVLM and the thoracic IML (intermediolateral) column of the spinal cord, which, in turn, would activate BAT and elevate body temperature. This interpretation is consistent with Bing et al.'s (1999) finding that the oral administration of moxonidine for 21 consecutive days increases BAT activity by 40% in both lean and obese Zucker rats.

Experiment 2

Method

Subjects

Twenty-four male Sprague-Dawley rats weighing approximately 275-325 g at the onset of the experiment served as subjects in Experiment 2. The rats were purchased, housed and treated as described in Experiment 1.

Surgical Procedures

The general surgical procedures in Experiment 2 were identical to those in Experiment 1, except that the indwelling cannulae were positioned in the fourth ventricle. The stereotaxic coordinates used were 11.6 mm posterior to Bregma, 1.0 mm lateral to the midline, and 7.0 mm ventral to the ventral surface of the skull (Paxinos & Watson, 1986).

Procedure

The experimental procedure was identical to that described in Experiment 1.

Results

Surgery. Of the 31 Sprague-Dawley rats that underwent surgery for Experiment 1, 24 contributed data to the study. Of these, 1 died during surgery, 2 lost their cannula head assembly before completion of the experiment, and 4 rats were eliminated from the analysis due to incorrect cannula placement.

Core Body Temperature (T_C). Table 2 describes the average baseline T_Cs for Experiment 2.

Moxonidine. Figure 4 (top panel) illustrates moxonidine's effect on T_C following vehicle pretreatment. A 3 x 3 (Dose x Time) analysis of variance with repeated meaures on Dose and Time was used to determine whether moxonidine influenced, in a dose-dependent manner, T_C after vehicle pretreatment. The analyses showed that moxonidine did not alter T_C in a dose-dependent fashion, $F(2, 4) = 0.574$, $p > .05$, but did elevate T_C over time, $F(2, 4) = 112.56$, $p < .01$. No dose x time interaction was apparent, $F(2, 4) = 0.88$, $p > .05$.

Figure 4 (lower panel) depicts the effect of moxonidine on T_C following efaroxan pretreatment. Efaroxan pretreatment did not produce a dose effect, $F(2, 4) = 0.02$, $p > .8$. Efaroxan pretreatment reduced the magnitude of the T_C increase over time; however, the increase in T_C across time remained significant, $F(2, 4) = 8.34$, $p < .05$. No dose by time interaction was observed, $F(2, 4) = 1.28$, $p > .05$.

Table 2

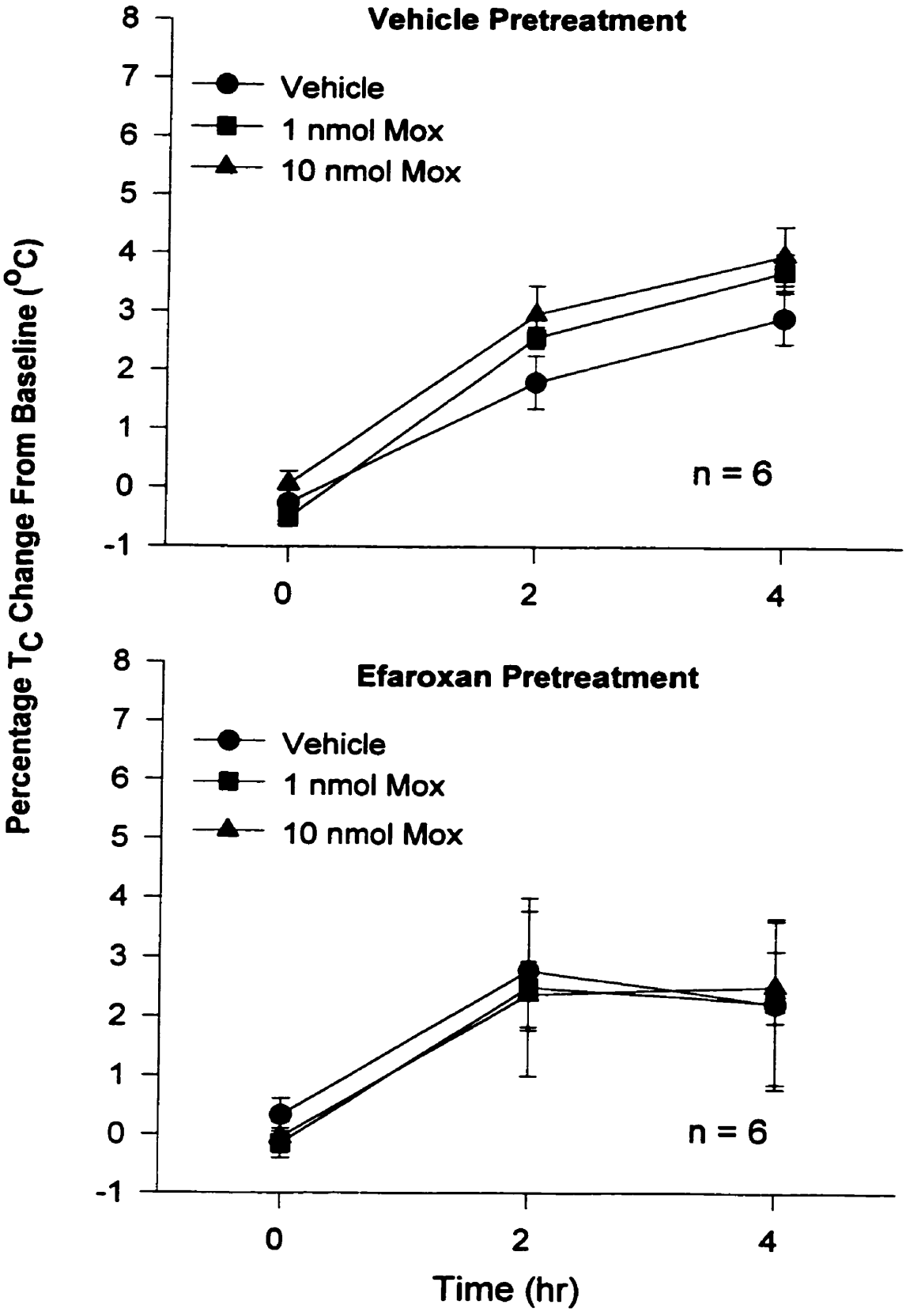
Mean and SE Preinjection Baseline Core Temperatures (T_C) For Experiment 2

Condition	T_C (^{O}C)
Vehicle Pretreatment	
Moxonidine	
0 nmol	37.15 ± 0.16
1 nmol	36.89 ± 0.10
10 nmol	36.87 ± 0.17
UK 14304	
0 nmol	37.12 ± 0.14
1 nmol	36.84 ± 0.08
10 nmol	37.04 ± 0.15
Efaroxan Pretreatment	
Moxonidine	
0 nmol	37.00 ± 0.27
1 nmol	36.86 ± 0.23
10 nmol	37.48 ± 0.31
SK&F 86466 Pretreatment	
UK 14304	
0 nmol	37.10 ± 0.21
1 nmol	37.18 ± 0.19
10 nmol	37.21 + 0.22

Note. 0 nmol is vehicle injection.

Figure Caption

Figure 4. Effect of fourth ventricle injection of moxonidine on core body temperature (T_C) following either vehicle or efaroxan pretreatment. In the top panel, while moxonidine elevated T_C across the experimental time period, no significant dose effect was observed. In the bottom panel, no significant dose effect occurred: however, efaroxan failed to block the moxonidine elevation in T_C over time. Data points (n = 6) represent the mean percentage change in T_C from baseline over the 4-hr; vertical lines depict standard errors of the means.


Vehicle Pretreatment
Vehicle
1 nmol Mox
10 nmol Mox
n = 6
Efaroxan Pretreatment
Vehicle
1 nmol Mox
10 nmol Mox
n = 6
Percentage T_C Change From Baseline (^oC)
Time (hr)
8
7
6
5
4
3
2
1
0
-1
0
2
4

UK 14304. Figure 5 (top panel) illustrates UK 14304's effect on T_C following vehicle pretreatment. A 3 x 3 (Dose x Time) repeated measures ANOVA was used to assess whether UK 14304 would influence T_C after saline pretreatment. The results in Figure 5 (top panel) show that following vehicle pretreatment, UK 14304 did not affect T_C in a dose dependent manner, F (2, 4) = 6.10, $p > .05$. However, a significant time effect, F (2, 4) = 53.7, $p < .01$ was present. No dose by time interaction, F (2, 4) = 2.26, $p > .05$ was observed.

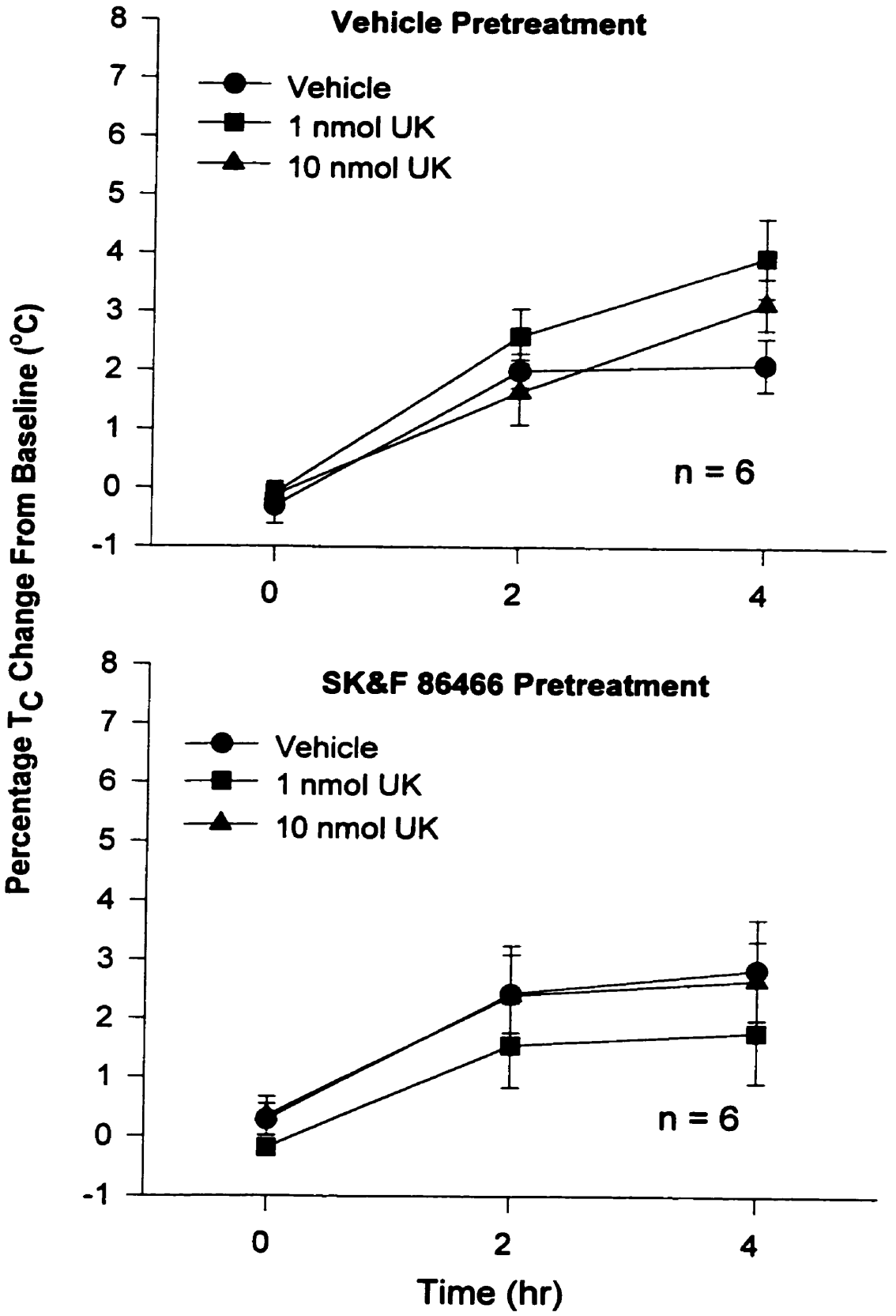
Figure 5 (lower panel) shows that pretreatment with SK&F 86466 blocked the hyperthermia observed over time following UK 14304 delivery, F (2, 4) = 3.54, $p > .05$. However, as with saline pretreatment, SK&F 86466 pretreatment did not elicit a UK 14304 dose main effect, F (2, 4) = 5.18, $p > .05$, or a dose by time interaction, F (2, 4) = 0.25, $p > .05$.

Discussion

The depressor and hypothermic effects of clonidine have traditionally been credited to the sympathoinhibitory action of medullary α_2-adrenergic. However, the literature subsequently suggested that these actions may instead be linked to activation of I_1-imidazoline receptors. While the effect of medullary I_1-imidazoline receptors on blood pressure has been vigorously investigated, the thermoregulatory role of I_1-imidazoline receptors has not been studied. Hence, Experiment 2 is the first study to assess the medullary contribution of moxonidine, an I_1-imidazoline receptor agonist, on body temperature. Given the evidence that both I_1-imidazoline and α_2-adrenergic agonists

Figure Caption

Figure 5. Effect of fourth ventricle injection of UK 14304 on core body temperature (T_C) following either saline or efaroxan pretreatment. In the top panel, UK 14304 elevated T_C across the experimental time period. No significant dose effect was observed. In the bottom panel, no significant dose effect occurred, however SK&F 86466 did block the UK 14304 elevation in T_C over time. Data points (n = 6) represent the mean percentage change in T_C from baseline over the 4-hr; vertical lines depict standard errors of the means.


Vehicle Pretreatment
Vehicle
1 nmol UK
10 nmol UK
n = 6
SK&F 86466 Pretreatment
Vehicle
1 nmol UK
10 nmol UK
n = 6
Percentage T_C Change From Baseline (°C)
Time (hr)
8
7
6
5
4
3
2
1
0
-1
0
2
4

reduce blood pressure and lower body temperature by blocking sympathetic output, it was predicted that moxonidine and UK 14304 would lower body temperature when delivered to the fourth ventricle. This prediction was not supported. Fourth ventricle microinjection of moxonidine and UK 14304 did not alter body temperature relative to the saline control. Similarly, neither pretreatment with efaroxan, an I_1-imidazoline receptor antagonist, nor SK&F 86466, an α_2-adrenergic receptor antagonist, altered body temperature.

The lack of an effect of both moxonidine and UK 14304 given in the 4^{th} ventricle on temperature regulation can be interpreted in several ways. First, the general increase in body temperature observed for all groups across the experimental period may represent the hyperthermic effects of handling. It cannot be excluded that the hyperthermic effects of handling masked any drug-induced thermal effects. A second interpretation centers on the selection of drug dose and the injection site. Although the doses used in the current protocol were derived from the experimental blood pressure literature, they may have been inappropriate for altering thermogenesis. In this regard, Ernsberger et al. (1995) demonstrated that the threshold dose of moxonidine sufficient to reduce blood pressure was 50 pmol when bilaterally injected into the RVLM of unconcious rats. Although the RVLM is approximately 1.5 mm from the fourth ventricle injection site, the high dose of moxonidine (10 nmol) used in our study was 500-fold greater than the dose used in Ernsberger et al.'s study and, therefore, should have been sufficient to reach cells in the RVLM. Finally, the possibility exists that the vascular and thermogenic effects of I_1-

imidazoline receptors are controlled by different medullary nuclei. Morrison and his colleagues (Morrison, 1999; Morrison, Sved, & Passerin, 1999) provided evidence that the sympathetic preganglionics controlling blood pressure, and those controlling body temperature, were dissociable. Using anesthetized rats, they measured postganglionic sympathetic nerve activity from either a small nerve bundle that innervates BAT, or from the splanchnic nerve, which predominately innervates vasculature. At normal body temperature, they observed negligible spontaneous sympathetic discharge to BAT. In contrast, the spontaneous output to the splanchnic nerve was robust. This finding suggested that the medullary sites innervating BAT were distinct from those innervating the vasculature beds controlling blood pressure. Morrison then monitored changes in the spontaneous discharge rate following reductions T_C, which was accomplished by turning off the heat source located beneath the animal. As T_C fell from 37.5 ^{O}C to 34.5 ^{O}C, large bursts of activity, nearly 500% above control values were recorded in the fibers innervating BAT. However, only a 19% increase in splanchnic nerve activity occurred following reductions in T_C. Morrison and his colleagues also found that the microinjection of bicuculline, a $GABA_A$ receptor antagonist, into the raphe pallidus produced a prompt and dramatic increase in sympathetic discharge to BAT, but only a small increase in splanchnic activity (see Figure 6). In contrast, bicuculline microinjection into the RVLM increased splanchnic nerve activity by 53% of control values, presumably through disinhibition, without changing sympathetic nerve activity to BAT. These observations suggest that the neural network responsible for generating

sympathetic discharge to BAT with hypothermia and bicuculline disinhibition may not be coupled to the network responsible for generating splanchnic sympathetic nerve activity. Thus, at the level of the medulla, neurons responsible for controlling sympathetic outflow to BAT may be dissociable from those regulating blood pressure. Therefore, the lack of moxonidine- or UK 14304-induced effects on body temperature may reflect the lack of I_1-imidazoline and α_2-adrenergic receptors in the raphe pallidus or that these pharmacological agents may not have diffused to the raphe pallidus, which is located approximately 1 mm ventral to the RVLM.

General Discussion

Attempts to understand the sympathetic control of the cardiovascular system stem in part from experimental observations using unconscious rats and therapeutic reports using clonidine. These findings suggested that, when activated, α_2-adrenergic receptors in the medulla, specifically the RVLM, selectively inhibit those sympathetic preganglionic fibers controlling arterial blood pressure. Many of these reports also demonstrated that clonidine-induced changes in blood pressure were accompanied by consistent changes in body temperature. Investigators eventually realized that clonidine also bound to medullary nonadrenergic imidazoline-preferring receptors, and that stimulating these imidazoline receptors mimicked clonidine's ability to reduce blood pressure. This evidence tempered the notion that clonidine's cardiovascular effects reflected the action of medullary α_2-adrenergic receptors with the realization that clonidine's cardiovascular effects may also reflect the action of medullary imidazoline

receptors. Since then most imidazoline research has focused on the role of medullary I_1-imidazoline receptors on the regulation of blood pressure. However, there have been few attempts to assess what role, if any, diencephalic I_1-imidazoline receptors exert on any other sympathetically mediated process. Moreover, no attempts have been made to determine what role imidazoline receptors exert on sympathetically mediated processes previously linked to the function of central α_2-adrenergic receptors, most notably the regulation of body temperature.

The present study is the first to investigate the relative contribution of α_2-adrenergic receptors and I_1-imidazoline binding sites, located in the medulla and diencephalon, towards regulating body temperature in conscious rats. Among other observations, the early findings that chronic and acute stimulation of purported α_2-adrenergic receptors reduced both blood pressure and body temperature, ostensibly through a medullary-induced sympathoinhibition, and findings that withdrawal from the chronic administration of clonidine elevated both blood pressure and body temperature, presumably through a medullary-induced sympathoexcitation, suggested that the sympathetic circuits controlling blood pressure contributed to the regulation of body temperature. In light of more recent evidence that chronic and acute stimulation of purported I_1-imidazoline binding sites reduce blood pressure through a medullary-induced sympathoinhibition, it was predicted that both moxonidine and UK 14304, relatively selective I_1-imidazoline and α_2-adrenergic agonists, respectively, when administered into either the third or fourth ventricle would lower body temperature. This prediction rested

on the tacit assumption that the sympathetic mechanisms regulating blood pressure and body temperature are functionally integrated, and sympathetic output controlled by medullary and diencephalic α_2-adrenergic and I_1-imidazoline receptors influence processes not traditionally associated with the regulation of blood pressure.

Experiment 1 investigated the influence of third ventricle administration of the I_1-imidazoline agonist, moxonidine, and the α_2-adrenergic agonist, UK 14304, on body temperature following pretreatment with either vehicle, efaroxan, an I_1-imidazoline antagonist, or SK&F 86466, an α_2-adrenergic antagonist. Moxonidine elevated body temperature in a dose-dependent and efaroxan-reversible fashion. In contrast, UK 14304 failed to alter body temperature. Though it is speculative, moxonidine-induced hyperthermia may reflect the thermogenic effect either of PGE_2, or increased food intake. The purported contribution of PGE_2 would be consistent with Romanovsky et al.'s (1993) finding that a late onset clonidine-induced hyperthermia administered in the hypothalamic preoptic area was blocked by indomethacin, a PGE_2 synthesis inhibitor, and not rauwolscine, a selective α_2-adrenergic antagonist. The developing awareness of the signal transduction pathway through which imidazoline functions also suggests that PGE_2 could contribute to moxonidine's hyperthermic action. In this regard, Separovic, Kester, and Ernsberger (1996) found that moxonidine induces the production of the secondary messenger diacylglyceride, which is the precursor to PGE_2. In contrast, the possibility that moxonidine's hyperthermic action could have resulted from diet-induced thermogenesis secondary to short-term increases in food intake has little direct

experimental support aside from unpublished observations from our lab that third ventricle microinjections of moxonidine produce short-term increases (6 hr) in food intake. Jackson and Nutt (1996) have also reported that imidazoline-based compounds like idazoxan and RX 801077, which are more specific for I_2-imidizoline receptors but bind marginally to I_1-imidazoline receptors, promote short-term increases in food-intake. Experiment 1 indicates that I_1-imidazoline receptors surrounding the third ventricle influence body temperature. The role exerted by these receptors in regulating body temperature may be different than expected from their sympathoinhibitory role in the RVLM. The observed hyperthermia indicates a net sympathoexcitation, rather than the sympathoinhibition noted in the medulla. These I_1-imidazoline receptors are evidently functionally distinct from α_2-adrenergic receptors, given that moxonidine, but not UK 14304, elevated core body temperature.

Experiment 2 examined the effect of the I_1-imidazoline agonist, moxonidine, and the α_2-adrenergic agonist, UK 14304, on body temperature when they were delivered to the fourth ventricle following pretreatment with either vehicle or SK&F 86466. Neither moxonidine nor UK 14304 elevated body temperature in a dose-dependent fashion. These results differ from what one might expect from the large body of evidence suggesting that both α_2-adrenergic and I_1-imidazoline receptors in the medulla, specifically the RVLM, reduce blood pressure by inhibiting sympathetic output. They could be interpreted as suggesting that α_2-adrenergic and I_1-imidazoline receptors surrounding the fourth ventricle do not influence body temperature. Alternatively, the

data may suggest that the medullary areas controlling blood pressure and body temperature are functionally and anatomically specific. This interpretation is based on an assumption that was not tested in this study, namely that the delivery of moxonidine or UK 14304 to the fourth ventricle would lower blood pressure. The negligible effects may also be partly attributed to inadequate doses of moxonidine and UK 14304, the fourth ventricle site of injection being too far from the RVLM, or to a combination of these considerations. These methodological considerations should be addressed in future studies.

One of the major problems encountered in investigating the sympathetic mechanisms of core body temperature is the complexity of the system. Thermoregulatory mechanisms have been demonstrated at all levels of the central nervous system, including the brain stem and spinal cord (Satinoff, 1974). Unfortunately, very little work has been done to investigate how they are integrated. Since the present study administered similar drugs to distinct, but integrated, brain areas, the regions surrounding the third and fourth ventricles, a unique opportunity exists for an integrative interpretation of sympathetic control of body temperature.

The recent work of Morrison and his colleagues (Morrison, 1999; Morrison, Sved, & Passerin, 1999), in conjunction with the purported mechanism of action of RVLM I_1-imidazoline receptors, may provide some integrative insight into the results of the present study. Morrison's findings showed that, at normal body temperature, spontaneous sympathetic output to BAT is minimal and may be controlled by the raphe pallidus, while

output to the vascular beds is robust and may originate in the RVLM. This anatomical dissociation, coupled with the reports that medullary I_1-imidazoline receptors are excitatory on sympathoinhibitory GABA, opioid, and serotonergic interneurons (Ernsberger et al., 1995; Head, 1999), suggests a plausible mechanism of moxonidine's hyperthermic action (see Figure 6). Delivery of moxonidine to the medulla would activate inhibitory interneurons which, in turn, would inhibit both the RVLM and raphe pallidus. Inhibition of the RVLM should markedly reduce the robust spontaneous activity to the vascular beds and thus lower blood pressure. In contrast, since spontaneous sympathetic output from the raphe pallidus is low, further inhibition would result in a negligible reduction in sympatheic output to BAT and minimal reduction in body temperature. This mechanism is supported by our observation that delivery of moxonidine to the fourth ventricle did not alter body temperature, and by reports in the literature that moxonidine delivered to the medulla lowers blood pressure (e.g., Nurminen, Culman, Haass, Chung, & Unger, 1998).

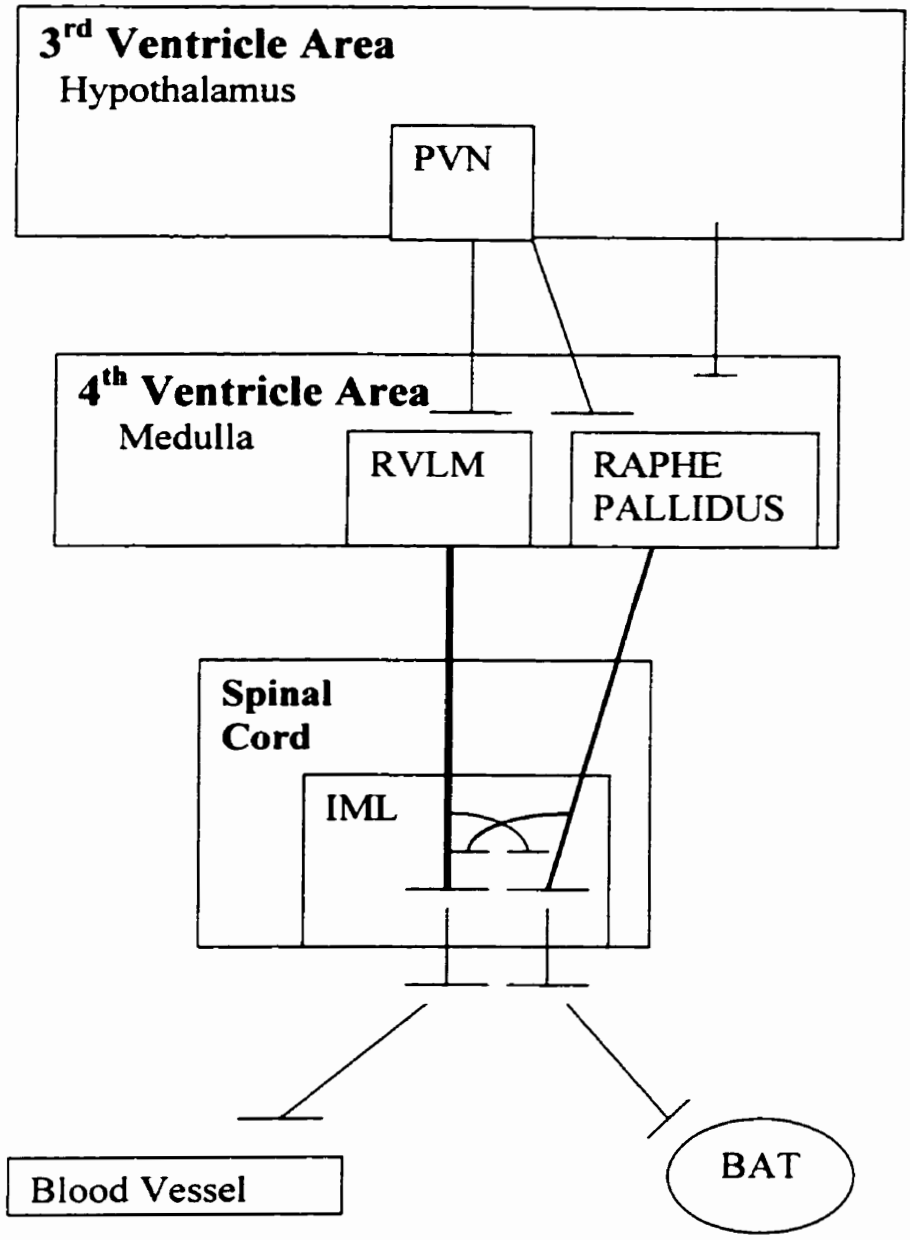
The delivery of moxonidine to the diencephalon in Experiment 1 may have altered the activity of caudal structures innervated by the hypothalamus, including the medulla (Koh & Ricardo, 1986). Specifically, Coote et al. (1998) have shown that chemical stimulation of neurons within the rat hypothalamic paraventricular nucleus (PVN) elicit excitatory responses in the RVLM, which elevate blood pressure. Assuming that hypothalamic I_1-imidazoline receptors, like those in the medulla, excite sympathoinhibitory interneurons, then administration of moxonidine to this area would

Figure Caption

Figure 6. Sympathetic control of BAT and the vasculature. Morrison and his colleagues (1999) suggest that the RVLM preferentially innervates IML neurons responsible for regulating blood pressure; while the raphe pallidus preferentially innervates those controlling BAT. At normal blood pressure and body temperature (T_C), spontaneous sympathetic output from the RVLM to the vasculature is robust (dark line), while output from the raphe pallidus to BAT is low. Therefore, direct inhibition of the RVLM would greatly reduce sympathetic output to the vasculature and significantly reduce blood pressure. In contrast, direct inhibition of the raphe pallidus, would further inhibit sympathetic output. This should only marginally reduce sympathetic output to BAT since it is already quite low. Assuming that the hypothalamic area regulates the RVLM and raphe pallidus by inhibition, then removing hypothalamic inhibition from the RVLM would only marginally increase RVLM sympathetic output, since it is already firing robustly. A slight increase in blood pressure would result. However, since the raphe pallidus is firing slowly, the removal of hypothalamic inhibition may result in a significantly increase in sympathetic output from the raphe pallidus. Thus, sympathetic output to BAT increases and T_C elevates.

BAT = brown adipose tissue; RVLM = rostral ventrolateral medulla;

IML intermediolateral spinal cord.


3rd Ventricle Area
Hypothalamus
PVN
4th Ventricle Area
Medulla
RVLM
RAPHE
PALLIDUS
Spinal
Cord
IML
Blood Vessel
BAT

reduce sympathetic output from the hypothalamus. Thus, medullary structures like the RVLM and raphe pallidus would become disinhibited. Since the RVLM already displays a high spontaneous sympathetic output, further disinhibition would tend to marginally increase output and blood pressure. Disinhibition of the raphe pallidus, however, which displays low spontaneous discharge rates, would be significant. The net effect would augment sympathetic discharge from the raphe pallidus to BAT and promote hyperthermia. While this interpretation is speculative, it is also consistent with the increases in blood pressure some investigators report with administrations of clonidine to the hypothalamus and surrounding areas (Kawasaki & Takasaki, 1986; Ebihara et al., 1993), and with our observation that delivery of moxonidine to the third ventricle elevates body temperature.

Prospectus

Collectively, the two experiments described above suggest that I_1-imidazoline receptors play a role in the regulation of body temperature. However, they also evoke considerations for follow-up studies. First, the I_1-imidazoline receptor agonist, moxonidine, and antagonist, efaroxan, bind marginally to α_2-adrenergic receptors. Thus, inadvertent stimulation of α_2-adrenergic in the vicinity of the third ventricle could have contributed to the hyperthermic effect of moxonidine observed in Experiment 1. This possibility should be explored blocking α_2-adrenergic receptors before administration of moxonidine. Second, although Experiement 1 demonstrated that moxonidine produced hyperthermia when delivered to the third ventricle, the precise neuronal site of action

remains unknown. Accordingly, an attempt should be made to identify what specific hypothalamic nuclei exert this hyperthermic action using selective I_1-imidazoline and α_2-adrenergic receptor and agonists. Third, should I_1-imidazoline receptors be responsible for moxonidine's hyperthermic action, an attempt should be made to understand the underlying behavioural or cellular process. For example, future studies should assess whether the hyperthermia is linked to either dietary thermogenesis, or the pyrogentic consequences of prostaglandin metabolism. Finally, the underlying theme of the current study focuses on how, and under what conditions, the brain mechanisms regulating body temperature interact with those regulating blood pressure. In this regard, future experimental designs should incorporate methods that simultaneously monitor blood pressure and body temperature while manipulating central pharmacology. Such data would significantly enhance our understanding of the central sympathetic mechanisms co-regulating blood pressure and body temperature.

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