The Influence of One-Kidney Goldblatt Hypertension and Cold Acclimation on Adrenergically-Induced Cardiovascular and Thermoregulatory Adjustments in Rats

A Dissertation

Presented to the

University of Manitoba

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

by

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August, 1987 

ISBN 0-315-37277-X

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A thesis 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

ACKNOWLEDGMENTS

I would like to express my deepest appreciation and kindest regards to my advisor, Dr. J. Roger Wilson, for his encouragement and support and for the innumerable discussions regarding my professional development. His knowledge was invaluable throughout my education and for that I remain indebted to him.

I would like to express my sincere gratitude to my committee members: Drs. D. Foster, R. MacArthur, D. Smyth, R. Tait, and L. Wilson, for their invaluable contribution to the design of this study and for the tremendous amount of time and effort devoted to the manuscript.

I would also like to thank Dr. D. Smyth for the use of his flame photometer.

I would like to express my sincere appreciation to the members of the Psychobiology laboratory for their untiring support and encouragement through all stages of this study.

Finally, I would like to thank my parents, Jan and Mary, for their patience and would like to express my deepest gratitude to James Caithness for the limitless support and understanding given for all my endeavors.

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Abstract

Cold acclimation (CA) and one-kidney Goldblatt (1K-GB) renovascular hypertension share certain features. For instance, there is increased resistance in vascular beds associated with the retention of core heat and functional hyperreactivity of adrenoceptors following either CA or the induction of 1K-GB hypertension. However, the effects of combined CA and 1K-GB hypertension remain unclear. The following four experiments assessed specific cardiovascular, thermophysiological, and thermobehavioral implications of 1K-GB hypertension, CA and catecholaminergic adrenoceptor stimulation. Experiment 1 examined whether $beta_2$-receptors contribute to isoproterenol (ISO)-induced thermogenesis in cold acclimated, normotensive and hypertensive rats. Twenty-four male rats were cold acclimated and adapted to mild physical restraint. Twelve were rendered hypertensive through nephrectomy and renal artery stenosis, and the remainder served as normotensive controls. Metabolic, thermal, and cardiovascular responses were assessed during infusions of ISO alone or combined with atenolol ($beta_1$-antagonist) or pindolol ($beta_2$-antagonist) following recovery from chronic catheterization of the descending aorta and right jugular vein. The increase in metabolism, body temperature and heart rate during ISO administration was attenuated by both antagonists. Thus, subsequent ISO administrations were preceded by pindolol to ensure predominantly $beta_1$-stimulation. Experiment 2 assessed whether $beta_1$-induced thermogenesis was potentiated by simultaneous stimulation of $alpha_1$-receptors. Sixty-four male rats were either cold- or non-cold-acclimated and rendered hypertensive or normotensive. All were

restraint-adapted. Metabolic, thermal and cardiovascular responses were assessed during individual or simultaneous infusions of ISO and the $alpha_1$-agonist, phenylephrine (PE) following recovery from catheterization of the descending aorta and right jugular vein. The increase in metabolism and body temperature during ISO was potentiated by the simultaneous administration of PE. Experiment 3 examined whether the $alpha_1$-mediated potentiation of ISO-induced thermogenesis was due to activation of specific receptors in thermogenic tissues, or a nonspecific pressor effect. Twenty-four male, cold acclimated, hypertensive or normotensive, restraint-adapted rats were chronically catheterized and tested as described for Experiments 1 and 2. Instead of infusing PE, the $alpha_2$-agonist guanabenz (GUANA) was administered along with ISO. Although GUANA and PE resulted in comparable pressor responses, the combined administration of ISO and GUANA failed to enhance metabolism or body temperature. Experiment 4 assessed whether the thermogenic potentiation in Experiment 2 could be reflected in heat escape responding. The animals were not restraint-adapted and had only their right jugular vein catheterized. Combined ISO and PE did not enhance the duration of heat escape responding above that observed during ISO. Thermobehavioral measures failed to translate the thermophysiological consequences of combined $alpha_1$- and $beta_1$-stimulation into differential thermoregulatory responses. Overall, both cold acclimation and 1K-GB hypertension increased baseline metabolism, perhaps due to a purported state of sympathoexcitation. Cold acclimation enhanced thermogenic reactivity to $beta_1$-stimulation but failed to alter alpha-reactivity.

Conversely, 1K-GB hypertension attenuated thermogenic responsivity to $beta_1$-stimulation, possibly due to a desensitization of the receptor during sympathoexcitation, but enhanced $alpha_2$-pressor reactivity. Few interactions of cold acclimation with 1K-GB hypertension were obtained. The results were further discussed in terms of underlying receptor and hormone mechanisms and sympathetic postganglionic activity.

The Influence of One-Kidney Goldblatt Hypertension and Cold Acclimation on Adrenergically-Induced Cardiovascular and Thermoregulatory Adjustments in Rats

Introduction

One model of body temperature regulation in endotherms suggests that this process involves three effector systems: metabolism, behavior and vasomotor state. One component of metabolism, facultative or nonshivering thermogenesis, involves the production and liberation of chemical energy, but does not involve shivering. Such metabolic adjustments may be evoked transiently by the acute exposure of an endotherm to either warm or cold ambient temperatures. However, chronic enhancement of metabolism is obtained by maintaining an animal in a cold ambient temperature for several weeks, a process regarded as cold acclimation. As early as 1956, Cottle and Carlson demonstrated that cold acclimation increased resting metabolic rate; whereas Jansky and Hart (1963) reported that cold acclimation increased the maximal metabolic response to acute cold exposure. Foster and Frydman (1978,1979) subsequently demonstrated that brown adipose tissue was the principal anatomical site of both the cold- and pharmacologically-induced increase in heat production in cold-acclimated rats. The brown adipose tissue-mediated nonshivering thermogenesis was presumably regulated through the local release of norepinephrine at sympathetic nerve endings and the predominant adrenergic receptor involved in the process was the $beta_1$ receptor (Depocas, Behrens, & Foster, 1978). However, more recent evidence suggests that $alpha_1$-receptors play a facilitative role in mediating brown adipose tissue thermogenesis.

For instance, Foster (1985) demonstrated that stimulation of $beta_1$-receptors with a thermogenic agent produced a large dose-dependent increase in oxygen consumption. However, when the $beta_1$-stimulation was combined with simultaneous $alpha_1$-excitation the increase in oxygen consumption was greater than that observed following $beta_1$- or $alpha_1$-stimulation alone. Hence, not only does cold acclimation increase metabolic responsiveness to $beta_1$-stimulation but there is strong evidence suggesting that the $alpha_1$-receptors are implicated in the nonshivering thermogenic response of cold-acclimated mammals. Thus, in response to both actue and chronic alterations in the ambient temperature, the metabolic rate of an endotherm is adjusted such that a normal body temperature is maintained.

The second component of this model of thermoregulation, behavior, is accomplished by both prepotent and learned operant responses. Weiss and Laties (1961) demonstrated that, when placed in a 2 ^{o}C environment, rats would press a lever in order to obtain warmth; whereas Lipton (1967) showed that when placed in a 43 ^{o}C, environment they would lever press to obtain cool air (Lipton, 1967). In both instances, the animals maintained a normal skin and core temperature. Behavioral thermoregulation may also be influenced through alterations in the metabolic component of this model. For example, Cox, Green, and Lomax (1975) observed that following the administration of a hypothermic agent, rats would increase their heat escape latency; whereas when treated with a hyperthermic agent the rats' heat escape latency decreased. Thus, rats defend a normal core temperature through behavioral means in response to external and

internal temperature challenges. Thermoregulatory behavior may also be altered through changes in the vasomotor state of the animal. For instance, Wilson and Fyda (in press) demonstrated that renovascular hypertensive rats, characterized by enhanced peripheral vascular resistance, exhibited augmented heat escape behavior when compared with normotensive controls. This finding may be accounted for by the impaired tissue conductance and hence, reduced thermolysis, which accompanied an increase in vasomotor tone. Thus, alterations in both metabolic rate and vasomotor tone are effective in evoking changes in thermoregulatory behavior such that a normal body temperature is defended.

The final component in the thermoregulatory model, the vasomotor state, involves the regulation of the temperature gradient between an animal and its environment. Numerous investigators have assessed the vasomotor consequences of exposing endotherms to varying ambient temperatures. For example, Berry, Montgomery, and Williams (1984) assessed tail blood flow in rats during a 30-min exposure to ambient temperatures ranging from 5 ^{o} to 40 ^{o}C. They found that when the ambient temperature was below 20 ^{o}C, vasoconstriction occurred abruptly in the tail vessels; whereas at ambient temperatures above 27 ^{o}C, tail vessels vasodilated. The vasoconstriction reduced tail blood flow and promoted heat retention; whereas the vasodilation promoted blood flow and facilitated heat dissipation. Although numerous investigations have assessed the vasomotor consequences of varying ambient temperatures, few researchers have examined the thermogenic consequences of chronic alterations in vasomotor tone. The most common method of evoking alterations in vascular resistance

is through the induction of hypertension. For instance, Fregly (1954) induced a renovascular form of hypertension and found that the survival rate of the rats was diminished when they were exposed for 4 hr to either a cold or hot environment. He posited that the reduced tolerance to a warm ambient temperature may reflect an impaired ability of the hypertensive animals to dissipate excess body heat due to their state of vasoconstriction. On the other hand, the reduced tolerance of hypertensive animals to a cold environment may be explained in terms of their state of hypothyroidism. Hypothyroid rats were reported to have a suppressed metabolic response to adrenergic stimulation (Fregly, 1975) and renovascular hypertensive has been associated with reduced beta-receptor responsivity. Since beta-receptor stimulation evokes thermogenesis, the hypertensive state may be secondarily associated with a reduced thermogenic responsivity to cold exposure. Thus, whereas on both sides of the thermoneutral zone there occur alterations in the vasomotor state which are designed to maintain a normal core temperature, experimentally-induced changes in vasomotor tone will alter thermoregulatory functioning such that the range of tolerable ambient temperatures is reduced. Thus, in general, the effector systems responsible for the maintenance of body temperature can operate independently of each other in response to alterations in ambient temperature. However, specific variations in either the metabolic or vascular components of this model may effect the functioning of the remaining variables, but in every instance, the operation of these variables serves to defend a normal body temperature.

One component of blood pressure control is total peripheral resistance. This is an index of vascular resistance to blood flow that reflects changes in blood vessel diameter. Accordingly, larger diameter vessels with low resistance facilitate blood flow; whereas smaller, high resistance vessels compromise blood flow (Levine, 1976). The diameter of the resistance vessels is under neurogenic control of sympathetic preganglionics. Through the local release of NE from sympathetic nerve endings, stimulation of $alpha_1$ and $alpha_2$ receptors on the resistance vessels will result in a dose-dependent vasoconstriction and an increase in total peripheral resistance (Hannah, Hamilton, & Reid, 1984).

In the absense of myocardial changes, a chronic increase in the vasomotor state of an animal produces arterial hypertension. However, arterial hypertension may be experimentally induced through several means, one of which involves the kidney and is called one-kidney Goldblatt (1K-GB) renovascular hypertension. One-kidney Goldblatt hypertension is characterized by a 22% increase in total peripheral resistance which asymptotes approximately 12-14 days after surgery (Bralet, Wepierre, & Bralet, 1973). This rise in total peripheral resistance was later demonstrated to reflect both an enhanced vascular contractility and a reduced threshold for vascular excitability following $alpha_1$-adrenergic stimulation (Gothberg, Hallback-Nordlander, Karlstrom, Ricksten, & Folkow, 1983). Investigators have also suggested that a sympathoexcitation could sustain, or at least participate in, the 1K-GB hypertension. For example, Tanaka, Seki, Fujii, Kurihara, and Ikeda (1982) observed an increase in cardiac NE turnover rate in 1K-GB hypertensives.

Similarly, Katholi, Winternitz, and Oparil (1982) demonstrated that 1K-GB animals had elevated plasma norepinephrine levels when compared to normotensive controls and that renal denervation reinstated normal blood pressure and plasma norepinephrine values. Thus, 1K-GB hypertension may be maintained, at least in part, through enhanced sympathetic activity, coupled with increased alpha-adrenergic sensitivity.

If one compares the processes involved in cold acclimation and 1K-GB hypertension, several similarities emerge. First, cold acclimation and 1K-GB hypertension may share vascular effector systems. The increased resistance of various vascular beds, characteristic of 1K-GB hypertension, may reduce heat dissipation by decreasing tissue conductance. The diminished tissue conductance may aid in the maintenance of a normal core temperature when an animal is housed at a low ambient temperature, as is the case during cold acclimation. Second, both cold acclimation and 1K-GB hypertension are characterized by enhanced sympathoexcitation, as reflected by elevated plasma norepinephrine levels and increased norepinephrine turnover in various organs such as the brown adipose tissue and the heart. Third, both cold acclimation and 1K-GB hypertension are associated with an enhanced adrenoceptor reactivity. Although both alpha- and beta- receptors are involved in cardiovascular regulation, only alpha-receptor responsivity has been reported to be enhanced in 1K-GB hypertension, whereas during cold acclimation, the beta-receptors have been demonstrated to be hyperreactive, with only recent reports suggesting a potentiating role for alpha-receptors. Despite the common features characterizing cold acclimation and

1K-GB hypertension, the interaction of these manipulations has received little systematic attention. Little is known about the effects of cold acclimation on the beta-adrenergic component of blood pressure maintenance or about the effects of 1K-GB hypertension on the alpha-adrenergic component of cold acclimation-induced thermogenesis. Thus, the purpose of this study was to assess whether 1K-GB renovascular hypertension influences the cardiovascular, thermophysiological, and thermobehavioral adjustments that accompany the thermogenic response of cold-acclimated rats during alpha-, beta-, or combined alpha- and beta-adrenoceptor stimulation.

Basic Components of Blood Pressure Regulation

Myocardial variables. The cardiovascular system is designed to accomplish two primary objectives: first, to supply oxygen and nutrients to all regions of the body and second, to maintain an adequate body temperature. To accomplish these objectives, blood must be actively distributed throughout the body, and the heart is responsible for distributing blood to various tissues and organs. The total amount of blood expelled by the heart per unit time is cardiac output (Levine, 1976). Two factors interact multiplicatively to regulate cardiac output: heart rate, the number of pulses per unit time, and stroke volume, the amount of blood ejected by each ventricle with each heart beat. Accordingly, if stroke volume is constant, cardiac output is proportional to alterations in heart rate (Smith & Kampine, 1984). For example, Vatner and Boettcher (1978) demonstrated that the heart rate, or chronotropic, consequences of an intravenous infusion of epinephrine resulted in a threefold increase in cardiac output in conscious rats. Thus, the myocardial component

of blood pressure regulation is comprised of cardiac output, which, in turn, is regulated to a large extent by heart rate.

Vascular variables. Total peripheral resistance, is comprised of resistance vessels--arteries and arterioles. These vessels are found in various vascular beds including cerebral, cardiac, muscular, visceral, renal, and cutaneous regions and each contributes to the sum total of peripheral resistance. Under conditions of exercise or thermal stress the vascular resistance in quadrupeds coordinates the distribution of blood flow to the brain, heart, and tissues acutely requiring oxygen, while it shunts blood away from tissues and organs not being used or less essential to the survival of the organism (Zelis, Flaim, Liedtke, & Nellis, 1981).

Adrenergic control of vasomotor tone. The distribution of blood flow to different vascular beds is largely controlled by sympathetic preganglionic motor neurons in the lateral horn of the spinal gray matter. Sympathetic activity may alter vasomotor tone, thereby contributing to either vasodilation or vasoconstriction of resistance vessels. The sympathetic postganglionic fibers exert their effects through the local release of norepinephrine at alpha- or beta-adrenergic receptors (Lands, Arnold, McAuliff, Luduena, & Brown, 1967). Bethelsen and Pettinger (1977) originally proposed that the alpha-adrenergic receptors may be subdivided into $alpha_1$-adrenoceptors, located postsynaptically on the vascular smooth muscle, and $alpha_2$-receptors, located presynaptically on peripheral sympathetic nerve endings or within the central nervous system. However, more recently, receptors with similar agonist/antagonist selectivity to the $alpha_2$-subtype have been identified

postsynaptically in the periphery (Hamilton & Reid, 1982).

The role of the alpha-adrenergic receptors in arterial blood pressure has been established at least since the early 1970s. For example, Clineschmidt, Geller, Govier, and Sjoerdsma (1970) investigated the reactivity of alpha-receptors to norepinephrine in the vascular smooth muscle of the rat. The aorta was removed, cut into strips, and placed in a muscle bath. The investigators then added graded doses of norepinephrine to the bath either alone or with the alpha-adrenergic blocking agent phentolamine. They found that the *in vitro* aortic strip preparation exhibited a dose-dependent contraction following norepinephrine administration and that this contraction could be prevented by pretreating the preparation with phentolamine. Norepinephrine was thus able to produce contraction of vascular smooth muscle through stimulation of alpha-adrenergic receptors.

Numerous studies have assessed the *in vivo* consequences of alpha-stimulation on mean arterial blood pressure (MABP) in rats. For example, Laverty, McGregor, and McQueen (1971) observed a 40% increase in MABP in response to and intravenous norepinephrine injection in conscious rats. However, given that norepinephrine can stimulate both alpha-and beta-adrenoceptors (Heymans and Neil, 1958) the rise in MABP could not be attributed to alpha-receptors alone. Accordingly, the investigators repeated the above procedure, except that 15 min following the norepinephrine administration they gave the rats a bolus intravenous injection of phenoxybenzamine, a potent alpha-receptor antagonist. The phenoxybenzamine reduced the increase in MABP (pressor) response to norepinephrine by more than 75%.

This reduction in MABP was thought to reflect a decrease in peripheral vascular resistance, since the latter diminished by 82% with no significant change in cardiac output. Laverty et al. (1971) concluded that the pressor reactivity accompanying systemic norepinephrine injections can be attributed to the activation of alpha-adrenergic receptors located on vascular smooth muscle.

Following the demonstration that alpha-adrenoceptors play a role in regulating vasomotor tone, Hannah, Hamilton, and Reid (1984) studied the pressor contribution of alpha-receptor subpopulations. They found that $alpha_1$- and $alpha_2$-adrenoceptor agonists given intravenously to conscious rabbits produced a dose-dependent pressor response. The response to the $alpha_1$-agonist phenylephrine was significantly attenuated by pretreatment with prazosin, an $alpha_1$-antagonist. Similarly, the pressor response to the $alpha_2$-agonist guanabenz was significantly reduced by pretreatment with idazoxan, an $alpha_2$-antagonist. Thus, peripheral stimulation of either $alpha_1$- or $alpha_2$-adrenoceptors elevated MABP, whereas inhibition of both alpha-receptor subtypes attenuated MABP.

Beta-adrenoceptors have also been implicated in regulating cardiovascular processes. Lands, Luduena, and Buzzo (1967) proposed the existence of two types of beta-adrenoceptors. They found that $beta_1$-receptors are located predominantly on the myocardium, although some are present on the vasculature, while $beta_2$-receptors are present on the vascular smooth muscle. Buckley et al. (1984) monitored the myocardial and vascular effects of an intravenously administered beta-agonist, isoproterenol, in swine. They found that it produced a 21% increase in heart rate (tachycardia) and a 19%

decrease in vascular resistance. The net effect was an 8% decrease in MABP. Moreover, the fact that the myocardial responses were due to $beta_1$-receptor activation was suggested by the decrease in heart rate (bradycardia) which followed $beta_1$-receptor blockade with practolol. Similarly, the finding that $beta_2$-receptor blockade with butoxamine resulted in an attenuation of the vasodilatory response supported the notion that the vasculature contains $beta_2$-receptors. Similar results had been obtained by Rockson, Homcy, Quinn, Manders, Haber, and Vatner (1981) in the dog. Thus, it appears that in quadrudpeds, isoproterenol administration increases myocardial, but decreases vascular activity through $beta_1$-and $beta_2$-receptor activation, respectively, with the net effect being a small decrease in MABP.

Sympathetic control of the cardiovascular system is centrally regulated predominantly by the baroreceptor reflex arch. The majority of baroreceptors are located in the carotid sinus and aortic arch. From here, primary baroreceptor afferents course through the glossopharyngeal and vagal nerves to synapse in the medial and commissural division of the Nucleus Tractus Solitarius (Palkovits, Mezey, & Zaborszky, 1979). Efferent fibers from the Nucleus of the Solitary Tract synapse in numerous medullary and supramedullary regions; however, for purposes of the present discussion, only two medullary regions will be discussed. One region which receives cardioinhibitory fibers from the Nucleus of the Solitary Tract is the Dorsal Motor Nucleus of the Vagus. From this area inhibitory fibers course through the vagal nerve to innervate the heart (Loewy & Neil, 1981; Spyer, 1981). The second area onto which efferent

fibers from the Nucleus Tractus Solitarius synapse is the A5 region. The A5 cell group has been shown to provide a dense noradrenergic input to the intermediolateral cell column between the first thoracic and second lumbar segments of the spinal cord (Loewy & McKellar, 1980; Loewy, McKellar, & Saper, 1979; Loewy & Neil, 1981). These noradrenergic efferents synapse on the preganglionic sympathetic fibers which, in turn, terminate on the postganglionic neurons which constitute the final stage in the baroreceptor reflex arch (Palkovits & Zaborszky, 1977). The postganglionic neurons, in turn, synapse on the vascular end organ of the cardiovascular system.

Baroreceptors code to the intensity of any mechanical deformation of the vasculature caused by a change in MABP (Kirchheim, 1976; Szulczyk & Trezebski, 1977). For instance, both common carotid occlusion distal to the site of the receptors and the administration of pressor agents increase the firing rate of the primary baroreceptor afferents (Spyer, 1981). The increased firing rate stimulates both the Nucleus of the Solitary Tract and A5 region (Neil & Loewy, 1981), and this, in turn, lowers vasomotor tone through a reduction in sympathetic preganglionic activity (Faiers, Calaresu, & Mogenson, 1975). Concurrently, the increased firing rate of the primary baroreceptor afferents stimulates the activity of the Dorsal Motor Nucleus of the Vagus (Nosaka, Yamamoto, & Yasunaga, 1979) which, increases the inhibitory action of the vagal nerve on the heart and produces a bradycardia (DeJong, Zandberg, & Bohus, 1975). The net effect of the baroreceptor reflex is to diminish MABP, given that the initial stimulus was an increase in MABP. Conversely, both common carotid occlusion proximal to the site of the receptors and

the administration of a depressor agent decrease the firing rate of the primary baroreceptor afferents (Spyer, 1981). The reduced firing rate inhibits both the Nucleus of the Solitary Tract and A5 region (Neil & Loewy, 1981), and this augments vasomotor tone through an elevation in sympathetic preganglionic activity (Faiers et al., 1975). Concurrently, the decreased firing rate of the primary baroreceptor afferents inhibits the activity of the Dorsal Motor Nucleus of the Vagus (Nosaka et al., 1979) which disinhibits the action of the vagal nerve on the heart and thus, results in a tachycardia (DeJong et al., 1975). The net effect of the baroreceptor reflex is to increase MABP.

One-Kidney Goldblatt Renovascular Hypertension

Induction and cardiovascular characteristics. Several models of experimental hypertension have been developed to assess the renal contribution to hypertension. One model was developed by Goldblatt, Lynch, Hanzal, and Summerville (1934), who found that chronic hypertension in dogs accompanied bilateral renal artery constriction. This model provided an easy and reliable method of simulating clinical renovascular hypertension. Since then, the techniques used to experimentally induce renovascular hypertension involve decreasing blood flow to one kidney with either the contralateral kidney removed (Goldblatt I or 1K-GB hypertension) or left intact (Goldblatt II or 2K-GB hypertension) (Leenen & DeJong, 1971).

Ledingham and Cohen (1964) found that 1K-GB hypertension in rats is accompanied by a transient elevation in cardiac output lasting five to seven days. Richardson, Fermoso, and Guyton (1964) proposed

that nephrectomy (excision of one kidney) and contralateral renal artery stenosis (reduced blood flow) promotes retention of sodium and water in rats. The resulting increase in blood volume elevates mean venous filling pressure, which accounts for the increase in cardiac output. Although these myocardial changes appear to be transient in nature, an increase in peripheral vascular resistance develops more slowly 12-14 days after surgery but persists for the duration of the preparation. Bralet, Wepierre, and Bralet (1973) reported that 1K-GB hypertension in rats is characterized by a 25% increase in MABP and a 22% elevation in peripheral vascular resistance. They also found that; whereas changes in regional circulation to muscle, brain, and heart did not contribute to the overall rise in peripheral resistance, vascular resistance in the splanchnic and abdominal skin regions increased by 31% and 50%, respectively, along with a 20% and 30% decrease in blood flow. Thus, the overall increase in peripheral resistance found in the 1K-GB hypertensive rat was felt to reflect regional changes in vasomotor state, with the splanchnic organs and skin regions accounting for over 80% of the total increase in peripheral resistance.

Such changes in vascular resistance accompanying 1K-GB hypertension could reflect either a lowered threshold for vascular excitability or an increase in vascular contractility. To dissociate the contribution of these processes, Gothberg, Hallback-Nordlander, Karlstrom, Ricksten, and Folkow (1983) assessed vascular sensitivity and force of contraction following graded infusions of NE in anesthetized, 1K-GB hypertensive rats. Vascular resistance was observed to increase by more than 60%. Moreover, the maximal

pressor response to alpha-adrenergic stimulation was increased by 35% for the hypertensive animals, while the threshold for alpha-stimulation was lowered by 25%. Accordingly, the increase in vascular resistance characteristic of 1K-GB hypertension is due to both enhanced vascular sensitivity, as reflected by the lowered excitation threshold, and a heightened contractility, as indicated by the increased pressor response to the same dose of norepinephrine when compared to the normotensive, nephrectomized controls.

Investigators have also attempted to identify the pathogenic mechanism(s) that account for the 1K-GB hemodynamic profile. For instance, Laragh, Sealey, Niarchos, and Pickering (1982) assessed the relative contribution of Angiotensin II, a pressor agent, and sodium balance in the 1K-GB hypertensive rat. Two weeks following the induction of 1K-GB hypertension, the rats were injected with an Angiotensin II blocker, Saralasin. Consistent with previous findings, Saralasin had no effect on MA BP, indicating that the hypertension was not Angiotensin II dependent. The 1K-GB hypertensive rats were then maintained for 4 weeks on a stringent dietary sodium restriction. During the initial stages of sodium deprivation, MABP decreased significantly, indicating that the 1K-GB form of hypertension was sodium dependent. However, over the course of the sodium deprivation the hypertension was gradually reinstated. Administration of Saralasin was now effective in reducing MABP to normal levels. Thus, with free access to sodium, the 1K-GB model of hypertension is basically sodium-volume dependent. However, when the volume support is removed through sodium deprivation, the hypertension becomes renin-angiotensin dependent. Renin-angiotensin

secretion, originally suppressed by the sodium-induced volume excess, rises markedly to replace the volume support and keep the MABP elevated. This suggests that if both the Angiotensin II and the volume factors could be blocked, then the development of 1K-GB hypertension would be prevented. However, Freeman, Davis, and Seymour (1982) found that rats maintained on a sodium-restricted diet and continuously infused with Saralasin still developed unabated hypertension, with only a 4 to 5 day delay in onset. Hence, although the renin-Angiotensin II and sodium-volume factors may play a transient, perhaps initiating role in the development of 1K-GB hypertension, the mechanisms governing its maintenance remain to be elucidated.

Peripheral neurogenic characteristics of 1K-GB renovascular hypertension. Given that 1K-GB hypertension develops in the absence of either Angiotensin II or sodium-water retention, the contribution of other factors was implicated. Reid, Dargie, Franklin, and Fraser (1977) had shown that the induction of 1K-GB hypertension in rats, increased circulating levels of plasma norepinephrine. This finding suggested a potential neurogenic contribution. Waeber et al. (1981) assessed the possibility of a sodium-induced activation of neurogenic mechanisms in 1K-GB hypertension. They examined the influence of a norepinephrine synthesis blocker, SK&F 64139, on the development of the hypertension, while they maintained the rats on either a regular or a low salt diet. The SK&F 64139 induced a more pronounced MABP decrease in the hypertensive rats maintained on a regular salt diet than those on a low salt diet. These observations indicated that in the 1K-GB hypertensive rat normal dietary sodium increases the

sympathetic role in blood pressure maintenance.

Tanaka, Seki, Fujii, Kurihara, and Ikeda (1982) investigated the rate of cardiac norepinephrine turnover during the development of 1K-GB hypertension in rabbits. Norepinephrine turnover in any tissue is defined as the rate of norepinephrine removal or renewal in the sympathetic nerve endings and its measurement commonly involves the intravenous injection of radioactively-labelled norepinephrine. The labelled norepinephrine rapidly equilibrates with the endogenous norepinephrine present in the nerve endings and since both norepinephrine components are released upon sympathetic stimulation, the rate of disappearance of labelled norepinephrine from a particular tissue is a measure of sympathoexcitation. Tanaka et al. (1982) found that cardiac norepinephrine turnover in the 1K-GB hypertensive rabbit was increased relative to normotensive control values, thereby supporting the idea than an increased sympathetic drive could sustain, or at least participate in, the 1K-GB hypertension.

Although enhanced sympathetic activity may play a role in 1K-GB hypertension, the specific nature of its contribution and the way it is expressed remains speculative. Katholi, Winternitz, and Oparil (1982) examined the effects of renal denervation or unclipping on the peripheral sympathetic postganglionics in 1K-GB hypertensive rats. Two weeks following the induction of 1K-GB hypertension, the animals underwent peripheral renal denervation, sham denervation, or unclipping. The 1K-GB hypertension elevated plasma norepinephrine levels relative to normotensive controls. However, renal denervation decreased systolic blood pressure; whereas unclipping the renal

artery reinstated MABP to normal levels. Moreover, both renal denervation and unclipping restored plasma norepinephrine to normal levels. Sham denervation in the 1K-GB animals had no effect on MABP or plasma norepinephrine levels. Katholi et al. (1982) concluded that the blood pressure lowering (depressor) effect of renal denervation or unclipping reflected the decline in plasma norepinephrine levels. Accordingly, the elevation of plasma norepinephrine observed in the 1K-GB hypertensive rat was felt to be secondary to the increased sympathetic neuronal activity which, in turn, is implicated in the maintenance of 1K-GB hypertension.

Thermoregulation

It is through the regulation of blood pressure that the circulation is capable of dissipating excess body heat, and thus controlling body temperature. The concept of set point is often used to describe the regulated body temperature. The set point is that temperature (or range of temperatures) around which the animal attempts to regulate its body temperature (Lipton, 1979). According to set-point theory of body temperature regulation there are four categories of body temperature: (a) normothermia-where set point and actual body temperature are essentially the same; (b) hypothermia-where set point may or may not be normal but actual body temperature is below this set point; (c) hyperthermia-where set point may or may not be normal but actual body temperature is higher than this set point; and (d) fever-where set point is raised and deep body temperature may or may not be raised to the same level (Kluger, 1979). When sensory inputs from the skin and core indicate that body temperature is greater than the set-point temperature, the animal

initiates effector responses which lower body temperature. Conversely, when the body temperature is lower than the set-point temperature, the effector responses tend to raise body temperature toward the set point.

Metabolic processes in thermoregulation. The metabolic component of thermoregulation is a chemical form of heat production which may be divided into two components. The first component consists of obligatory or basal metabolism, which is the rate of heat production seen when a fasted endotherm is resting in a thermoneutral environment. It serves to maintain the basic energy demands of the organism and is generally unresponsive to changes in the environment. The second component consists of facultative or regulatory nonshivering thermogenesis. This is defined as that increase in metabolic rate from the basal state seen when an endotherm is exposed to temperatures below thermoneutrality, when activity increases, or when food is ingested (Jansky, 1973). It is facultative thermogenesis, which allows the organism to regulate its body temperature, and which will be the focus of the remainder of this section.

Facultative nonshivering thermogenesis may be evoked by exposing an endotherm to a cold ambient temperature and is characterized by an increase in oxygen consumption and carbon dioxide production. Cottle and Carlson (1956) demonstrated that when the ambient temperature was decreased from 30 to 10 ^{o}C, rats increased their oxygen consumption within 10 min. The rise in oxygen consumption asymptoted within 40 min after the start of cooling and was 20% greater than control values. Hsieh, Carlson, and Gray (1957) increased the severity of

the ambient temperatures to 5 °C and found that rats, which were prevented from shivering through the administration of curare, a nicotinic neuromuscular blocker, still exhibited an elevation in oxygen consumption within 10 min of cold exposure. The maximal rise in oxygen consumption was 25% greater than that of control values and during the 90-min exposure to 5 °C the rats maintained a normal core temperature of 36.5 °C. Thus, when exposed to moderately cold ambient temperatures, rats were able to maintain an adequate core temperature primarily by increasing nonshivering thermogenesis. Jansky and Hart (1963) then examined both the latency and duration of nonshivering thermogenesis in rats exposed to a potentially lethal ambient temperature, -20 °C. A rapid rise in oxygen consumption was sustained for approximately 120 min, along with the maintenance of a normal core temperature. However, if exposure to -20 °C was prolonged to 200 min, all animals succumbed to hypothermia. It appears that rats housed in a normal ambient temperature are capable of rapidly evoking nonshivering thermogenesis upon cold exposure and of sustaining this increase for variable durations depending on the extent of the thermal challenge.

To assess the adaptive processes involved in chronic exposure to moderate cold, cold acclimation procedures are often employed. Cold acclimation commonly involves maintaining endotherms at an ambient temperature of about 5 °C for 3 to 4 weeks. Cottle and Carlson (1956) were among the first investigators to assess the metabolic effects of cold acclimation in rats. These investigators found that the resting metabolic rate of these animals in an ambient temperature of 30 °C was 20% greater than that of the warm-acclimated rats at

the same temperature. When the room temperature was lowered from 30 °C to 10 °C, the cold-acclimated rats increased their oxygen consumption within 10 min and reached an asymptote within 60 min. The maximal elevation in oxygen consumption obtained in the cold-acclimated animals at 10 °C was 100% greater than that seen at 30 °C and was 64% greater than that observed in the non-cold-acclimated animals in the cold ambient temperatures.

Jansky and Hart (1963) examined whether the mortality rate seen in warm-acclimated rats exposed to -20 °C could be reduced by prior cold acclimation. Rats were housed for 3 weeks at 6 °C and then subjected to gradually diminishing temperatures. Whereas all warm-acclimated animals succumbed to hypothermia following 200 min at -20 °C, the cold-acclimated rats not only survived but sustained a 62% elevation in oxygen consumption relative to warm-acclimated animals and thereby prevented hypothermia. Comparable mortality rates in the cold-acclimated rats within an equivalent time span could be obtained only when the ambient temperature plummeted to -40 °C. These findings suggest that cold acclimation increases both resting metabolic rate and metabolic responsiveness to acute cold exposure, resulting in better survival rates in cold environments. Thus, nonshivering thermogenesis may act as an acute or chronic mechanism to enhance survival when the environmental temperature falls below the thermoneutral zone.

The role of brown adipose tissue in nonshivering thermogenesis. Numerous researchers have investigated the nature of one of the underlying effector systems in nonshivering thermogenesis, brown adipose tissue. This is a highly vascularized, large cell-single

nucleus tissue, comprising only 1 to 5% of body weight in cold-acclimated animals and is located in interscapular, axillary, and paraspinal regions and within the thoracic and abdominal cavities (Nedergaard & Lindberg, 1982). Foster and Frydman (1978, 1979) were among the first to provide firm evidence that brown adipose tissue was the predominant effector organ involved in nonshivering thermogenesis. In their studies, blood flow, cardiac output, and fractional distribution of cardiac output to various brown adipose tissue locations, along with arterio-venous oxygen differences and oxygen consumption were assessed in cold- and warm-acclimated rats during norepinephrine- and cold-induced thermogenesis. When cold- and warm-acclimated rats were compared at rest, fractional distribution of cardiac output to brown adipose tissue, brown fat blood flow, arterio-venous oxygen difference across brown adipose tissue, and oxygen consumption was was greater for the cold- than for the non cold-acclimated rats. When the animals were compared during norepinephrine infusions and during cold exposure, the magnitude of the change in these variables increased, however, the rise was greater for the cold-acclimated than in the non cold-acclimated animals. The investigators correlated the increase in blood flow to brown adipose tissue with the rise in arterio-venous oxygen difference across brown adipose tissue and found a linear correlation coefficient, r = .97. Since, the rise in blood flow to brown fat is very closely correlated with an elevation in oxygen consumption of brown fat and it exhibited nearly a 50 fold increase in blood flow, the investigators suggested that the oxygen consumption of brown adipose tissue contributes 60% or more to the thermogenic response

of cold-acclimated rats to norepinephrine and between 65-80% to the total increase in heat production of cold-exposed, cold-acclimated rats. Thus, brown adipose tissue constitutes the principal anatomical site of the cold- and norepinephrine-induced increase in heat production in cold-acclimated rats, as well as making a significant contribution to the thermogenesis seen in warm-acclimated rats.

The role of the sympathetic nervous system in nonshivering thermogenesis. The finding that cold acclimation increased metabolic heat production provided an impetus for understanding its neurogenic control. Hsieh et al. (1957) showed that cold-acclimated, curarized rats exposed to an ambient temperature of 5 ^{o}C exhibited an increase in oxygen consumption sufficient to maintain a normal core temperature. However, sympathetic ganglionic blockade with hexamethonium lowered oxygen consumption to values 30% below those observed at 30 ^{o}C, led to a progressive hypothermia, and blocked or diminished the initial rise in oxygen consumption seen upon cold exposure. This finding suggested that the cold-induced increase in nonshivering thermogenesis was sympathetically-mediated. Other studies have yielded results consistent with this interpretation. For instance, cold exposure has been shown to elevate urinary norepinephrine levels in rats (Leduc, 1961), plasma norepinephrine levels in dogs (Bergh, Hartley, Landsberg, & Ekklom, 1979; Therminarias, Chripaz, & Tanche, 1979), and preganglionic sympathetic nerve discharge in cooled rabbits (Iriki, Riedel, & Simon, 1971). More recently, Young, Saville, Rothwell, and Stock (1982) examined the changes in norepinephrine turnover in brown adipose tissue and

heart of rats following acute or chronic cold exposure. Exposure to 4 °C for 6 hr doubled the rate of norepinephrine turnover in the heart and led to a 4- to 12-fold potentiation of norepinephrine turnover in brown adipose tissue, relative to control values for rats maintained at 22 °C. However, exposure to 4 °C for 9 days prior to the turnover test resulted in a 105% rise in cardiac norepinephrine turnover and a 109% increase in BAT norepinephrine turnover, when compared to controls. Thus, either acute or chronic exposure to a cold ambient temperature is capable of elevating sympathetic activity, with the magnitude of the increase being proportional to the duration of cold exposure.

Because it seemed reasonably established that cold-induced increases in nonshivering thermogenesis are sympathetically mediated, Depocas, Behrens, and Foster (1978) evaluated the interrelation of arterial plasma norepinephrine concentration and the thermogenic response in warm (28 °C) and cold (6 °C) acclimated rats. Norepinephrine was infused intravenously at doses ranging from 2.5 to 10.0 ng/min/g·[74] until a steady state plasma level was obtained. Then oxygen consumption, rectal temperature, and plasma norepinephrine concentration were determined for 30 min. Stimulation of sympathetic noradrenergic receptors produced a dose-dependent facilitation of thermogenesis and rectal temperature. Specifically, while norepinephrine infusions increased oxygen consumption, the elevation seen in the cold-acclimated animals was 3 times that seen in the warm-acclimated rats. Rectal temperature began to rise in both groups within 10 min of the start of the norepinephrine infusion and by 27 min the rectal temperature of the cold-acclimated animals

was 2.2 ^{o}C greater than that of the warm-acclimated rats (39.2 vs. 37.0 ^{o}C). Thus, although noradrenergic stimulation could induce thermogenesis, the specific nature of the receptor contribution remained unclear.

To elucidate the receptor characteristics of brown adipose tissue-mediated nonshivering thermogenesis, Flaim, Horwitz, and Horowitz (1977) conducted a three-part study examining the effects of alpha- and beta-adrenergic agonists and antagonists on interscapular brown fat temperature in cold-acclimated rats. In the first part brown adipose tissue temperature was measured in response to intravenously administered phenylephrine and isoproterenol, alpha- and beta-adrenergic agonists, respectively. Both adrenergic agonists elicited a biphasic temperature response comprised of an initial drop within 1.5 min, superceded by a rise that peaked within 4 min and lasted for 5 min. The dose-dependent nature of the hypothermia accompanying phenylephrine administration presumably reflected alpha adrenoceptor-induced vasoconstriction of interscapular brown adipose tissue. This would jeopardize the delivery of oxygen needed for metabolic heat production. In contrast, the dose-dependent nature of isoproterenol-induced hyperthermia indicates a beta-receptor contribution. The specific process contributing to this increase remains speculative, but it appeared to reflect beta-receptor-induced thermogenesis. In the second part, brown adipose tissue temperature response to electrical stimulation of the nerves supplying the left interscapular brown fat pad was assessed in the presence of the alpha- and beta-adrenergic antagonists, phentolamine and propranolol. Electrical stimulation produced a biphasic temperature

response similar to that induced neuropharmacologically. Intravenous administration of phentolamine buffered the amplitude of both phases of the temperature response, while propranolol diminished only the hyperthermia. The fact that beta-blockade had little effect on the first, hypothermic, phase of the temperature response supported the earlier contention that it was alpha-adrenergically mediated. However, an alpha-receptor participation in the subsequent temperature rise was suggested by the observation that both alpha- and beta-blockade were effective in attenuating this response. In the last part of the Flaim et al. (1977) study, brown adipose tissue temperature was examined in response to a combined phenylephrine and isoproterenol administration. The results showed that the initial temperature drop was approximately 40% less than what one might anticipate from alpha-adrenoceptor stimulation alone. Moreover, the amplitude of the subsequent temperature rise was nearly 40% greater than the sum of the thermogenic effects elicited separately by the alpha-and beta-agonists. This apparent synergism in the temperature rise following combined alpha- and beta-receptor stimulation may indicate a role for alpha-receptors in what is typically thought of as a beta-receptor phenomenon.

More recently, Foster (1985) conducted several experiments identifying the adrenergic receptors contributing to neuropharmacologically-induced alterations in oxygen consumption of anesthetized, cold-acclimated rats. The first experiment examined the influence of selective $alpha_1$- and $alpha_2$-antagonists on the thermogenic response to intravenously infused norepinephrine. The $alpha_1$-antagonist, prazosin, was found to inhibit the

norepinephrine-induced increase in oxygen consumption by almost 50% as compared to the 24% inhibition induced by the $alpha_2$-antagonist, yohimbine, suggesting that the contribution of $alpha_2$-receptors was negligible. The second experiment was designed to determine whether the inhibition of norepinephrine-induced thermogenesis by prazosin was related to its selective affinity for $alpha_1$-receptors. Foster reasoned that if this were the case then prazosin would not only dampen norepinephrine thermogenesis by competitive inhibition of $alpha_1$-receptors, but the thermogenesis would be reinstated in the presence of renewed selective $alpha_1$-stimulation. Thus, once prazosin had suppressed the norepinephrine-induced increase in oxygen consumption, phenylephrine, an $alpha_1$-agonist, was administered intravenously. As anticipated, the prazosin-induced thermogenic inhibition was reversed by phenylephrine, thereby reinforcing the hypothesis that $alpha_1$-stimulation played at least a supportive role in brown adipose tissue thermogenesis. Foster (1985) also suggested that another way of altering the alpha-adrenoceptor participation in the adrenergic activation of brown adipose tissue thermogenesis was to test the prediction that activation of beta-receptors with isoproterenol would be an insufficient stimulus for a full thermogenic response unless alpha-receptors were also simultaneously stimulated. Hence, he conducted two additional experiments. The third experiment assessed the oxygen consumption response to beta-receptor stimulation with isoproterenol alone. Intravenous administration of isoproterenol in cold-acclimated rats produced a large dose-dependent increase in oxygen consumption. This, in conjunction with Nedergaard and Lindberg's (1982)

observation of a high density $beta_1$-receptor population on brown fat cells, suggested a major thermogenic role for $beta_1$-receptor stimulation of brown adipose tissue. The fourth, and final, experiment assessed the oxygen consumption response to combined alpha-and beta-receptor stimulation. The results showed that simultaneous administration of phenylephrine and isoproterenol greatly enhanced oxygen consumption relative to the response obtained with either pharmacological agent alone. This enhanced thermogenesis supported Foster's contention that the rise in oxygen consumption normally seen following selective beta-stimulation was not maximal unless the $alpha_1$-receptors were simultaneously stimulated. Thus, while activation of the brown adipose tissue-$beta_1$-adrenoceptors is the predominant effector mechanism by which thermogenesis is elicited, the simultaneous activation of $alpha_1$-receptors not only plays a supportive role, but also exerts an important potentiating effect.

Vascular processes in thermoregulation. In addition to metabolic processes involved in thermoregulation, changes in environmental temperature may alter the animal's cardiovascular processes. The vascular component of circulatory assisted heat transport controls the temperature gradient between the animal and its environment (Richards, 1973). The amount of heat that may be dissipated from the animal depends thermal conductance, or the amount of heat flow across a tissue per unit difference between core and skin temperature. Accordingly, vasoconstriction is related to a reduction in blood flow, in tissue conductance, and in the amount of heat dissipated from the animal; whereas vasodilation is associated with an increase

in blood flow, in tissue conductance, and in the amount of heat lost to the environment (Richards, 1973).

Several studies have investigated regional differences in vasomotor state and, hence, tissue conductance, in endotherms placed in varying environmental temperatures. Rand, Burton, and Ing (1965) provided some insight into the critical contribution of the rat's tail for temperature-induced alterations in the vasomotor state. They used rats acclimated to either 11 ^{o} or 20 ^{o}C for 4 weeks and measured tail blood flow and heat loss upon exposure to environmental temperatures between 17 ^{o} to 33 ^{o}C. The results showed that between 27 ^{o} to 30 ^{o}C an abrupt vasodilation occurred which allowed for a 15-20 fold increase in blood flow when compared to that seen at lower temperatures and a marked elevation in heat loss that was proportional to the blood flow. The heat loss accompanying vasodilation was about 60 cal/min/100 ml of tail or about 4.7 cal/min. Since the metabolic rate of the rat at 33 ^{o}C is about 28.5 cal/min, in warm surroundings the tail is able to dissipate approximately 17% of the total heat production through a vasodilation-induced increase in thermal conductivity.

More recently, Raman, Roberts, and Vanhuyse (1983) studied the thermal control of tail blood flow in restrained rats. The tail was maintained at temperatures varying from 15 to 42 ^{o}C, while body temperature was cyclically cooled and warmed to 35 or 40 ^{o}C, respectively. During this time, tail blood flow was monitored and the value of R, the ratio of thermal conductance to tail blood flow which indicates the amount of heat exchanged between the tail and the environment, was calculated. They found that during increases in

body temperature, tail blood flow rose in proportion to the rise in rectal temperature; however, the absolute blood flow depended on tail temperature. Moreover, the value of R was dependent on tail temperature but was independent of rectal temperature. At a tail temperature of 20 ^{o}C, the mean value of R is small, about .27. Since a small R value indicated that only a small amount of the heat brought to the tail is being transferred to the environment, only a small portion of the venous return was apparently shunted through the superficial veins. At 30 ^{o}C, the R equalled .45, suggesting a progressively increasing superficial venous flow. At 42 ^{o}C, the R was much higher, .92, indicating that a very high proportion of the venous flow was distributed to the superficial vessels. Thus, redistribution of blood flow within the rat tail varies with the tail temperature and secondarily with temperatures elsewhere within the core of the rat. At low tail temperatures most of the venous blood is shunted to deep veins, reducing tail heat transfer, while at higher tail temperatures, more of the venous blood travels in superficial veins, tending to increase tail heat transfer. The tail then is capable of altering both its vasomotor state and its blood flow distribution such that the amount of heat dissipated is appropriate for the specific ambient temperature.

Berry, Montgomery, and Williams (1984) quantified the cutaneous vasomotor and thermoregulatory responses in anesthetized rats over a wide range of environmental temperatures. Tail blood flow and tail skin and core temperatures were monitored in response to a 30 min exposure to one of five ambient temperatures ranging from 5 ^{o} to 40 ^{o}C. They found that all indices of thermoregulation were at a

minimum in rats exposed to an ambient temperature of 5 °C and at a maximum when exposed to 40 °C. Thus, a general increase in tail blood flow and in tail skin and core temperatures was observed with progressive elevations in the ambient temperature. The investigators concluded that as the ambient temperature falls below 20 °C, vasoconstriction occurs abruptly in the tail vessels. This reduces tail temperature and blood flow, diminishes thermal conductivity, and, thus, promotes heat retention. On the other hand, at ambient temperatures between 27 ° and 30 °C, vasodilation occurs abruptly in the tail vessels. This elevates tail temperature and blood flow, enhances thermal conductivity, and facilitates heat dissipation.

Given that the vasomotor state of an endotherm is controlled by sympathetic preganglionics, the possibility that adrenergic stimulation may affect the vascular adjustments to thermal challenges was examined. Fregly, Field, Nelson, Tyler, and Dasler (1977) examined the vascular responsiveness of cold-acclimated rats to specific alpha-and beta-adrenergic agonists. Rats were housed for 8 weeks at either 5 °C or housed at 25 °C. Twenty-four hours prior to the testing, all animals were removed from their home cages and allowed to rest undisturbed in a 25 °C room, during which time baseline measures of tail skin and core temperature were obtained. Isoproterenol (beta-agonist) or phenylephrine (alpha-agonist) was then administered subcutaneously, and tail skin and core temperatures were measured for an additional 2 hr. The lowest dose of isoproterenol, 28 ug/kg, increased tail skin temperature about 1.5 ° and 3 °C in the control and cold-acclimated rats, respectively, while core temperature in both groups rose only

slightly. The highest dose of isoproterenol, 139 ug/kg, produced a marked elevation in both tail skin (7.5 ^{o} vs. 9 ^{o}C) and core (.5 ^{o} vs. 2 ^{o}C) temperatures in the control versus cold-acclimated rats. Conversely, the alpha-agonist phenylephrine had no effect on the tail skin or core temperature of either the cold-acclimated or non cold-acclimated rats. Thus, cold acclimation enhanced beta-receptor responsiveness to isoproterenol, as reflected by the greater increase in core temperature, and by the larger rise in tail skin temperature.

Since various investigators have demonstrated that the vascular processes involved in both hypertension and thermoregulation are under control of the beta-adrenergic receptors, Katovich, Fregly, and Barney (1978) assessed the responsiveness of tail skin temperature to beta-adrenergic stimulation in renal hypertensive and normotensive rats. Eleven weeks after the induction of 1K-Grollman hypertension, a renovascular model similar to the 1K-GB form, the animals were injected subcutaneously with varying doses of isoproterenol. Measurements of core and tail skin temperature were made for an additional 2 hr. The lowest dose of isoproterenol administered to either the hypertensive or normotensive control animals, 15 ug/kg, increased both core and tail skin temperatures above the pretreatment level. However, the rise in tail skin temperature was significantly greater in the normotensive than in the hypertensive group. For the normotensive rats the highest dose of isoproterenol, 45 ug/kg, produced a 6.3 ^{o}C elevation of tail skin temperature above predrug levels; whereas for the hypertensive animals, an increase of only 4.3 ^{o}C occurred. Core temperature did not differ between the

groups but did rise upon administration of isoproterenol. At all doses of isoproterenol, the rise in tail skin temperature in the renal hypertensive group was significantly attenuated with respect to that of the normotensive control group. Katovich et al. (1978) speculated that the reduced vascular responsiveness of the tail to isoproterenol in the hypertensive animals may reflect either a reduction in beta-adrenergic responsiveness or a competing alpha-adrenergic reactivity characteristic of one-kidney renovascular hypertension (Gothberg et al., 1983). In any event, a reduced beta-responsiveness would constitute possible impairment of the vascular mechanisms contributing to thermoregulation in hypertensive animals.

Behavioral thermoregulation. The final component of the present model of body temperature control is comprised of behavior. Behavioral thermoregulation consists of numerous motor responses designed to maintain a normal core temperature in the face of alterations in the ambient temperature. In addition to prepotent thermoregulatory behaviors which sustain body temperature, animals may be trained to perform an operant task in order to obtain thermal reinforcement. During the last 20 years operant behavioral methods have been extensively used as a means of assessing temperature regulation. In the 1950s it was observed that when animals were exposed to hot temperatures their work rate for a cold temperature increased; while, conversely, exposure to cold elevated their work rate for heat (Carlton & Marks, 1958; Weiss, 1957). In more recent years, operant behavioral methods have been combined with artificial displacements of local temperatures of various thermosensitive sites in the body and with a variety of nonthermal stimuli, including the

administration of pharmacological agents that influence thermoregulation.

In one of the earliest studies which employed operant thermoregulation, Weiss and Laties (1961) examined the relationship between body temperature and a response which provided a peripheral source of heat to the skin. In the first experiment, shaved rats that had been maintained at 25 °C were placed in a chamber cooled to 2 °C. Inside the chamber was a lever which, when pressed, would turn on a heat lamp for 5 s. After approximately 5 hr in the 2 °C chamber the rats began to press the lever at a steady and substantial rate, which they maintained for the duration of the experiment. The investigators suggested that this operant behavior was evoked when a certain lower body temperature had been reached. In the second experiment they examined whether the decline in body temperature was the reason for the long response latency. Fourteen pairs of shaved rats were used: Half were preexposed to 5 °C for 5 hr, while the remainder were kept at 25 °C. All rats were then subjected to 2 °C for 16 hr and lever pressing was monitored. The results showed that rats preexposed to a cold room temperature had a response latency that was 3 hr less than that seen in animals preexposed to a normal room temperature. The difference in latency occurred presumably because the former group began the experiment with a body temperature closer to the threshold needed to induced cold-escape responding. In the third study, they measured both lever pressing and subcutaneous temperature, while the rat was in a 2 °C chamber. They chose to measure subcutaneous temperature instead of core temperature due to technical limitations precluding the measurement

of core temperature in a freely-moving animal and because they posited that subcutaneous temperature would be quite sensitive to individual heat reinforcements. At the start of the test, subcutaneous temperature was around 35 ^{o}C. From this point it dropped to approximately 27 ^{o}C at which time the rat began to work steadily for heat. Once stable responding was attained, subcutaneous temperature rose back to 35 ^{o}C, where it was maintained for the remainder of the test. Thus, Weiss and Laties (1961) suggested that the initiation of thermoregulatory behavior may be due to a drop in subcutaneous temperature of approximately 8 ^{o}C; whereas the maintenance of the behavior was perhaps due to the regulation of a normal (35 ^{o}C) subcutaneous temperature.

In a subsequent experiment, Lipton (1967) examined whether rats could operantly maintain a normal core temperature when exposed to a hot ambient temperature. Rats were placed in a chamber maintained at 43 ^{o}C by the presence of a heat lamp for 1 hr/day over 45 consecutive days. Inside the chamber was a lever which, when pressed, turned off the heat lamp and turned on a ventilation fan for 10 s. During each heat-escape test, body temperature was assessed immediately before and after the test session and the number of heat-escape responses made was monitored. The number of heat-escape responses made during the 45 test sessions was fairly constant, and the mean body temperature following the completion of all test sessions was comparable to that obtained prior to the tests. Accordingly, rats subjected to a hot environment are able to maintain a normal core temperature by thermobehavioral means.

While it was apparent that behavioral responses in both cold and hot environments contribute to regulating body temperature, the behavioral consequences of changes in internal temperature, while in a thermoneutral environment, remained unclear. However, Adair (1971) investigated the thermobehavioral consequences of manipulating rectal temperature. Squirrel monkeys were restrained in a chair and taught to pull a chain in order to select between two air temperatures, 10 and 50 ^{o}C. When the response was acquired, the animals alternated between the two temperatures to achieve an average air temperature of 35 to 36^{o}C. Once behavioral responding had stabilized, rectal temperature of the monkeys was raised or lowered every 10 min, through the use of rectally implanted thermodes. During this manipulation, skin and selected air temperatures were monitored. When rectal temperature was lowered to 33 ^{o}C, the monkeys compensated by raising the air, and thereby skin, temperature. Conversely, when the rectal temperature was raised to 43 ^{o}C, the monkeys lowered the air and skin temperatures. Smaller displacements of the rectal temperature produced air and skin temperature changes in the appropriate direction but of a proportionately smaller magnitude. Thus, not only do alterations in environmental temperatures result in behavioral responses designed for the maintenance of core temperature, but manipulations of rectal temperature alter behavioral thermoregulation such that normal core and skin temperatures are strived for.

More recently, investigators have extended the range of experimental manipulations which may influence behavioral thermoregulation to include the influence of pharmacological agents

that affect body temperature. For instance, Cox, Green, and Lomax (1975) assessed the latency to escape a heat lamp following systemically administered hypo- or hyperthermic agents. The experimental protocol consisted of placing the rats in a long, narrow chamber above which was located a movable heat lamp. After 5 min in the 23 ^{o}C chamber the heat lamp was moved over the animal and the experimenter turned it on. Heat escape latency, operationally defined as the time elapsed for the rat to move outside the perimeter of the visible infrared beam of the heat lamp, was recorded. Following escape, the lamp was switched off for an intertrial interval equal to the response latency. This sequence of heating, escape, and intertrial interval was repeated twice more; and, thus, each test session was comprised of three heat-escape sequences. This three-sequence experimental protocol was done initially in the absence of any pharmacological manipulation and then was repeated following a single intraperitoneal injection of either N-methyldiphenhydramine, a peripherally acting hypothermic agent which suppresses metabolism, or tri-iodothyronine, a peripherally acting hyperthermic agent which augments metabolism. After the pharmacological manipulation the experimenters delayed placing the rats into the test chamber until the drug had its desired thermolytic effect. Following N-methyldiphenhydramine, the rats exhibited the characteristic decline in rectal temperature and an increase in heat-escape latency. Conversely, tri-iodothyronine induced a rise in rectal temperature and reduced heat escape latency. These adjustments in behavior tended to return body temperature toward its preinjection value. Accordingly, pharmacologically-induced

disruptions in body temperature produced compensatory behavioral adjustments in heat exposure which, in turn, served to defend normal body temperature.

Statement of the Problem

The autonomic control of circulation serves a dual function: distribution of nutrients to the tissues and maintenance of thermal homeostasis. Organisms may typically alternate between these two functions such that nutrient supply may be compromised in order to serve immediate thermoregulatory requirements or thermoregulation may be jeopardized in order to fulfill imperative nutrient demands. However, structural and functional changes in the circulatory system, such as those that occur during hypertension, may disrupt the interdependent workings of the two functions. Moreover, adaptive processes such as cold acclimation may also influence the autonomic control of the two functions. The interaction of processes such as hypertension and cold acclimation has received little systematic investigation. This is somewhat surprising since the induction of cold acclimation and renovascular hypertension share certain features. For example, in either instance there is a functional hyperreactivity of adrenoceptors, a generalized sympathoexcitation, and an altered vasomotor state in those vascular beds commonly associated with heat transport (Bralet et al., 1973; Foster & Frydman, 1979). Thus, the purpose of this study was to assess whether 1K-GB renovascular hypertension and cold-acclimation interact to influence the cardiovascular, thermophysiological, and thermobehavioral adjustments during alpha-, beta-, or combined alpha- and beta-adrenoceptor stimulation.

The enhancement of thermogenesis with beta-stimulation obtained in cold-acclimated rats is generally viewed as secondary to $beta_1$-stimulation of brown adipose tissue. However, the fact that the available beta-agonists are relatively nonspecific renders the $beta_2$-receptor subpopulation as a potential confounding influence on isoproterenol-induced thermogenesis. Accordingly, to assess the influence of either cold acclimation or renovascular hypertension on $beta_1$-induced thermogenesis one must first identify, then preclude, the inadvertent contribution of $beta_2$-receptors towards isoproterenol-induced thermogenesis in cold acclimation rats. The strategy employed in Experiment 1 to accomplish this preliminary objective was to assess in cold-acclimated rats the thermogenic consequences of isoproterenol in the presence or absence of selective $beta_1$-(atenolol) or $beta_2$-(pindolol) antagonists. The reasoning was that the total abolition of isoproterenol-induced thermogenesis following pretreatment with a selective $beta_1$-antagonist, coupled with a negligible reduction after pretreatment with a selective $beta_2$-antagonist, would imply a negligible contribution of $beta_2$-receptors. It was anticipated that in the cold-acclimated normotensive rat isoproterenol-induced thermogenesis and hyperthermia would be blocked with a $beta_1$-antagonist (atenolol); whereas the $beta_2$ (pindolol)-antagonist would exhibit little, if any, buffering influence. In the cold-acclimated 1K-GB rat this profile would also prevail except that the underlying isoproterenol-induced thermogenesis and hyperthermia would be attenuated due to the reduced beta-responsivity reported for 1K-GB rats (Fregly, 1954). One would

also predict that isoproterenol would reduce MABP, but whether $beta_1$- or $beta_2$-blockers would differentially affect this depressor response remained speculative. The influence of 1K-GB hypertension on this latter effect was conjectural given the paucity of information about myocardial, vascular, or brown adipose tissue receptors in this, or any model of hypertension. The observations in thermogenesis established guidelines for the use of a $beta_2$-receptor antagonist as a pretreatment in subsequent studies with isoproterenol to selectively stimulate $beta_1$-receptors.

Traditionally, $beta_1$-receptors located on the brown adipose tissue have been functionally linked to nonshivering thermogenesis. Yet, the myocardium is also a significant thermogenic organ (Vary, Reibel, & Neely, 1981), whose $beta_1$-adrenoceptors contribute to cardiovascular homeostasis (Crass, Shipp, & Pieper, 1975). One might anticipate then that cold acclimation in the normotensive rat might potentiate not only the thermogenic consequences of $beta_1$-adrenergic stimulation but its tachycardic responsiveness as well. A similar line of logic may be applied to the classical notion of smooth muscle $alpha_1$-adrenoceptors. Such receptors are linked functionally to the control of the resistance side of circulation, whose state of hyperreactivity is associated with the 1K-GB model of renovascular hypertension (Gothberg et al., 1983). One might anticipate then that 1K-GB hypertension in the non-cold-acclimated rat might potentiate the vascular reactivity to $alpha_1$-receptors which, in turn, will have not only cardiovascular but also antithermolytic effects. Furthermore, the induction of either $beta_1$- or $alpha_1$-hyperreactivity could represent an underlying

shared physiochemical process such that functional alterations in two or more subpopulations of adrenoceptors may coevolve. The development of hypertension might then be accompanied by enhanced thermogenic sensitivity, while the process of cold acclimation could potentiate those vascular substrates typically associated with hypertension. The same reasoning would suggest that the simultaneous induction of cold acclimation and 1K-GB hypertension would promote a thermally altered endotherm by inducing thermogenic hyperreactivity and compromising vascular means of thermolysis. The findings that (a) cold acclimation potentiates the thermogenic consequences of $alpha_1$-stimulation (Foster, 1985) and (b) hypertension is accompanied by altered thermal sensitivity (Fregly, 1954) are certainly consistent with this idea.

From the considerations discussed above emerge several specific questions. First, will the development of increased reactivity to $beta_1$-induced thermogenesis accompanying cold acclimation (a) enhance the vascular responsiveness to $alpha_1$-agonists in the normotensive rat, (b) exacerbate the characteristic $alpha_1$-pressor responsiveness of 1K-GB hypertension, or (c) unmask a latent thermogenic contribution of $alpha_1$-receptors following stimulation with an $alpha_1$-agonist when presented alone or concurrently with a $beta_1$-agonist? Second, will the induction of 1K-GB hypertension alter sensitivity to $beta_1$-induced thermogenesis in the non-cold- or cold-acclimated rat? Third, even in the absence of any change in catecholaminergic-induced thermogenesis, does the maintenance of 1K-GB hypertension jeopardize thermolysis and, thereby, facilitate hyperthermia in either the non-cold- or cold-acclimated rat? To

address these questions, Experiment 2 proposed to monitor cardiovascular, metabolic, and body temperature responses during the systemic infusion of either $alpha_1$ (phenylephrine)-, $beta_1$ (isoproterenol)-, or combined $alpha_1$ (phenylephrine)- and $beta_1$ (isoproterenol)-agonists. The adrenoceptor agents will be administered in a non-cold- or cold-acclimated and normotensive or 1K-GB hypertensive state. It is expected that cold acclimation will enhance the thermogenic role of alpha-adrenoceptors, and this enhancement should be most evident when administered concurrently with the $beta_1$-agonist, isoproterenol. The induction of 1K-GB hypertension was expected to be accompanied by the purported alpha-mediated pressor hyperreactivity and by altered thermogenic and tachycardic responsiveness to the $beta_1$-agonist. The overall response profile was anticipated to be exacerbated with cold acclimation or combined alpha- and beta-stimulation.

Any net thermal imbalance that occurred as a result of the processes of cold acclimation, 1K-GB hypertension, or adrenoceptor stimulation discussed in the above experiments would tend to alter tolerance to a warm environment. If warm tolerance is actually reduced, as would be the case if obligatory thermogenesis is enhanced and thermolysis is suppressed, then superimposing a mild heat challenge might render an otherwise innocuous thermophysiological adjustment into a significant thermal perturbation. This would, in turn, tend to evoke compensatory operant thermoregulatory means of preventing the progressive development of hyperthermia. Accordingly, Experiment 4 proposed to assess, in a warm challenge situation, the thermobehavioral implications of the design discussed for

Experiment 2. Specifically, the following questions were addressed: First, given that cold acclimation promotes sympathetically-mediated thermogenesis, then if one evokes this response with alpha- and/or beta-adrenoceptor stimulation in a normotensive, warm-exposed rat, will the animal exhibit a greater tendency to behaviorally offset hyperthermia than the non-cold-acclimated controls? If so, is this tendency augmented with the induction of 1K-GB hypertension? Second, since the 1K-GB rat has an enhanced vascular and pressor hyperreactivity to alpha-agonists and may exhibit reduced thermolysis, will the alpha-adrenoceptor elevation of MABP in the 1K-GB rat result in a greater increase in heat escape behavior in a mild heat challenge than that obtained in the normotensive control? To address these questions the duration and frequency of heat escape responding for a cool convective reinforcement were monitored during the systemic infusion of either $alpha_1$ (phenylephrine)-, $beta_1$ (isoproterenol)-, or combined $alpha_1$ (phenylephrine)- and $beta_1$ (isoproterenol)-agonists. The adrenoceptor agents will be administered in a non-cold- or cold-acclimated and normotensive or 1K-GB hypertensive state. It is anticipated that in thenon cold-acclimated, normotensive rat the duration of lever pressing will be increased following $alpha_1$- or $beta_1$-agonist administration and will be synergistically enhanced when these agents are jointly infused. This latter effect would reflect an additional $alpha_1$-mediated augmentation of brown adipose tissue thermogenesis. The presence of either cold acclimation or 1K-GB hypertension should also increase the duration of heat escape responding to either agonist administration alone or in combination,

and this tendency will be potentiated even further in the cold-acclimated-1K-GB rat. No group differences in frequency of lever pressing are anticipated.

One premise of Experiments 2 and 4 is that cold acclimation alone, or in combination with 1K-GB renovascular hypertension, will tend to facilitate alpha$_1$-induced hyperthermia. The fact that alpha$_1$-adrenoceptors are established vasoconstrictor agents obfuscates somewhat the interpretation of any resulting changes in rectal temperature or heat escape behavior since they could be secondary to either increased brown adipose tissue-mediated thermogenesis or vasoconstrictor-induced compromised thermolysis. Access to the relevant metabolic and cardiovascular parameters may clarify this issue. However, another way to dissociate the thermogenic from the thermolytic consequences of alpha$_1$-induced thermophysiological and behavioral adjustments is to neuropharmacologically induce increased vascular resistance similar to that obtained with alpha$_1$-stimulation without triggering thermogenesis. In this regard, the smooth muscle component of blood pressure regulation is also controlled by alpha$_2$-adrenoceptors, with alpha$_2$-stimulation resulting in an increased peripheral vascular resistance. The resulting elevation in MABP reflects the contribution of a subpopulation of adrenoceptors comparable to alpha$_1$-receptors but occurs without thermogenesis. Accordingly, if hyperthermia emerges in Experiment 2, then the question of whether such changes can be attributed to alpha$_1$-induced thermogenesis, or alpha$_1$-compromised thermolysis, or some synergistic interaction of the two remains unanswered. To address this issue Experiment 3

examined, in cold-acclimated normotensive and hypertensive rats, the thermophysiological effects of systemically-infused $beta_1$ (isoproterenol)-, $alpha_2$ (guanabenz)-, or combined $beta_1$ (isoproterenol)- and $alpha_2$ (guanabenz)-agonists. The protocol for Experiment 3 was identical to that described for Experiment 2 except for the specific neuropharmacological agents used. The following general results were anticipated: The thermogenic, hyperthermic and pressor effects of ISO will once again be obtained in the normotensive rat and altered in the 1K-GB rat. Infusion of the $alpha_2$-agonist will have a negligible effect on thermogenesis in the normotensive rat, but it may evoke a vasoconstrictor-mediated hyperthermia in the 1K-GB rat. The pressor consequences of $alpha_2$-stimulation will be comparable to that observed upon $alpha_1$-stimulation. The joint infusion of ISO and the $alpha_2$-agonist should have no potentiating effect on the hyperthermic and pressor response obtained in both normotensive and hypertensive cold-acclimated rats.

General Method

Subjects

One hundred and seventy-six male, Sprague-Dawley rats weighing 70-90 g were used. The animals were initially housed in individual wire-mesh cages with free access to standard rat chow (Wayne F6 Rodent Blox: starch, 45.5%, protein, 24.0%, moisture, 12.2%, ash,7.9%, fat, 6.0%, fiber, 4.5%) and water. The colony room was maintained on a 9/15 hr light/dark cycle (lights on 0700-1600) at 23 $\pm$ 2 ^{o}C.

Apparatus

Metabolic thermal testing. A metabolic chamber was located inside a temperature-controlled cabinet maintained at 23 ^{o}C. A schematic representation of the apparatus is illustrated in Figure 1. The metabolic chamber was a Plexiglas cylinder, 37 cm long with an internal diameter of 13.8 cm. The cylinder contained an exit for temperature probes and an arterial/jugular catheter interface coupler. Two Plexiglas plates (21 x 21 x 1.2 cm) provide an air-tight seal for the metabolic chamber and contain air inlet and outlet ports. Dry air passed through the chamber at a rate of 1000 ml/min. Expired gases were dried by passage through Drierite and analyzed for carbon dioxide and oxygen content using a Beckman LB-2 Medical Gas Analyzer and a Beckman OM-11 Oxygen Analyzer, connected in series. The calibrations procedure used for the expired gas analyzers is presented in Appendix A. The formulae used for determining oxygen consumption and carbon dioxide production are provided in Appendix B. Prior to oxygen consumption detemination, but following carbon dioxide analysis, the expired gases were passed

through a soda lime (6 to 12 mesh) tube to remove carbon dioxide. Carbon dioxide and oxygen content were continuously recorded on a dual channel Omniscribe recorder (Fisher Recordall Series 5000). Although expired gases were continuously monitored, they were only recorded every 10 min. Given that the metabolic testing apparatus had a 95% clearance time of 16 min, and that the recorded metabolic rate did not take into account this relatively long clearance time, the metabolic response as a function of time must be viewed with caution. Rectal temperature (via a YSI 402 probe inserted 4 cm), tail and interscapular skin temperatures (via YSI 409 probes taped to the skin) and ambient temperature (via a YSI 401 probe) inside the metabolic chamber were recorded with a YSI telethermometer (Model 46TUC, Yellow Springs Instruments Inc.). The aortic catheter was connected to a Gould Statham pressure transducer (Model P23Gb) with Intramedic polyethylene tubing (PE100), and BP was recorded on a Grass Model 5B polygraph. Heart rate was monitored by connecting heart rate electrodes with wire leads to a polygraph preamplifier (Model 5P1) and was determined by counting the number of pulsatile pen deflections in a 5-s interval. In order to assess evaporative heat loss, the Drierite tube through which the expired gases passed was weighed to the nearest .001 g on a Sartorius balance (Model 2604) before and after each period of testing.

Behavioral thermal testing. Operant thermoregulation was assessed in a convective thermal controller. The convective thermal controller is a forced convection system in which the animal is bathed in low-humidity air of controlled temperature and velocity. Air circulates over the animal in an operant chamber, but the

temperature of the air is behaviorally controlled. As illustrated in Figure 2, two continuously circulating air systems are arranged so that one system (e.g., warm air) is circulated through the chamber, while the other system (e.g., cool air) is shunted through a bypass. When the animal presses a manipulandum, two valves that control the direction of air flow rotate 90°. The air flow circulating through the operant chamber is then diverted through the bypass, while the air moving through the bypass is re-routed to the operant chamber. This closed system permits changes in chamber temperature to occur from one stable state to another in only the time it takes the valves to rotate (.3 s) and air to reach the animal. The components of the convective thermal controller are interconnected by an insulated sheet-metal duct (ID=15.2 cm). The air temperature is controlled by one of two 30.5 cm^2 thermal sources: the hot box containing a 240 VAC heating coil and the cold box housing several rows of copper tubing heat exchangers. Manually operated thermostats control the temperature (+.5 °C) of the heater coils in the hot box or of a duct heater located upstream from the refrigerator freon heat exchanger.

The operant chamber is 48 x 31 x 28 cm and is constructed of insulated sheet-metal and Plexiglas. Baffles are located at each end to prevent lamination of air flow. The animal is supported on a 1.6 cm plastic mesh floor that prevents rapid heat exchange, which may occur at the surfaces of animals exposed to more conductive materials. The manipulandum is a thermally-insulated lever measuring 5 x 3 x 1 cm and is located 2 cm above the plastic mesh floor on the wall opposite the chamber door. A feces trap beneath the plastic

mesh floor contains sawdust. The ambient temperature of the convective thermal controller was 37 °C at the start of the test session, and the thermal reinforcement consisted of a 17 °C air flow through the chamber.

Surgical Procedures

Induction of 1K-GB renovascular hypertension or sham operation. All surgeries and recovery periods occurred in an ambient temperature of 21 °C. Thirteen days after arrival in the laboratory, one half of the animals were randomly assigned to the 1K-GB renovascular hypertensive group; whereas the remaining animals were assigned to the normotensive sham-operated group. All animals were removed from their home cages, anesthetized with sodium pentobarbital (Allen & Hansburys, 60 mg/Kg i.p.), and shaved on the right side in preparation for a right laparotomy. After the kidney was exposed and cleared of surrounding fascia, the renal artery was sealed with a double ligation of 00 silk surgical suture and the kidney was excised. Following a 24 hr recovery period the animals were returned to their home cages in either the 6 ° or 23 °C room. Seventy-two hours after the nephrectomy, the 1K-GB animals were removed from their home cages, anesthetized, and subjected to a left laparotomy, following which the left kidney was lifted and the renal vasculature cleared of surrounding tissue. A solid silver clip (4 x 2 x 1.6 mm) with a slit of a diameter of .2 mm was applied to the renal artery, proximal to the aorta. Leenen and DeJong (1971) have demonstrated that this technique is effective in producing a 60% increase in MABP. The sham-operated animals underwent a left laparotomy and the renal vasculature was cleared of surrounding tissue. However, a

silver clip was not applied to the renal artery. Twenty-four hours following surgery the animals were returned to their home cages.

Aortic catheterization. Twenty-four days after arrival all animals used for metabolic thermal testing were implanted with chronically indwelling, descending aortic catheters. The aortic catheters have a Teflon tip (Small Parts Inc., OD-.034", ID-.022") with a blunt tapered end, attached to 45 cm of Tygon tubing (OD-.75 cm, ID-.15 cm). The connection was made by soaking 1 to 2 cm of the Tygon in 1,2-dichloroethane (Fisher Scientific Co. Ltd.) for 4 to 5 min and inserting the Teflon tip approximately 1 cm into the Tygon tubing. The completed catheter was flushed with distilled water and left to dry overnight. One day before aortic catheterization the catheter was filled with TDMAC heparin complex (2%, Polysciences Inc.), allowed to soak for 30 min, and then dried by infusing air through it.

For purposes of surgery the animals were removed from their home cages and anesthetized with sodium pentobarbital (60 mg/Kg i.p.). A 2-3 cm incision was made through the midabdominal region. The intestines were gently retracted, and the descending aorta, approximately 2 cm caudal to the left renal artery, was exposed and isolated. A 20-cm length of 00 silk surgical suture was wound around the aorta and tied very loosely. The aorta was gently lifted with curved forceps, the suture was pulled to temporarily occlude blood flow, and a small puncture was made with a 27 ga stainless steel hypodermic needle. The Teflon tip of the catheter was inserted 1.2 cm into the aorta via this puncture and blood flow was reestablished. The catheter was then anchored to surrounding

muscles. The catheter was filled with heparinized isotonic saline (100 U/ml, Sigma Chemical Co.) and closed off with a stainless steel obturator.

Jugular catheterization. A .25 ml jugular catheter (PE50, Intramedic, OD.038", ID.025") was implanted while the animal was still under sodium pentobarbital anesthesia from the aortic catheterization. The right jugular vein was exposed by making a 2-cm incision anterior to the clavicle. The jugular vein was isolated from the surrounding tissue, lifted slightly with forceps, and punctured with a 22 ga stainless steel hypodermic needle. The catheter was inserted 3 cm into the vein through the puncture and ligated to the vein rostral and caudal to the point of insertion. The catheter was also ligated to the surrounding muscle for anchorage, led subcutaneously to the dorsal side, and externalized at the nape of the neck. After the jugular catheter was filled with heparinized isotonic saline (100 U/ml) and was closed off with a stainless steel obturator, both catheters were taped to an Elizabethan collar secured to the animal's neck.

Following catheterization, bilateral heart rate electrodes were implanted in the sides of the rat. Each electrode consisted of a 2.5 cm length of stainless steel suture that was led subcutaneously for approximately 1 cm, below the rats' forelimbs. The free ends of each electrode were wound around each other, hiding the ends of the wire.

Thirty-six hours following surgery the rats were returned to their home cages either in a 6 or 23 ^{o}C room. Both catheters were flushed once daily with .3 ml of 100 U/ml heparinized isotonic

saline solution to prevent clotting.

Carotid catheterization. All animals in Experiment 3 underwent a right carotid catheterization. The animals were removed from their home cages and anesthetized with sodium pentobarbital (60 mg/Kg, i.p.). A 1-cm incision was made medially on the ventral surface of the neck. The sternohyoideus and sternomastoideus muscles were gently separated from the trachea, and the right carotid artery was exposed. The artery was cleared of connective tissue with care being taken not to damage the vagus and cervical sympathetic nerves, which overlie the carotid. The carotid was lifted with curved forceps to temporarily occlude blood flow. A small puncture was made in the artery with a 27 ga. needle, 1.5 cm of a PE50 catheter was inserted into the carotid and blood flow was reestablished. The catheter was anchored in place to the surrounding muscles, and given that the carotid catheterization was an acute procedure, the wound was covered with several saline-soaked gauze sponges.

Post-operative care. All incisions were sutured with 00 silk surgical suture. A local analgesic (2% Xylocaine Hydrochloride, Astra) and a topical antibacterial cream (Furacin, .2%, Austin) were applied to the wound, and an intramuscular injection of procaine penicillin (Ethacilin, Rogar STB, 1.5 ml/Kg) was given to each animal following every surgery.

Urinary sodium and potassium assay

Following restraint adaptation on Day 21 the animals in Experiment 1 and Experiment 2 were placed in individual wire mesh cages located in a 23 ^{o}C room. The cages were specially designed to collect urine. During the 24-hr period that the urine was

collected the animals had free access to tap water and approximately 20 g of food. The collected urine was filtered through gauze sponges to remove any food particles and was stored at -20 ^{o}C until analyzed.

For purposes of analysis the urine was thawed, diluted with distilled water in a 1:10 ratio, and vortexed for 3 s. A Beckman Klina Flame Photometer (Beckman Instruments Co.) was calibrated with Beckman K^{+}-Na^{+} Standard (100:100 mMol/L) and the urines were analyzed for sodium and potassium content, expressed as mEq/L.

Statistical analyses

A priori linear contrasts were performed on interactions specifically hypothesized in the statement of the problem. In particular, interactions involving hypertensive status, cold acclimation status, and combined hypertensive and cold acclimation status for metabolic and cardiovascular variables in Experiment 2 and for heat escape behavior in Experiment 4 were tested in an a priori manner.

For baseline data in Experiment 1 and 3, dependent variables were analyzed using 2 x 2 x 2 x 5 (BP Status x Drug Group x Test Session x Time) mixed effects ANOVAs with repeated measures on the last factor, whereas those of Experiment 2 were analyzed using 2 x 2 x 2 x 2 x 5 (BP Status x Acclimation Status x Drug Group x Test Session x Time) and those of Experiment 4 were analyzed using 2 x 2 x 2 x 2 x 6 (BP Status x Acclimation Status x Drug Group x Test Session x Time) mixed effects ANOVAs with repeated measures on the last factor.

Data obtained during the drug administration were broken down into three analyses each. For Experiment 1 the first analysis

compared ISO and atenolol with ISO and pindolol using 2 x 2 x 5 (BP Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last factor. The second and third analyses for Experiment 1 compared ISO alone with ISO and atenolol and ISO alone with ISO and pindolol, respectively, each using 2 x 2 x 5 (BP Status x Drug Group x Time) mixed effects ANOVAs with repeated measures in the last two factors. For Experiment 2 the first analysis compared ISO alone with PE alone using 2 x 2 x 2 x 5 (BP Status x Acclimation Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last factor. The second and third analyses for Experiment 2 compared ISO alone with ISO and PE and PE alone with ISO and PE, respectively, each using 2 x 2 x 2 x 5 (BP Status x Acclimation Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last two factors. For experiment 3 the first analysis compared ISO alone with GUANA alone using 2 x 2 x 5 (BP Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last factor. The second and third analyses for Experiment 3 compared ISO alone with ISO and GUANA and GUANA alone with ISO and GUANA, respectively, each using 2 x 2 x 5 (BP Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last two factors. Finally, for Experiment 4 the first analysis compared ISO alone with PE alone using 2 x 2 x 2 x 6 (BP Status x Acclimation Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last factor. The second and third analyses for Experiment 4 compared ISO alone with ISO and PE and PE alone with ISO and PE, respectively, each using 2 x 2 x 2 x 6 (BP Status x Acclimation Status x Drug Group x Time) mixed effects ANOVAs with

repeated measures on the last two factors.

For all dependent variables, the percentage change from baseline during the drug administrations was calculated for each time interval according to the following formula:

percentage change = $((time_i - \text{mean baseline})/\text{mean baseline}) \times 100$

where the mean baseline is the last three values obtained during the 50 min baseline period, and time consisted of the five 10-min sampling intervals during the drug infusion period. The percentage change from baseline data were then analyzed in three ways for each experiment. For the first experiment, percentage change from baseline for ISO and atenolol was compared with the percentage change from baseline for ISO and pindolol using 2 x 2 x 5 (BP Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last factor. The second and third analyses consisted of comparing the percentage change from baseline for ISO alone with the percentage change from baseline for ISO and pindolol, respectively, using 2 x 2 x 5 (BP Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last two factors. For Experiment 2, the first analysis compared percentage change from baseline for ISO alone with the percentage change from baseline for PE alone using 2 x 2 x 2 x 5 (BP Status x Acclimation Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last factor. The second and third analyses for Experiment 2 compared the percentage change from baseline for ISO alone with the percentage change from baseline for ISO and PE and the percentage change from baseline for PE alone with the percentage change from baseline for ISO and PE, respectively, each using a 2 x 2 x 2 x 5 (BP Status x Acclimation

Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last two factors. For Experiment 3, the first analysis compared percentage change from baseline for ISO alone with the percentage change from baseline for GUANA alone using 2 x 2 x 5 (BP Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last factor. The second and third analyses compared the percentage change from baseline for ISO alone with the percentage change from baseline for ISO and GUANA and the percentage change from baseline for GUANA alone with the percentage change from baseline for ISO and GUANA, respectively, each using 2 x 2 x 5 (BP Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last two factors. Finally, the first analysis for Experiment 4 compared the percentage change from baseline for ISO alone with the percentage change from baseline for PE alone using 2 x 2 x 2 x 6 (BP Status x Acclimation Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last factor. The second and third analyses compared the percentage change from baseline for ISO alone with the percentage change from baseline for ISO and PE and the percentage change from baseline for PE alone with the percentage change from baseline for ISO and PE, respectively, each using 2 x 2 x 2 x 6 (BP Status x Acclimation Status x Drug Group x Time) mixed effects ANOVAs with repeated measures on the last two factors.

Follow-up tests consisted of performing trend analyses on all significant time main effects and interactions. Significant interactions were further probed by performing linear contrasts. For all analyses, probability levels were set at $p<.01$. A more conservative alpha level was used in order to diminish the probability of Type I error.

Experiment 1. Contribution of $Beta_1$- and $Beta_2$-Adrenoceptors to Isoproterenol-Induced Thermogenesis in Cold Acclimated, One Kidney Goldblatt Hypertensive and Normotensive Control Rats.

Method

Subjects

Twenty-four male, Sprague-Dawley rats initially weighing 70-90 g were used. The animals were housed in a 23 ^{o}C colony room maintained on a 9/15 hr light/dark cycle with free access to standard rat chow and water.

Procedure

Pretest procedure. Two days after arrival in the laboratory all animals began 24 days of CA in a walk-in refrigerator. During CA the animals were housed in individual polypropylene cages lined with approximately 300 g of wood-chip bedding and allowed free access to standard rat chow and water. The walk-in refrigerator was maintained at 6±1 ^{o}C and kept on a 9/15 hr light/dark cycle (lights on 0700-1600).

On the 10th day of CA the animals were removed from the walk-in refrigerator, and a right nephrectomy was performed as described in the surgical manipulation section. On the 14th day of CA the animals were randomly assigned to either a 1K-GB hypertensive group (n=12) or a sham-operated group (n=12). The 1K-GB rats had a solid silver clip applied to the left renal artery; whereas the sham animals underwent a left laparotomy, but no renal clip was applied. Following a 24 hr recovery at 21 ^{o}C the animals were returned to their home cages in the walk-in refrigerator. Starting on the 16th day of CA, all animals underwent adaptation to mild physical restraint for a 6 day

period while in the walk-in refrigerator. On the first two days of restraint adaptation (Days 16-17 of CA), the animals were placed in Plexiglas restrainers for 1 hr/day, starting at 0700. On Days 3-4 (Days 18-19 of CA), the duration of restraint was increased to 2 hr/day. Finally, on Days 5-6 of adaptation (Days 20-21 of CA), the animals were restrained for 4 hr/day in Biodec restrainers, designed to avoid heat accumulation by the animal. During this time they also had a rectal probe (YSI 402 probe, Fisher Scientific Co. Ltd., vinyl coated, 2.4 mm diameter) inserted 4 cm into their rectums. This procedure served to adapt the animals to the probe placement used during metabolic thermal testing. At the end of restraint on Day 21 the animals had their urines collected for a 24-hr period prior to catheterizations or metabolic thermal testing.

On Day 22 of CA the animals underwent the chronic catheterization of their descending aortas and right jugular veins as previously described in the surgical manipulation section. The animals were allowed a 36-hr recovery period in a 21 ^{o}C room and a further 12-hr recovery period in the 6 ^{o}C walk-in refrigerator. Forty-eight hours following catheterization, metabolic testing commenced.

Metabolic testing procedure. Following post-operative recovery the animals' metabolic and cardiovascular responses to systemic administration of isoproterenol (ISO), combined ISO and atenolol (a $beta_1$-antagonist), and combined ISO and pindolol (a $beta_2$-antagonist) were assessed. Three animals were tested each day, commencing at 0700 hr.

For purposes of metabolic testing the animals were assigned to one of two drug conditions: (a) administration of ISO alone or

combined with a $beta_1$-antagonist or (b) administration of ISO alone or combined with a $beta_2$-antagonist. This assignment of animals resulted in the following subgroups. A 1K-GB group that received (a) ISO alone on one test session and combined ISO and a $beta_1$-antagonist on the other test session, (1K-GB-B_1, $\underline{n}$=6) or (b) ISO alone and then combined with a $beta_2$-antagonist, (1K-GB-B_2, $\underline{n}$=6); a sham group that received (a) ISO alone and then ISO combined with a $beta_1$-antagonist (sham-B_1, $\underline{n}$=6) or (b) ISO alone and then combined with a $beta_2$-antagonist (sham-B_2, $\underline{n}$=6). Testing on the ISO and combined ISO-beta-blocker conditions were conducted across two test sessions with a 46 hr rest period interpolated between the sessions.

The drug solutions, consisting of ISO ((-1)-isoproterenol hydrochloride, 0.5 ug/kg/min), atenolol (dl-atenolol hydrochloride, 3.0 mg/kg), and pindolol (1-(1H-Indol-4-yloxyl)-3-(isopropylamino)-2-propranolol, 10 ug/kg), (all from Sigma Chemical Co. Ltd.), were weighed on a Sartorius balance (Model 2604) and dissolved in sterile .15M saline. All drug doses were expressed in the hydrochloric acid form. The ISO solution also contained .1% ascorbic acid to retard oxidation. A fresh solution was made before each infusion and the pH of the solution was monitored. Although it is generally acknowledged that a dose-response design is preferable to a single dose study, there was a pragmatic reason for using only one dose of each drug. Repeated administrations of ISO were shown in pilot work to either debilitate or kill the animal. Since it was desired to make within subject comparisons across various drug combinations, the numer of ISO infusions were kept at a minimum in order to promote the health

of the animal.

For purposes of metabolic testing the animals were placed in a Biodec restrainer and inserted into a metabolic chamber located in a controlled temperature cabinet maintained at 23 ^{o}C. Following the placement of the interscapular and tail skin and rectal temperature probes, the connections of the heart rate electrodes, and of the aortic catheter to the pressure transducer, the metabolic chamber was sealed and the animal was allowed a 90-min stabilization period. Ten min recordings of oxygen consumption, carbon dioxide production, rectal temperature, interscapular skin temperature, tail skin temperature, BP and heart rate were monitored for the last 50 minutes of stabilization and constituted the baseline period for each test session. Following baseline, the drug infusion period commenced. For the rats receiving the $beta_1$-antagonist, drug infusions consisted of ISO (.5 ug/kg/min) on one test session and ISO (.5 ug/kg/min) preceeded 10 min earlier by a bolus injection of atenolol (3.0 mg/kg) on the remaining test session. Animals given the $beta_2$-blocker received ISO (.5 ug/kg/min) alone and ISO (.5 ug/kg/min) preceeded 10 min earlier by a bolus injection of pindolol (10 ug/kg). The dose of ISO used was reported effective in inducing thermogenesis by Foster (1985); whereas the doses of atenolol and pindolol were reported to effectively block $beta_1$- and $beta_2$-receptors, respectively, by Lands et al. (1967). The order of drug administration was counterbalanced across the two test sessions.

The infusion process adhered to a standard protocol. When the infusion was preceeded by a bolus injection, the jugular catheter

was filled with .25 ml of the injectate 10 min prior to the end of the baseline period. Upon completion of baseline, the injectate was introduced into the animal by filling the jugular catheter with .25 ml of the infusate, thereby displacing the injectate. At this point the jugular catheter, filled with the infusate, was attached to a 10-cc syringe located in an infusion pump. The 10-cc syringe was filled with heparinized saline. Ten minutes following the administration of the injectate, the infusate was intorduced into the animal by infusing .25 ml of heparinized saline at a rate of .005 ml/min into the jugular catheter for a duration of 50 min. This procedure allowed the animal to receive the infusate while filling the jugular catheter with heparinized saline. When only an infusion of ISO was given the jugular catheter was filled with .25 ml of the infusate 10 min prior to the end of baseline. The jugular catheter was attached to a 10-cc synringe filled with heparinized saline and located in an infusion pump. The infusate was introduced into the animal following completion of baseline by infusing .25 ml of heparinized saline at a rate of .005 ml/min into the jugular catheter for a duration of 50 min. This procedure allowed the animal to receive the infusate while filling the jugular catheter with heparinized saline. During the drug administration period, 10-min recordings of oxygen consumption, carbon dioxide production, rectal temperature, interscapular and tail skin temperatures, BP and heart rate were obtained. Following completion of the infusion the animals were returned to their home cages in the walk-in refrigerator and allowed a 46 hr rest period before being tested for a second time.

Following completion of the second test session the animals were sacrificed with an overdose of sodium pentobarbital (60 mg/kg i.p.).

Results

Body Weight Analysis

There were no significant differences in the body weights between the 1K-GB hypertensive (M=261.1±14.6g) and the sham normotensive (M=263.6±4.4g) group.

Metabolic Rate

Baseline. No difference across test sessions was obtained for either oxygen consumption (VO_2) or carbon dioxide production (VCO_2). However, as depicted in Table 1, both indices of metabolic rate were marginally elevated in the 1K-GB animals when compared with the sham normotensive animals, $F(1,20)=4.92$, $p<.04$ for VO_2 and $F(1,20)=5,37$, $p<.03$ for VCO_2. Moreover, there was a general tendency for VO_2 and VCO_2 to be elevated at the start of baseline, $F(4,80)=7.10$, $p<.0001$ and $F(4,80)=9.73$, $p<.0001$, respectively. However, Figure 3 shows that these metabolic variables reached an asymptote at a lower level by the 20th min of baseline, as revealed by a significant quadratic component in the trend analysis, $F(1,20)=10.37$, $p<.004$. Analysis of baseline RQ, (.83), a ratio of VCO_2 to VO_2, revealed no significant BP status, time main effects, or interactions.

Isoproterenol Alone. Isoproterenol had no differential thermogenic effect on 1K-GB or sham rats. Isoproterenol increased VO_2 and VCO_2 over time, $F(4,80)=23.57$, $p<.0001$ and $F(4,80)=28.72$, $p<.0001$, respectively (Figure 3), in a quadratic manner for both VO_2, $F(1,20)=36.06$, $p<.0001$ and VCO_2, $F(1,20)=19.50$, $p<.0001$.

The percentage change from baseline over time for both metabolic variables also increased, $F(4,80)=13.89$, $p<.0001$ for VO_2 and $F(4,80)=22.23$, $p<.0001$ for VCO_2. Linear contrasts suggested that both variables stabilized within 30 min of the start of the infusion, $F(1,4)=4.43$, $p<.04$ and $F(1,40)=10.10$, $p<.002$, respectively. Moreover, this tendency to rise over time was marginally dependent on the BP status of the animals, $F(4,80)=2.52$, $p<.05$ for VO_2 (Figure 4) and $F(4,80)=3.00$, $p<.02$ for VCO_2, in that both were attenuated within the first 30 min of infusion for the 1K-GB hypertensive group. Respiratory quotient tended to increase over time, (.89 to .93), $F(4,80)=5.23$, $p<.0009$, and trend analysis revealed a linear component for this rise, $F(1,20)=13.51$, $p<.002$.

Isoproterenol with a $Beta_1$-Antagonist (Atenolol) versus Isoproterenol with a $Beta_2$-Antagonist (Pindolol). Neither VO_2 or VCO_2 differed as a function of the beta-antagonist used with ISO, nor did the induction of 1K-GB hypertension have a differential effect on the metabolic response to these agents. However, 1K-GB hypertension produced an attenuated percentage change in VO_2, $F(1,20)=12.99$, $p<.0018$, and VCO_2, $F(1,20)=18.86$, $p<.0003$. Isoproterenol, with either beta-antagonist, did increase both VO_2 and VCO_2 as a function of time (Figure 3), $F(4,80)=15.91$, $p<.0001$ and $F(4,80)=21.44$, $p<.0001$, respectively, with this increase being expressed in the percentage change from baseline data, $F(4,80)=24.28$, $p<.0001$ for VO_2 and $F(4,80)=32.34$, $p<.0001$ for VCO_2. Trend analysis of both VO_2 and VCO_2 of time yielded significant quadratic components, $F(1,20)=13.38$, $p<.002$ for VO_2 and $F(1,20)=9.66$, $p<.006$ for VCO_2, respectively, whereas, linear

contrasts suggested that metabolism stabilized within 30-40 min after the start of the infusions, $F(1,4)=1.04$, $p<.031$ and $F(1,4)=2.81$, $p<.010$, for VO_2 and VCO_2, respectively. Moreover, the tendency for percentage change in VO_2 (as depicted in Figure 5) and VCO_2 to increase over time was attenuated in the 1K-GB hypertensives, $F(4,80)=3.56$, $p<.01$ for VO_2 and $F(4,80)=4.98$, $p<.0012$ for VCO_2. Analysis of RQ yielded a tendency to increase over time across both drug manipulations, (M=.83 to .91), $F(4,80)=5.73$, $p<.0004$, and this rise never did asymptote, as revealed in a significant linear component in the trend analysis, $F(1,20)=8.89$, $p<.007$.

Isoproterenol Alone versus Isoproterenol with a Beta$_1$-Antagonist (Atenolol). When collapsed across the drug manipulations, the induction of 1K-GB hypertension had no overall effect on metabolism. As presented in Figure 3, the thermogenic increase in both VO_2 and VCO_2 obtained with ISO were suppressed when the ISO infusion was preceeded by a beta$_1$-antagonist, atenolol, $F(1,10)=9.21$, $p<.013$ and $F(1,10)=17.32$, $p<.002$, respectively. This suppression was also expressed in the percentage change from baseline data for VO_2, $F(1,20)=15.50$, $p<.003$ and for VCO_2, $F(1,20)=14.09$, $p<.004$. Both indices of metabolic rate increased during both infusion routines, $F(4,40)=9.16$, $p<.0001$ for VO_2 and $F(4,40)=14.07$, $p<.0001$ for VCO_2. Trend analysis yielded a significant quadratic component for the rise in VO_2 and VCO_2, $F(1,10)=22.04$, $p<.0008$ and $F(1,10)=20.67$, $p<.0011$, respectively; whereas linear contrasts showed that both variables reached a steady state within 30-40 min of the start of the infusion period, $F(1,4)=1.37$, $p<.024$ and $F(1,4)=3.04$, $p<.008$, for VO_2 and VCO_2,

respectively. Analysis of RQ revealed a tendency to increase as a function of time across both drug infusions, (.87 to .93), $F(4,40)=5.87$, $p<.0008$. Trend analysis revealed a significant linear component, $F(1,10)=5.87$, $p<.009$, suggesting that RQ failed to reach a steady state of responding during the drug infusions.

Isoproterenol Alone versus Isoproterenol with a Beta$_2$-Antagonist (Pindolol). When collapsed across drug infusions, 1K-GB hypertension marginally attenuated the percentage change in metabolism, $F(1,20)=5.01$, $p<.049$ for VO_2 and $F(1,20)=9.28$, $p<.012$ for VCO_2. However, as displayed in Figure 3, VCO_2 was moderately suppressed when the ISO infusion was preceeded by beta$_2$-blockade, $F(1,10)=7.95$, $p<.018$. Both VO_2 and VCO_2 increased as a function of time, $F(4,40)=35.08$, $p<.0001$ and $F(4,40)=38.97$, $p<.0001$, and exhibited a quadratic component $F(1,10)=26.56$, $p<.0004$ and $F(1,10)=17.90$, $p<.002$. Moreover, the increase in VO_2 (Figure 6) and VCO_2 over time was less for the 1K-GB hypertensives than for the sham normotensives, $F(4,40)=5.78$, $p<.0009$ for VO_2 and $F(4,40)=4.10$, $p<.007$ for VCO_2. Linear contrasts indicated that the difference between the two groups was apparent within the first 10 min of the infusion, $F(1,4)=3.23$, $p<.035$ for VO_2 and $F(1,4)=10.85$, $p<.001$ for VCO_2. Respiratory quotient was also suppressed when the ISO infusion was preceeded by beta$_2$-blockade, $F(1,10)=18.63$, $p<.002$. As a function of time, RQ also tended to increase, (.85 to .91), $F(4,40)=6.60$, $p<.0004$, in a linear fashion, $F(1,10)=10.41$, $p<.009$.

Body Temperature Indices

Baseline. No difference in rectal temperature (Tr),

interscapular skin temperature (Tis), or tail skin temperature (Tts) developed between the two test sessions. However, as illustrated in Figure 7, there was a differential change in Tr between the 1K-GB and sham animals over time, $F(4,80)=9.55$, $p<.0001$. Linear contrasts revealed that the 1K-GB animals had a higher Tr for only the first 20 min of baseline, $F(1,4)=3.67$, $p<.04$.

Isoproterenol Alone. The induction of 1K-GB hypertension only marginally suppressed the percentage change from baseline for Tr in response to ISO, $F(1,20)=23.72$, $p<.0001$. However, Tr and Tts increased over time, $F(4,80)=62.41$, $p<.0001$ and $F(4,80)=45.53$, $p<.0001$, respectively, (Figure 8), and these results were paralleled by an increase in percentage change from baseline over time for Tr, $F(4,80)=61.88$, $p<.0001$, and Tts, $F(4,80)=44.85$, $p<.0001$. Trend analysis yielded a quadratic component for both variables, $F(1,20)=5.55$, $p<.03$ and $F(1,20)=5.95$, $p<.02$, respectively, whereas linear contrasts suggested that Tr and Tts stabilized within 30 min of the start of the ISO infusion, $F(1,4)=5.30$, $p<.02$ and $F(1,40)=4.99$, $p<.03$, respectively. It was also apparent that the increase in Tr over time depended on the BP status of the animals, $F(4,80)=6.71$, $p<.0001$, and that this finding was also evident in the percentage change from baseline for Tr, $F(4,80)=6.66$, $p<.0001$. The rise in Tr during ISO was attenuated in the 1K-GB group when compared with the sham animals (Figure 9) and linear contrasts suggested that the difference between the two groups was apparent within the first 30 min of the infusion routine, $F(1,40)=5.75$, $p<.02$.

Isoproterenol with a $Beta_1$-Antagonist (Atenolol) versus Isoproterenol with a $Beta_2$-Antagonist (Pindolol). No index of

body temperature differed as a function of the beta-antagonist used with ISO, nor did the induction of 1K-GB hypertension have a differential effect on the thermogenic response to these agents. However, both Tr (Figure 8) and Tts increased during the administration of ISO combined with either antagonist, $F(4,80)=14.61$, $p<.0001$ and $F(4,80)=19.48$, $p<.0001$ respectively. This increase was paralleled by a similar rise in percentage change from baseline for Tr, $F(4,80)=14.66$, $p<.0001$, and Tts, $F(4,80)=19.59$, $p<.0001$. Trend analysis yielded a significant linear component for both variables, $F(1,20)=17.26$, $p<.0005$ and $F(1,20)=31.12$, $p<.0001$, respectively, suggesting that both Tr and Tts continued to increase for the duration of the infusion period. However, the increase in percentage change from baseline in Tr tended to be lower in the 1K-GB hypertensive rats, $F(4,80)=2.66$, $p<.038$ relative to their normotensive controls.

Isoproterenol Alone versus Isoproterenol with a Beta$_1$ (Atenolol)-Antagonist. When collapsed across drug infusions, 1K-GB hypertension had no effect on indices of body temperature. However, there was a significant BP Status x Drug interaction for the percentage change in Tr, $F(1,10)=9.18$, $p<.013$. As shown in Table 2, when ISO alone was administered, the 1K-GB animals exhibited a lower Tr response than did the sham animals, $F(1,1)=21.37$, $p<.0001$. However, during combined ISO and beta$_1$-blockade infusions, the values for the two groups were virtually identical, $F(1,1)=0.55$, $p<.461$. Moreover, as evident in Figure 8, only the increase in Tts was suppressed when ISO was preceeded with a beta$_1$-blocker, $F(1,10)=9.95$, $p<.01$. Both Tr and Tts demonstrated a tendency to

increase during the drug infusions, $F(4,40)=25.86$, $p<.0001$ for Tr and $F(4,40)=28.96$, $p<.0001$ for Tts. However, the increase for Tr was greater during ISO alone than during ISO with the $beta_1$-blocker, $F(4,40)=4.76$, $p<.0031$. Trend analysis of the overall tendency for Tr and Tts to increase over time yielded a linear component, $F(1,10)=31.78$, $p<.0002$ and $F(1,10)=51.84$, $p<.0001$, respectively, implying that neither variable attained asymptotic levels during the drug infusions. In addition, the 1K-GB and sham groups exhibited a differential increase in Tr over time, $F(4,40)=7.07$, $p<.0002$. As illustrated in Figure 10, Tr for the 1K-GB animals stablized within 30 min of the start of the infusions, $F(1,4)=3.73$, $p<.006$, whereas the sham animals exhibited a steady increase, $F(1,4)=1.37$, $p<.024$.

Isoproterenol Alone versus Isoproterenol with a $Beta_2$-Antagonist (Pindolol). The induction of 1K-GB hypertension had no effect on the three indices of body temperature. However, as depicted in Figure 8, pretreatment with the $beta_2$-antagonist suppressed the ISO-induced rise in Tr, $F(1,10)=10.56$, $p<.009$, and to a lesser extent Tts, $F(1,10)=7.92$, $p<.018$. Both Tr, $F(4,40)=23.13$, $p<.0001$, and Tts, $F(4,40)=25.82$, $p<.0001$, increased during the infusions. This trend was linear for both Tr, $F(1,10)=27.52$, $p<.0004$, and Tts, $F(1,10)=58.48$, $p<.0001$. Furthermore, the increase in Tr over time was marginally greater during ISO alone than when ISO was paired with the $beta_2$-blocker, $F(4,40)=3.41$, $p<.017$. This finding was also expressed in the percentage change data, $F(1,20)=23.23$, $p<.0007$. Moreover, as shown in Table 2, when ISO was administered alone, the 1K-GB group had a lower percentage change in Tr than did the shan animals, $F(1,1)=16.18$, $p<.0001$. However,

pretreatment with the beta$_2$-blocker lowered the percentage change in Tr selectively in the sham animals rendering the two groups statistically similar, $F(1,1)=1.41$, $p<.238$.

Cardiovascular Parameters

Baseline. No differences in mean arterial blood pressure (MABP) or heart rate (HR) were obtained across the two test sessions. As depicted in Table 1, renal clipping in the 1K-GB group increased MABP, $F(1,20)=51.25$, $p<.0001$, but only marginally increased HR, $F(1,20)=5.61$, $p<.03$.

Isoproterenol Alone. Mean arterial blood pressure remained significantly elevated for the 1K-GB group when compared with the sham animals, $F(1,20)=20.13$, $p<.0002$, Table 1, however, HR did not differ between the two groups in response to ISO. The percentage change in HR was attenuated in the hypertensive animals relative to the normotensive controls, $F(1,20)=9.45$, $p<.006$, Table 2.

Isoproterenol with a Beta$_1$-Antagonist (Atenolol)- versus Isoproterenol with a Beta$_2$-Antagonist (Pindolol). Neither MABP nor HR differed as a function of which drug combination was administered. However, when collapsed across drug manipulations, 1K-GB animals continued to exhibit a significantly higher BP, $F(1,20)=29.13$, $p<.0001$, but only marginally lower HR, $F(1,20)=5.97$, $p<.024$, as compared with the sham controls.

Isoproterenol Alone versus Isoproterenol with a Beta$_1$-Antagonist (Atenolol). There was a tendency for MABP to decrease as a function of time across both drug conditions, $F(4,40)=3.51$, $p<.015$, as illustrated in Figure 11. Trend analysis revealed a moderate quadratic component, $F(1,10)=5.31$, $p<.04$, and

linear contrasts suggested that after 20 min of either infusion routine, MABP had stabilized, $F(1,4)=5.75$, $p<.02$. On the other hand, HR was lower when the ISO infusion was preceeded by a $beta_1$-antagonist, $F(1,10)=16.91$, $p<.002$, and the percentage change in HR was also suppressed, $F(1,20)=8.68$, $p<.015$. Moreover, when collapsed across drug conditions, a smaller percentage change in HR was observed in the 1K-GB animals, $F(1,20)=10.24$, $p<.009$.

Isoproterenol Alone versus Isoproterenol with a $Beta_2$-Antagonist (Pindolol). The administration of a $beta_2$-blocker prior to ISO did not alter the magnitude of the ISO-induced depressor response or tachycardia (Figure 11). When collapsed across drug infusion routines, the 1K-GB animals had a higher MABP than did the sham animals, $F(1,10)=91.67$, $p<.0001$. However, 1K-GB hypertension did not affect the myocardial response to ISO, either alone or with the $beta_2$-antagonist.

Evaporative Heat Loss Analysis

The induction of 1K-GB hypertension had no overall effect on evaporative heat loss (EHL). However, EHL was significantly increased following all drug administrations (M=2.2 ml/50 min) when compared with baseline (M=1.9 ml/50 min), $F(1,20)=12.95$, $p<.002$.

Urinary Volume, Sodium, and Potassium Analysis

Analysis of urinary volume (Ms=27.5 vs. 27.7 ml), sodium (Ms=64.5 vs. 61.7 mEq/l), and potassium (Ms=151.8 vs. 155.0 mEq/l) content revealed no significant difference between the 1K-GB and the sham groups.

Discussion

The enhancement of thermogenesis with beta-stimulation obtained in cold-acclimated rats is generally viewed as arising from $beta_1$-stimulation of brown adipose tissue. However, the fact that the available beta-agonists are relatively nonspecific renders the $beta_2$-receptor subpopulation as a potential confounding influence on isoproterenol-induced thermogenesis. Accordingly, to assess the influence of 1K-GB renovascular hypertension on $beta_1$-induced thermogenesis one must first identify, and then preclude, the inadvertent contribution of $beta_2$-receptors towards isoproterenol-induced thermogenesis in cold-acclimated rats. The strategy employed in Experiment 1 to accomplish this objective was to assess the thermogenic consequences of isoproterenol in the presence or absence of selective $beta_1$- or $beta_2$-antagonists in cold-acclimated, hypertensive and normotensive rats.

Comparison of baseline values suggested that the experimental protocol introduced minimal stress or testing artifact. First, the absense of differences between the two test sessions suggests that the drug manipulations on the first day of testing did not alter baseline or drug responses during the second test session. Second, baseline metabolic rate and body temperatures were within the normal range of variability reported for cold-acclimated rats (Depocas et al., 1978; Flaim et al., 1977; Foster, 1985; Fregly et al., 1977). This suggests that (a) the restraint adaptation used during cold acclimation habituated the animals to the metabolic testing procedure, and (b) neither cold acclimation nor the surgical manipulations jeopardized the maintenance of an adequate metabolic

rate or body temperature. Third, baseline blood pressure and heart rate for the sham normotensive animals were within a generally accepted normotensive range (Leenen & DeJong, 1971), suggesting that neither cold acclimation nor the restraint procedure caused blood pressure to approach a hypertensive range.

The response profiles accompanying adrenergic stimulation included the following general features. Isoproterenol increased metabolic rate, rectal and tail skin temperatures, and heart rate, but reduced blood pressure. The thermogenic response to isoproterenol is consistent with its beta-receptor stimulating action on brown adipose tissue (Foster, 1985; Fregly, 1975). The increased rectal and tail skin temperatures are presumably secondary to the isoproterenol-induced thermogenesis (Flaim et al., 1977; Fregly et al., 1977). The rise in tail skin temperature and the depressor response may also reflect vascular beta-receptor mediated vasodilation (Berry et al., 1984; Himori et al., 1981). The tachycardic response to isoproterenol agreed with the findings of Buckley et al. (1984). They attributed the increase in heart rate following isoproterenol administration to its $beta_1$-stimulating effects since Lands et al. (1967) had earlier documented the presence of $beta_1$-receptors on the myocardium. The general paucity of changes in interscapular skin temperature may be an artifact of the experimental procedure. Most studies assessing interscapular skin temperature use subcutaneously-implanted thermocouples (e.g., Flaim et al., 1977). Instead, the procedure used in the present study was to tape a banjo probe to the interscapular skin surface. The difficulty experienced in maintaining an adequate skin contact may

have jeopardized the validity of the measure. Nevertheless, the observed isoproterenol-induced increase in interscapular skin temperature is consistent with Flaim et al.'s (1977) report that isoproterenol-induced thermogenesis elevates brown adipose tissue temperature, which, in turn, may be reflected as an increase in interscapular skin temperature.

Pretreatment with a $beta_1$-antagonist, atenolol, suppressed the isoproterenol-induced thermogenesis, the rise in rectal and tail skin temperature and the tachycardia. The attenuated thermogenesis supports Nedergaard and Lindberg's (1982) contention that brown fat thermogenesis is largely $beta_1$-mediated. Similarly, the diminished rectal and tail skin temperature response would be anticipated as a logical consequence of the suppression of isoproterenol-induced thermogenesis (Hart, 1971). Finally, the dampened isoproterenol-induced tachycardia supports the contention that isoproterenol stimulates cardiac $beta_1$-receptors (Lands et al., 1967). However, even in the presence of atenolol, isoproterenol increased metabolic rate and body temperature. The persistent thermogenesis suggests that (a) either the $beta_1$-antagonist was ineffective in blocking all $beta_1$-receptors or (b) other receptor populations participate in mediating thermogenesis. It is probable that atenolol effectively blocked $beta_1$-receptors given Himori et al.'s (1981) finding that it has a 300:1 affinity for $beta_1$- over $beta_2$-receptors, has a half-life (approximately 8.5 hr) far exceeding the 50 min infusion period used in this experiment, and effectively blocked cardiac $beta_1$-receptors, as assessed by the suppression of isoproterenol-induced tachycardia. Viewed together,

these observations suggest that other receptor populations may contribute to isoproterenol-induced thermogenesis.

In this regard, $beta_2$-antagonism with pindolol also reduced the isoproterenol-induced rise in metabolism and body temperature. Given pindolol's relative $beta_2$-receptor specificity (Himori et al., 1981), it seems likely that its attenuation of isoproterenol-induced thermogenesis reflected a $beta_2$-contribution to nonshivering thermogenesis rather than from inadvertent $beta_1$-antagonism. Moreover, the failure of pindolol to suppress the isoproterenol-induced tachycardia supports the findings that cardiac beta-receptors are predominantly $beta_1$ (Lands et al., 1967). Thus, the ability of the $beta_1$-antagonist to suppress the isoproterenol-induced increase in metabolism, body temperature and heart rate and of the $beta_2$-antagonist to attenuate only the metabolic and body temperature response suggests that another receptor population, perhaps $beta_2$, contributes to brown adipose tissue mediated thermogenesis. Hence, it may be concluded that, in order to evoke only $beta_1$-mediated thermogenesis with isoproterenol, the $beta_2$-receptors should be antagonized.

The drug manipulations increased evaporative heat loss relative to baseline levels. Schmidt-Nielsen et al. (1970) reported that evaporative heat loss dissipates excess body heat to the environment. This process is increased when rats were challenged in a warm environment or when administered a thermogenic agent. In the present study, isoproterenol was an effective thermogenic agent. Accordingly, the observed increase in evaporative heat loss may have served to buffer the isoproterenol-induced increase in rectal

temperature. In the cold-acclimated rats, this rise in evaporative heat loss was apparently unaltered by the presence of Goldblatt hypertension.

Several noteworthy characteristics of the 1K-GB animals were apparent during baseline recordings. The hypertensive animals had a transiently elevated metabolic rate and rectal temperature that persisted for the first 20 min; whereas their blood pressures and heart rates were chronically increased throughout the test session. This profile is consistent with the 1K-GB's purported state of sympathoexcitation, as assessed by the elevation in cardiac NE turnover (Tanaka et al., 1982) and plasma NE levels within 10 days of the induction of 1K-GB hypertension (Katholi et al., 1982; Reid et al., 1977). The sympathetic postganglionic release of NE could mediate nonshivering thermogenesis (Depocas et al., 1978), activate vascular $alpha_1$-receptors to augment peripheral resistance (Hannah et al., 1984), and stimulate cardiac $beta_1$-receptors to increase heart rate (DeJong et al., 1975).

In the 1K-GB animals the isoproterenol-induced increase in metabolic rate, rectal, interscapular, and tail skin temperatures, and heart rate was attenuated relative to the sham animals. This suppressed response may reflect an upper limit to the effects of isoproterenol. The law of initial values (Wilder, 1957) suggests that an autonomic response to stimulation is a function of the prestimulus level of responding. The higher this level, the smaller the response to a function-increasing stimulus. Accordingly, the reduced isoproterenol responsiveness for the 1K-GB animals may be an artifact of the elevated physiological baselines, thus, reflecting

the law of initial values. Alternatively, Rockson et al. (1981) reported that chronic NE stimulation suppressed the responsiveness of adrenoceptors to subsequent exogenous administration of epinephrine. If the chronically elevated plasma NE level of the 1K-GB animal (Katholi et al., 1982; Reid, et al., 1977) continuously activates the adrenergic receptors, then the exogenous administration of isoproterenol, a beta-agonist, might result in an attenuated metabolic responsiveness. This possibility is consistent with Fregly's (1954) report of a reduced beta-receptor reactivity in renovascular hypertensive rats.

From the results of Experiment 1 it may be concluded that a receptor population other than $beta_1$--perhaps $beta_2$--contributes to brown adipose tissue-mediated thermogenesis. Thus, to ensure the exclusive activation of $beta_1$-mediated thermogenesis with isoproterenol, the $beta_2$-receptors should be blocked. Furthermore, although the 1K-GB hypertensives were hyporesponsive to the thermogenic and cardiovascular effects of isoproterenol, they were not differentially responsonsive to either beta-antagonist. Hence, $beta_2$-antagonism itself should not influence the specific effect of 1K-GB hypertension on $beta_1$-induced thermogenesis.

Experiment 2. Effects of One-Kidney Goldblatt Hypertension and Cold Acclimation on Thermophysiological and Pressor Reactivity during Systemic Phenylephrine, Isoproterenol, and Combined Phenylephrine and Isoproterenol Infusions in Rats.

Method

Subjects

Sixty-four male, Sprague-Dawley rats initially weighing 70-90 g were used. The animals were housed and fed in a manner identical to that described for Experiment 1.

Procedure

Pretest procedure. Two days after their arrival in the laboratory half of the animals began 24 days of CA in a walk-in refrigerator as described in Experiment 1. The remaining animals (nCA) were housed in individual wire mesh cages located in a colony room maintained at 23+1 ^{o}C and kept on a 9/15 hr light/dark cycle (light on 0700-1600).

During CA or nCA, half of the animals were randomly assigned to the 1K-GB hypertensive group (1K-GB-CA, n=16 and 1K-GB-nCA, n=16) whereas the remaining animals were assigned to the sham normotensive group (sham-CA, n=16 and sham-nCA, n=16). The 1K-GB induction and sham operation followed the protocol described in the surgical procedures section and followed the time course discussed in Experiment 1. As described in Experiment 1, all animals underwent restraint adaptation, had 24 hr urine samples collected, and had their descending aortas and right jugular veins catheterized. Forty-eight hours following catheterization, metabolic testing commenced.

Metabolic testing. For purposes of metabolic testing the four groups were assigned to one of two drug conditions: (a) administration of either ISO or phenylephrine (PE) alone and (b) administration of ISO combined with PE. All animals receiving the ISO administrations were pretreated with pindolol, the beta$_2$-antagonist. This assignment of animals resulted in the following subgroups. A 1K-GB-CA group that received (a) ISO alone on one test session and combined ISO and PE on the other test session (1K-GB-CA-ISO, n=8) or (b) PE alone and then combined ISO and PE (1K-GB-CA-PE, n=8); a 1K-GB-nCA group that received (a) ISO alone and then combined ISO and PE (1K-GB-nCA-ISO, n=8) or (b) PE alone and then combined ISO and PE (1K-GB-nCA-PE, n=8); a sham-CA group that received (a) ISO alone and then combined ISO and PE (sham-CA-ISO, n=8) or (b) PE alone and then combined ISO and PE (sham-CA-PE, n=8); and finally a sham-nCA group that received (a) ISO alone and then combined ISO and PE (sham-nCA-ISO, n=8) or (b) PE alone and then combined with ISO and PE (sham-nCA-PE, n=8).

Following recovery from the catheterizations, the metabolic and cardiovascular responses to intravenous drug administrations were assessed over two test sessions. Three animals were tested each day commencing at 0700 hr. The drug solutions, consisting of ISO ((-1)-isoproterenol hydrochloride, .5 ug/kg/min), pindolol (1(1H-Indol-4-yloxyl)-3-(isopropylamino)-2-propranol, 10 ug/kg), and PE (l-phenylephrine hydrochloride, 10 ug/kg/min) were weighed on a Sartorius Balance and dissolved in sterile .15M saline. All doses were expressed in the hydrochloric acid form. The dose of PE used was shown by Foster (1985) to be without thermogenic properties and

was reported to produce a moderate pressor response (Bethelsen & Pettinger, 1977). All ISO administrations, whether alone or in combination with PE were preceeded with a .25 ml bolus injection of the $beta_2$ blocker pindolol. Solutions were made immediately before each infusion and the pH of the solutions was monitored. The order of drug administration was counterbalanced across the test sessions.

For purposes of metabolic testing the animals were set up in the metabolic chamber as described in Experiment 1. The animals were allowed 90 min to stabilize, the last 50 min of which constituted the baseline period. Infusions of ISO and PE combined and ISO alone or PE alone occurred in the manner described in Experiment 1. During both baseline and drug administration periods, 10-min recordings of oxygen consumption, carbon dioxide production, rectal temperature, interscapular and tail skin temperatures, BP and heart rate were taken. Between the first and second test session the animals were returned to their home cages located in either the walk-in refrigerator or the 23 ^{o}C colony room and allowed a 46-hr rest period. Following the completion of the second test session all animals were euthanized with an overdose of sodium pentobarbital (60 mg/kg, i.p.).

Results

Body Weight Analysis

There were no group differences in the body weights between the 1K-GB hypertensive (M=255.2 g) and the sham normotensive (M=260.7 g) group or between the CA (M=246.5 g) and the nCA (M=239.6 g) group.

Metabolic Rate

Baseline. No differences in baseline were observed for oxygen

consumption (VO_2), carbon dioxide production (VCO_2), or respiratory quotient (RQ) between the two test sessions. As shown in Tables 3 and 4, neither 1K-GB hypertension nor CA altered baseline metabolism. A BP Status x Acclimation Status interaction occurred for VO_2, $F(1,56)=8.87$, $p<.0043$. Analysis of the data summarized in Figure 12 shows (a) that in the nCA condition, the 1K-GB group exhibited a lower VO_2 than the sham controls, $F(1,1)=14.43$, $p<.0002$; (b) that the CA process augmented the VO_2 for the 1K-GB group, $F(1,1)=7.21$, $p<.008$, but reduced VO_2 for the sham controls, $F(1,1)=48.94$, $p<.0001$. Consequently, when rats were cold acclimated, their VO_2 profiles were reversed, such that the 1K-GB group's VO_2 was higher than that of the controls, $F(1,1)=34.59$, $p<.0001$. When collapsed across BP and acclimation status, baseline VO_2 increased over time, $F(4,228)=34.23$, $p<.0001$ (Figure 13). Trend analysis revealed a quadratic component for this increase, $F(1,56)=11.03$, $p<.0016$, with VO_2 stabilizing after 30 min of baseline, $F(1,4)=8.65$, $p<.0003$. Moreover, the tendency for VO_2 to increase was dependent on the BP status of the animals, $F(4,228)=4.50$, $p<.0016$. As illustrated in Figure 14, the 1K-GB animals had a higher VO_2 for the last 30 min of baseline relative to the sham controls, $F(1,4)=16.52$, $p<.0001$. Respiratory quotient decreased over time, $Ms=.81$ to $.70$, $F(4,228)=4.52$, $p<.0016$, in a linear fashion, $F(1,56)=129.01$, $p<.0001$.

Isoproterenol Alone versus Phenylephrine Alone. Isoproterenol increased VO_2 and VCO_2, $F(1,56)=15.65$, $p<.0001$ and $F(1,56)=12.79$, $p<.0007$, respectively; whereas PE did not affect either variable. This finding was paralleled in the percentage change data for VO_2,

$F(1,56)=15.16$, $p<.0003$, and for VCO_2, $F(1,56)=26.38$, $p<.0001$. Collapsed across both drugs, VO_2 and VCO_2 increased over time, $F(4,228)=19.70$, $p<.0001$ and $F(4,228)=4.28$, $p<.0023$, respectively. Trend analysis revealed that the increase over time followed a linear trend for VO_2, $F(1,56)=31.62$, $p<.0001$, but was quadratic for VCO_2, $F(1,56)=5.06$, $p<.028$, with VCO_2 stabilizing within 30 min of infusion. Moreover, this time function for VO_2, $F(4,228)=11.13$, $p<.0001$, and VCO_2, $F(4,228)=5.81$, $p<.0002$, was drug-dependent. For the last 30 min of the infusion, VO_2, $F(1,4)=7.28$, $p<.007$, and VCO_2, $F(1,4)=6.27$, $p<.013$, increased more during the ISO than PE infusion (Figure 13). Respiratory quotient decreased over time, Ms=.72 to .69, $F(4,228)=7.99$, $p<.0001$, in a linear manner, $F(1,56)=14.60$, $p<.0003$.

Isoproterenol Alone versus Isoproterenol Combined with Phenylephrine. As depicted in Figure 13, both VO_2, $F(1,28)=10.01$, $p<.0037$, and VCO_2, $F(1,28)=4.33$, $p<.05$, were greater for the combined ISO and PE injections than for ISO alone. When the drug infusions were collapsed, the percentage change in VO_2, $F(1,28)=10.54$, $p<.003$, and VCO_2, $F(1,28)=5.09$, $p<.03$, depended on the acclimation status of the animals, with the CO animals increasing metabolic rate more than the nCO animals. Analysis of a BP Status x Acclimation Status interaction, $F(1,28)=15.77$, $p<.0005$, Figure 15, showed that when non-cold-acclimated, the 1K-GB animals had a lower VO_2 than the sham controls, $F(1,1)=13.80$, $p<.0002$; whereas, this difference is reversed for the cold-acclimated animals, $F(1,1)=54.89$, $p<.0001$. Moreover, a BP Status x Acclimation Status x Drug interaction, $F(1,28)=14.05$, $p<.0008$, showed that during ISO, the

VO_2 response was comparable across all BP and CA manipulations (Figure 16). However, the responsiveness to combined ISO and PE injections was potentiated in the 1K-GB-CA rats, $F(1,1)=29.76$, $p<.0001$, and in the sham-nCA animals, $F(1,1)=32.13$, $p<.0001$. Thus, the 1K-GB rats exhibited an increased sensitivity during CA, and this trend emerged again when ISO and PE were combined. It was also apparent that VO_2 and VCO_2 increased over time, $F(4,112)=74.80$, $p<.0001$ and $F(4,112)=31.28$, $p<.0001$, respectively, in a quadratic fashion, $F(1,28)=40.66$, $p<.0001$ for VO_2 and $F(1,28)=44.63$, $p<.0001$ for VCO_2, and stabilized within 30 min of the start of the infusion, $F(1,4)=10.96$, $p<.001$ for VO_2 and $F(1,4)=12.41$, $p<.0005$ for VCO_2. Conversely, RQ decreased over time, (M=.74 to .71), $F(4,112)=6.28$, $p<.0001$, in a quadratic fashion, $F(1,28)=5.25$, $p<.029$, and stabilized within 30 min of the infusion, $F(1,4)=1.94$, $p<.016$.

Phenylephrine Alone versus Isoproterenol Combined with Phenylephrine. As illustrated in Figure 13, PE infusions evoked a lower VO_2 and VCO_2 than did combined ISO and PE, $F(1,28)=44.41$, $p<.0001$ and $F(1,28)=43.81$, $p<.0001$, respectively. The VO_2, $F(4,112)=40.39$, $p<.0001$, and VCO_2, $F(4,112)=27.11$, $p<.0001$, increased as a function of time. Quadratic components were obtained for VO_2, $F(1,28)=20.55$, $p<.0001$ and VCO_2, $F(1,28)=31.97$, $p<.0001$; whereas, linear contrasts showed that both VO_2, $F(1,4)=9.42$, $p<.0002$, and VCO_2, $F(1,4)=8.22$, $p<.004$, reached a steady state within 30 min of the start of the infusion. For both VO_2 and VCO_2 the time function varied with the drug manipulation, $F(4,116)=39.00$, $p<.0001$ and $F(4,116)=33.28$, $p<.0001$, respectively, in that the thermogenesis was greater during combined ISO and PE than

during PE alone. Moreover, the BP status of the animals had a further differentiating effect on the drug dependent increase in VO_2 (Figure 17) and VCO_2 over time, $F(4,116)=3.74$, $p<.007$ and $F(4,116)=4.35$, $p<.003$, respectively. In either instance, the 1K-GB animals tended to have a lower metabolic rate within 30 min relative to the Sham animals, $F(1,4)=6.67$, $p<.01$ for VO_2 and $F(1,4)=4.76$, $p<.03$ for VCO_2.

Body Temperature Indices

Baseline. There were no differences in either rectal temperature (Tr), interscapular skin temperature (Tis), or tail skin temperature (Tts) for baseline between the two test sessions. Although the process of CA did not have an overall effect on any index of body temperature, 1K-GB hypertension marginally increased Tr, $F(1,56)=5.70$, $p<.020$ (Table 3). A BP Status x Acclimation Status interaction occurred for Tts, $F(1,56)=13.44$, $p<.0005$. Analysis of the data summarized in Figure 18 shows that when the rats were not cold acclimated, the 1K-GB group exhibited a higher Tts, $F(1,1)=51.15$, $p<.0001$, whereas, when they were cold acclimated, the Tts for the 1K-GB group was suppressed, $F(1,1)=50.16$, $p<.0001$, but augmented for the sham controls, $F(1,1)=45.64$, $p<.0001$. Baseline Tts also increased over time, $F(4,228)=16.70$, $p<.0001$, in a linear manner, $F(1,56)=19.71$, $p<.0001$.

Isoproterenol Alone versus Phenylephrine Alone. Although no drug effect was obtained for Tis or Tts, Tr increased from 36.7 to 37.2 ^{o}C during ISO administration, but was stable at 35.9 ^{o}C during PE, $F(1,56)=32.17$, $p<.0001$ (Figure 19). Both Tr, $F(4,228)=5.45$, $p<.0003$, and Tts, $F(4,228)=19.02$, $p<.0001$, increased over time in a

quadratic fashion, $F(1,56)=6.49$, $p<.014$ for Tr and $F(1,56)=9.08$, $p<.004$ for Tts. Linear contrasts suggested that Tr, $F(1,4)=1.06$, $p<.03$, and Tts, $F(1,4)=8.80$, $p<.003$, stabilized within 30 min. For both Tr, $F(4,228)=46.98$, $p<.0001$, and Tts, $F(4,228)=11.86$, $p<.0001$, the increase over time was greater during ISO than during PE administrations. This finding was similarly expressed in the percentage change for Tr, $F(4,228)=46.24$, $p<.0001$, and for Tts, $F(4,228)=12.48$, $p<.0001$.

Isoproterenol Alone versus Isoproterenol Combined with Phenylephrine. No effects of drug manipulation were obtained for Tr, Tis, or Tts. However, the percentage change from baseline in Tts exhibited a marginal BP Status x Acclimation Status interaction, $F(1,28)=7.07$, $p<.013$ (see Figure 20), which reflected a lower Tts for the 1K-GB animals than for the sham animals, when non-cold-acclimated, $F(1,1)=14.98$, $p<.0001$, but an increased Tts response in the 1K-GB rats following CA, $F(1,1)=10.46$, $p<.001$. Generally, Tr, $F(4,112)=73.70$, $p<.0001$, and Tts, $F(4,112)=48.23$, $p<.0001$, increased over time with a quadratic trend, $F(1,28)=37.50$, $p<.0001$ and $F(1,28)=15.39$, $p<.0005$, respectively. The time function for Tr was dependent on the pharmacological manipulation, $F(4,108)=5.65$, $p<.0003$, with the increase in Tr being greater during combined ISO and PE administration than during ISO alone, $F(1,4)=7.45$, $p<.007$ (Figure 19). The time function for Tts varied with the acclimation status, $F(4,112)=3.67$, $p<.008$, with the increased Tts being greater for the cold-acclimated animals by the 30th min, $F(1,4)=6.92$, $p<.009$, but being attenuated towards the latter part of the infusion (Figure 21).

Phenylephrine Alone versus Isoproterenol Combined with Phenylephrine. The Tr response evoked by PE (M=36.0 ^{o}C) was lower than that obtained with combined ISO and PE infusions, (M=37.2 ^{o}C), $F(1,28)=24.91$, $p<.0001$. Generally, Tr and Tts increased over time, $F(4,112)=19.40$, $p<.0001$ and $F(4,112)=14.18$, $p<.0001$, respectively, in a linear manner, $F(1,28)=25.86$, $p<.0001$ and $F(1,28)=20.79$, $p<.0001$, respectively. For Tr, $F(4,116)=75.72$, $p<.0001$, Tis, $F(4,116)=3.34$, $p<.013$, and Tts, $F(4,116)=13.00$, $p<.0001$, the increase over time was greater during combined ISO and PE infusions than when PE was administered alone (Figure 19 for Tr and Tts).

Cardiovascular Parameters

Baseline. There were no differences in either mean arterial blood pressure (MABP) or heart rate (HR) for baseline between the two test sessions. Although the process of CA had no effect on baseline BP or HR, Table 3 shows that the 1K-GB group increased BP and HR, $F(1,56)=154.40$, $p<.0001$ and $F(1,56)=8.12$, $p<.006$, respectively. Both baseline BP, $F(4,228)=7.14$, $p<.0001$, and HR, $F(4,228)=7.74$, $p<.0001$, also decreased over time, with a linear trend for both BP, $F(1,56)=11.71$, $p<.0012$, and for HR, $F(1,56)=15.67$, $p<.0002$ (Figure 22).

Isoproterenol Alone versus Phenylephrine Alone. The 1K-GB animals generally had a higher MABP (M=177.6 mmHg) than did the sham animals, (M=119.6 mmHg), $F(1,56)=102.12$, $p<.0001$. However, the 1K-GB animals exhibited a somewhat lower percentage change in MABP, $F(1,56)=5.20$, $p<.03$, and HR, $F(1,56)=2.56$, $p<.04$, when compared with the sham group. As depicted in Figure 22, the MABP level in response to PE was greater, $F(1,56)=21.35$, $p<.0001$, but the HR was lower,

$F(1,56)=78.97$, $p<.0001$, than that observed for ISO. Moreover, as illustrated in Figure 23, the MABP response to ISO and PE was dependent on the CA status of the animals, $F(1,56)=7.36$, $p<.009$. During ISO administration, the cold- acclimated animals showed a lower MABP than did the non-cold-acclimated group, $F(1,1)=39.15$, $p<.0001$, whereas, during PE, the cold-acclimated group had a higher MABP than did the non-cold-acclimated animals, $F(1,1)=7.66$, $p<.006$ (Table 4). However, for both the cold, $F(1,1)=39.15$, $p<.0001$, and non-cold-acclimated animals, $F(1,1)=7.66$, $p<.006$, MABP was greater for PE than for ISO administrations .

Isoproterenol Alone versus Isoproterenol Combined with Phenylephrine. Both MABP and HR differed as a function of the BP status. Mean arterial blood pressure was higher, $F(1,28)=52.62$, $p<.0001$; (Ms=168.6 vs. 106.3 mmHg); whereas HR was marginally lower, $F(1,28)=6.11$, $p<.02$, for the 1K-GB group than for the sham controls and (Ms=510 vs. 552 bpm). Moreover, the 1K-GB animals exhibited a lower percentage change in HR than did the sham animals, $F(1,28)=17.99$, $p<.0002$.

Phenylephrine Alone versus Isoproterenol Combined with Phenylephrine. Mean arterial blood pressure for the 1K-GB group was greater than that observed for the sham group, (Ms=178 vs. 112 mmHg), $F(1,28)=54.57$, $p<.0001$. Figure 22 shows that MABP was greater, $F(1,28)=44.44$, $p<.0001$, but HR was less, $F(1,28)=154.62$, $p<.0001$, when PE was administered alone than when ISO and PE were combined.

Evaporative Heat Loss Analysis

Neither 1K-GB hypertension nor cold acclimation affected

evaporative heat loss. However, EHL was increased following combined ISO and PE (M=2.7 ml/50 min), F(1,56)=10.73, p<.002, relative to ISO (M=2.2 ml/50 min) or PE (M=2.0 ml/50 min) administration alone. Evaporative heat loss was also increased following all drug administrations relative to baseline values (M=1.7 ml/50 min), F(1,56)=107.05, p<.0001.

Urinary Volume, Sodium, and Potassium analysis

Analysis of urinary volume revealed an increase for the CA animals (M=20.5 ml/24hr) compared to the nCA group (M=16.0 ml/24hr), F(1,59)=4.54, p<.037. There was no main effect of either blood pressure or acclimation status on the urinary sodium concentration; however, BP and CA did interaction, F(1,59)=5.33, p<.025. Although there were no differences between the 1K-GB and sham animals when they were cold acclimated, (Ms=90 vs. 77 mEq/l), F(1,1)=1.37, p<.247, in the non-cold-acclimated condition, the sham animals had a significantly greater sodium concentration (M=110.7 mEq/l) than did the 1K-GB rats (M=86.9 mEq/l), F(1,1)=4.35, p<.004 (Figure 24). No differences for urinary potassium concentration were obtained.

Discussion

Beta$_1$-receptors on brown adipose tissue are functionally linked to nonshivering thermogenesis; whereas those on the myocardium contribute to cardiovascular homeostasis. Vascular alpha$_1$-receptors control the resistance side of circulation; however, their stimulation appears to potentiate beta$_1$-mediated thermogenesis. The cardiovascular effects of processes such as cold acclimation, which enhance beta$_1$-mediated nonshivering thermogenesis, are unknown, and the thermogenic consequences of hypertension,

which often potentiates $alpha_1$-controlled vasomotor tone, remain to be elucidated. Thus, the purpose of Experiment 2 was threefold. First, to assess whether Foster's (1985) finding in presumably normotensive rats of an enhanced thermogenic response to isoproterenol when phenylephrine was administered concurrently could be extended to non-cold-acclimated, 1K-GB hypertensive and sham normotensive rats. Second, to assess whether the development of increased reactivity to $beta_1$-induced thermogenesis accompanying cold acclimation would (a) unmask a latent thermogenic contribution of $alpha_1$-receptors during stimulation with an $alpha_1$-agonist and (b) enhance cardiovascular responsiveness to an $alpha_1$-agonist in the normotensive rat. Third, to examine whether 1K-GB hypertension will (a) alter the sensitivity to $beta_1$-induced thermogenesis in cold- or non-cold-acclimated rats and (b) jeopardize thermolysis, thereby facilitating hyperthermia in either cold- or non-cold-acclimated rats. To accomplish these objectives, cardiovascular, metabolic, and body temperature responses were monitored during the systemic infusion of either $alpha_1$-, $beta_1$-, or combined $alpha_1$- and $beta_1$-agonists in cold- or non-cold-acclimated, hypertensive or normotensive rats.

Analysis of baseline values revealed several features consistent with those observed for Experiment 1. First, there were no baseline differences between the two test sessions. Second, baseline metabolism and body temperatures were within the normal range of variability reported for cold and non-cold acclimated animals. Third, baseline blood pressures and heart rates for the sham animals were within a generally accepted normotensive range. These findings

were discussed in Experiment 1 and that discussion remains appropriate for the present experiment.

The response profiles resulting from adrenergic stimulation include several general features. Isoproterenol increased metabolic rate, rectal and tail skin temperatures, and heart rate, but it decreased blood pressure. These responses to isoproterenol are consistent with the findings of Experiment 1 and the discussion presented for those observations are applicable to the present experiment. Phenylephrine did not change metabolic rate or body temperature indices from baseline levels. These data support Foster's (1985) contention that phenylephrine alone is nonthermogenic. Phenylephrine did, however, increase blood pressure and decrease heart rate. The pressor effects support Hannah et al.'s (1984) finding that phenylephrine produced an $alpha_1$-mediated vasoconstriction, which, in turn, increased blood pressure. Thus, the phenylephrine-induced pressor response observed in this experiment probably reflects an $alpha_1$-mediated increase in total peripheral resistance. The bradycardia is consistent with the report by Wasserstrum and Herd (1977) in the squirrel monkey. They found that phenylephrine, which has no direct myocardial effects (Varma, Johnsen, Sherman, & Youmans, 1960), decreased heart rate. Wasserstrum and Herd (1977) suggested that the phenylephrine-induced increase in peripheral vascular resistance triggered a baroreceptor-induced bradycardia. This might account for the bradycardia observed in the present experiment.

When isoproterenol and phenylephrine were combined, metabolic rate and rectal and tail skin temperatures increased above those seen during the individual administration of either agent. This result is consistent with those of Flaim et al. (1977) and Foster (1985) that metabolism and body temperature indices increase more during the concurrent stimulation of $alpha_1$- and $beta_1$-receptors than during the activation of either receptor population alone. The general consensus from these findings is that, although $beta_1$-receptors may play an activating role in brown adipose tissue-mediated thermogenesis, the concurrent stimulation of $alpha_1$-receptors may potentiate this effect. This potentiating effect was also obtained in non-cold-acclimated and 1K-GB hypertensive and sham normotensive rats.

The thermogenesis and increased tail skin temperature observed with isoproterenol, presented either alone or with phenylephrine, was potentiated by cold acclimation. These findings and those from studies using related adrenoceptor agents (Depocas et al., 1978; Fregly et al., 1977) lend credence to the notion that cold acclimation may hypersensitize brown adipose tissue $beta_1$-receptors. No evidence that cold acclimation potentiated the thermogenic contribution of, or the cardiovascular responsiveness to, $alpha_1$-stimulation with an $alpha_1$-agonist in the normotensive rat was obtained.

The drug manipulations increased evaporative heat loss relative to baseline levels. This finding is in accordance with those reported in Experiment 1 and the discussion therein is appropriate for the present experiment.

The finding that the urine volume excreted in 24 hr was greater in the cold-acclimated animals supports Fregly's (1954) findings. He interpreted the increased output as secondary to an enhanced fluid intake in the cold-acclimated animals. Observations of fluid consumption in the present study, although not quantified, would support this notion. Urinary sodium concentration was jointly influenced by both the blood pressure and acclimation status of the animals. When non-cold acclimated, the 1K-GB hypertensives had a lower urinary sodium concentration suggesting they retained more sodium than their sham counterparts. Laragh et al. (1982) demonstrated that during the early stages of a stringent dietary sodium restriction, blood pressure was significantly reduced. These findings suggested that the 1K-GB preparation promotes the retention of sodium and water, which, in turn, elevates blood volume and initiates hypertension. Similarly, since the 1K-GB animals in the present study were apparently retaining sodium, their elevation in blood pressure may have resulted from an enhanced blood volume. During cold acclimation, the sodium concentration in the urine of sham animals was reduced such that it failed to differ from that observed in the 1K-GB rats. The increased volume of urine excreted by the cold-acclimated animals may account for this finding. If the animals are maintaining a constant sodium balance and, thus, are excreting the same absolute quantity of sodium, then the increased urinary output would diminish sodium concentration. Alternatively, if cold acclimation results in sodium retention, then the animals would excrete less sodium, and when coupled with the increased volume of excreted urine, sodium concentration would decline. Further

investigation is required to determine which of these explanations is more viable.

The 1K-GB animals had an elevated baseline oxygen consumption, rectal temperature, blood pressure, and heart rate. The 1K-GB animals are characterized by an increase in cardiac NE turnover (Tanaka et al., 1982) and an elevated plasma NE level (Katholi et al., 1982; Reid et al., 1977). These findings suggest a sympathoexcitation, the consequences of which may (a) increase peripheral vascular resistance through stimulation of vascular alpha-receptors and hence increase blood pressure and (b) activate cardiac $beta_1$-receptors promoting tachycardia. Moreover, since the sympathetic postganglionic release of NE mediates nonshivering thermogenesis (Depocas et al., 1978; Hsieh et al., 1957; Young et al., 1982), the increased baseline metabolism in the 1K-GB group could reflect the chronic stimulation of brown adipose tissue beta-receptors. The increased rectal temperature of the 1K-GB animal probably reflects this enhanced thermogenesis (Hart, 1971). The increased peripheral vascular resistance characteristic of the 1K-GB rat (Bralet et al., 1973) may also compromise tissue conductance (Raman et al., 1983), thereby promoting a higher rectal temperature by impairing thermolysis.

Baseline oxygen consumption was jointly influenced by the blood pressure and acclimation status of the animals. In the sham group, cold acclimation lowered baseline oxygen consumption. This finding deviates from the reports of Foster and Frydman (1978) who found that cold acclimation enhanced baseline metabolism. However, the present study used restrained and conscious, rather than anesthetized

rats. Few, if any, studies have assessed the effects of mild physical restraint or adaptation to such restraint on the metabolic rate of cold acclimated animals. Such procedures may exert a selective effect on metabolism under conditions of cold acclimation. Restrained, conscious animals were used to avoid the artifacts of anesthesia on metabolic and cardiovascular variables. Brezenoff (1973) and DeCastro (1979) assessed the effect of pentobarbital anesthesia on the cardiovascular and metabolic response to intravenously administered norepinephrine. They found that a deep anesthesia led to a 10% to 30% reduction in the pressor response and a 20% diminution of the metabolic response to NE when compared with conscious controls. Thus, a deep pentobarbitone anesthesia tends to suppress cardiovascular and thermogenic responsiveness to adrenergic stimulation. Rectal temperature also declined during anesthesia. To the extent that (a) rectal temperature reflects metabolic rate and (b) the adrenoceptors involved in the cardiovascular responses are comparable to those mediating metabolism, the use of sodium barbital anesthesia by Foster and Frydman (1978) may have suppressed metabolism, at least in the cold-acclimated condition. Perhaps then, the cold acclimation-induced elevation in metabolism observed in their study is an artifact of the anesthetic. This possibility would, however, counter the prevailing notion that cold acclimation elevates baseline metabolism and hence, the issue awaits further investigation. In the present study, the hypertensive, cold-acclimated rats had an elevated baseline oxygen consumption. This is consistent with reports that cold acclimation increases baseline metabolic rate (Cottle & Carlson, 1956; Foster & Frydman,

1978, 1979). A cold acclimation-induced increase in sympathetic activity (Hsieh et al., 1957) is thought to stimulate $beta_1$-receptors located on brown adipose tissue (Nedergaard & Lindberg, 1982) and, thus, to promote thermogenesis (Depocas et al., 1978; Foster, 1985). Thus, in the present study, cold acclimation may have enhanced oxygen consumption in the 1K-GB animals through a rise in sympathetic activation of brown adipose tissue.

The suppressed metabolic and myocardial responsiveness to isoproterenol alone or combined with phenylephrine in the 1K-GB hypertensives may reflect an attenuated sensitivity of $beta_1$-receptors located on brown adipose tissue and myocardium. This attenutation of beta-responsivity in the 1K-GB animals may be explained through the studies of Rockson et al. (1981), Katholi et al. (1982), and Fregly (1954) as discussed in Experiment 1. The fact that hypertension did not alter rectal temperature responsiveness does not preclude a reduced beta-adrenoceptor interpretation of the attenuated rise in thermogenesis. The increased vascular resistance sustaining the renovascular hypertension (Bralet et al., 1973) would tend to reduce tissue conductance (Raman et al., 1983) and, thereby, buffer drops in rectal temperature that would otherwise accompany a suppressed thermogenesis. Hence, although the 1K-GB animals tended to exhibit a lower thermogenic response to adrenergic stimulation, a reduced thermolysis, or enhanced heat accumulation, may prevent a concomitant reduction in rectal or skin temperatures.

It may be concluded from Experiment 2 that Foster's (1985) finding of an $alpha_1$-potentiation of $beta_1$-mediated thermogenesis in cold-acclimated rats may be extended to non-cold-acclimated and

1K-GB hypertensive rats. Moreover, cold acclimation failed to promote a thermogenic contribution by $alpha_1$-receptors and failed to enhance cardiovascular responsivity to $alpha_1$-stimulation. One-kidney Goldblatt hypertension attenuated both thermogenic and cardiovascular responsiveness to $beta_1$-stimulation in cold- and non-cold-acclimated rats. However, despite the thermogenic attenuation, 1K-GB hypertension promoted hyperthermia, possibly due to impaired thermolysis. Finally, cold acclimation and 1K-GB hypertension did not interact in any systematic manner to influence either the cardiovascular or thermoregulatory systems.

Experiment 3. Cardiovascular and Thermophysiological Adjustments Accompanying Systemic $Beta_1$-, $Alpha_2$-, and Combined $Beta_1$- and $Alpha_2$-Stimulation in Cold-Acclimated, 1K-GB Hypertensive and Normotensive Control Rats.

Method

Subjects

Twenty-four male, Sprague-Dawley rats initially weighing 70-90 g were used. The animals were housed and fed in a manner identical to that described for Experiment 1.

Procedure

Pretest procedure. Two days after their arrival in the laboratory all animals began 24 days of CA in a walk-in refrigerator as described in Experiment 1. During CA the animals were assigned to either the 1K-GB hypertensive or sham normotensive group. The 1K-GB induction and sham operation followed the protocol described in the surgical procedures section. As described for Experiment 1, all animals underwent restraint adaptation and had their descending aorta and right jugular vein catheterized. Metabolic testing commenced forty-eight hours following catheterization.

Metabolic testing. For purposes of metabolic testing the animals were further assigned to one of two drug conditions that were conducted over separate test sessions: (a) Administration of either ISO or Guanabenz (GUANA) (an $alpha_2$-agonist) alone; (b) administration of ISO combined with GUANA. All animals receiving the ISO administrations were pretreated with pindolol, the $beta_2$-antagonist. This assignment of animals resulted in the following subgroups: a 1K-GB group that received (a) ISO alone on

one test session and combined ISO and GUANA on the other test session (1K-GB-ISO, n=6) or (b) GUANA alone and the combined ISO and GUANA (1K-GB-GUANA, n=6) and a sham group that received (a) ISO alone and then combined with GUANA (sham-ISO, n=6), or (b) GUANA alone and then combined with ISO (sham-GUANA, n=6).

Following recovery from the catheterizations, the animals' metabolic, thermogenic, and cardiovascular responses to intravenous administration of ISO, GUANA, and combined ISO and GUANA were assessed. Three animals were tested each day commencing at 0700 hr, and a 46-hr rest period was interpolated between the first and last test session. The protocol used for metabolic testing was identical to that described for Experiment 1 except for the agents used. On one test session either GUANA alone (1-(2,6-dichlorobenzylidenamino) guanadine, 100 ug/kg/min) or ISO alone (.5 ug/kg/min) was infused whereas on the other test session, combined ISO and GUANA was administered. The doses for all drugs were expressed in the hydrochloric acid form. The dose of GUANA was reported by Hannah et al. (1984) to produce a moderate pressor response. The order of drug administration over the two test sessions was counterbalanced. The dependent variables monitored were identical to those described for Experiment 1, and the animals were sacrificed at the end of the second test session with an overdose of sodium pentobarbital.

Results

Body Weight

There were no differences in body weight between the 1K-GB hypertensive (M=242.1 g) and the sham normotensive (M=256.8 g) groups.

Metabolic Rate

Baseline. No differences in oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were obtained between the two test sessions. Oxygen consumption increased over time, $F(4,80)=50.26$, $p<.0001$, (Figure 25) in a quadratic fashion, $F(1,20)=17.80$, $p<.0004$, and stabilized within 30 min of the start of baseline, $F(1,4)=19.19$, $p<.0001$. Respiratory quotient decreased over time, $F(4,80)=22.16$, $p<.0001$, in a quadratic fashion, $F(1,20)=7.59$, $p<.012$, and stabilized within 30 min, $F(1,4)=10.15$, $p<.002$.

Isoproterenol Alone versus Guanabenz Alone. Isoproterenol increased both VO_2, $F(1,20)=17.06$, $p<.0005$, and VCO_2, $F(1,20)=23.90$, $p<.0001$; whereas GUANA was without effect (see Figure 25). Both VO_2, $F(1,20)=14.28$, $p<.0012$, and VCO_2, $F(1,20)=23.21$, $p<.0001$, were suppressed in the 1K-GB hypertensives relative to the sham controls, and this finding was expressed in the precentage change data for VO_2, $F(1,20)=15.21$, $p<.0009$, and for VCO_2, $F(1,20)=20.80$, $p<.0002$. Moreover, both VO_2, $F(4,80)=14.86$, $p<.0001$, and VCO_2, $F(4,80)=6.18$, $p<.0002$, increased over time. This increase developed in a quadratic fashion for VO_2, $F(1,20)=12.01$, $p<.0024$, and stabilized within the first 30 min of the infusion, $F(1,4)=1.84$, $p<.178$. A linear trend occurred for VCO_2, $F(1,20)=9.75$, $p<.005$. For VO_2 (depicted in Figure 26), $F(4,80)=11.28$, $p<.0001$, and VCO_2, $F(4,80)=7.55$, $p<.0001$, the increase over time was less for the 1K-GB animals than for the sham group, with the difference between the groups being apparent within 20 min of the start of the infusion, $F(1,4)=9.91$, $p<.002$, for VO_2 and $F(1,4)=12.07$, $p<.0007$ for VCO_2. The increase over time for

VO_2, $F(4,80)=18.26$, $p<.0001$, and VCO_2, $F(4,80)=11.08$, $p<.0001$, was also greater during ISO than during GUANA infusion (Figure 25).

Isoproterenol Alone versus Isoproterenol Combined with Guanabenz. No drug main effect was observed for metabolism (see Figure 25). As Figure 27 shows, the 1K-GB hypertension tended to suppress both VO_2, $F(1,10)=5.10$, $p<.047$, and VCO_2, (not shown), $F(1,10)=9.11$, $p<.013$. Both VO_2, $F(4,40)=44.26$, $p<.0001$, and VCO_2, $F(4,40)=14.00$, $p<.0001$, increased over time in a quadratic fashion, $F(1,10)=9.94$, $p<.01$ and $F(1,10)=6.46$, $p<.029$, respectively, and both stabilized within 30 min of the infusion, $F(1,4)=15.30$, $p<.0002$ for VO_2 and $F(1,4)=19.43$, $p<.0001$ for VCO_2. Moreover, this increase over time in VO_2, $F(4,40)=8.21$, $p<.0001$, and VCO_2, $F(4,40)=7.66$, $p<.0001$, was lower in the 1K-GB animals.

Guanabenz Alone versus Isoproterenol Combined with Guanabenz. Combined ISO and GUANA increased both VO_2, $F(1,10)=26.00$, $p<.0005$, and VCO_2, $F(1,10)=21.14$, $p<.001$, whereas GUANA alone had no effect. Moreover, VO_2, $F(4,40)=20.98$, $p<.0001$, and VCO_2, $F(4,40)=10.24$, $p<.0001$, increased over time in a linear fashion, $F(1,10)=32.86$, $p<.0002$ for VO_2 and $F(1,10)=23.76$, $p<.0006$ for VCO_2. This increase for VO_2, $F(4,40)=36.04$, $p<.0001$ and VCO_2, $F(4,40)=19.51$, $p<.0001$, was greater during combined ISO and GUANA than during GUANA alone (Figure 25). Linear contrasts revealed that this difference between the drugs was apparent within 20 min of the start of the infusions, $F(1,4)=8.45$, $p<.004$ for VO_2 and $F(1,4)=14.52$, $p<.0002$ for VCO_2.

Body Temperature Indices

Baseline. No difference in rectal temperature (Tr), interscapular skin temperature (Tis), and tail skin temperature (Tts) occurred during baseline between the two test sessions. The 1K-GB animals had a lower Tis and Tts than did the sham animals, $F(1,20)=52.41$, $p<.0001$ and $F(1,20)=28.03$, $p<.0001$, respectively (see Table 5). Rectal temperature, $F(4,80)=10.69$, $p<.0001$, and Tts, $F(4,80)=15.07$, $p<.0001$, increased over time (see Figure 28), in a linear fashion $F(1,20)=28.75$, $p<.0001$, and $F(1,20)=24.13$, $p<.0001$, for Tr and Tts, respectively. Figure 29 shows that this time function for Tts was dependent on the BP status, $F(4,80)=7.29$, $p<.0001$, with the increase for the 1K-GB group being smaller than for the sham animals, $F(1,4)=16.32$, $p<.0001$.

Isoproterenol Alone versus Guanabenz Alone. Isoproterenol increased rectal temperature ($M=38.1\ ^{o}C$); whereas GUANA had no effect, ($M=36.8\ ^{o}C$), $F(1,20)=32.87$, $p<.0001$ (see Figure 28). Goldblatt hypertension lowered Tr, $F(1,20)=13.12$, $p<.0017$, Tis, $F(1,20)=34.87$, $p<.0001$, and Tts, $F(1,20)=16.06$, $p<.0007$, relative to the sham animals. These variables also increased over time, $F(4,80)=34.68$, $p<.0001$ for Tr, $F(4,80)=12.98$, $p<.0001$ for Tis, and $F(4,80)=4.31$, $p<.0033$ for Tts in a linear fashion, $F(1,20)=56.65$, $p<.0001$, $F(1,20)=17.12$, $p<.0005$, and $F(1,20)=5.93$, $p<.024$, respectively. Figure 30 represents the mean Tr for 1K-GB and sham rats as a function of time. The increase in Tr, $F(4,80)=12.88$, $p<.0001$, and Tts, $F(4,80)=6.64$, $p<.0001$, depended on the BP status of the animals, with the increase being less for the 1K-GB animals and apparent within 30 min of the start of the infusions, $F(1,4)=8.03$,

$p<.007$ for Tr and $F(1,4)=10.94$, $p<.0013$ for Tts. This finding was paralleled by the percentage change in Tr, $F(4,80)=12.65$, $p<.0001$, and Tts, $F(4,80)=6.38$, $p<.0002$.The increase in Tr, $F(4,80)=32.45$, $p<.0001$, Tts, $F(4,80)=3.70$, $p<.0081$, and Tis, $F(4,80)=10.24$, $p<.0001$, over time was drug-dependent, with all three variables increasing more during ISO, than during GUANA, infusion.

Isoproterenol Alone versus Isoproterenol Combined with Guanabenz. No drug main effect was observed for body temperature indices (see Figure 28). However, the percentage change in Tr was marginally lower for the 1K-GB animals ($M=1.1\%$), $F(1,10)=8.31$, $p<.016$, than for the sham animals ($M=2.7\%$). Rectal temperature, $F(4,40)=60.59$, $p<.0001$ and Tts, $F(4,40)=8.72$, $p<.0001$ increased over time in a linear fashion, $F(1,10)=126.86$, $p<.0001$ and $F(1,10)=11.00$, $p<.008$, respectively. Moreover, the increase in Tr, $F(4,40)=11.63$, $p<.0001$, and Tts, $F(4,40)=5.37$, $p<.002$, over time was attenuated in the 1K-GB hypertensives within 20 min of the start of the infusion, $F(1,4)=8.29$, $p<.005$ for Tr and $F(1,4)=10.40$, $p<.002$ for Tts.

Guanabenz Alone versus Isoproterenol Combined with Guanabenz. The combined administration of ISO and GUANA increased both Tr, $F(1,10)=10.97$, $p<.008$, and Tts, $F(1,10)=14.80$, $p<.003$; whereas GUANA alone had no effect (Figure 28). The 1K-GB hypertensives exhibited lower Tis, $F(1,10)=23.92$, $p<.0006$, and Tts, $F(1,10)=12.45$, $p<.006$, than the sham animals. Body temperatures increased over time, $F(4,40)=37.24$, $p<.0001$ for Tr, $F(4,40)=34.98$, $p<.0001$ for Tis, and $F(4,40)=7.89$, $p<.0001$ for Tts, with a linear trend, $F(1,10)=49.38$, $p<.0001$, $F(1,10)=64.86$, $p<.0001$, and $F(1,10)=11.11$, $p<.008$, respectively. The increase over time for Tr was suppressed for the

1K-GB rats, $F(4,40)=4.55$, $p<.004$, by the 20th min of the infusion, $F(1,4)=5.84$, $p<.017$ (see Figure 31). In addition, a Drug x Time interaction was apparent for Tr, $F(4,40)=34.25$, $p<.0001$, Tis, $F(4,40)=20.29$, $p<.0001$, and Tts, $F(4,40)=4.41$, $p<.005$. In all instances, the increase was greater during combined ISO and GUANA than during GUANA alone (Figure 28 for Tr and Tts), and was apparent by the 30th min of infusion, $F(1,4)=9.10$, $p<.003$ for Tr, $F(1,4)=1.97$, $p<.037$ for Tis, and $F(1,4)=5.22$, $p<.024$ for Tts.

Cardiovascular Parameters

Baseline. No differences in mean arterial blood pressure (MABP) or heart rate (HR) were obtained between the two test sessions. One-kidney Goldblatt hypertension increased both MABP and HR, as displayed in Table 5, $F(1,20)=530.74$, $p<.0001$ $F(1,20)=18.06$, $p<.0004$, respectively. Moreover, MABP, $F(4,80)=12.44$, $p<.0001$, and HR, $F(4,80)=3.77$, $p<.0007$, decreased over time in a linear manner, $F(1,20)=22.53$, $p<.0001$ and $F(1,20)=8.00$, $p<.01$, respectively (see Figure 32).

Isoproterenol Alone versus Guanabenz Alone. Isoproterenol alone resulted in a lower MABP, $F(1,20)=31.91$, $p<.0001$ but a higher HR, $F(1,20)=86.24$, $p<.0001$, than did GUANA alone (see Figure 32). Mean arterial blood pressure was generally higher for the 1K-GB animals, $F(1,20)=139.54$, $p<.0001$, but was drug dependent, $F(1,20)=16.58$, $p<.0006$. Moreover, a BP Status x Drug interaction was obtained for the percentage change in HR, $F(1,20)=9.62$, $p<.006$ (Table 6). Although HR did not differ between the 1K-GB and sham animals during GUANA infusions, the 1K-GB animals showed a suppressed percentage change in HR during ISO administration relative to the sham rats,

$F(1,1)=115.03$, $p<.0001$. Isoproterenol produced a marginal difference between the 1K-GB and Sham animals, $F(1,1)=5.68$, $p<.0188$, whereas, upon GUANA administration, MABP was greater for the 1K-GB animals than for the sham group, $F(1,1)=575.96$, $p<.0001$, (Table 5).

Isoproterenol Alone versus Isoproterenol Combined with Guanabenz. There were no between drug differences in MABP or HR (Figure 32). However, 1K-GB hypertension increased MABP relative to the sham controls, $F(1,10)=68.62$, $p<.0001$. Moreover, the percentage change in HR was less for the 1K-GB animals ($M=9.6\%$) than for the sham counterparts ($M=45.8\%$), $F(1,10)=34.89$, $p<.0001$.

Guanabenz Alone versus Isoproterenol Combined with Guanabenz. Mean arterial blood pressure was higher, $F(1,10)=37.94$, $p<.0001$, whereas HR was lower, $F(1,10)=84.33$, $p<.0001$, for GUANA alone than for combined ISO and GUANA (Figure 32). When collapsed across drug infusions, MABP was higher for the 1K-GB animals, $F(1,10)=207.68$, $p<.0001$. A BP Status x Drug interaction, $F(1,10)=13.25$, $p<.005$, showed that for both GUANA, $F(1,1)=797.08$, $p<.0001$, and combined ISO and GUANA, $F(1,1)=258.53$, $p<.0001$, MABP was greater for the 1K-GB animals (Table 5), although the magnitude of the difference was greater during GUANA alone, $F(1,1)=797.08$, $p<.0001$.

Evaporative Heat Loss Analysis

Evaporative heat loss (EHL) was greater for the drug infusions ($M=2.6$ ml/50 min) than for the baseline period ($M=1.6$ ml/50 min), $F(1,20)=63.10$, $p<.0001$. Moreover, combined administration of ISO and GUANA increased EHL ($M=3.2$ ml/50 min) relative to that observed for either ISO ($M=2.9$ ml/50 min) or GUANA ($M=1.9$ ml/50 min) alone, $F(1,20)=23.73$, $p<.0001$.

Discussion

One premise of Experiment 2 was that cold acclimation alone, or in combination with 1K-GB hypertension, would facilitate $alpha_1$-induced hyperthermia. The fact that $alpha_1$-adrenoceptors mediate vasoconstriction obfuscates the interpretation of any resulting changes in rectal temperature since it could be secondary to either increased brown adipose tissue-mediated thermogenesis or vasoconstrictor-induced reduction in tissue conductance. One way to dissociate the thermogenic from the thermolytic consequences of $alpha_1$-stimulation is to neuropharmacologically increase vascular resistance similar to that obtained with $alpha_1$-stimulation without triggering thermogenesis. In this regard, $alpha_2$-stimulation increases peripheral vascular resistance but does so without stimulating thermogenesis. Accordingly, Experiment 3 assessed whether the hyperthermia observed in Experiment 2 could attributed to $alpha_1$-induced thermogenesis, or $alpha_1$-compromised thermolysis, or some synergistic interaction of the two. To address this issue, Experiment 3 examined, in cold-acclimated normotensive or hypertensive rats, the thermophysiological effects of systemically infused isoproterenol, guanabenz, or combined isoproterenol and guanabenz.

Several general characteristics of baseline values were evident. First, there were no baseline differences between the two test sessions. Second, baseline metabolic rate and body temperatures were within the normal range of variability reported for cold acclimated rats. Third, baseline blood pressure and heart rate for the sham normotensive animals were within a generally accepted normotensive

range. These findings are consistent with those reported in Experiment 1 and hence, the discussion presented in that experiment is applicable to the present experiment.

The response profiles resulting from adrenergic stimulation included several general features. Isoproterenol increased metabolic rate, rectal and tail skin temperatures, and heart rate, whereas it decreased blood pressure. Given that the effects of isoproterenol are in accordance with those presented in Experiment 1, the discussion presented in that experiment is appropriate here.

The finding that guanabenz did not alter metabolic rate or body temperature indices from baseline levels is consistent with Foster's (1985) finding that alpha$_2$-antagoism only marginally altered NE-induced thermogenesis and supports his contention that alpha-receptor stimulation is nonthermogenic. Guanabenz, however, increased blood pressure and decreased heart rate. The pressor effects support Hannah et al.'s (1984) finding that guanabenz produced an alpha$_2$-mediated vasoconstriction which, in turn, increased blood pressure. Thus, the rise in blood pressure observed in the present experiment probably reflects an alpha$_2$-mediated increase in total peripheral resistance. The bradycardia is consistent with Spyer's (1981) report that regardless of whether blood pressure was increased mechanically or pharmacologically, it initiates a baroreceptor-mediated reflexive bradycardia. Thus, the bradycardia observed in the present experiment may be baroreceptor-mediated.

The thermogenic and body temperature response to isoproterenol was not potentiated when isoproterenol and guanabenz were

co-administered. Foster (1985) found that the thermogenic response to intravenously administered NE was reduced by only 24% following $alpha_2$-antagonism, and suggested that, of the alpha-adrenoceptor subtypes, only the $alpha_1$ population exerts an important role in brown adipose tissue thermogenesis. Accordingly, given the lack of $alpha_2$-mediated potentiation or antagonism on thermogenesis, $alpha_2$-adrenoceptors seem to exert little, if any, effect on this beta-mediated response.

The drug manipulations also increased evaporative heat loss relative to baseline levels. This finding is in accordance with the results of Experiment 1 and the discussion presented therin is applicable in the present experiment.

Several features of the 1K-GB animals emerged during baseline. For instance, 1K-GB rats exhibited lower interscapular and tail skin temperatures. Skin temperature is an index of vasomotor tone (Raman et al., 1983), with lower temperatures reflecting a vasoconstriction-induced reduction in tissue conductance. Since the 1K-GB model of hypertension is characterized by an increase in peripheral vascular resistance (Bralet et al., 1973), this state of vasoconstriction may reduce tissue conductance and decrease skin temperatures. The 1K-GB animals also exhibited a higher arterial blood pressure and heart rate than did the normotensive controls. This profile may be accounted for by the 1K-GB's purported state of sympathoexcitation, as assessed by the elevation in cardiac NE turnover (Tanaka et al., 1982) and plasma NE level (Katholi et al., 1982; Reid et al., 1977). The sympathetic postganglionic release of NE could activate vascular $alpha_1$-receptors to augment peripheral

resistance (Hannah et al., 1984), stimulate cardiac $beta_1$-receptors and, thus, evoke a tachycardia.

In the 1K-GB animals the increase in metabolic rate, rectal and tail skin temperature, and heart rate following isoproterenol, either alone or with guanabenz, was attenuated. This suppression in beta-receptor responsivity is consistent with that observed in Experiment 1 and may be explained through consideration of the studies by Katholi et al. (1982), Rockson et al. (1981), and Tanaka et al. (1982), as described in the first experiment.

The 1K-GB hypertensives also had a greater pressor response to guanabenz, similar to that reported with NE stimulation by Gothberg et al. (1983). They found that the elevated vascular resistance sustaining the 1K-GB hypertension was due to both enhanced vascular sensitivity and contractility to alpha-adrenergic stimulation. However, they failed to specify which alpha-receptor subtype may be involved in this enhanced reactivity. Perhaps the $alpha_2$-receptors in the 1K-GB hypertensive rat were the most likely candidate for hyperreactivity, as suggested by the enhanced pressor response to guanabenz obtained in the present experiment.

From the results of Experiment 3 it appears that, unlike the $alpha_1$-adrenoceptors, $alpha_2$-stimulation had no thermogenic effect and does not potentiate $beta_1$-mediated thermogenesis or the resulting hyperthermia. However, $alpha_2$-stimulation produces a pressor response comparable to that observed with $alpha_1$-activation. Both $alpha_1$- and $alpha_2$-stimulation increases blood pressure through an elevation in vascular resistance which, in turn, reduces tissue conductance and promotes heat retention. The fact

that the pressor response was comparable between $alpha_1$- and $alpha_2$-stimulation suggests a similar degree of vasoconstriction and, hence, compromised thermolysis. Thus, the $alpha_1$-potentiation of $beta_1$-mediated thermogenesis and hyperthermia found in Experiment 2 was probably not due to a compromised thermolysis. Instead, the $alpha_1$-receptors appeared to play an enhancing role in what is typically thought to be a $beta_1$-mediated response.

Experiment 4. Effect of 1K-GB Hypertension and Cold Acclimation on Heat Escape Responding during Systemic Phenylephrine, Isoproterenol, and Combined Phenylephrine and Isoproterenol Infusions in Rats.

Method

Subjects

Sixty-four male, Sprague-Dawley rats initially weighing 70-90 g were used. The animals were housed in a manner identical to that described for Experiment 1.

Procedure

Pretest procedure. Two days after their arrival in the laboratory all animals were trained to behaviorally thermoregulate in the convective thermal controller. Shaping the operant thermoregulatory response was accomplished through successive approximations. This shaping involved placing the animal in the convective thermal controller during a heat challenge (37 ^{o}C) and initially reinforcing, with a 17 ^{o}C air current, those responses which approximated the lever press. This procedure was continued until the animal would respond unassisted without becoming hyperthermic. The responding was monitored until the rats exhibited an average duration of lever pressing of 120 s for each 10-min interval. Thereafter the animals were allowed a 3-hr period during which they controlled the ambient temperature of the convective thermal controller through unassisted operant responding. The animals were considered trained if, after the 3-hr period, they demonstrated no evidence of hyperthermia, that is, their rectal temperature was less than 38.5 ^{o}C and if the average duration of lever pressing was 150 s/10 min. Following shaping the animals were

assigned to one of four conditions as described in Experiment 2: (a) 1K-GB cold-acclimated, (n=16), (b) 1K-GB non-cold-acclimated, (n=16), (c) sham cold-acclimated, (n=16), and (d) sham non-cold-acclimated, (n=16). Whereas, in Experiment 2, all animals were restraint adapted and had their aortic and right jugular vein catheterized, the animals in Experiment 4 were not restraint adapted and had only their right jugular veins catheterized. Forty-eight hours following catheterization, heat escape testing commenced.

Heat escape response testing procedure. Heat escape responding was assessed over two test sessions in response to the intravenous administration of ISO, PE and combined ISO and PE. Every ISO administration was preceeded by a bolus injection of the $beta_2$-antagonist, pindolol. Three animals were tested each day with a 46-hr rest period interpolated between the first and last test session. During each test session, the duration and frequency of lever pressing was obtained every 10 min, and rectal temperature was assessed immediately prior to and following testing in the convective thermal controller. The drug solutions, consisting of ISO ((-)-isoproterenol hydrochloride, .5 ug/kg/min), pindolol (1-(1H-Indol-4-yloxyl)-3-(isopropylamino)- 2-propranol, 10 ug/kg), and PE (l-phenylephrine hydrochloride, 10 ug/kg/min) were weighed on a Sartorius balance and dissolved in .15M sterile saline. All drug doses were expressed in the hydrochloric acid form. A fresh solution was made before each infusion, and the pH of the solution was monitored.

Prior to the initiation of testing, a very light stainless steel tether (Model 56-1456, Ealing Scientific Ltd.) was secured to the

animal's Elizabethan collar and the jugular catheter was threaded through the tether. Once the animal was inside the convective thermal controller, the jugular catheter was connected to a single channel fluid swivel (Model 56-1316, Harvard Infusion Swivel) located at the top of the cabinet, and which, in turn, was connected to an infusion pump (Sage Triple Channel Syringe Pump, Model 355, Fisher Scientific Co. Ltd.) via a polyethylene tube (PE100). The ambient temperature inside the operant chamber was set at 37 ^{o}C and the animal allowed a 100 min stabilization period, the last 60 min of which constituted baseline. The drug infusion occurred in the manner described for Experiment 1, except that the duration of drug infusion was 60 min. Immediately prior to and following completion of each test session, rectal temperature was assessed through the insertion of a rectal probe (YSI 402 probe, Fisher Scientific Co. Ltd.) 4 cm into the rectum for 10 s.

Cardiovascular assessment

Upon completion of the second test session the right carotid artery was catheterized as described in the surgical procedure section. Mean arterial blood pressure and heart rate were obtained by attaching the carotid catheter to a Gould Statham pressure transducer (Model P23Gb), which, in turn, was connected to a Grass polygraph (Model 79D). Blood pressure and heart rate were determined as five individual recordings recorded over a 30-min period while the animal was anesthetized. Following completion of MABP and heart rate assessment the animals were sacrificed with an overdose of sodium pentobarbital administered via the carotid catheter.

Results

Body Weight Analysis

There were no differences in body weight between the 1K-GB (M=255.2 g) and the sham (M=260.8 g) rats or between the cold-acclimated (M=246.5 g) and the non cold-acclimated (M=239.6 g) groups.

Duration and Frequency of Lever Press

Baseline. There were no differences in duration of lever press between the two test sessions, nor did the duration change over time (Figure 33). The 1K-GB hypertensives stabilized at a lower duration of lever press than the sham normotensives, $F(1,56)=30.34$, $p<.0001$. Cold acclimation also suppressed the duration of lever press relative to the nCA animals, $F(1,56)=8.68$, $p<.005$ (Table 7). Although there were no differences in frequency of lever press between the two test sessions, lever press frequency diminished over time, $F(5,285)=24.16$, $p<.0001$ (Figure 34), in a quadratic fashion, $F(1,56)=10.55$, $p<.002$. However, linear contrasts suggested that by the 40th min of baseline, frequency stabilized, $F(1,5)=9.32$, $p<.002$.

Isoproterenol Alone versus Phenylephrine Alone. The duration of lever press tended to be greater for ISO than for PE, $F(1,56)=6.15$, $p<.016$ (Figure 33). When collapsed across drugs, both 1K-GB hypertension, $F(1,56)=6.16$, $p<.016$, and cold acclimation, $F(1,56)=17.34$, $p<.0001$, showed lower duration of lever press relative to their respective controls. Analysis of a Drug x Time interaction, $F(5,285)=3.23$, $p<.007$, revealed that response frequency was greater during ISO for only the initial 40 min, $F(1,5)=8.99$, $p<.003$, after which it was exceeded by the response rate obtained during PE

infusions, $F(1,5)=2.92$, $p<.033$ (Figure 34).

Isoproterenol Alone versus Isoproterenol Combined with Phenylephrine. There was no drug main effect for the duration of lever press (Figure 33). The CA animals (M=230.6 s/10 min) had a shorted duration of lever press than did the nCA animals (M=274.3 s/10 min), $F(1,28)=0.86$, $p<.004$. No main effects or interactions were obtained for frequency of responding.

Phenylephrine Alone versus Isoproterenol Combined with Phenylephrine. The duration of lever press responding was shorter for PE alone (M=210.5 s/10 min) than for combined ISO and PE infusion (M=272.3 s/10 min), $F(1,28)=45.74$, $p<.0001$. Both the 1K-GB, $F(1,28)=11.29$, $p<.0023$, and the CA, $F(1,28)=5.78$, $p<.023$, animals pressed for shorter durations than did their respective controls. No main effects or interactions were obtained for frequency of responding.

Rectal Temperature

Baseline. There were no differences in baseline rectal temperature (Tr) between the two test sessions. Rectal temperature was higher in the 1K-GB hypertensives relative to the sham controls $F(1,56)=23.89$, $p<.0001$. Conversely, cold acclimation reduced baseline Tr (Table 7), $F(1,56)=134.77$, $p<.0001$. A BP Status x Acclimation Status interaction, $F(1,56)=17.01$, $p<.0001$, indicated that Tr was greater for the 1K-GB animals only in the non-cold-acclimated condition, $F(1,1)=44.98$, $p<.0001$ (see Figure 35).

Isoproterenol Alone versus Phenylephrine Alone. Rectal temperature obtained immediately after the drug infusion routine was higher following ISO (M=37.0 ^{o}C) than following PE (M=36.8 ^{o}C)

administration, $F(1,56)=29.30$, $p<.0001$. Thus, the percentage change in Tr was .34 during ISO; whereas during PE it was -.34. The CA animals ($M=36.8$ ^{o}C) had a lower Tr than did the nCA group ($M=37.0$ ^{o}C), $F(1,56)=27.78$, $p<.0001$.

Isoproterenol Alone versus Isoproterenol Combined with Phenylephrine. There was no drug main effect for Tr. The CA animals ($M=36.9$ ^{o}C) had a lower Tr when compared with the nCA group ($M=37.2$ ^{o}C), $F(1,28)=29.99$, $p<.0001$, whereas, the 1K-GB animals ($M=37.1$ ^{o}C) had a higher Tr than the sham animals ($M=37.0$ ^{o}C), $F(1,28)=8.35$, $p<.007$. A BP Status x Drug interaction, $F(1,28)=5.39$, $p<.028$, indicated that, following ISO, Tr did not differ between the 1K-GB and sham groups, $F(1,1)=0.03$, $p<.872$; whereas, following combined ISO and PE infusion, Tr was greater for the 1K-GB animals than for the sham controls, $F(1,1)=8.53$, $p<.005$ (Table 7).

Phenylephrine Alone versus Isoproterenol Combined with Phenylephrine. Rectal temperature was less following PE ($M=36.8$ ^{o}C) than following combined ISO and PE infusions ($M=37.2$ ^{o}C), $F(1,28)=48.32$, $p<.0001$. The CA animals ($M=36.9$ ^{o}C) had a lower Tr than did the nCA group ($M=37.0$ ^{o}C), $F(1,28)=9.44$, $p<.005$.

Cardiovascular parameters

Post-heat escape response testing. Analysis of the data presented in Table 8 revealed that MABP, $F(1,56)=1344.21$, $p<.0001$, and HR $F(1,56)=24.36$, $p<.0001$, was higher for the 1K-GB group. Similarly, both variables were elevated for the cold acclimated animals, $F(1,56)=15.10$, $p<.0003$ for MABP and $F(1,56)=14.80$, $p<.0003$ for HR. A BP Status x Acclimation Status was apparent for MABP (Figure 36), $F(1,56)=61.37$, $p<.0001$ and HR (Figure 37),

$F(1,56)=14.64$, $p<.0003$. For the sham animals, both variables did not vary between the cold and non-cold acclimated conditions, whereas, for the 1K-GB group, MABP, $F(1,1)=112.27$, $p<.0001$, and HR, $F(1,1)=49.84$, $p<.0001$, was greater in the cold acclimated condition. Furthermore, HR for the 1K-GB animals was greater than that seen for sham animals only in the cold acclimated condition, $F(1,1)=87.59$, $p<.0001$.

Discussion

Although both cold acclimation and 1K-GB hypertension tend to elevate rectal temperature through enhanced thermogenesis and impaired thermolysis, respectively, the thermobehavioral consequences of these processes remain unknown. Similarly, adrenoceptor stimulation generally promotes an increase in rectal temperature which was potentiated by cold acclimation but attenuated by 1K-GB hypertension. However, the behavioral consequences of the adrenergic stimulation, whether alone or combined with cold acclimation or 1K-GB hypertension, remain undefined. Thus, the purpose of Experiment 4 was three fold. The first was to assess whether the thermogenic effects of isoproterenol, the antithermolytic consequences of phenylephrine, and the synergistic results of combined isoproterenol and phenylephrine would be reflected in appropriate thermobehavioral response profiles. The second was to assess whether the thermogenic consequences of cold acclimation would be expressed as altered heat escape responding and whether adrenergic stimulation would enhance this behavior. The third was to examine whether the antithermolytic effects of 1K-GB hypertension would be translated into an enhanced heat escape response and whether adrenoceptor stimulation would

alter the response profile. To address these questions, the duration and frequency of heat escape responding for a cool convective reinforcement were monitored in cold or non-cold-acclimated, 1K-GB hypertensive or normotensive rats during the systemic infusion of either $alpha_1$ (PE)-, $beta_1$ (ISO)-, or combined $alpha_1$- and $beta_1$-agonists.

Several general characteristics of baseline responding emerged. First, there were no baseline differences between the two test sessions suggesting that the drug manipulations on the first day of testing did not alter baseline or drug responding for the second test session. Second, rectal temperatures were within the normal range of variability reported for both cold- and non-cold-acclimated rats (Depocas et al., 1978; Fregly et al., 1977). This finding suggests that neither cold acclimation nor the surgical manipulations rendered the animals unable to maintain an adequate rectal temperature. Third, the general absence of differences in frequency of bar pressing suggests that neither the surgical, thermal, nor pharmacological manipulations altered the animals' ability to lever press or general activity level.

The response profiles resulting from adrenergic stimulation include several general features. For instance, isoproterenol increased rectal temperature, whereas phenylephrine was either without effect or, when combined with isoproterenol, failed to enhance rectal temperature above that seen during isoproterenol alone. The isoproterenol effect is consistent with the results of Experiments 1 to 3 and may have resulted from (a) its thermogenic effect (Foster, 1985) and (b) a failure to behaviorally offset the

thermogenesis. This latter possibility is consistent with several studies (Adair, 1971; Cox et al., 1975; Lipton, 1967; Weiss & Laties, 1961), which found that endotherms were able to behaviorally attenuate, but not eliminate, changes in core temperature accompanying environmental or pharmacological thermal challenges.

Heat escape duration increased following isoproterenol administration. This result is generally consistent with Cox et al.'s (1975) finding that thermogenic agents reduce the latency to escape a hot ambient temperature. Thus, the thermogenic effect of isoproterenol may have similarly caused the animals to increase their heat escape responding. Although Experiment 2 demonstrated that phenylephrine was ineffective in inducing thermogenesis, the drug slightly increased the duration of heat escape responding. The elevation in vasomotor tone accompanying phenylehprine (Hannah et al., 1984) tends to reduce tissue conductance (Raman et al., 1983), which, in turn, jeopardizes thermolysis (Rand et al., 1965). Endotherms may offset the hyperthermic consequence of a reduced thermolysis by behaviorally lowering the ambient temperature (Wilson & Fyda, 1987). Thus, the phenylephrine-induced increase in response duration may be a behavioral consequence of the compromised thermolysis accompanying the increased peripheral resistance. Although Experiment 2 revealed an enhanced metabolism during combined administration of isoproterenol and phenylephrine, this effect was not translated into differential thermoregulatory behavior. There are two viable explanations for this finding. First, the behavioral measure used may not have been sufficiently sensitive to detect the changes in thermoregulatory behavior evoked with the drug regimen.

Second, the duration of heat escape responding observed with isoproterenol may have approached a biological ceiling that could hamper behavioral potentiation otherwise evident with the combined administration of isoproterenol and phenylephrine. This latter interpretation seems unlikely given the moderate duration of responding (232/600 s) that emerged with isoproterenol.

Several characteristics of cold-acclimated animals were apparent during baseline. For instance, cold acclimation elevated blood pressure and heart rate. The pressor response is consistent with Fregly's (1954) finding that a 20-day chronic exposure to 5 ^{o}C increases mean systolic blood pressure from 157 to 175 mmHg. This increase may reflect a residual cold-induced vasoconstriction. However, the fact that in both Fregly's (1954) report and in the present experiment, the rats were at room temperature for at least 30 min prior to the blood pressure determination would tend to reinstate normal vasomotor tone. A more plausible interpretation is that the sympathoexcitatory consequences of cold acclimation (Leduc, 1961; Therminarias et al., 1979; Young et al., 1982) promote a chronically elevated peripheral resistance through stimulation of vascular alpha-receptors and, thereby, increase blood pressure. Similarly, this sympathoexcitation may chronically stimulate cardiac beta$_1$-receptors to produce tachycardia.

Cold-acclimated animals exhibited a lower rectal temperature throughout baseline; whereas the duration of heat escape responding was transiently lowered for only the first 10 min of baseline. The reduced rectal temperature may have little to do with cold acclimation itself, but rather the fact that rectal temperature was

obtained shortly after removal from the walk-in-cooler may account for its reduction. The transient suppression in duration may be a secondary result of the diminished rectal temperature. Although endotherms will use behavioral means of regulating a normal core and skin temperature when thermally challenged (Adair, 1971; Carlton & Marks, 1958; Lipton, 1967), the behavior is influenced by their body temperature immediately prior to testing. For instance, Weiss and Laties (1961) demonstrated that rats preexposed to 5 ^{o}C for 5 hr prior to testing showed a shorter latency to escape a 2 ^{o}C environment than did animals preexposed to 25 ^{o}C. The difference in latency presumably occurred because the former group began the experiment with a body temperature closer to the threshold needed to induce cold escape. In the present experiment, the animals preexposed to 6 ^{o}C were then tested at 37 ^{o}C. Perhaps the transient reduction in escape responding was a consequence of their rectal temperature being below the threshold needed to induce heat escape. Thus, the net effect of the low responding in this heat challenge situation would be to reinstate normal rectal temperature. However, it should be kept in mind that the actual difference in rectal temperature between the cold- and non cold-acclimated rats was only .1 ^{o}C, a value below the sensitivity of the measuring device. Hence, the appropriateness of explaining the suppression in heat escape responding in terms of the lowered rectal temperature in the cold-acclimated animals must be viewed with caution. Once this end was accomplished, a higher response profile would be needed to prevent hyperthermia. This increased response profile may reflect itself in the elevation in duration of heat escape responding such

that, for the majority of baseline, there was no difference between the cold and the non-cold-acclimated animals.

Cold acclimation attenuated the behavioral responsiveness to adrenoceptor stimulation. This finding is contrary anticipations based on an enhanced metabolic response to these agents. However, a plausible explanation for the finding may lie in the fact that rectal temperature was lower for the cold-acclimated group. Several investigators (e.g., Adair, 1971; Cox et al., 1975) have demonstrated that when rectal temperature is lowered, rats increase their latency to escape a warm ambient temperature. Similarly, the suppressed response duration obtained during adrenoceptor stimulation may reinstate normal rectal temperature. Thus, the reduced heat escape responding in the cold-acclimated animals during adrenergic stimulation may reflect a lowered rectal temperature and may not indicate a suppressed responsiveness to the drugs.

Several features of the 1K-GB animals were observed during baseline. The induction of hypertension and tachycardia in the 1K-GB model was comparable to that reported by Bralet et al. (1973) and Leenen and DeJong (1971), attesting to the effectiveness of the surgical procedures used in this experiment. During baseline, the 1K-GB animals had a higher rectal temperature than did the sham normotensive group. This increase may be interpreted in two ways. First, Goldblatt hypertension is sustained by an increase in peripheral vascular resistance (Bralet et al., 1973). Such a rise in vascular resistance could reduce tissue conductance, impair thermolysis (Raman et al., 1983), and thereby enhance rectal temperature. Second, Experiment 2 found that the 1K-GB animal has

an elevated baseline metabolic rate. Since rectal temperature covaries with metabolism (Hart, 1971), the hypertensives' higher rectal temperatures may be secondary to their enhanced metabolism.

The 1K-GB animals also exhibited a lower baseline duration of heat escape responding. Since hyperthermia promotes behavioral attempts to reduce core temperature (Adair, 1971; Lipton, 1967; Weiss & Laties, 1961) one would assume that this principle would apply to the behavioral consequences of 1K-GB hypertension. However, the Goldblatt animals seemed to sustain an ambient temperature which augments, rather than offsets, a high rectal temperature. One viable explanation that is consistent with both the earlier metabolic data and the increased rectal temperature, is suggested by the set-point theory of body temperature regulation. This theory states that body temperature is regulated at some centrally determined level. If an alteration in thermoregulatory set-point occurs such that body temperature is lower, an endotherm will evoke both behavioral and physiological means of increasing rectal temperature (Kluger, 1979). For instance, Wilson, Wilson, and DiCara (1977) observed a chronic increase in the core temperatures of spontaneously hypertensive rats, albeit in the absence of an increase in basal metabolic rate, and thermoregulatory responding designed to sustain a warmer ambient temperature than their inbred normotensive controls. This behavioral profile was interpreted as reflecting a centrally elevated thermoregulatory set-point. Thus, the reduced heat escape responding of the 1K-GB hypertensive rats in the present experiment may reflect a similar increase in thermoregulatory set-point.

The 1K-GB animals also exhibited a lower duration of heat escape responding to adrenergic stimulation. There are two possible explanations for this finding. First, it may be a continuation of the suppressed duration observed during baseline. Second, Cox et al. (1975) demonstrated that in response to a thermogenic agent, rats exhibited compensatory behavioral adjustments in heat exposure which, in turn, served to defend normal body temperature. The three earlier experiments demonstrated a lower metabolic rate and rectal temperature in the 1K-GB hypertensives during isoproterenol, suggesting that isoproterenol was a less effective thermogenic agent in the 1K-GB animals. If this is the case, then the behavioral compensation for isoproterenol may have been attenuated accordingly. Thus, the presence of 1K-GB hypertension did alter the thermobehavioral profile seen during adrenergic stimulation, but did so in a way that promoted hyperthermia.

The results of Experiment 4 suggest that: (a) the potentiation of isoproterenol-induced nonshivering thermogenesis through the combined administration of isoproterenol and phenylephrine is not translated into a thermobehavioral effect. (b) Cold acclimation fails to enhance heat escape responding above that observed for non-cold-acclimated rats, either during baseline or drug infusions. This result may reflect the experimental procedure. Since the cold-acclimated animals were behaviorally tested immediately after being removed from the walk-in cooler, their rectal temperatures were lower than the non-cold-acclimated rats. As a result, perhaps the cold-acclimated rats lowered their heat escape responding in an attempt to elevate their rectal temperatures. (c) The 1K-GB animals also

exhibited lowered heat escape responding despite their elevated rectal temperatures. This finding may reflect a centrally elevated set-point for body temperature regulation, but such a possibility awaits further investigation.

General Discussion

The results from Experiments 1 to 3 demonstrated that beta-adrenoceptor stimulation with isoproterenol led to an increase in nonshivering thermogenesis, as assessed by oxygen consumption and carbon dioxide production, and also rectal and tail skin temperature responses. Similar results were obtained by Flaim et al. (1977), Foster (1985), and Fregly et al. (1977). Foster and Frydman (1978, 1979) demonstrated that the beta-adrenergic component of nonshivering thermogenesis was mediated primarily through brown adipose tissue, and Nedergaard and Lindberg (1982) later showed that specifically the $beta_1$-receptor subtype was responsible for mediating this response. Recent evidence, however, has indicated that a receptor population other than the $beta_1$-subtype may play a role in nonshivering thermogenesis.

One purpose of this four part study was to assess whether a receptor population other than the $beta_1$-subtype contributed to brown adipose tissue-mediated thermogenesis. It was hypothesized that more than one beta-adrenoceptor subtype may be involved in nonshivering thermogenesis, and this hypothesis was supported by the results of Experiment 1. Both $beta_1$- and $beta_2$-blockade with relatively specific antagonists were equally effective in attenuating isoproterenol-induced thermogenesis. Moreover, even in the presence of the beta-antagonists, isoproterenol still increased metabolic rate and rectal temperature. These results suggested that a receptor population other than $beta_1$- or $beta_2$-receptors is involved in nonhsivering thermogenesis.

In this regard, Arch et al. (1984) assessed the relative effectiveness of specific beta-adrenoceptor agonists in evoking brown adipose tissue-mediated lipolysis in the rat. In the first part of their study they compared the lipogenic:tachycardic ($beta_1$):tracheal relaxing ($beta_2$) efficacy of various $beta_1$- and $beta_2$-agonists. In response to isoproterenol, a nonselective beta-agonist, they observed a relative potency of 1:7:4; whereas following salbutamol, a $beta_2$-agonist, the relative potencies were 1:2.8:88. The apparent selectivity of salbutamol for tracheal relaxation is consistent with its classification as a $beta_2$-agonist. Moreover, the ineffectiveness of salbutamol to induce lipolysis suggests that the brown adipocyte beta-adrenoceptor is different from the tracheal $beta_2$-receptor. However, the possibility that the myocardial and adipocyte receptors were identical could not be excluded by these results. Although isoproterenol and salbutamol were 2.8 to 7-fold more potent as myocardial than lipolytic stimulants, this action could be due to the fewer beta-adrenoceptors in brown adipocytes. If the beta-adrenoceptors in brown adipose tissue and myocardium are identical, then a lower number of beta-receptors in the adipocytes would result in all beta-agonists being more potent stimulants of heart rate than lipolysis. In the second part of their study, Arch et al. (1984) found that novel beta-adrenoceptor agonists selectively stimulate lipolysis. Specifically, BRL 28410, 35113, and 37344 were, respectively, 21-, 28-, and 400-fold more potent as stimulants of brown adipocyte lipolysis than as stimulants of heart rate. Indeed, BRL 37344 was 5-fold more potent than isoproterenol as a stimulant

of lipolysis, but 523-fold less potent as a stimulant of heart rate. Therefore, the brown adipocyte beta-adrenoceptor is probably not of the $beta_1$-subtype. They suggested that the beta-adrenoceptors in brown adipocytes do not conform to the $beta_1/beta_2$ classification but rather, may represent a new type of receptor, the $beta_3$-adrenoceptor, which mediates brown adipose tissue activity. Although the results of Experiment 1 imply that a receptor population other than $beta_1$ or $beta_2$ may be involved in mediating thermogenesis, whether this population is comprised of the $beta_3$-receptors suggested by Arch et al. (1984) awaits further investigation.

A second purpose of this study was to (a) confirm Foster's (1985) finding that simultaneous stimulation of $alpha_1$- and beta-receptors potentiated thermogenesis above that seen during beta-stimulation alone, (b) extend these findings from the cold-acclimated animal to the non-cold-acclimated, 1K-GB hypertensive rat, and (c) determine whether simultaneous stimulation of $alpha_2$-receptors along with $beta_1$-activation would potentiate thermogenesis. It was hypothesized that Foster's (1985) finding would be confirmed and that it could be extended beyond the cold-acclimated animal. Moreover, $alpha_2$-stimulation was not anticipated to enhance the thermogenesis seen during isoproterenol administration. These hypotheses were verified in Experiments 2 and 3.

Stimulation of $beta_1$-receptors with isoproterenol evoked nonshivering thermogenesis, and the simultaneous activation of $alpha_1$-receptors potentiated the effect. These findings are consistent with the findings of Foster (1985) and also support the

results of Flaim et al. (1977). In addition, although $alpha_2$-stimulation produced a pressor response of a comparable magnitude to that observed during $alpha_1$-stimulation, concurrent stimulation of $beta_1$- and $alpha_2$-receptors failed to enhance thermogenesis above that seen during $beta_1$-stimulation alone. The consensus of these findings was that the $alpha_1$-induced potentiation of $beta_1$-mediated nonshivering thermogenesis was due to a specific $alpha_1$-agonism and not to a nonspecific pressor effect of alpha-stimulation. This finding agrees with Foster's (1985) finding that the thermogenic response to intravenously administered NE was reduced by only 24% following $alpha_2$-antagonism, and with his contention that, of the alpha-adrenoceptor subtypes, only the $alpha_1$-population exerts an important role in brown adipose tissue-mediated thermogenesis. However, in addition to the reports of Flaim et al. (1977) and Foster (1985) based on cold-acclimated animals, this $alpha_1$-mediated potentiation of nonshivering thermogenesis was extended from cold-acclimated animals to non-cold-acclimated, conscious, hypertensive and normotensive rats. This generalization implies that the enhancing effect of $alpha_1$-receptors on metabolism is not unique to a cold-acclimated animal; and, hence, even when brown adipose tissue is in an inactive state, $alpha_1$-receptors play a role in what has typically been thought of as a $beta_1$-mediated process.

A third purpose of this study was to assess whether cold acclimation would (a) result in the reported increase in beta-adrenoceptor responsivity and (b) unmask a latent role for $alpha_1$-receptors in brown adipose tissue-mediated thermogenesis.

It was anticipated that cold acclimation would enhance both beta- and $alpha_1$-adrenoceptor responsivity to adrenergic agents. Experiment 2 confirmed the increased beta-receptor responsivity but failed to support a potentiation of $alpha_1$-involvement in nonshivering thermogenesis. Although the $alpha_1$-potentiation of $beta_1$-mediated nonshivering thermogenesis extended beyond the cold-acclimated rat, only the cold-acclimated animal exhibited enhanced thermogenic responsivity to $beta_1$-stimulation. The biochemical processes by which cold acclimation enhance nonshivering thermogenesis are not completely understood; however, several mechanisms have been identified. Cold acclimation increases the activity of the sympathetic preganglionics (Hsieh et al., 1957; Young et al., 1982), which leads to the enhanced release of NE (Depocas et al., 1978) and the chronic stimulation of brown adipose tissue beta-receptors (Nedergaard & Lindberg, 1982). Stimulation of these beta-receptors initially increases the metabolism of triglycerides found in the brown adipocytes (Nedergaard & Cannon, 1984). Within a few hours the intracellular stores of the triglycerides are depleted with a concomitant rise in the activity of lipoprotein lipase (Carneheim, Nedergaard, & Cannon, 1984). This increase in lipoprotein lipase metabolizes the circulating triglycerides and thus, provides a source of fatty acids for brown adipose tissue. Through the synthesis and metabolism of fatty acids that brown adipose tissue evokes a chronic elevation in thermogenesis. How beta-stimulation can effect an increase in lipoprotein lipase activity is unknown, but this increase in activity can be mimicked by the injection of beta-adrenergic agonists and can be totally

abolished by beta-antagonism (Carneheim et al., 1984). Although cold acclimation enhanced $beta_1$-receptor responsivity to adrenergic stimulation, it failed to alter the potentiating role of the $alpha_1$-receptors to nonshivering thermogenesis seen in the non-cold- acclimated rats. This result is somewhat inconsistent with the cold acclimation-induced enhancement of $alpha_1$-mediated cellular processes in brown adipose tissue (Nedergaard et al., 1986), but it is possible that the cellular events may not be directly translated into a measurable metabolic response.

A fourth purpose of this study was to assess whether 1K-GB hypertension would (a) jeopardize thermolysis and, thereby, facilitate hyperthermia and (b) alter sensitivity to $beta_1$-adrenoceptor stimulation. It was anticipated that 1K-GB hypertension would promote hyperthermia and would alter $beta_1$-receptor responsivity. These hypotheses were verified by the results of Experiments 1 to 3. Contrary to the cold-acclimated animals, the 1K-GB renovascular hypertensive rats exhibited a higher baseline metabolic rate and rectal temperature. The 1K-GB animals are characterized by sympathoexcitation, as suggested by their increase in cardiac norepinephrine turnover (Tanaka et al., 1982) and elevated plasma norepinephrine level (Katholi et al., 1982; Reid et al., 1977). Since the sympathetic postganglionic release of norepinephrine mediates nonshivering thermogenesis (Depocas et al., 1978; Hsieh et al., 1957; Young et al., 1982), the increased baseline metabolism in the 1K-GB group could reflect the chronic stimulation of brown adipose tissue beta-receptors. The increased rectal temperature of the 1K-GB animal may reflect its enhanced metabolic

rate (Hart, 1971). Alternatively, the increased peripheral vascular resistance characteristic of the 1K-GB rat (Barlet et al., 1973) may reduce tissue conductance (Raman et al., 1983), thereby promoting a higher rectal temperature through impaired thermolysis.

The 1K-GB hypertensive also exhibited a reduced thermogenic--rectal and tail skin temperature--and heart rate responsivity to $beta_1$-stimulation. Although this reduction may reflect a desensitization of the beta-receptors, which may accompany sympathoexcitation (Field, Janis, & Triggle, 1973), an alternate explanation exists.

Several investigations revealed that treatments which reduce the activity of the thyroid gland prevent the development of renovascular hypertension (Fregly , 1958, 1959). However, since a direct role of the thyroid gland in renal hypertension was still not evident, Fregly & Gonzalez (1961) assessed its activity during the development of renovascular hypertension in rats. In their study, rats made hypertensive with latex encapsulation of both kidneys had radioactively labelled iodide injected from 1 to 33 weeks after encapsulation. For 48 hr after injection, the rats' metabolic rates were monitored and urines were collected. The rats were killed by decapitation, and the thyroid gland was removed and weighed. They found that blood pressure was elevated within 2 weeks of encapsulation, metabolism was generally increased by the third week, and thyroid weight was significantly increased by the eleventh week. Moreover, by the eleventh week of encapsulation, the amount of labelled iodide in the urine was less for the hypertensive animals. Fregly and Gonzalez (1961) suggested that this finding may indicate

an increase in circulating iodide which, in turn, would reduce the secretory activity of the thyroid gland. Thus, they suggested that the renovascular hypertensives may be characterized by a reduction in the activity of the thyroid gland.

To further test this possibility, Fregly (1971) repeated his 1961 study with the addition of another measure of thyroid activity. In this study, the necks of the unanesthetized, renal hypertensive rats were held over a scintillation counter either 8 or 24 hr after the injection of radioactively labelled iodide. This procedure served as an index of the uptake of iodide by the thyroid gland. Blood pressure was increased within 2 weeks of renal encapsulation; whereas iodide uptake by the thyroid gland and urinary excretion of labelled iodide was decreased by the seventh week. These results were consistent with those of his earlier study and suggested a reduction in thyroid gland function during the development of renovascular hypertension.

In assessing the effects of hypothyroidism in rats, it was observed that these animals were unable to maintain their body temperature when acutely exposed to 5 or 7 ^{o}C in spite of a large increase in the rate of secretion of epinephrine and norepinephrine (Fregly, Iampietro, & Otis, 1961; Lutherer, Fregly, & Anton, 1969). It was suggested that the increased heat loss of cold exposed hypothyroid rats at a time when catecholamine secretion rate was elevated may reflect a reduced responsivness to adrenergic stimulation. Fregly, Nelson, Resch, Field, and Lutherer (1975) then assessed the responsiveness of hypothyroid rats to beta-adrenoceptor stimulation. In this study, the rats were either euthyroid, were

rendered hypothyroid by the administration of the antithyroid drug aminotriazole, or received aminotriazole in conjunction with thyroxine, hence, mimicking the euthyroid controls. Since tail skin temperature may be used as an index of beta-receptor responsivity, Fregly et al. (1975) assessed this parameter in response to the subcutaneous administration of isoproterenol. They found that in both the control condition and in the animals receiving aminotriazole and thyroxine, isoproterenol induced a 5 ^{o}C increase in tail skin temperature within 50 min of the injection. In contrast, the animals receiving the antithyroid drug exhibited only a 3 ^{o}C rise in tail skin temperature. Moreover, the fact that pretreating the animals with the beta-antagonist propranolol, resulted in a failure of isoproterenol to increase tail skin temperature strengthens the contention that the increase in tail skin temperature in the rat is a beta-mediated response. Similarly, the administration of aminotriazole to rats, at the dose used, produced hypothyroidism. This hypothyroidism was apparent from the findings of a reduced systolic blood pressure with an increased thyroid weight in the aminotriazole treated rats. Thus, the findings of Fregly et al (1975) suggest that hypothyroidism reduced beta-adrenoceptor responsiveness to exogenously administered adrenergic agents. Since renovascular hypertension has been associated with a hypothyroid state, and hypothyroidism is linked to a reduced beta-responsivity, the reduced thermogenic and cardiovascular responsivity to $beta_1$-stimulation in the 1K-GB hypertensive rat may similarly reflect a reduction in thyroid activity.

A fifth purpose of this study was to determine whether the combined induction of cold acclimation and 1K-GB hypertension would alter cardiovascular and thermogenic response profiles following adrenergic stimulation. Experiment 2 demonstrated a virtual absence of an interaction between the acclimation and blood pressure status. This finding may reflect the opposing beta-receptor responsivity present in the two factors. Field et al. (1973) demonstrated that the interaction of processes that individually alter sympathetic activity will result in an additive change in that activity. To the extent that this finding may be applied to receptor responsivity, the opposing effects of cold acclimation and 1K-GB hypertension on beta-receptor reactivity may ameliorate each other when the two processes are combined. Hence, the paucity of significant interactions between cold acclimation and 1K-GB hypertension is anticipated.

The general purpose of the behavioral study was to assess whether the metabolic and cardiovascular consequences of the manipulations used in Experiment 2 could be translated into differential heat escape profiles. The specific findings of the behavioral experiment were discussed earlier and, in general, failed to reflect many of the metabolic observations as behavioral responding. This may be due to a relative insensitivity of the convective thermal controller to detect the slight changes in behavior which may have occurred or it may reflect a biological ceiling to behavioral responding which could not be potentiated.

One finding that does warrant further discussion and that may attest to the effectiveness of behavior as a thermoregulatory

process is that, although the animals were not completely successful in ameliorating the thermogenic consequences of adernergic stimulation, they did attenuate the hyperthermic effects of the agents. For instance, in response to isoproterenol, rectal temperatures ranged from 37.0 ^{o} to 38.7 ^{o}C for Experiments 1 to 3; whereas in the behavioral study, rectal temperatures varied from 36.9 ^{o} to 37.2 ^{o}C. Similarly, in response to combined isoproterenol and phenylephrine, rectal temperature ranged from 37.2 ^{o} to 37.6 ^{o}C for Experiment 2; whereas in Experiment 4 it varied from 37.0 ^{o} to 37.2 ^{o}C. This attenuation in the hyperthermic effect of thermogenic agents is consistent with the findings of Cox et al. (1975). They found that the administration of agents which promote either hyper- or hypothermia evoked behavioral responses designed to defend a normal core temperature but that the defence was not 100% effective. In the present study, the fact that behavior did maintain rectal temperature within a narrower range around baseline values suggests that heat escape responding was an effective means of regulating body temperature.

The results of the present study imply that 1K-GB hypertensive rats may be thermally maladapted to a cold environment. In the non-cold-acclimated condition, the induction of 1K-GB hypertension resulted in a 5% mortality rate; however, this was increased to 20% when the animals were cold acclimated. The reduced survival rate of hypertensive animals in a cold ambient temperature may relate to their reduced beta-receptor responsivity to adrenergic stimulation. Cold exposure leads to the rapid release of norepinephrine from the sympathetic preganglionics, and endotherms normally respond to this

norepinephrine by increasing thermogenesis. However, the 1K-GB animals have a reduced beta-receptor responsivity; and, hence, the sympathetic release of norepinephrine evokes a lower thermogenic response than that observed in normotensive animals. If the cold exposure is continued, then this reduced thermogenic response to circulating norepinephrine will lead to hypothermia and eventually death.

Conversely, Wilson et al. (1977) suggested that a hypertensive rat may be thermally maladapted to a warm environment. This was postulated primarily because of the moderate hyperthermia observed in the hypertensive animals and because of their relative inability to cope with a mild form of heat stress. The investigators suggested that this maladaptation resulted from a reduced thermolysis which often accompanies an increase in peripheral vascular resistance. If one considers the implications of both a reduced heat tolerance reported by Wilson et al. (1977) and a reduced cold tolerance present in the current findings, it would seem reasonable to postulate that a hypertensive animal may have a narrower range of temperatures at which survival is optimized. This narrower range of survival temperatures may indicate that the thermoneutral zone is diminished for a hypertensive endotherm. The validity of this suggestion awaits further investigation.

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Table 1
Mean (+SEM) Respiratory, Thermal, and Cardiovascular Variables Collapsed across Time during Baseline and Drug Infusions for 1K-GB Hypertensive and Sham Normotensive Rats

Group	VO_2	VCO_2	RQ	Tr	Tis	Tts	MABP	HR
				Baseline[a]				
1K-GB	3.16(.06)	2.43(.04)	.86(.07)	36.8(.07)	31.1(.20)	23.9(.16)	183(3.24)	420(4.97)
Sham	2.69(.04)	2.16(.03)	.81(.01)	36.6(.06)	31.9(.18)	24.3(.13)	118(1.31)	386(3.64)
	*	*					**	*
				Isoproterenol[a]				
1K-GB	3.46(.09)	3.02(.07)	.88(.01)	37.3(.10)	31.5(.24)	27.9(.46)	140(4.09)	558(6.40)
Sham	3.55(.07)	3.41(.07)	.96(.01)	38.0(.13)	33.0(.25)	29.4(.44)	100(1.75)	590(4.10)
		*		*	*		**	
				Isoproterenol with Atenolol[b]				
1K-GB	3.35(.13)	2.70(.08)	.82(.02)	37.3(.09)	30.2(.27)	25.1(.42)	144(6.63)	465(10.88)
Sham	2.77(.09)	2.67(.09)	.96(.01)	37.1(.16)	30.4(.35)	26.6(.57)	99(2.49)	542(15.00)
				Isoproterenol with Pindolol[b]				
1K-GB	3.31(.09)	2.74(.11)	.83(.02)	36.8(.16)	30.6(.68)	25.8(.61)	165(4.41)	482(20.88)
Sham	3.33(.16)	2.73(.10)	.84(.03)	36.8(.19)	32.7(.33)	26.7(.66)	101(2.68)	564(7.06)

Note. VO_2 = oxygen consumption (ml O_2/g/hr), VCO_2 = carbon dioxide production (ml CO_2/g/hr), RQ = respiratory quotient (VCO_2/VO_2), Tr = rectal temperature (^oC), Tis = interscapular skin temperature (^oC), Tts = tail skin temperature (^oC), MABP = mean arterial blood pressure (mmHg), HR = heart rate (bpm).

[a] $n = 12$; [b] $n = 6$; * $p < .05$, ** $p < .01$.

Table 2

Mean (+SEM) Percentage Change from Baseline for Respiratory, Thermal, and Cardiovascular Variables Collapsed across Time during Drug Infusions for 1K-GB Hypertensive and Sham Normotensive Rats

Group	VO_2	VCO_2	RQ	Tr	Tis	Tts	MABP	HR
				Isoproterenol[a]				
1K-GB	15.29(3.20)	32.45(3.47)	14.46(1.93) *	1.71(.25) **	-.59(.73) **	14.51(2.73)	-23(1.79) *	35(3.38) **
Sham	39.44(3.92)	64.24(4.96)	17.89(1.82)	3.60(.39)	2.21(.54)	19.95(2.95)	-15(1.91)	53(2.25)
				Isoproterenol with Atenolol[b]				
1K-GB	1.71(1.52)	11.15(2.26)	9.51(1.78)	.55(.19)	-1.29(.41)	6.71(1.68)	-18(1.36)	10(3.77)
Sham	14.60(4.49)	32.79(4.86)	16.39(1.45)	.87(.35)	.54(.41)	8.23(2.06)	-14(1.41)	33(2.94)
				Isoproterenol with Pindolol[b]				
1K-GB	7.20(3.04)	11.62(3.59)	-6.05(6.97)	1.23(.27)	.85(.36)	8.89(2.37)	-14(1.04)	21(2.46)
Sham	14.59(4.24)	24.91(5.07)	8.89(2.01)	.69(.35)	.19(.43)	10.89(2.40)	-16(1.39)	58(3.33)

Note. VO_2 = oxygen conusmption, VCO_2 = carbon dioxide production, RQ = respiratory quotient, Tr = rectal temperature, Tis = interscapular skin temperature, Tts - tail skin temperature, MABP = mean arterial blood pressure, HR = heart rate.

[a] $n = 12$; [b] $n = 6$; * $p < .05$, ** $p < .01$.

Table 3

Mean (+SEM) Respiratory, Thermal, and Cardiovascular Variables Collapsed across the Cold- and non Cold-Acclimated Groups and Time during Baseline and Drug Infusions for 1K-GB Hypertensive and Sham Normotensive Rats

Group	VO_2	VCO_2	RQ	Tr	Tis	Tts	MABP	HR
				Baseline[a]				
1K-GB	3.34(.04)	2.50(.03)	.76(.01)	36.7(.05) *	32.1(.11)	24.6(.17)	185(1.85) **	428(2.77) **
Sham	3.25(.04)	2.36(.03)	.75(.01)	36.4(.04) *	32.8(.12)	24.3(.12)	114(1.27) **	402(2.99) **
				Isoproterenol[b]				
1K-GB	4.13(.09)	2.78(.06)	.68(.01)	37.4(.09)	31.5(.32)	27.9(.45) *	159(2.27)	505(10.99)
Sham	3.56(.09)	2.66(.05)	.77(.02)	37.1(.10)	32.6(.24)	27.4(.40) *	111(3.02)	559(5.86)
				Phenylephrine[b]				
1K-GB	3.55(.09)	2.31(.05)	.67(.02)	35.9(.14)	31.4(.22)	26.6(.36)	196(3.24)	392(5.83)
Sham	3.45(.09)	2.36(.05)	.71(.02)	36.0(.09)	32.2(.32)	24.9(.39)	128(2.37)	380(7.18)
				Isoproterenol with Phenylephrine[a]				
1K-GB	4.59(.08)	3.31(.07)	.72(.01)	37.6(.09)	32.9(.19)	28.2(.36)	169(3.33) **	526(4.12) *
Sham	4.63(.09)	3.23(.06)	.71(.01)	37.2(.09)	33.2(.09)	27.6(.29)	99(2.10) **	547(3.43) *

Note. VO_2 = oxygen consumption (ml O_2/g/hr), VCO_2 = carbon dioxide production (ml CO_2/g/hr), RQ = respiratory quotient (VCO_2/VO_2), Tr = rectal temperature (^{o}C), Tis = interscapular skin temperature (^{o}C), Tts = tail skin temperature (^{o}C), MABP = mean arterial blood pressure (mmHg), HR = heart rate (bpm).

[a] $n = 16$; [b] $n = 8$; * $p < .05$, ** $p < .01$.

Table 4

Mean (+SEM) Respiratory, Thermal, and Cardiovascular Variables Collapsed across the 1K-GB Hypertensive and Sham Normotensive Groups and Time during Baseline and Drug Infusions for Cold-Acclimated (CA) and non Cold-Acclimated (nCA) Rats

Group	VO_2	VCO_2	RQ	Tr	Tis	Tts	MABP	HR
				Baseline[a]				
CA	3.21(.04)	2.36(.03)	.75(.01)	36.6(.05)	32.3(.13)	24.3(.13)	149(2.53)	414(2.95)
nCA	3.38(.04)	2.50(.03)	.75(.01)	36.5(.04)	32.6(.10)	24.6(.16)	150(2.56)	417(3.00)
				Isoproterenol[b]				
CA	3.85(.09)	2.80(.06)	.74(.01)	37.4(.10)	32.4(.31)	28.1(.42)	131(3.62)	541(6.69)
nCA	3.84(.09)	2.64(.05)	.71(.02)	37.0(.09)	31.7(.26)	27.2(.43)	139(3.91)	523(11.25)
				Phenylephrine[b]				
CA	3.37(.07)	2.21(.04)	.68(.02)	35.9(.12)	31.5(.29)	26.0(.34)	169(4.92)	385(7.44)
nCA	3.63(.09)	2.46(.05)	.70(.02)	36.0(.11)	32.1(.25)	25.4(.44)	156(4.52)	387(5.59)
				Isoproterenol with Phenylephrine[a]				
CA	4.54(.08)	3.29(.07)	.73(.01)	37.5(.09)	32.9(.31)	28.3(.30)	130(3.91)	541(3.76)
nCA	4.68(.09)	3.24(.06)	.70(.01)	37.4(.08)	33.3(.16)	27.7(.35)	138(3.94)	532(3.96)

Note. VO_2 = oxygen consumption (ml O_2/g/hr), VCO_2 = carbon dioxide production (ml CO_2/g/hr), RQ = respiratory quotient (VCO_2/VO_2), Tr = rectal temperature (^{o}C), Tis = interscapular skin temperature (^{o}C), Tts = tail skin temperature (^{o}C), MABP = mean arterial blood pressure (mmHg), HR = heart rate (bpm).

[a] $n = 16$; [b] $n = 8$; * $p < .01$.

Table 5
Mean (+SEM) Respiratory, Thermal, and Cardiovascular Variables Collapsed across Time during Baseline and Drug Infusions for 1K-GB Hypertensive and Sham Normotensive Rats

Group	VO_2	VCO_2	RQ	Tr	Tis	Tts	MABP	HR
				Baseline[a]				
1K-GB	2.88(.04)	2.15(.03) *	.75(.01) **	36.8(.04)	29.5(.15) **	23.4(.09) **	190(2.12) **	456(1.75) **
Sham	3.02(.05)	2.45(.04)	.82(.01)	36.8(.07)	34.5(.17)	25.8(.18)	105(1.34)	395(5.08)
				Isoproterenol[b]				
1K-GB	3.59(.12)	2.62(.05)	.74(.01)	37.5(.09)	30.4(.29)	25.9(.60)	155(3.78) *	516(5.71)
Sham	4.48(.13)	3.51(.13)	.78(.01)	38.7(.16)	36.2(.26)	30.0(.63)	89(4.07)	561(9.41)
				Guanabenz[b]				
1K-GB	2.66(.07)	1.81(.05)	.68(.01)	36.6(.02)	29.4(.19)	22.6(.09)	239(4.18) **	424(4.17)
Sham	3.52(.12)	2.61(.08)	.74(.01)	37.0(.12)	34.0(.60)	27.4(.48)	102(4.06)	397(11.19)
				Isoproterenol with Guanabenz[a]				
1K-GB	3.97(.08)	2.76(.05) *	.70(.01) *	37.5(.06)	30.5(.23) **	25.8(.41) **	163(3.08) **	508(5.65) *
Sham	4.26(.10)	3.19(.08)	.75(.01)	37.7(.18)	34.9(.14)	30.1(.48)	85(1.42)	548(5.62)

Note. VO_2 = oxygen consumption (ml O_2/g/hr), VCO_2 = carbon dioxide production (ml CO_2/g/hr), RQ = respiratory quotient (VCO_2/VO_2), Tr = rectal temperature (^{o}C), Tis = interscapular skin temperature (^{o}C), Tts = tail skin temperature (^{o}C), MABP = mean arterial blood pressure (mmHg), HR = heart rate (bpm).
[a] $n = 12$; [b] $n = 6$; * $p < .05$, ** $p < .01$.

Table 6

Mean (+SEM) Percentage Change from Baseline for Respiratory, Thermal, and Cardiovascular Variables Collapsed across Time during Drug Infusions for 1K-GB Hypertensive and Sham Normotensive Rats

Group	VO_2	VCO_2	RQ	Tr	Tis	Tts	MABP	HR
				Isoproterenol[b]				
1K-GB	21.61(1.97)	11.64(1.03)	-7.81(1.27)	.98(.13)	2.17(.47)	7.69(1.90)	-24(1.58)**	11(1.32)**
Sham	40.14(3.73)	36.26(3.27)	-2.39(1.08)	2.98(.43)	2.44(.35)	14.15(2.29)	-18(1.43)**	48(3.80)**
				Guanabenz[b]				
1K-GB	-4.98(1.58)	-1.29(1.19)	4.09(1.11)	-.21(.06)	-.47(.19)**	-.79(.20)	26(1.73)**	-5(.73)
Sham	9.99(2.62)	3.48(2.27)	-5.41(1.64)	.86(.14)	1.18(.28)**	5.18(1.15)	5(3.37)**	4(2.59)
				Isoproterenol with Guanabenz[a]				
1K-GB	26.65(1.79)*	20.93(1.85)*	-4.37(.88)	1.57(.13)	3.33(.26)**	9.39(1.41)	-9(1.72)	12(1.31)**
Sham	37.01(2.53)*	32.05(2.38)*	-3.43(.87)	2.71(.33)	1.09(.27)**	14.07(1.82)	-15(1.25)	40(2.01)**

Note. VO_2 = oxygen consumption, VCO_2 - carbon dioxide production, RQ = respiratory quotient, Tr = rectal temperature, Tis = interscapular skin temperature, Tts = tail skin temperature, MABP = mean arterial blood pressure, HR = heart rate.

[a] $n = 12$; [b] $n = 6$; * $p < .05$, ** $p < .01$.

Table 7

Mean (+SEM) heat escape responding and rectal temperature across baseline and drug infusions for 1K-GB hypertensive, Sham normotensive, cold acclimated (CA), and non-cold acclimated (nCA) rats

Group	Duration	Frequency	Tr
	Baseline[a]		
1K-GB	157.4(5.95)	5.8(.27)	36.9(.02)
Sham	195.4(7.94) **	4.8(.26)	36.8(.02) **
CA	166.3(6.82)	5.6(.26)	36.8(.01)
nCA	186.6(7.29) **	5.0(.28)	37.0(.02) **
	Isoproterenol[b]		
1K-GB	223.4(14.39)	4.2(.43)	37.0(.05)
Sham	242.3(14.57)	3.7(.36)	37.0(.08) **
CA	208.5(13.57)	3.5(.33)	36.9(.04)
nCA	257.3(14.99)	4.4(.45)	37.2(.05) *
	Phenylephrine[b]		
1K-GB	195.4(15.12)	3.6(.36)	36.8(.04)
Sham	223.5(16.89)	3.3(.34)	36.8(.05)
CA	194.4(15.19)	3.4(.36)	36.7(.04)
nCA	224.4(16.80)	3.6(.34)	36.8(.05)
	Isoproterenol with Phenylephrine[a]		
1K-GB	250.3(10.39)	3.9(.25)	37.2(.03)
Sham	293.3(12.14) **	3.3(.23)	37.0(.05) **
CA	252.4(10.19)	3.6(.26)	37.0(.02)
nCA	291.2(12.34) **	3.6(.23)	37.2(.05) **

Note. Duration (s/10 min; Frequency (# lever presses/10 min); Tr (^{o}C).

[a] n=16; [b] n=8. * $p < .05$; ** $p < .01$.

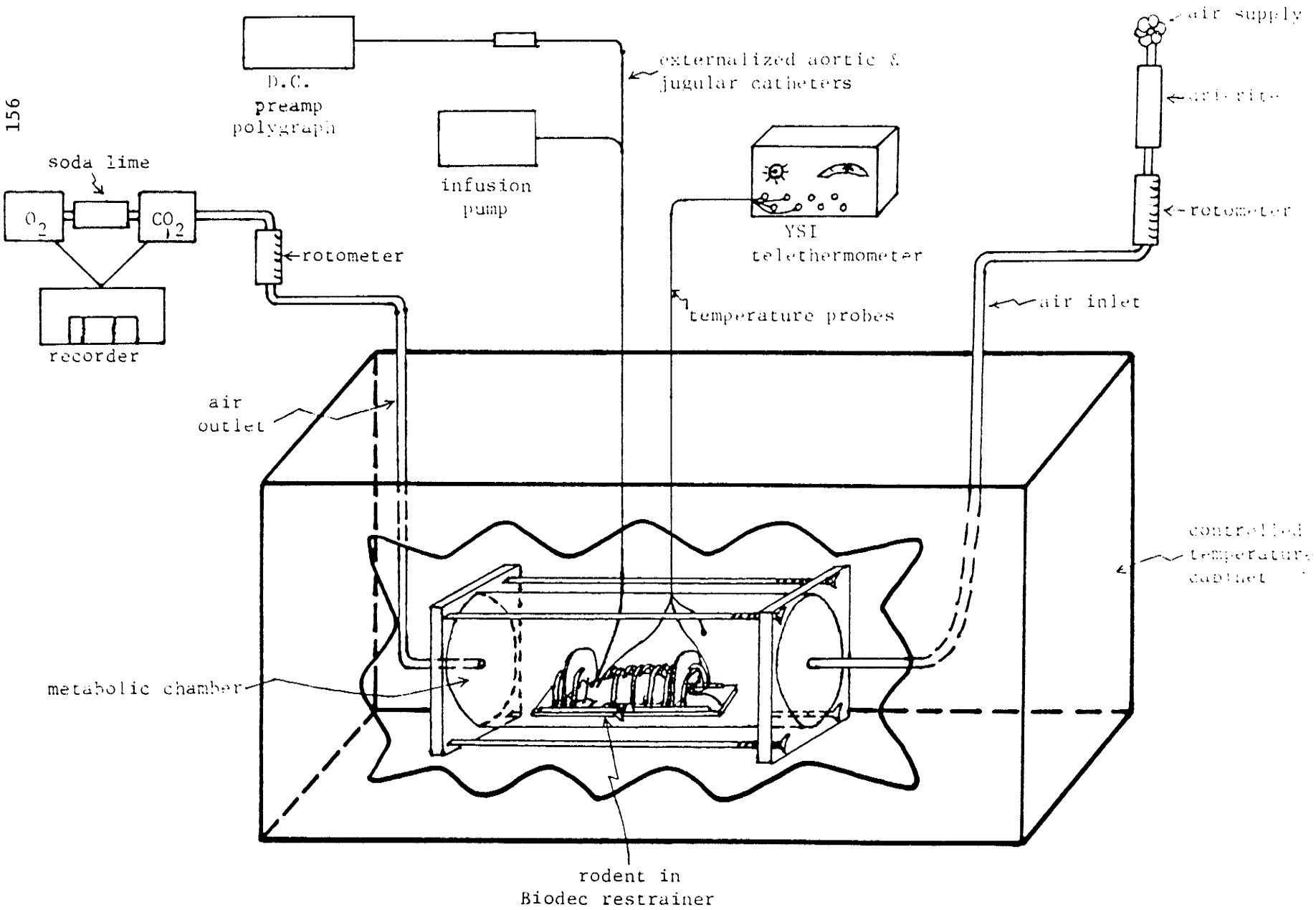
Table 8
Mean (+SEM) post-test cardiovascular parameters for 1K-GB hypertensive, Sham normotensive, cold acclimated (CA), and non-cold acclimated (nCA) rats

Group	MABP		HR	
1K-GB	164(1.23)	**	353(1.28)	**
Sham	89(1.40)		316(.97)	
CA	130(1.30)	*	349(1.12)	*
nCA	122(1.33)		321(1.14)	

Note. MABP (mmHg); HR (bpm).

* $p < .01$; ** $p < .0001$

Figure 1. Schematic representation of the apparatus used for metabolic testing.


D.C.
preamp
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rotometer
rotometer
temperature probes
air inlet
recorder
air
outlet
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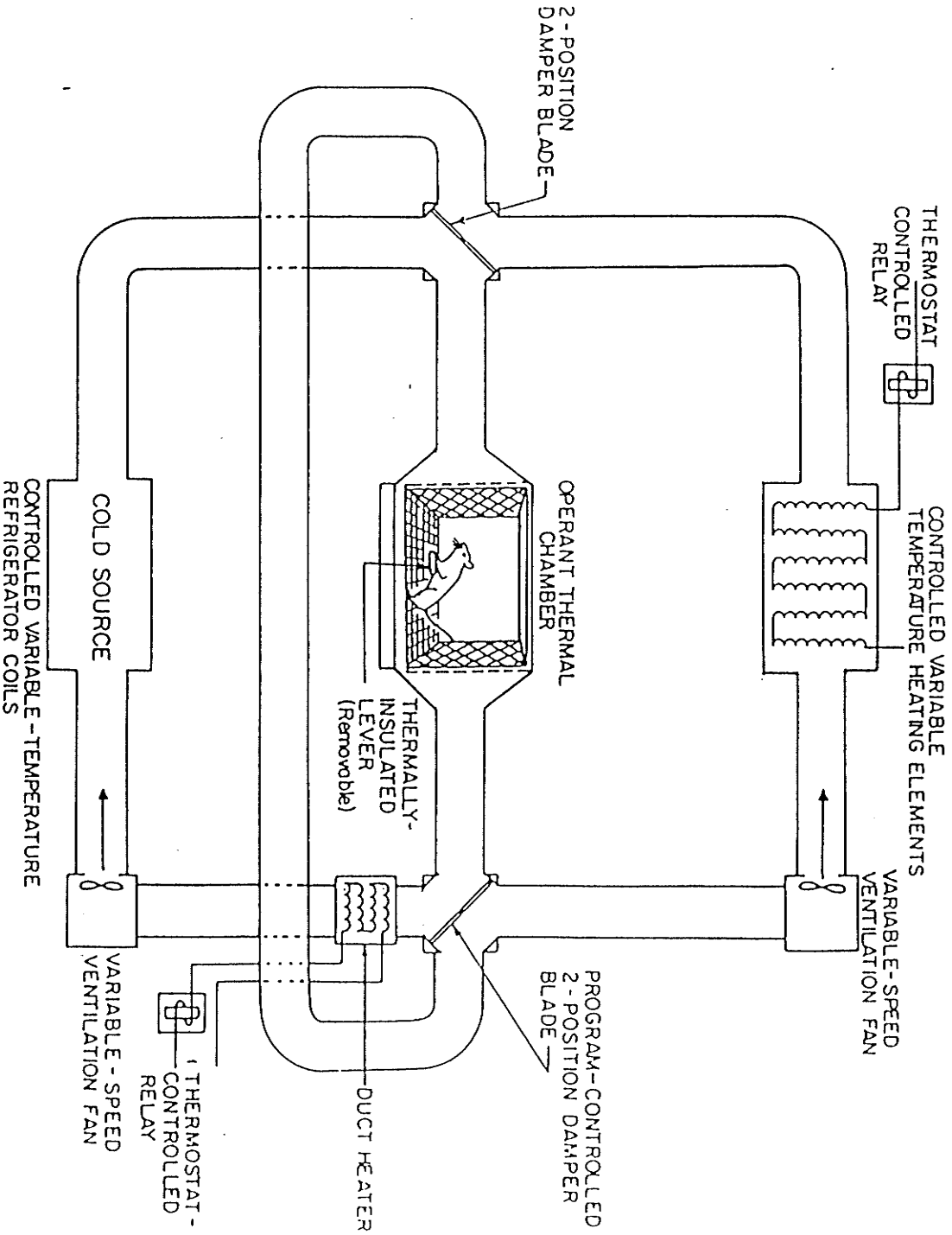
cabinet
metabolic chamber
rodent in
Biodec restrainer

Figure 2. Schematic representation of the apparatus used for heat escape response testing: The Convective Thermal Controller.



CONVECTIVE THERMAL CONTROLLER (CTC)

Figure 3. Mean (±SEM) oxygen consumption ($ml O_2/g/hr$) and carbon dioxide production ($ml CO_2/g/hr$) collapsed across the 1K-GB hypertensive and sham normotensive rats as a function of time for baseline, and for the administration of isoproterenol, isoproterenol with the $beta_1$-antagonist and isoproterenol with the $beta_2$-antagonist.

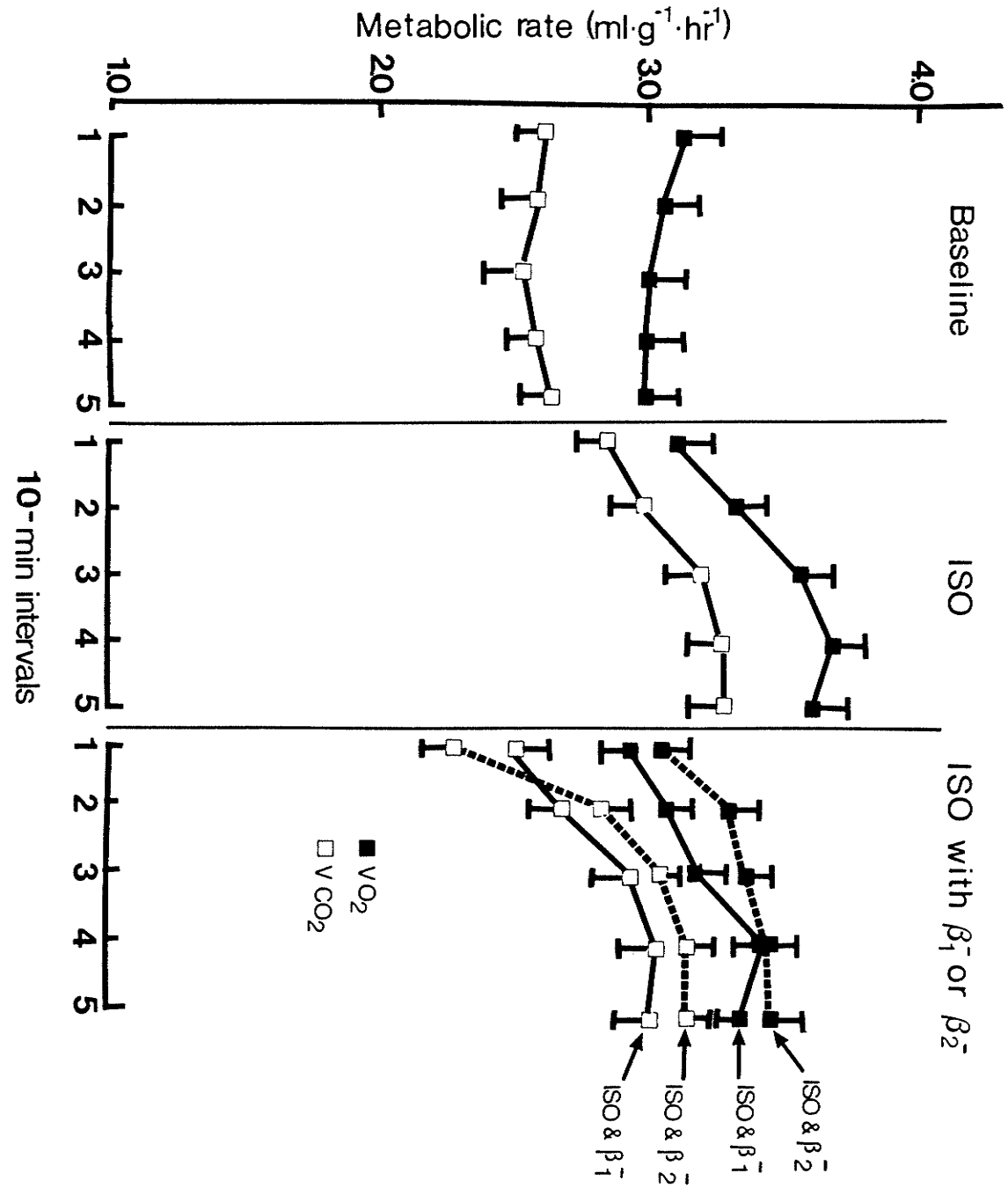

Metabolic rate (ml·g⁻¹·hr⁻¹)
1.0
2.0
3.0
4.0
Baseline
ISO
ISO with β1⁻ or β2⁻
ISO & β1⁻
ISO & β2⁻
ISO & β1⁻ & β2⁻
■ VO2
□ VCO2
1 2 3 4 5
10-min intervals

Figure 4. Mean (±SEM) oxygen consumption ($ml O_2/g/hr$) as a function of time during isoproterenol administrations for 1K-GB hypertensive and sham normotensive rats.

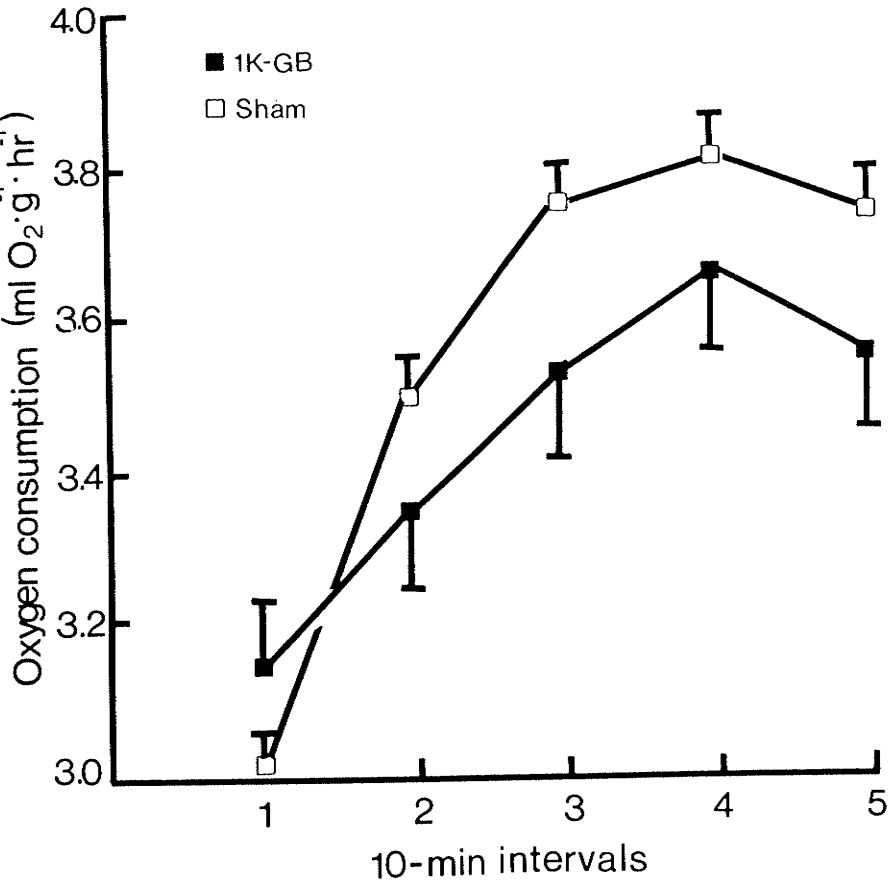

4.0
3.8
3.6
3.4
3.2
3.0
Oxygen consumption (ml $O_2 \cdot g^{-1} \cdot hr^{-1}$)
1K-GB
Sham
1
2
3
4
5
10-min intervals

Figure 5. Mean (±SEM) percentage change from baseline for oxygen consumption collapsed across both beta-antagonists as a function of time for 1K-GB hypertensive and sham normotensive rats.

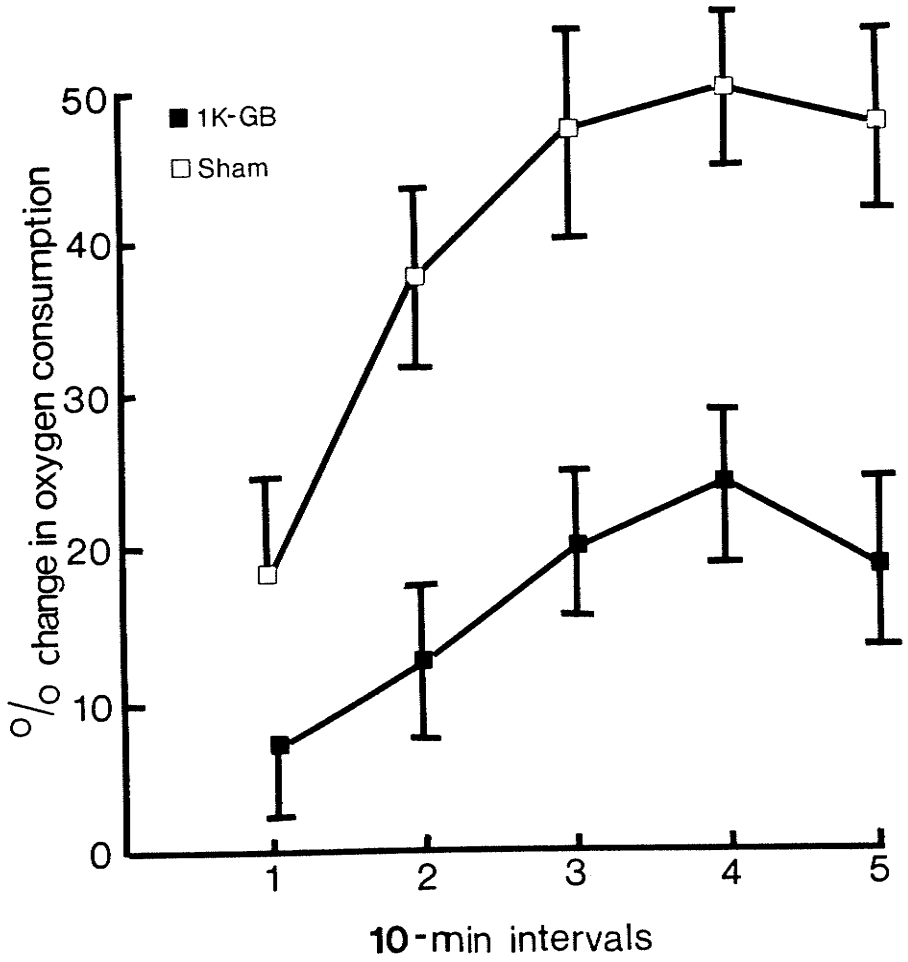

50
40
30
20
10
0
■ 1K-GB
□ Sham
% change in oxygen consumption
1
2
3
4
5
10-min intervals

Figure 6. Mean (±SEM) oxygen consumption ($ml O_2/g/hr$) collapsed across isoproterenol and combined isoproterenol and pindolol administrations as a function of time for 1K-GB hypertensive and sham normotensive rats.

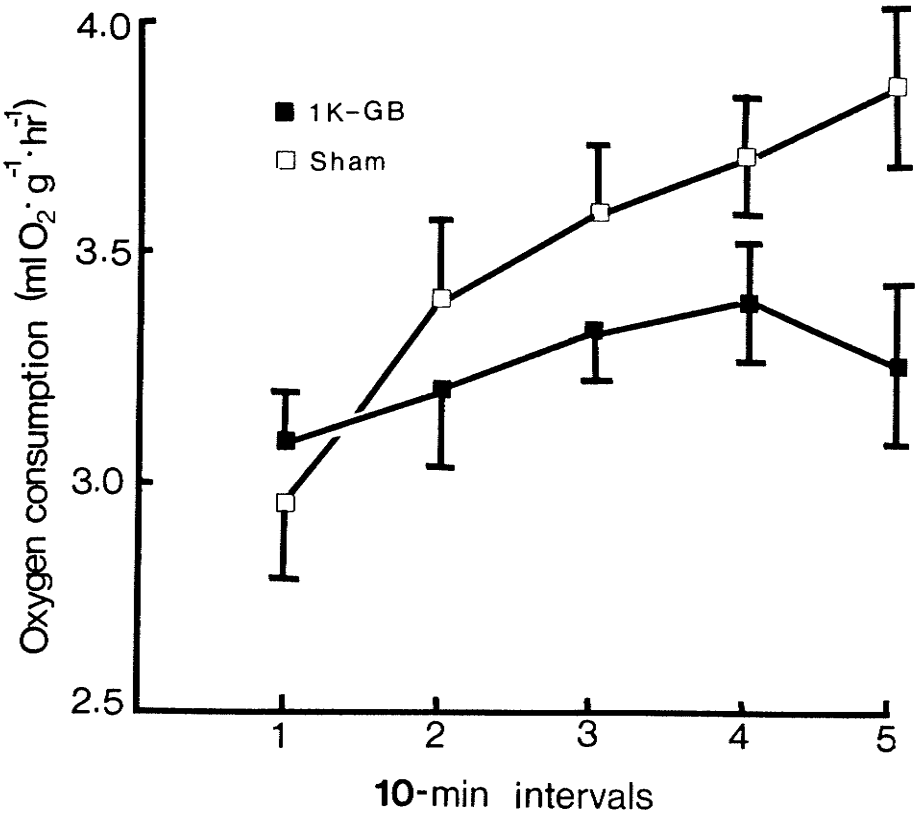

4.0
3.5
3.0
2.5
1K-GB
Sham
Oxygen consumption (ml $O_2 \cdot g^{-1} \cdot hr^{-1}$)
1
2
3
4
5
10-min intervals

Figure 7. Mean (±SEM) baseline rectal temperature (^{o}C) as a function of time for 1K-GB hypertensive and sham normotensive rats.

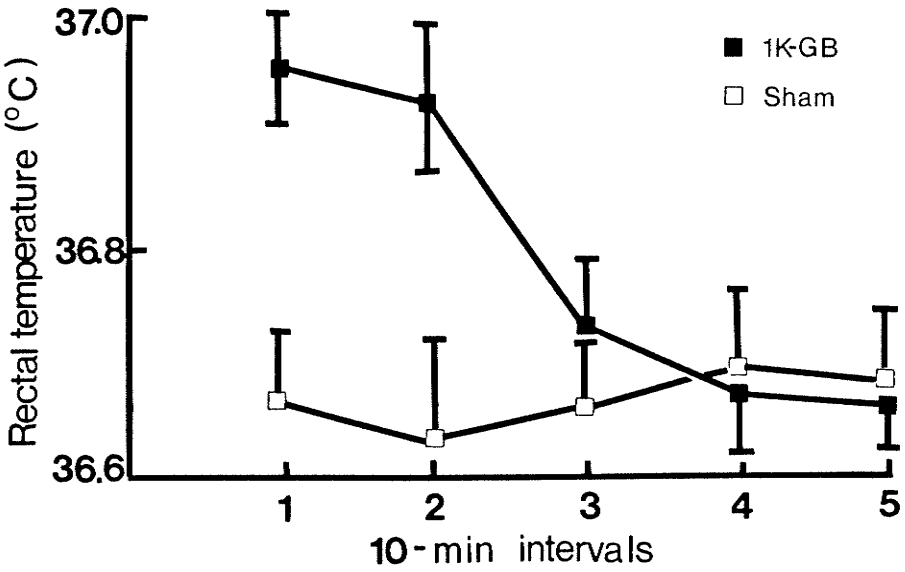

37.0
36.8
36.6
Rectal temperature (°C)
1K-GB
Sham
1
2
3
4
5
10-min intervals

Figure 8. Mean (±SEM) rectal temperature (^{o}C) and tail skin temperature (^{o}C) collapsed across the 1K-GB hypertensive and sham normotensive rats as a function of time for baseline, and for the administration of isoproterenol, isoproterenol with the $beta_1$-antagonist, and isoproterenol with the $beta_2$-antagonist.

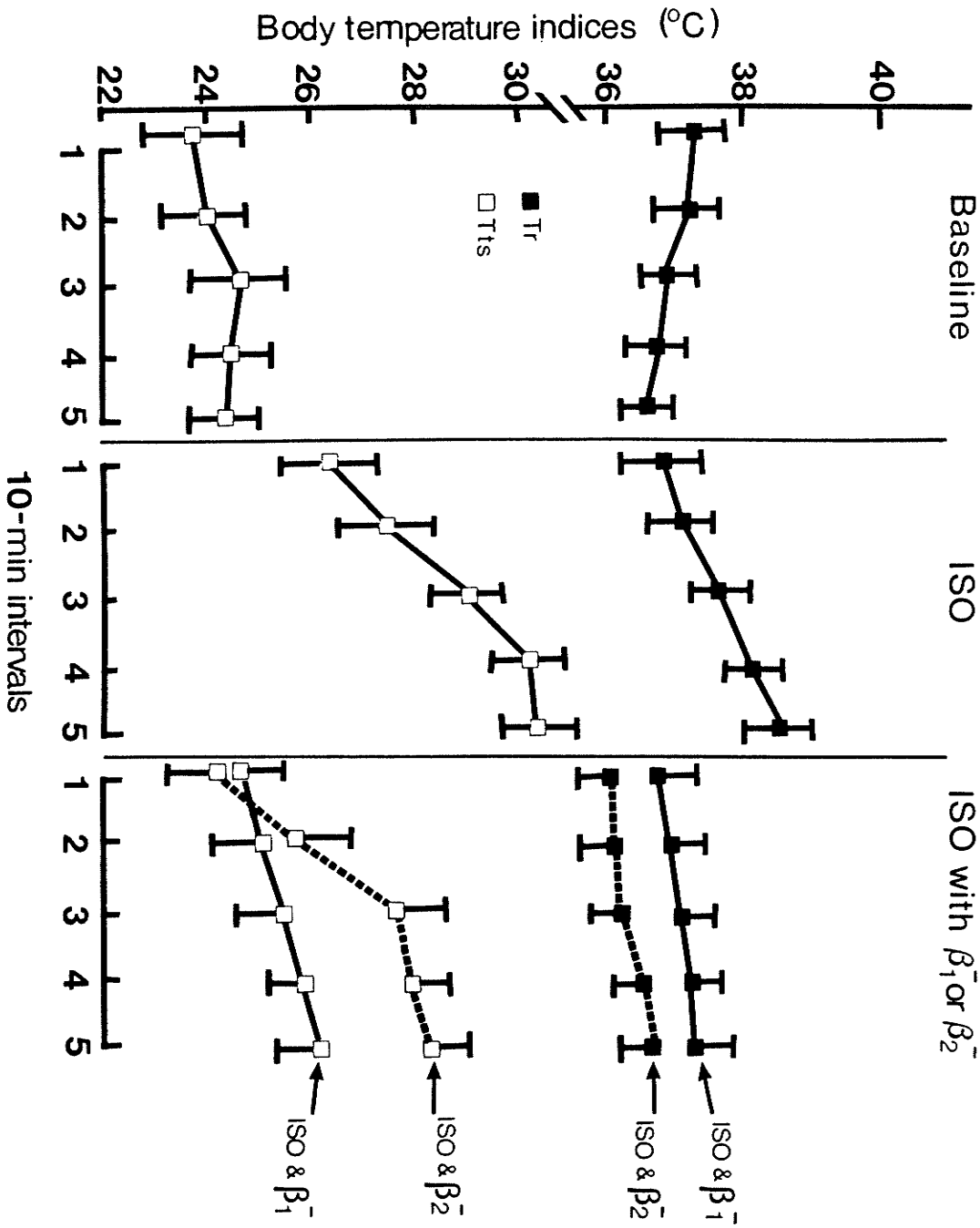

Body temperature indices (°C)
22
24
26
28
30
36
38
40
Tr
Tts
Baseline
ISO
ISO with β_1^- or β_2^-
1 2 3 4 5
10-min intervals
ISO & β_1^-
ISO & β_2^-
ISO & β_1^-
ISO & β_2^-

Figure 9. Mean (±SEM) rectal temperature (^{o}C) as a function of time during isoproterenol infusions for 1K-GB hypertensive and sham normotensive rats.

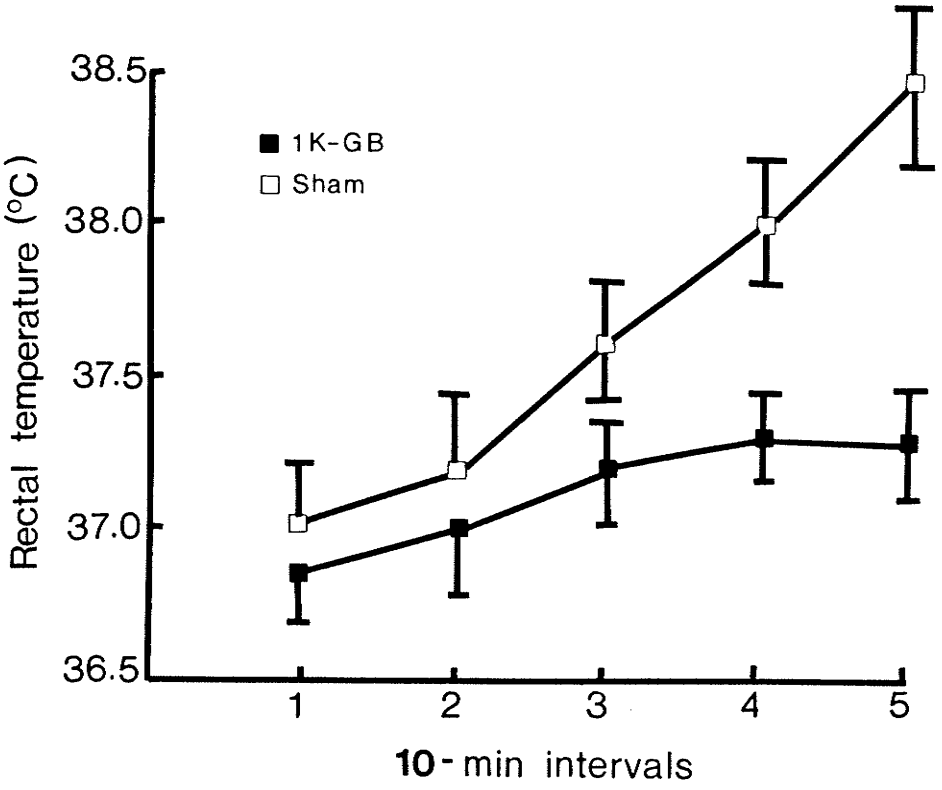

38.5
38.0
37.5
37.0
36.5
Rectal temperature (°C)
1K-GB
Sham
1
2
3
4
5
10-min intervals

Figure 10. Mean (±SEM) rectal temperature (^{o}C) collapsed across isoproterenol and combined isoproterenol and atenolol infusions as a function of time for 1K-GB hypertensive and sham normotensive rats.

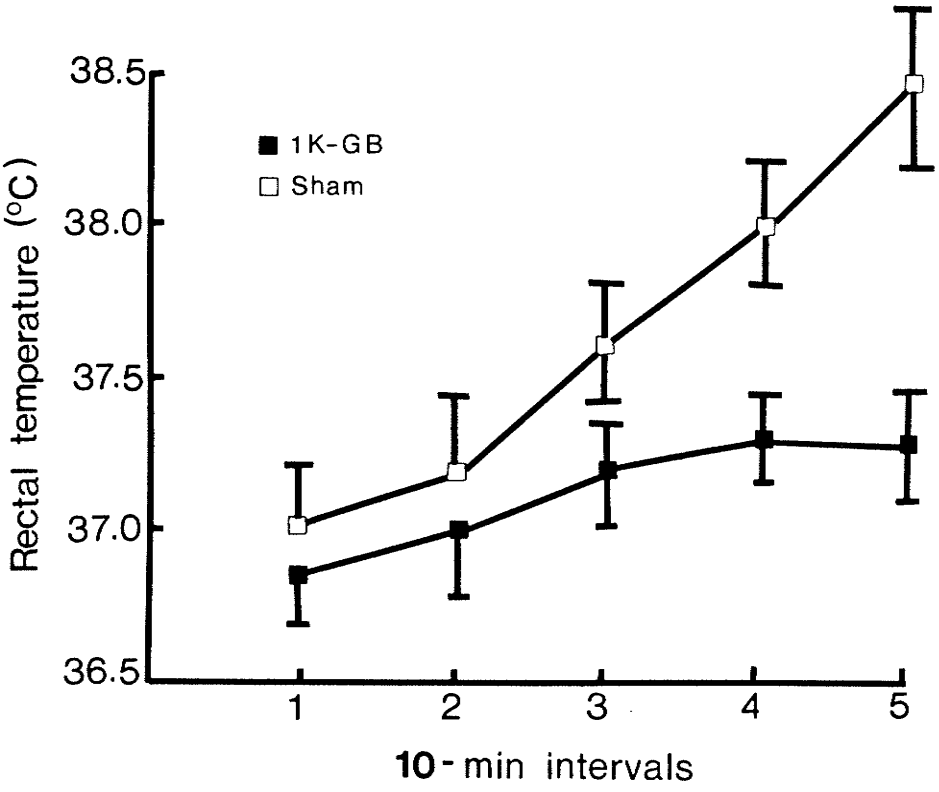

38.5
38.0
37.5
37.0
36.5
Rectal temperature (°C)
1K-GB
Sham
1
2
3
4
5
10-min intervals

Figure 11. Mean (±SEM) arterial blood pressure (mmHg) and heart rate (bpm) collapased across the 1K-GB hypertensive and sham normotensive rats as a function of time for baseline, and for the administration of isoproterenol, isoproterenol with the $beta_1$-antagonist, and isoproterenol with the $beta_2$-antagonist.

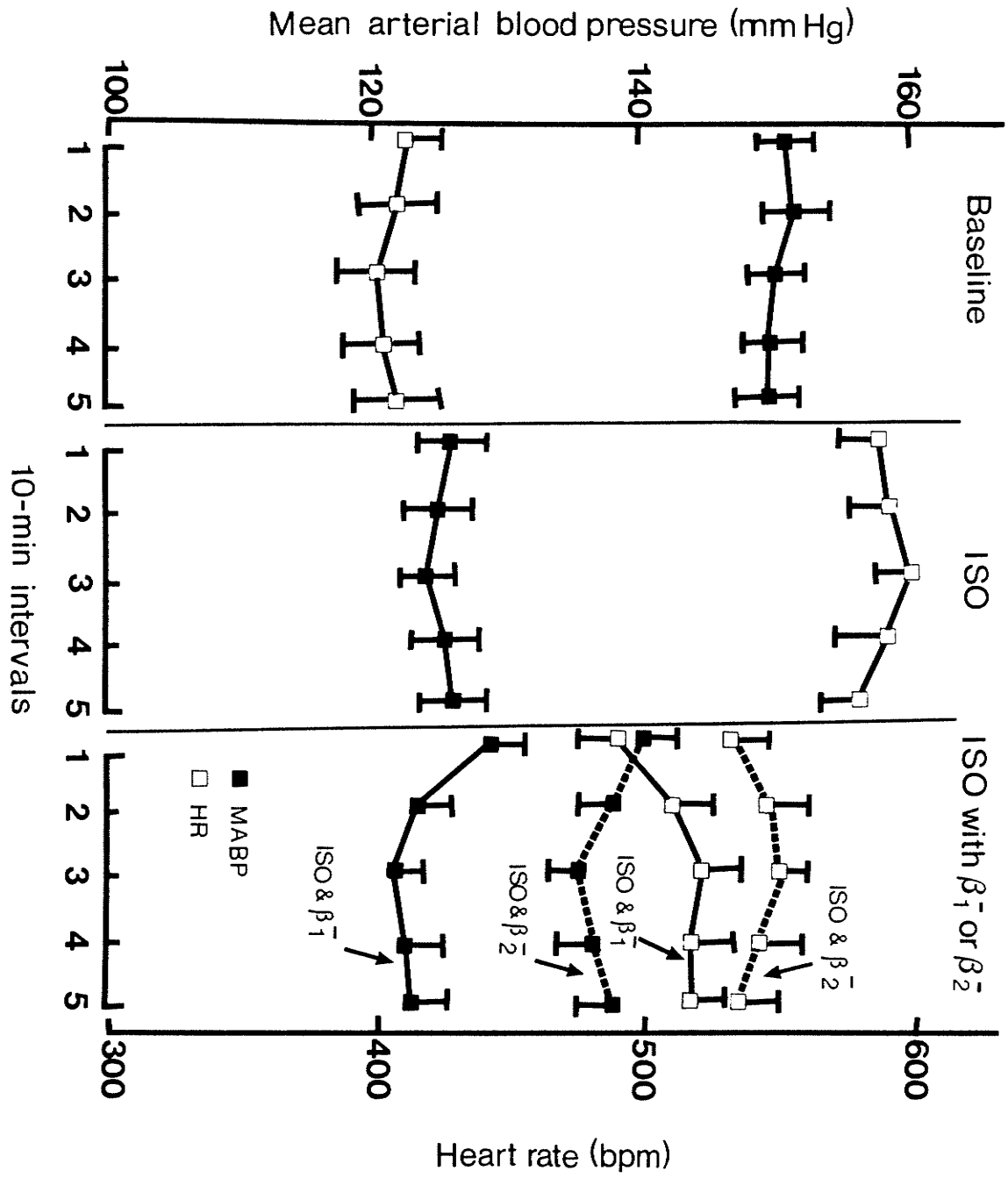

Mean arterial blood pressure (mm Hg)
100
120
140
160
Baseline
ISO
ISO with β_1^- or β_2^-
1 2 3 4 5
10-min intervals
MABP
HR
ISO & β_1^-
ISO & β_2^-
ISO & β_1^-
ISO & β_2^-
300
400
500
600
Heart rate (bpm)

Figure 12. Mean (±SEM) baseline oxygen consumption (mlO_2/g/hr) as a function of the blood pressure and acclimation status of the animals.

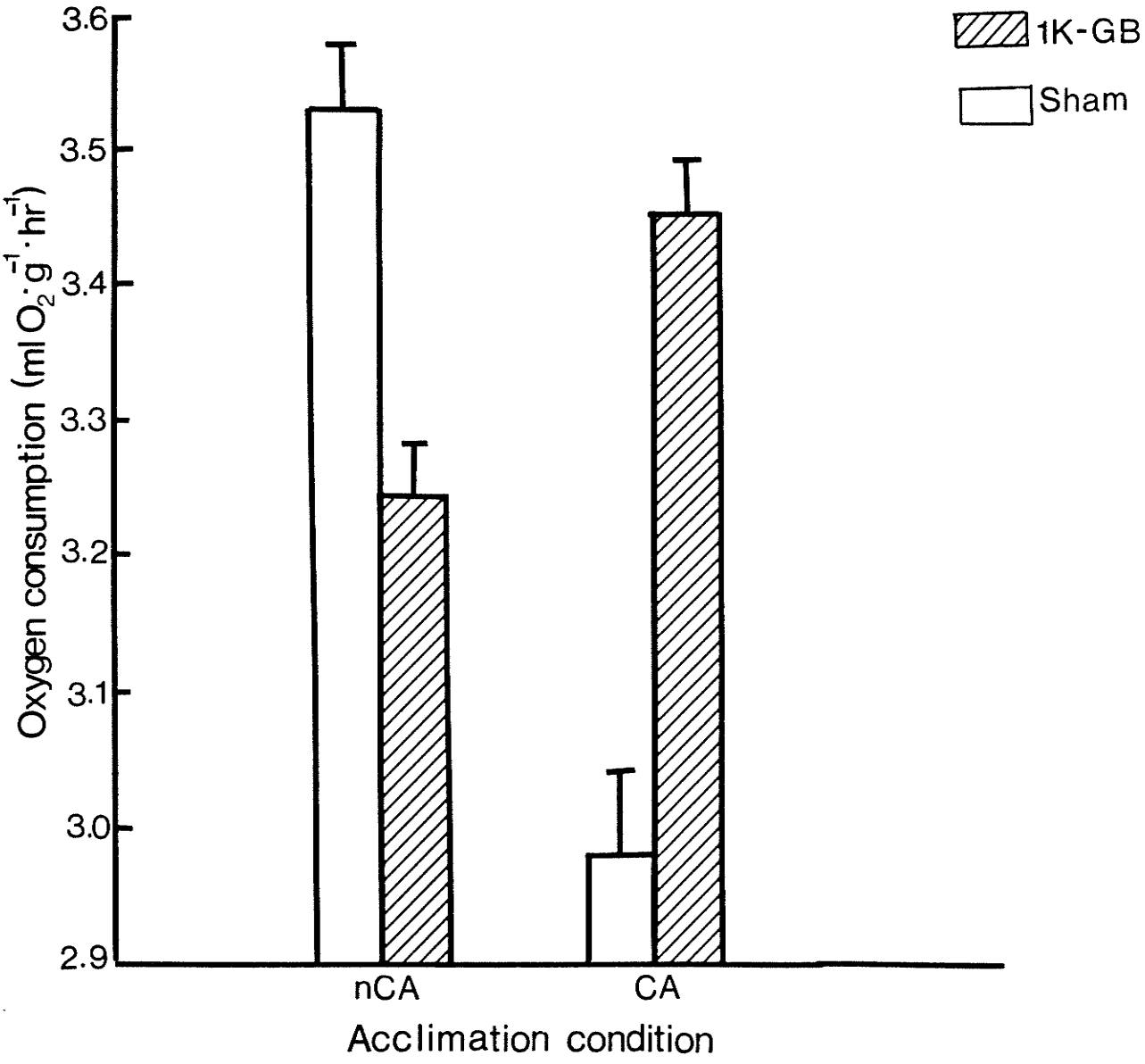

1K-GB
Sham
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
Oxygen consumption (ml $O_2^{-1} \cdot g \cdot hr^{-1}$)
nCA
CA
Acclimation condition

Figure 13. Mean (±SEM) oxygen consumption ($ml O_2/g/hr$) and carbon dioxide production ($ml CO_2/g/hr$) collapsed across the 1K-GB hypertensive, sham normotensive, cold-acclimated, and non cold-acclimated groups as a function of time for baseline and for the administration of isoproterenol, phenylephrine, and combined isoproterenol and phenylephrine.

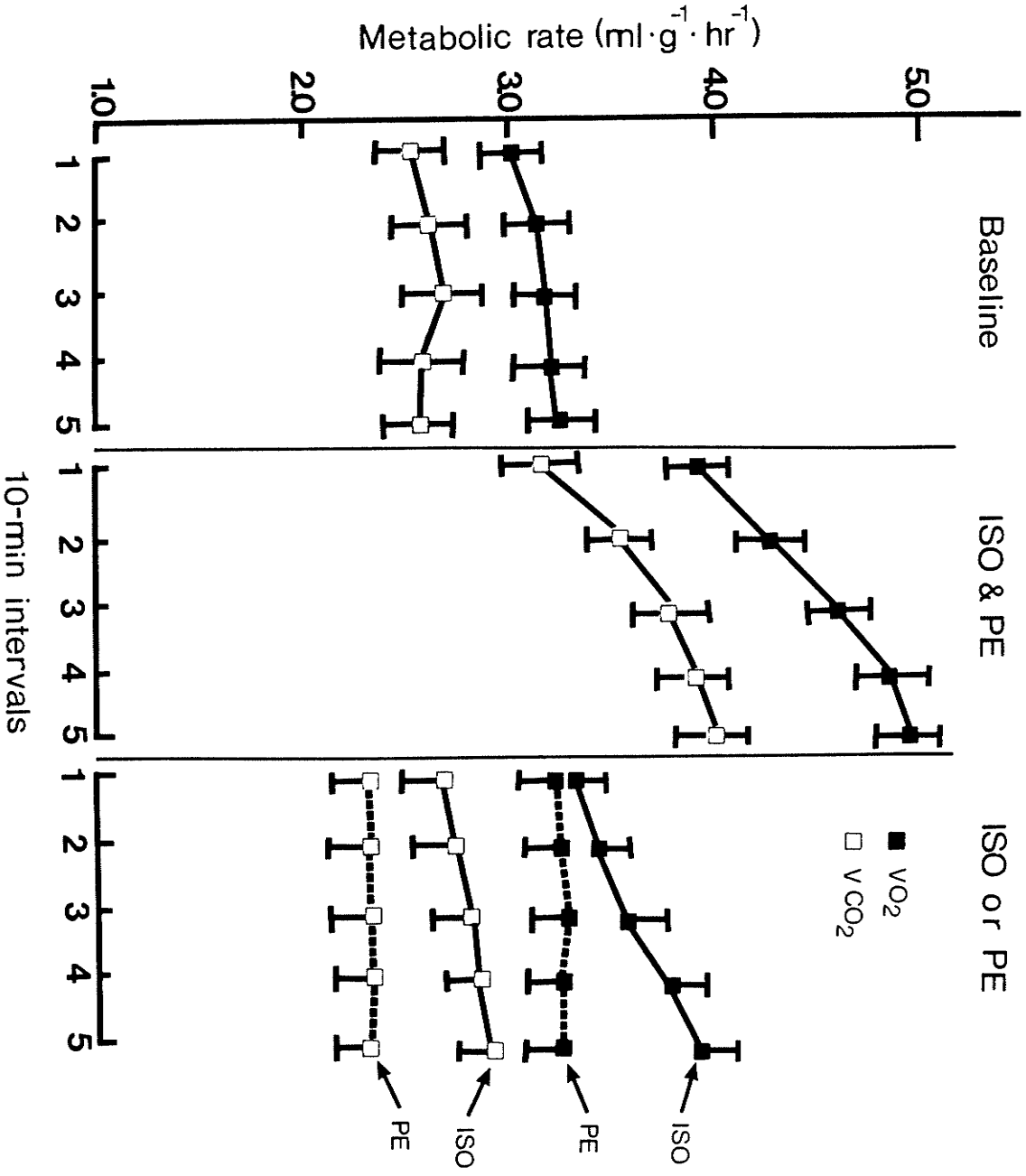

Metabolic rate (ml·g⁻¹·hr⁻¹)
1.0
2.0
3.0
4.0
5.0
Baseline
ISO & PE
ISO or PE
■ VO2
□ VCO2
1 2 3 4 5
10-min intervals
ISO
PE
ISO
PE

Figure 14. Mean (±SEM) baseline oxygen consumption ($ml O_2/g/hr$) collapsed across the cold- and non cold-acclimated groups as a function of time for 1K-GB hypertensive and sham normotensive rats.

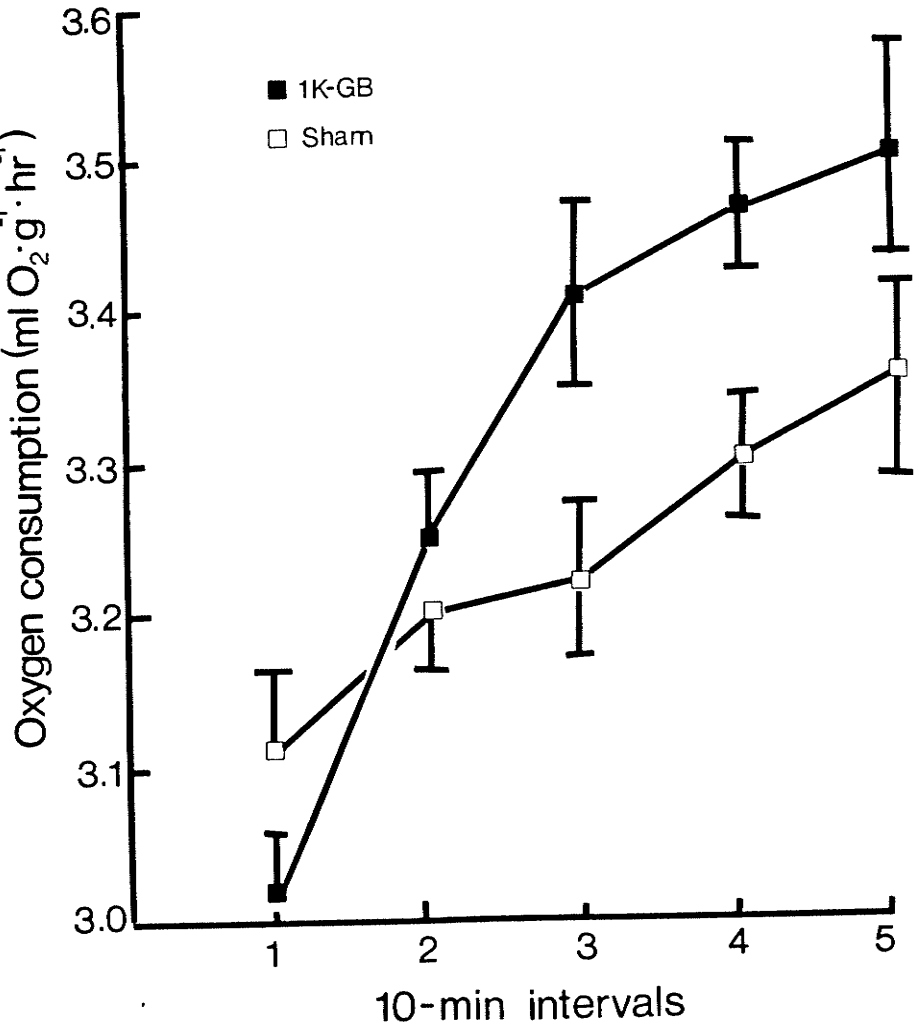

3.6
3.5
3.4
3.3
3.2
3.1
3.0
1K-GB
Sham
Oxygen consumption (ml $O_2 \cdot g^{-1} \cdot hr^{-1}$)
1
2
3
4
5
10-min intervals

Figure 15. Mean (±SEM) oxygen consumption ($ml O_2/g/hr$) collapsed across the administration of isoproterenol and combined isoproterenol and phenylephrine as a function of the blood pressure and acclimation status of the rats.

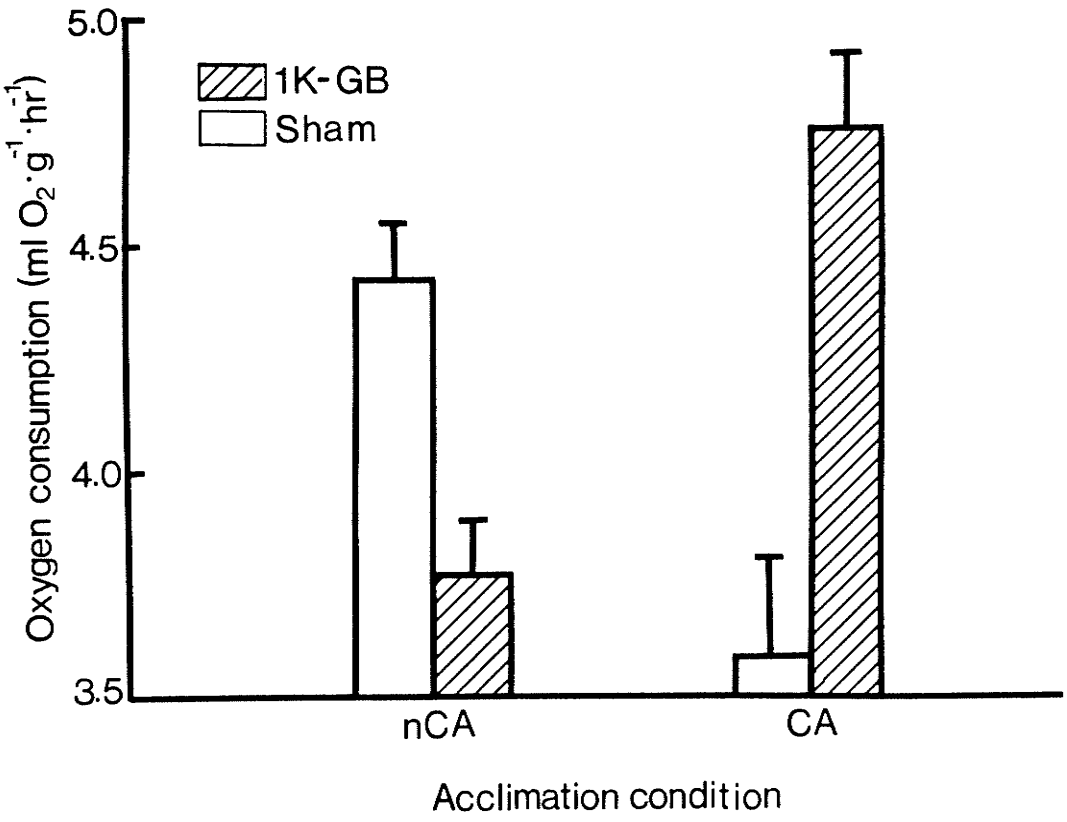

5.0
4.5
4.0
3.5
1K-GB
Sham
Oxygen consumption (ml $O_2 \cdot g^{-1} \cdot hr^{-1}$)
nCA
CA
Acclimation condition

Figure 16. Mean (±SEM) oxygen consumption (mlO_2/g/hr) as a function of the administration of isoproterenol alone or combined with phenylephrine for cold-acclimated 1K-GB hypertensive and sham normotensive and non cold-acclimated 1K-GB hypertensive and sham normotensive rats.

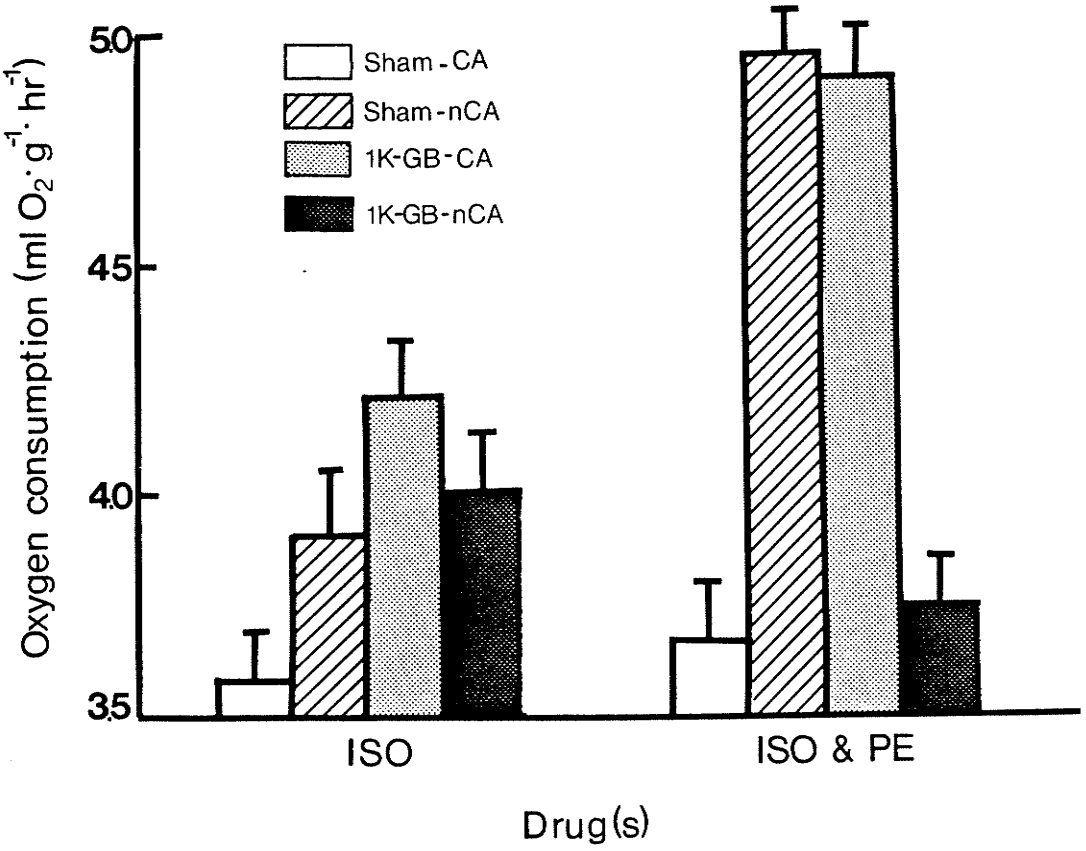

Sham-CA
Sham-nCA
1K-GB-CA
1K-GB-nCA
Oxygen consumption (ml $O_2 \cdot g^{-1} \cdot hr^{-1}$)
5.0
4.5
4.0
3.5
ISO
ISO & PE
Drug(s)

Figure 17. Mean (±SEM) oxygen consumption (mlO_2/g/hr) collapsed across the cold- and non cold-acclimated groups as a function of time during phenylephrine and combined isoproterenol and phenylephrine administrations for 1K-GB hypertensive and sham normotensive rats.

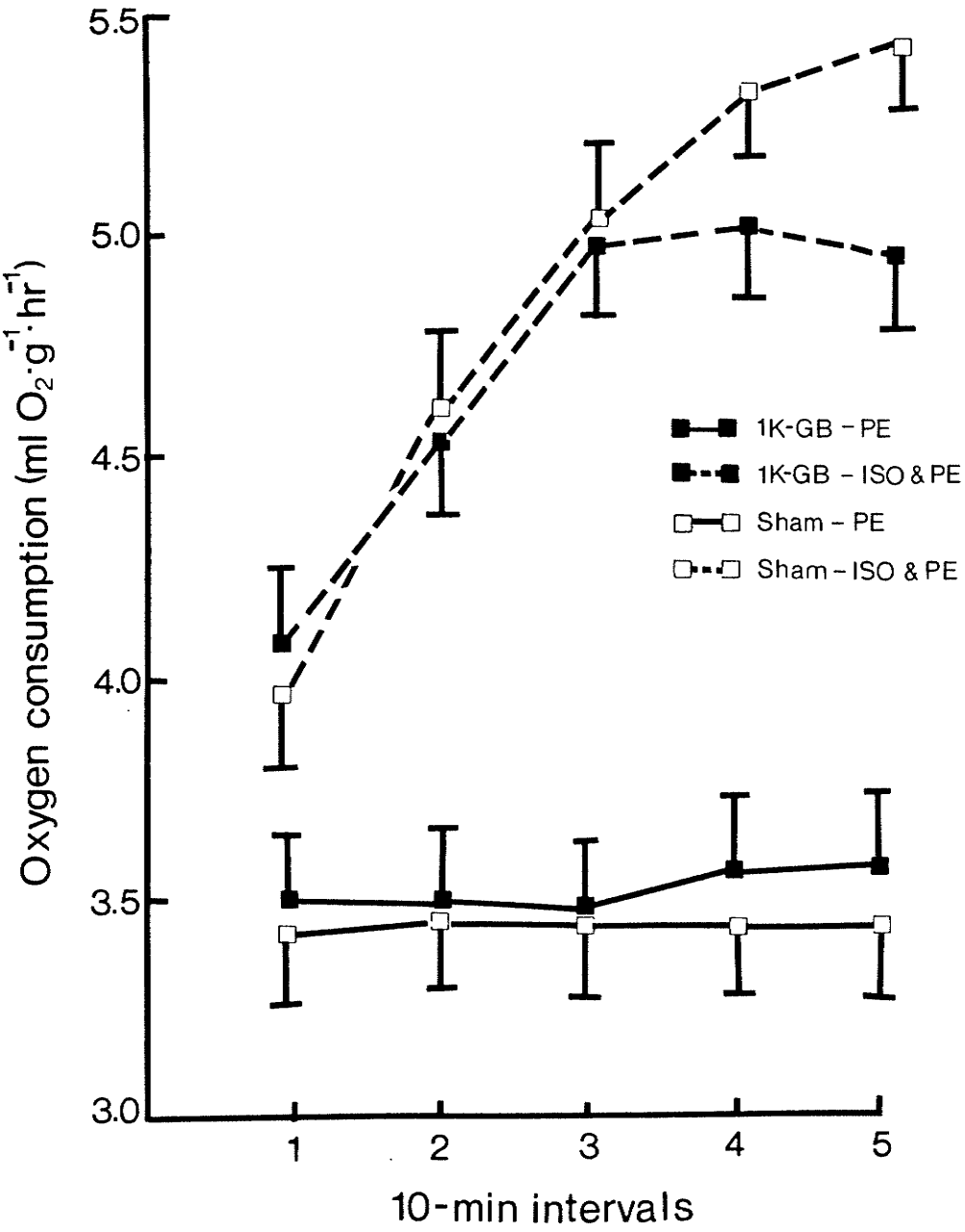

5.5
5.0
4.5
4.0
3.5
3.0
Oxygen consumption (ml $O_2 \cdot g^{-1} \cdot hr^{-1}$)
1K-GB – PE
1K-GB – ISO & PE
Sham – PE
Sham – ISO & PE
1
2
3
4
5
10-min intervals

Figure 18. Mean (±SEM) baseline tail skin temperature (^{o}C) as a function of the blood pressure and acclimation status of the animals.

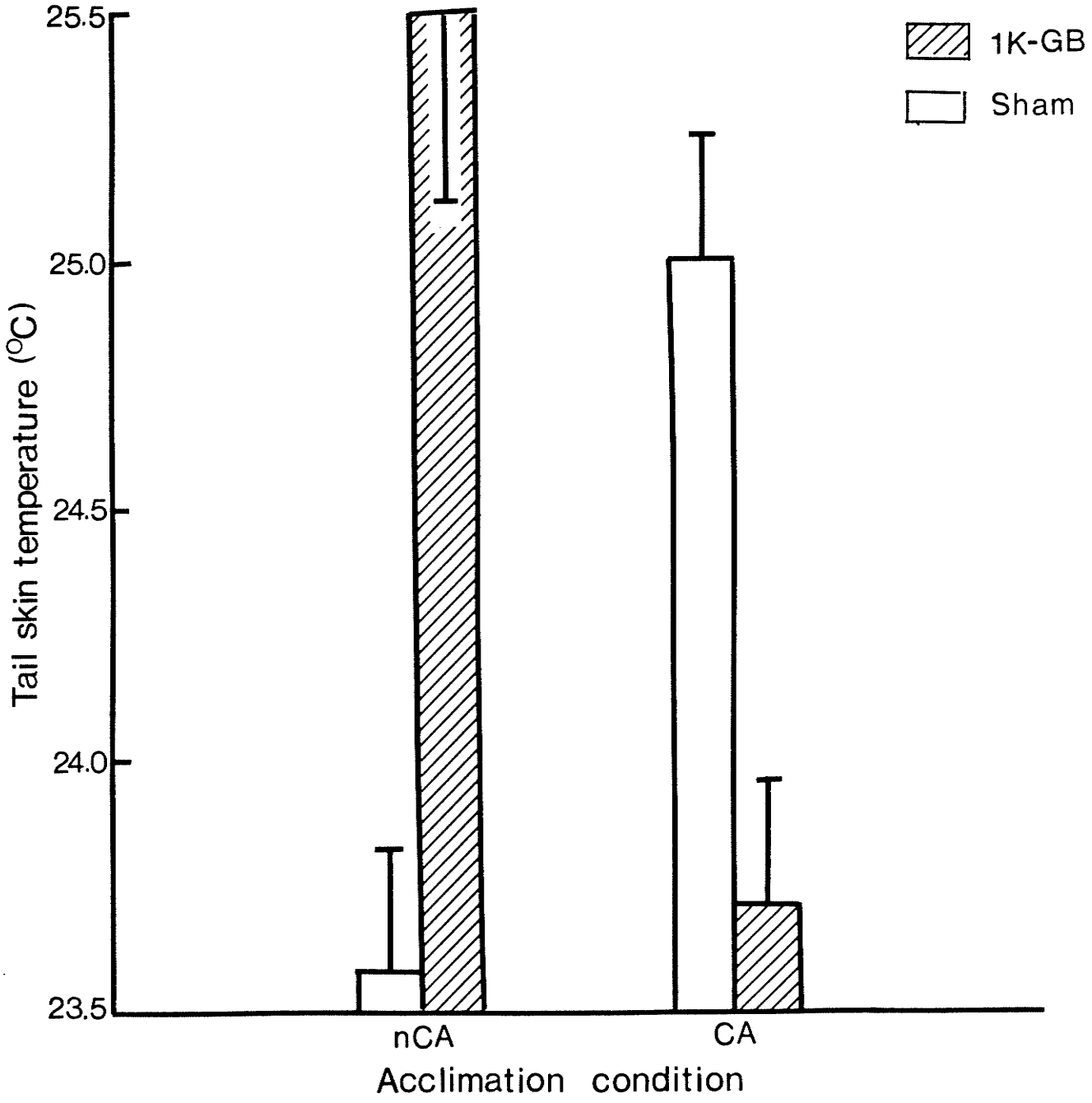

25.5
25.0
24.5
24.0
23.5
Tail skin temperature (°C)
1K-GB
Sham
nCA
CA
Acclimation condition

Figure 19. Mean (±SEM) rectal temperature (°C) and tail skin temperature (°C) collapsed across the 1K-GB hypertensive, sham normotensive, cold-acclimated, and non cold-acclimated groups as a function of time for baseline, and for the administration of isoproterenol, phenylephrine, and combined isoproterenol and phenylephrine.

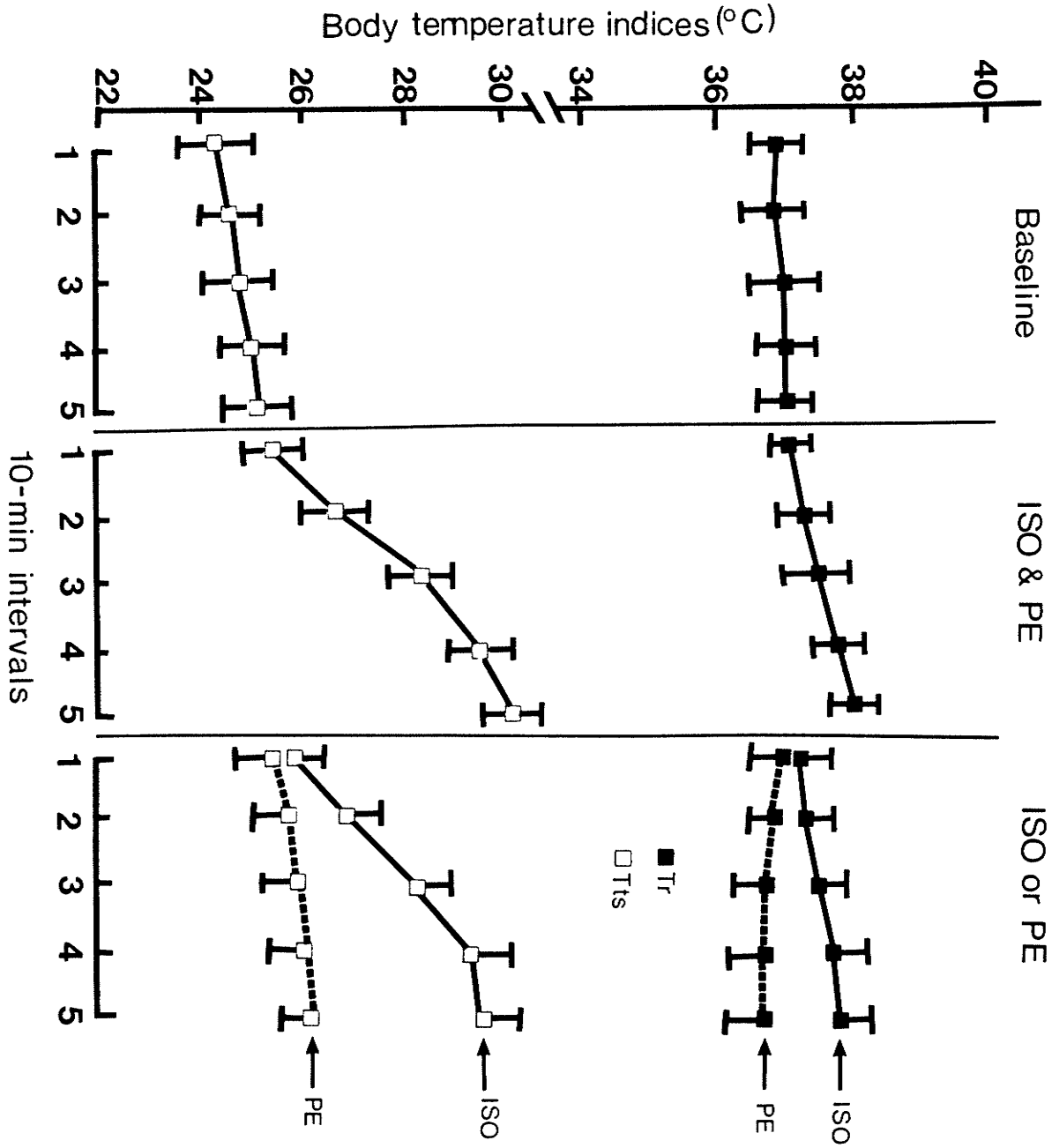

Body temperature indices (°C)
40
38
36
34
30
28
26
24
22
Baseline
ISO & PE
ISO or PE
Tr
Tts
ISO
PE
ISO
PE
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
10-min intervals

Figure 20. Mean (±SEM) percentage change from baseline for tail skin temperature collapsed across the administration of isoproterenol and combined isoproterenol and phenylephrine as a function of the blood pressure and acclimation status of the rats.

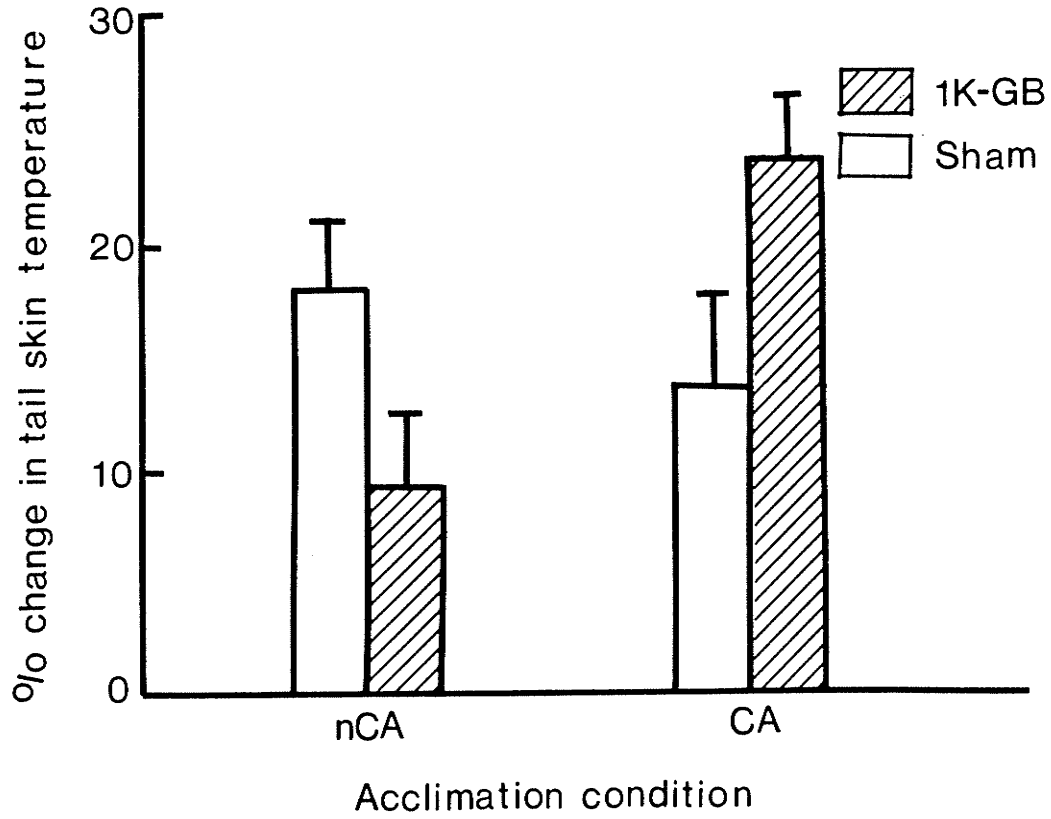

30
20
10
0
% change in tail skin temperature
1K-GB
Sham
nCA
CA
Acclimation condition

Figure 21. Mean (±SEM) tail skin temperature (^{o}C) collapsed across the administration of isoproterenol and combined isoproterenol and phenylephrine as a function of time for cold- and non cold-acclimated rats.

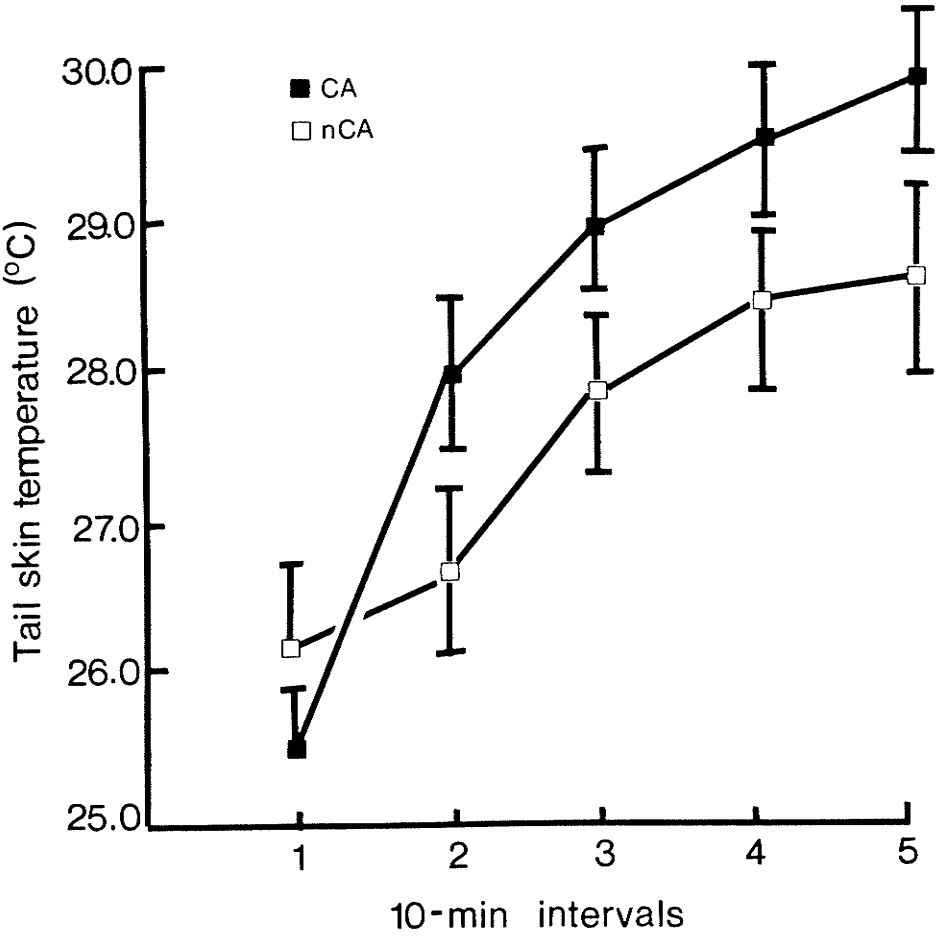

30.0
29.0
28.0
27.0
26.0
25.0
Tail skin temperature (°C)
CA
nCA
1
2
3
4
5
10-min intervals

Figure 22. Mean (±SEM) arterial blood pressure (mmHg) and heart rate (bpm) collapsed across the 1K-GB hypertensive, sham normotensive, cold-acclimated, and non cold-acclimated groups as a function of time for baseline. and for the administration of isoproterenol, phenylephrine, and combined isoproterenol and phenylephrine.

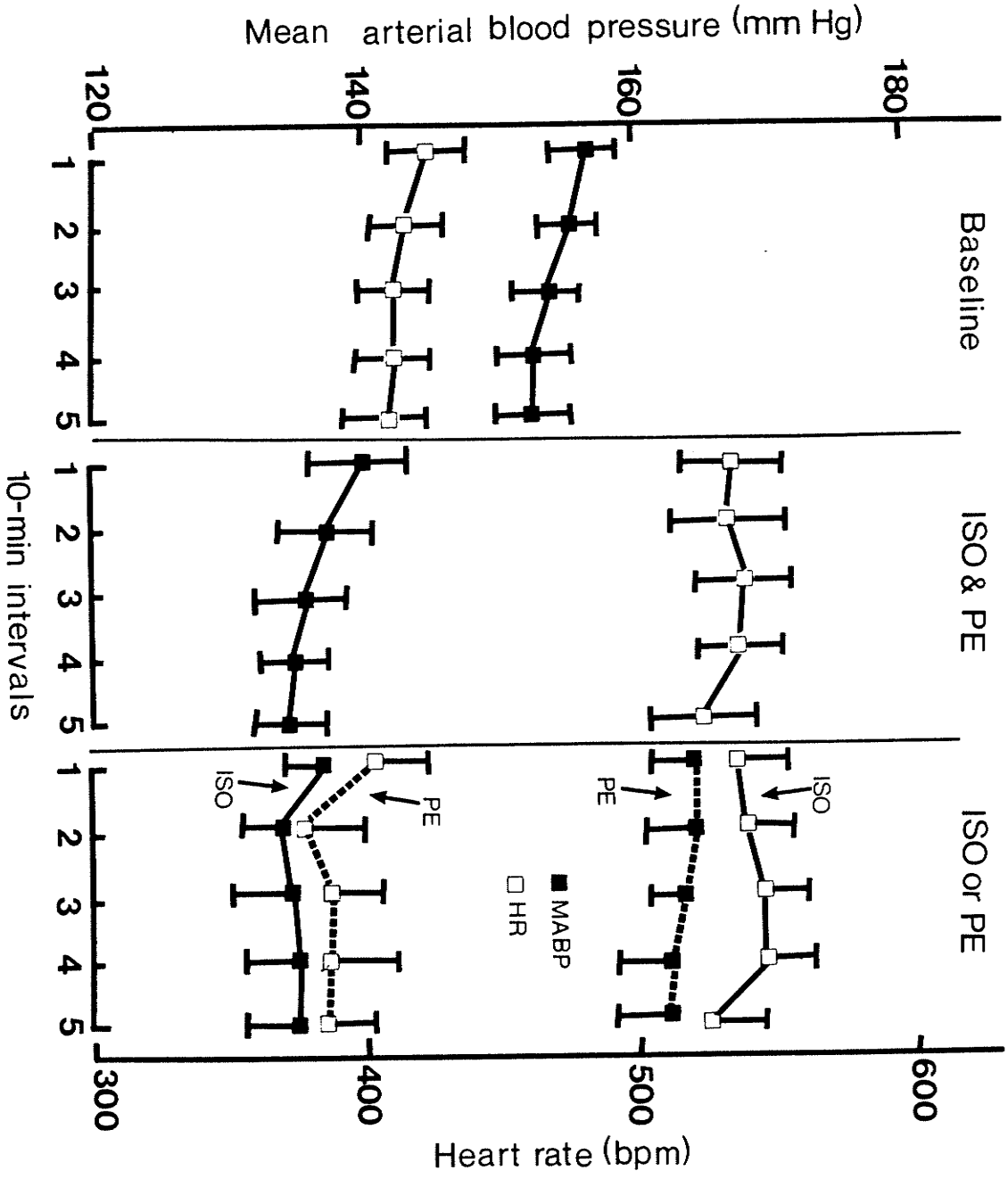

Mean arterial blood pressure (mm Hg)
120
140
160
180
Baseline
ISO & PE
ISO or PE
10-min intervals
1
2
3
4
5
ISO
PE
PE
ISO
MABP
HR
300
400
500
600
Heart rate (bpm)

Figure 23. Mean(±SEM) arterial blood pressure (mmHg) collapsed across the 1K-GB hypertensive and sham normotensive groups as a function of isoproterenol or phenylephrine administrations for cold- and non cold-acclimated rats.

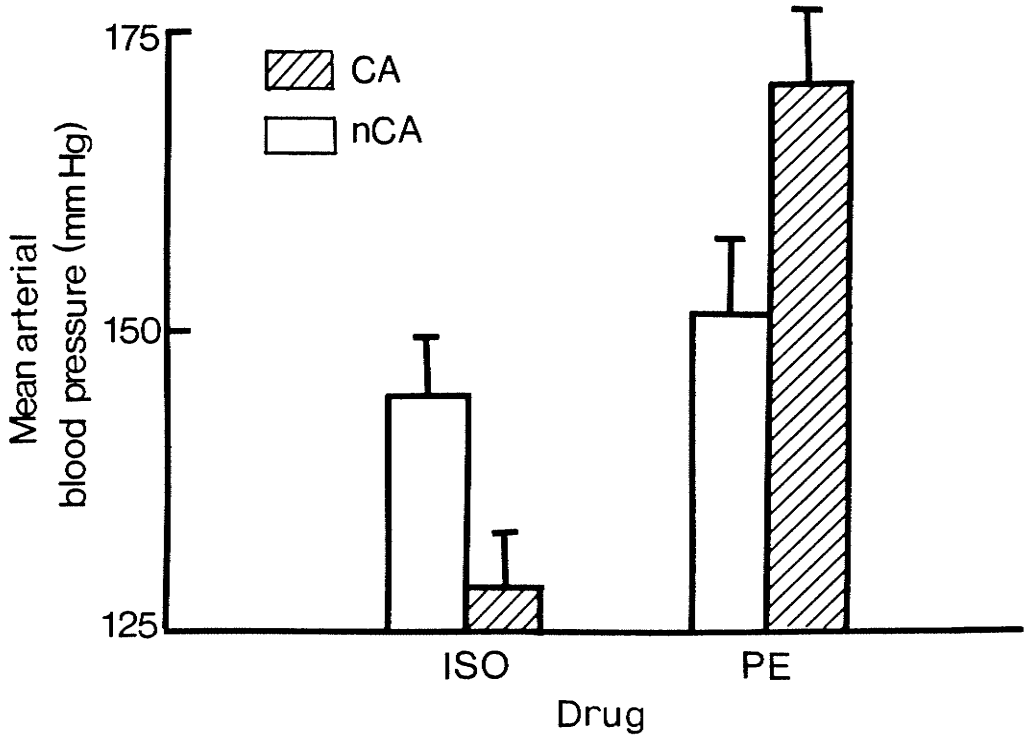

175
150
125
Mean arterial
blood pressure (mm Hg)
CA
nCA
ISO
PE
Drug

Figure 24. Mean (±SEM) urinary sodium concentration as a function of the blood pressure and acclimation status of the rats.

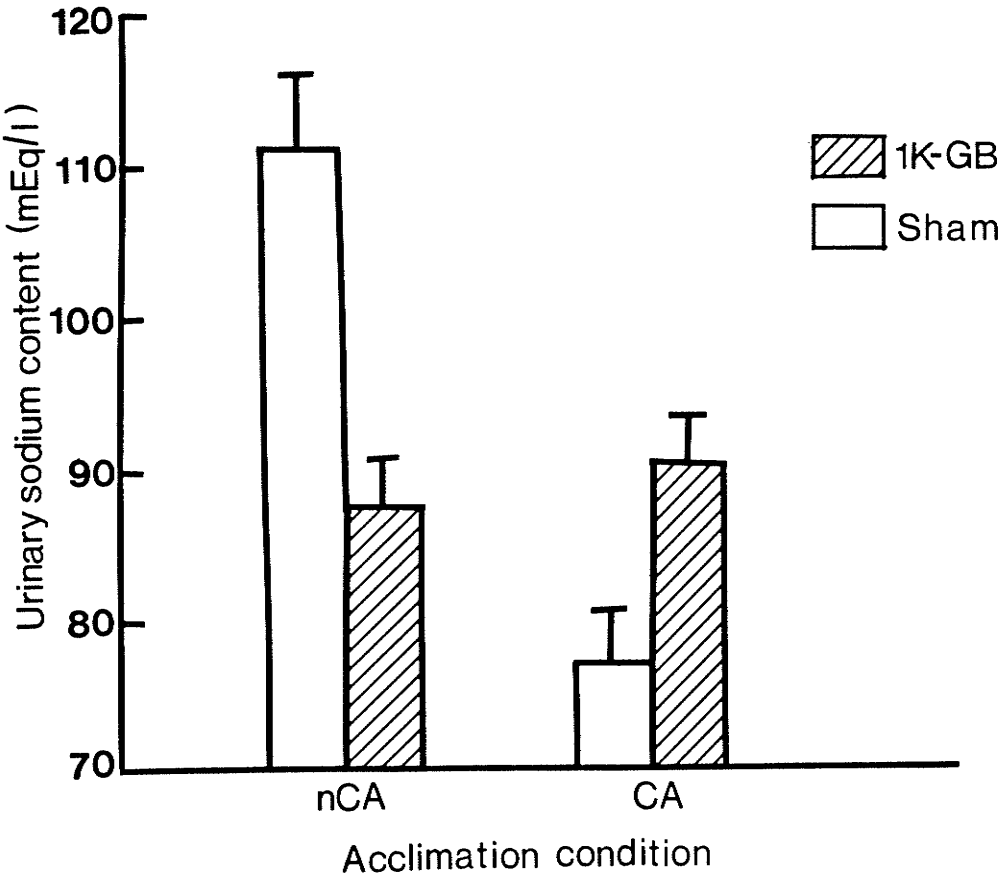

120
110
100
90
80
70
Urinary sodium content (mEq/l)
1K-GB
Sham
nCA
CA
Acclimation condition

Figure 25. Mean (±SEM) oxygen consumption ($ml O_2/g/hr$) and carbon dioxide production ($ml CO_2/g/hr$) collapsed across the 1K-GB hypertensive and sham normotensive rats as a function of time for baseline, and for the administration of isoproterenol, guanabenz, and combined isoproterenol and guanabenz.

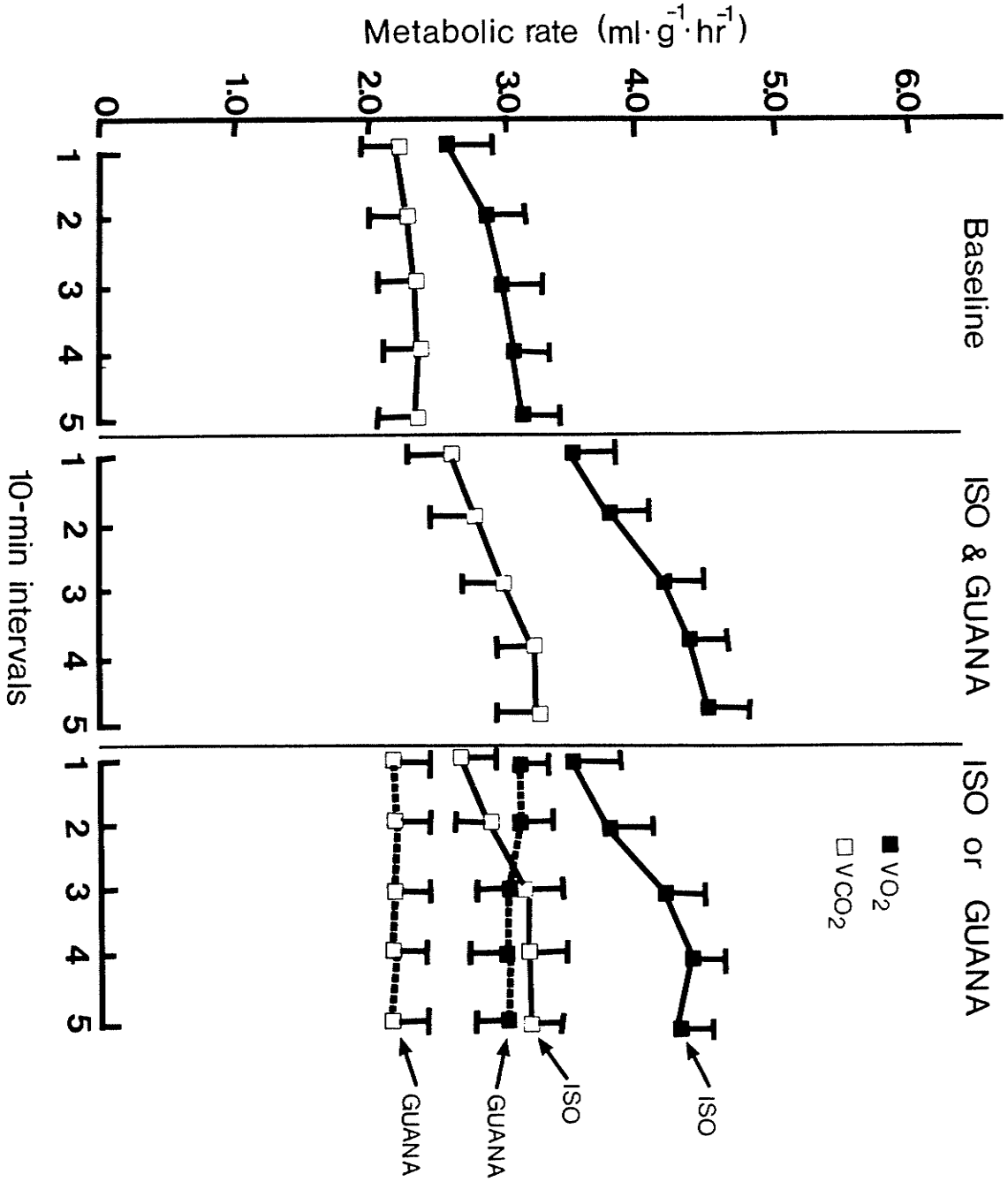

Metabolic rate (ml·g⁻¹·hr⁻¹)
6.0
5.0
4.0
3.0
2.0
1.0
0
Baseline
ISO & GUANA
ISO or GUANA
■ VO2
□ VCO2
ISO
GUANA
ISO
GUANA
1 2 3 4 5
10-min intervals

Figure 26. Mean (±SEM) oxygen consumption (mlO_2/g/hr) collapsed across isoproterenol and guanabenz administrations as a function of time for 1K-GB hypertensive and sham normotensive rats.

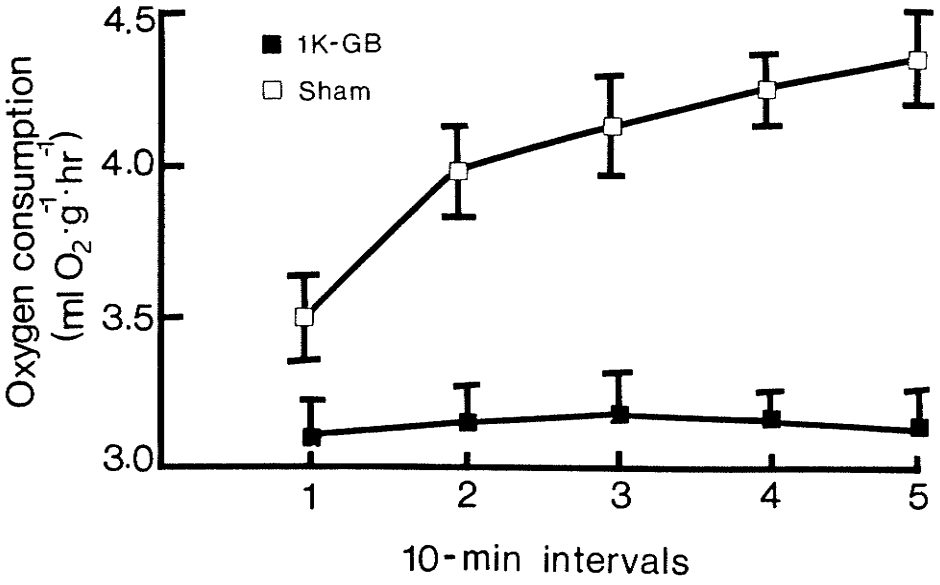

4.5
4.0
3.5
3.0
1K-GB
Sham
Oxygen consumption
(ml $O_2 \cdot g^{-1} \cdot hr^{-1}$)
1
2
3
4
5
10-min intervals

Figure 27. Mean (±SEM) oxygen consumption ($ml O_2/g/hr$) collapsed across isoproterenol and combined isoproterenol and guanabenz administrations as a function of time for 1K-GB hypertensive and sham normotensive rats.

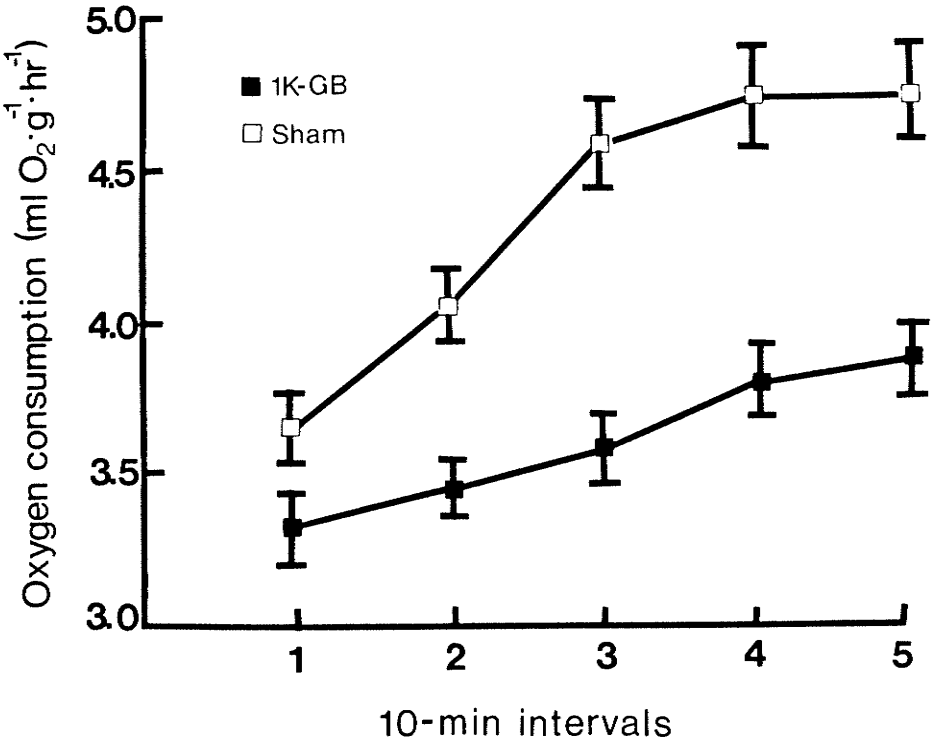

5.0
4.5
4.0
3.5
3.0
Oxygen consumption (ml $O_2 \cdot g^{-1} \cdot hr^{-1}$)
1K-GB
Sham
1
2
3
4
5
10-min intervals

Figure 28. Mean (±SEM) rectal temperature (°C) and tail skin temperature (°C) collapsed across the 1K-GB hypertensive and sham normotensive rats as a function of time for baseline, and for the administration of isoproterenol, guanabenz, and combined isoproterenol and guanabenz.

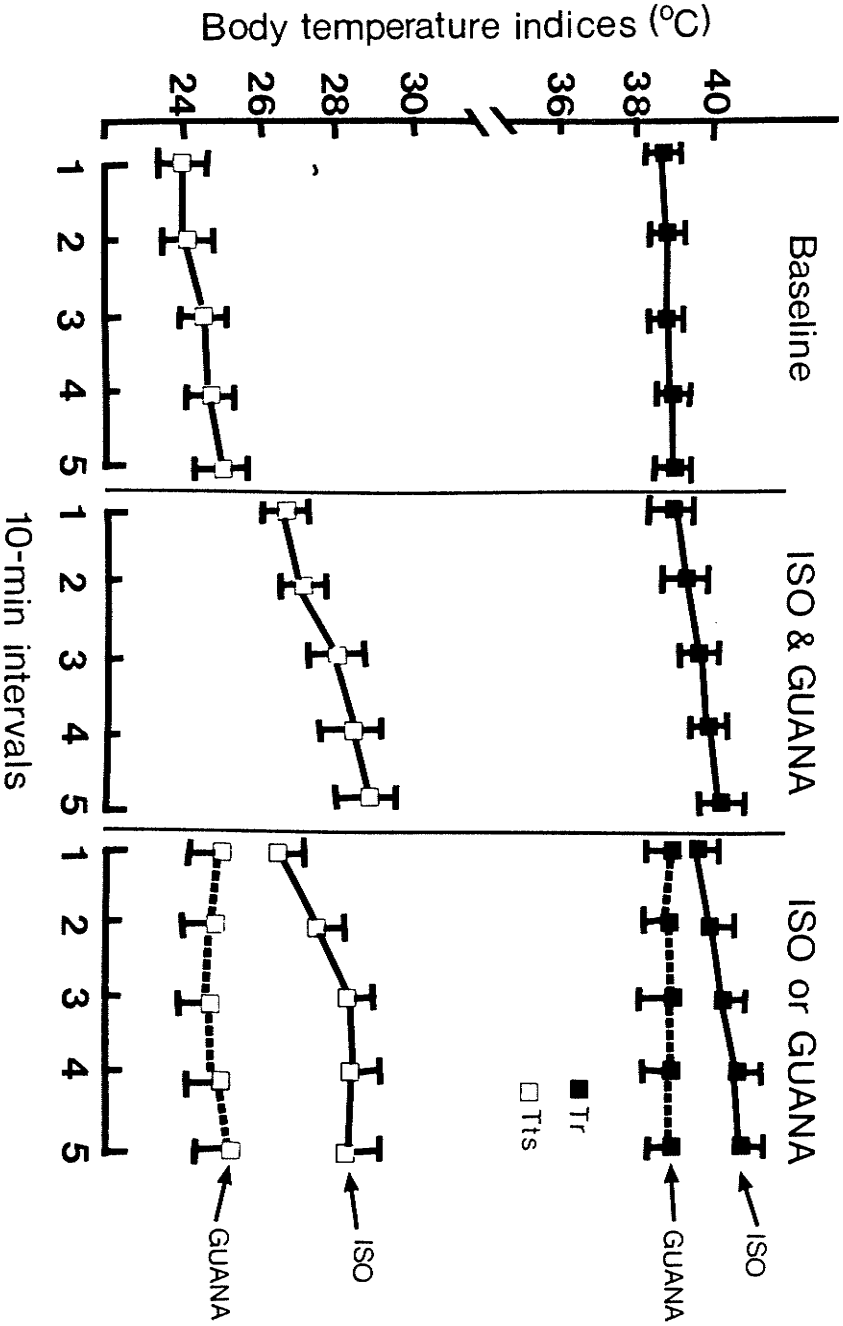

Body temperature indices (°C)
24
26
28
30
36
38
40
Baseline
ISO & GUANA
ISO or GUANA
1 2 3 4 5
10-min intervals
■ Tr
□ Tts
ISO
GUANA
ISO
GUANA

Figure 29. Mean (±SEM) baseline tail skin temperature (^{D}C) as a function of time for 1K-GB hypertensive and sham normotensive rats.

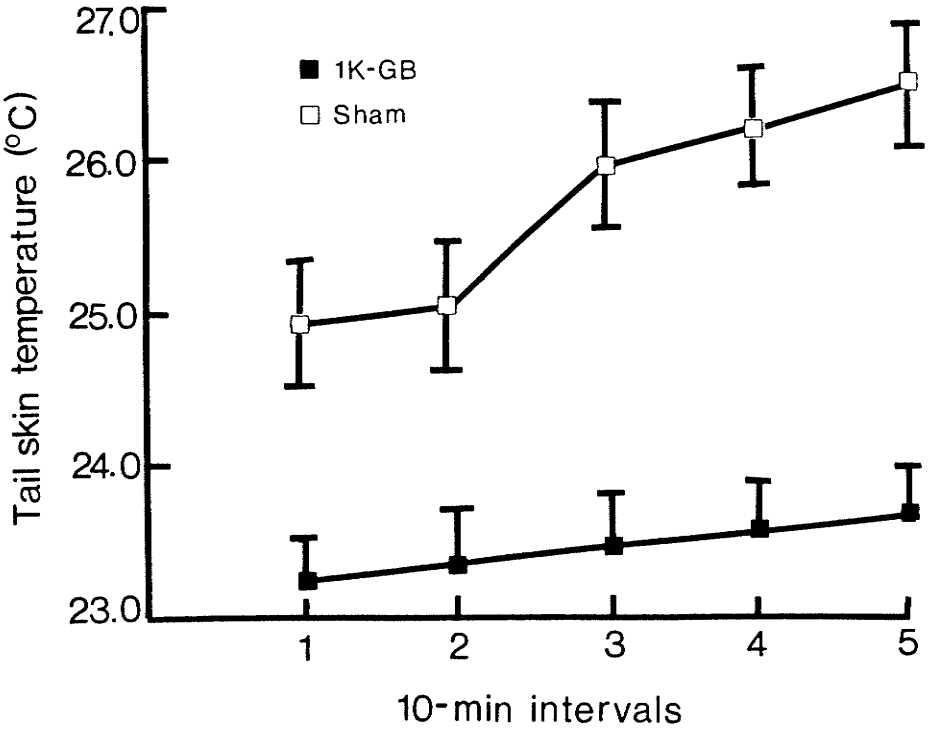

27.0
26.0
25.0
24.0
23.0
Tail skin temperature (°C)
1K-GB
Sham
1
2
3
4
5
10-min intervals

Figure 30. Mean (±SEM) rectal temperature (^{o}C) collapsed across isoproterenol and guanabenz administrations as a function of time for 1K-GB hypertensive and sham normotensive rats.

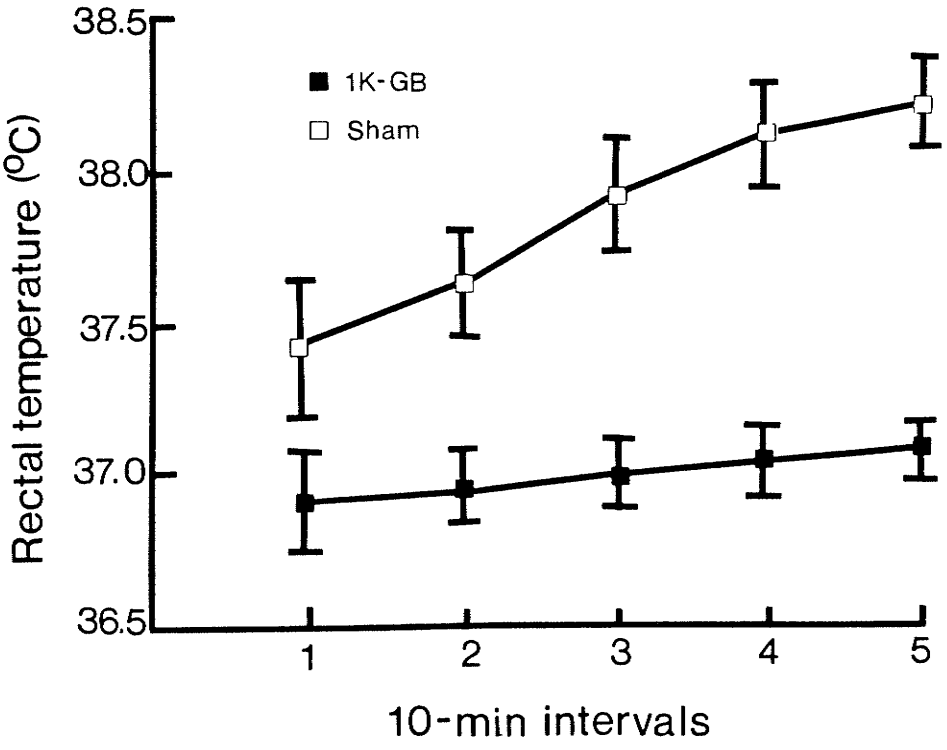

38.5
38.0
37.5
37.0
36.5
Rectal temperature (°C)
1K-GB
Sham
1
2
3
4
5
10-min intervals

Figure 31. Mean (±SEM) rectal temperature (^{o}C) collapsed across isoproterenol and combined isoproterenol and guanabenz administrations as a function of time for 1K-GB hypertensive and sham normotensive rats.

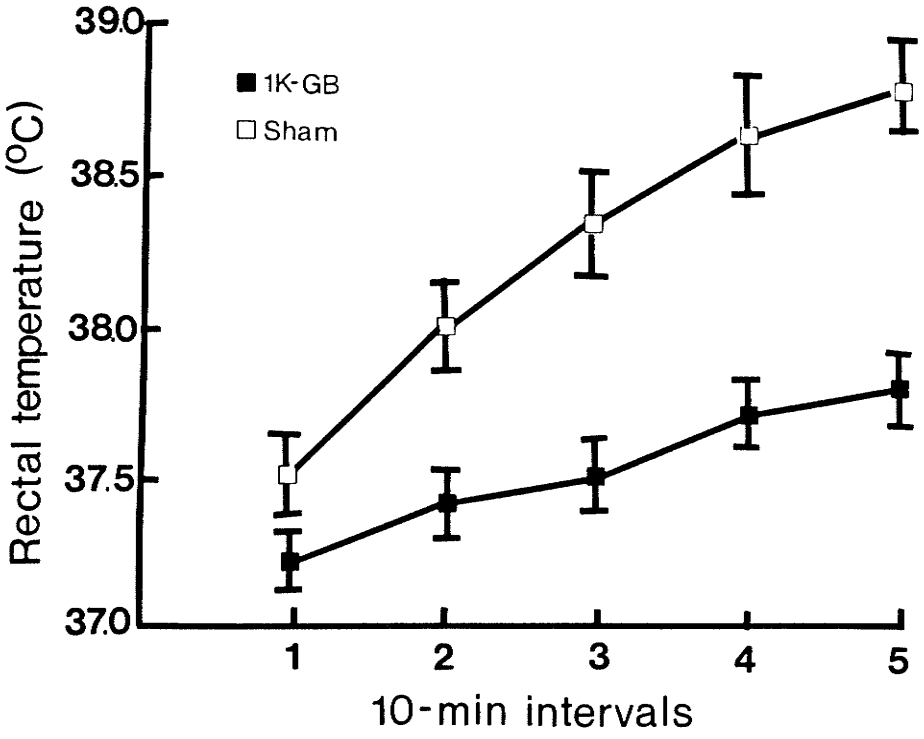

39.0
38.5
38.0
37.5
37.0
Rectal temperature (°C)
1K-GB
Sham
1
2
3
4
5
10-min intervals

Figure 32. Mean (±SEM) arterial blood pressure (mmHg) and heart rate (bpm) collapsed across the 1K-GB hypertensive and sham normotensive rats as a function of time for baseline, and for the administration of isoproterenol, guanabenz, and combined isoproterenol and guanabenz.

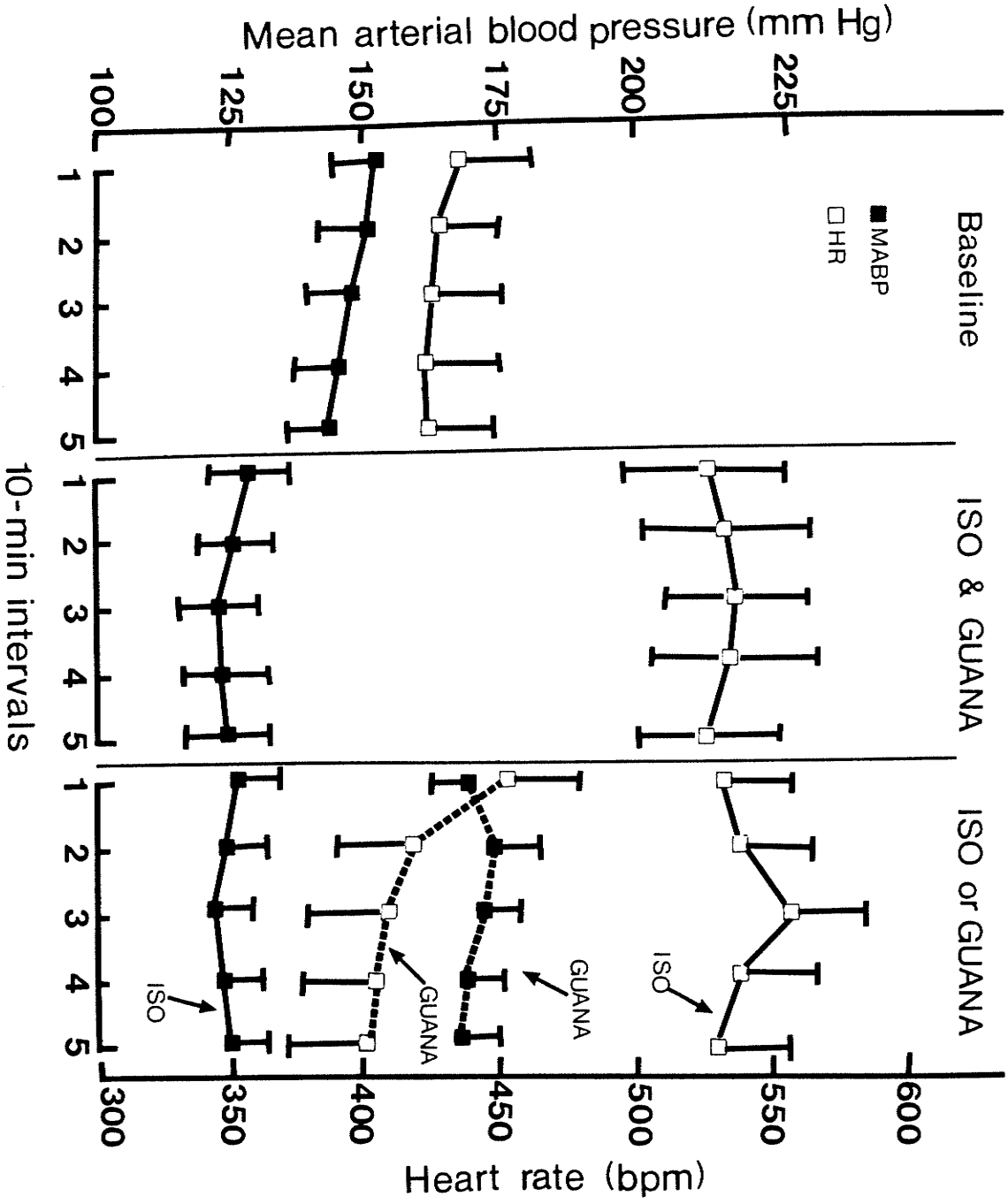

Mean arterial blood pressure (mm Hg)
100
125
150
175
200
225
MABP
HR
Baseline
ISO & GUANA
ISO or GUANA
10-min intervals
1
2
3
4
5
ISO
GUANA
GUANA
ISO
300
350
400
450
500
550
600
Heart rate (bpm)

Figure 33. Mean (±SEM) duration of heat escape responding (s/10 min) collapsed across the 1K-GB hypertensive, sham normotensive, cold-acclimated, and non cold-acclimated rats as a function of time for baseline, and for the administration of isoproterenol phenylephrine, and combined isoproterenol and phenylephrine.

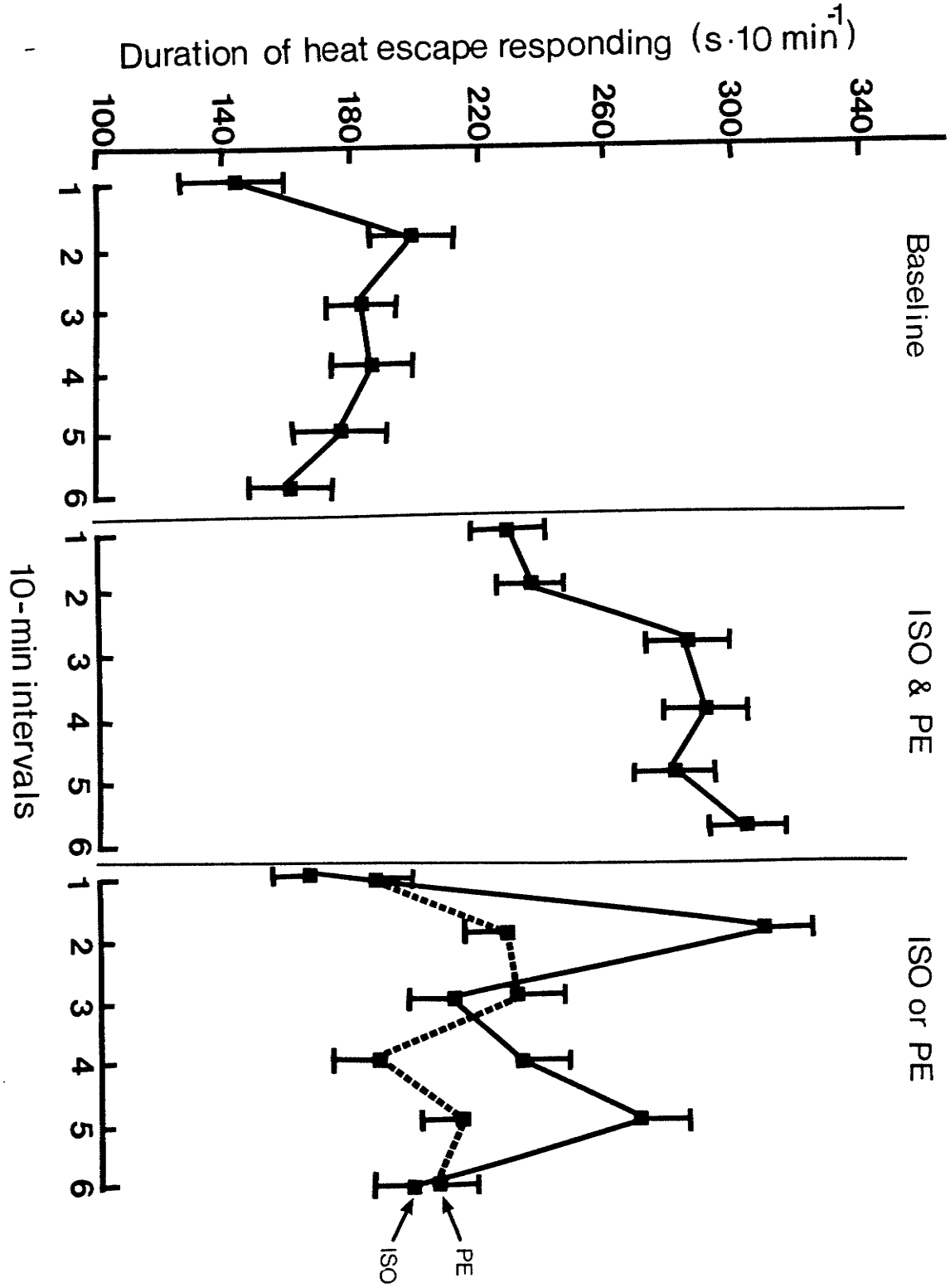

Duration of heat escape responding (s·10 min-1)
100
140
180
220
260
300
340
Baseline
ISO & PE
ISO or PE
10-min intervals
1
2
3
4
5
6
ISO
PE

Figure 34. Mean (±SEM) frequency of heat escape responding (# of lever presses/10 min) collapsed across the 1K-GB hypertensive, sham normotensive, cold-acclimated, and non cold-acclimated rats as a function of time for baseline, and for the administration of isoproterenol, phenylephrine, and combined isoproterenol and phenylephrine.

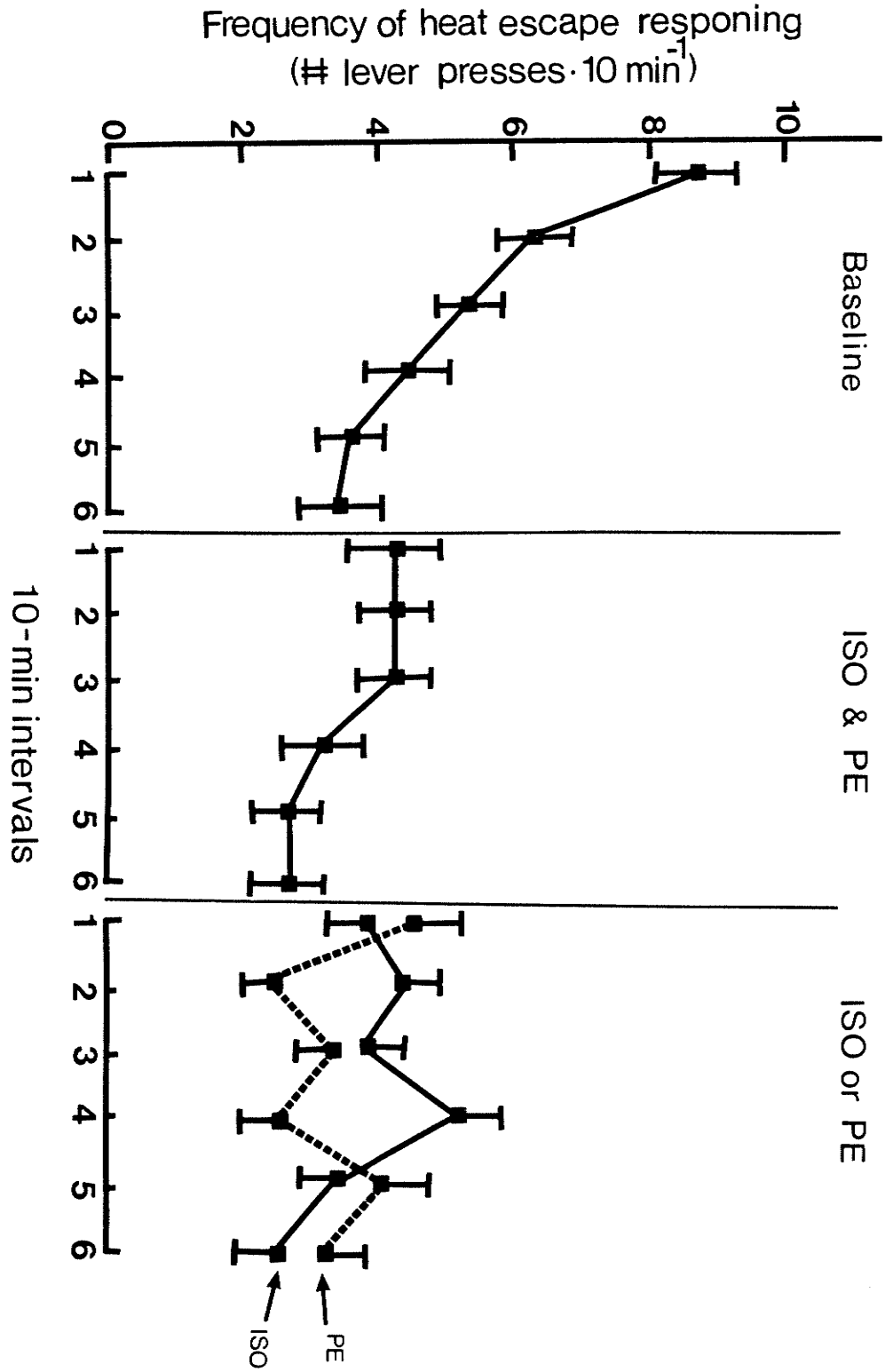

Frequency of heat escape responing
(# lever presses · 10 min⁻¹)
0
2
4
6
8
10
1
2
3
4
5
6
10-min intervals
Baseline
ISO & PE
ISO or PE
ISO
PE

Figure 35. Mean (±SEM) baseline rectal temperature (^{o}C) as a function of the blood pressure and acclimation status of the rats.

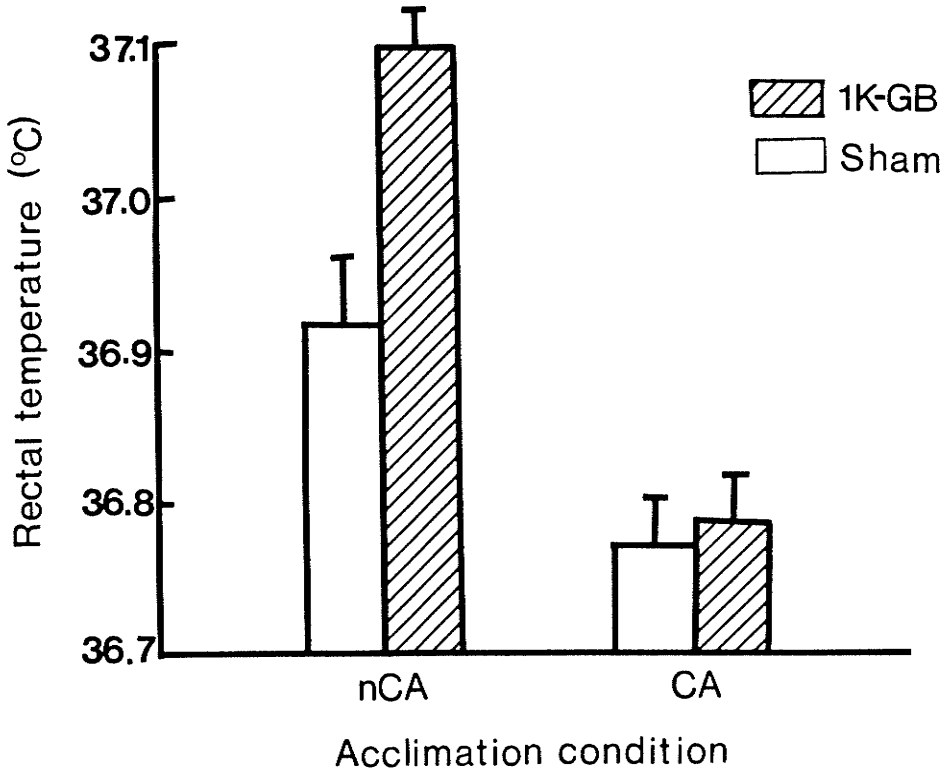

37.1
37.0
36.9
36.8
36.7
Rectal temperature (°C)
1K-GB
Sham
nCA
CA
Acclimation condition

Figure 36. Mean (±SEM) arterial blood pressure (mmHg) as a function of the blood pressure and acclimation status of the rats.

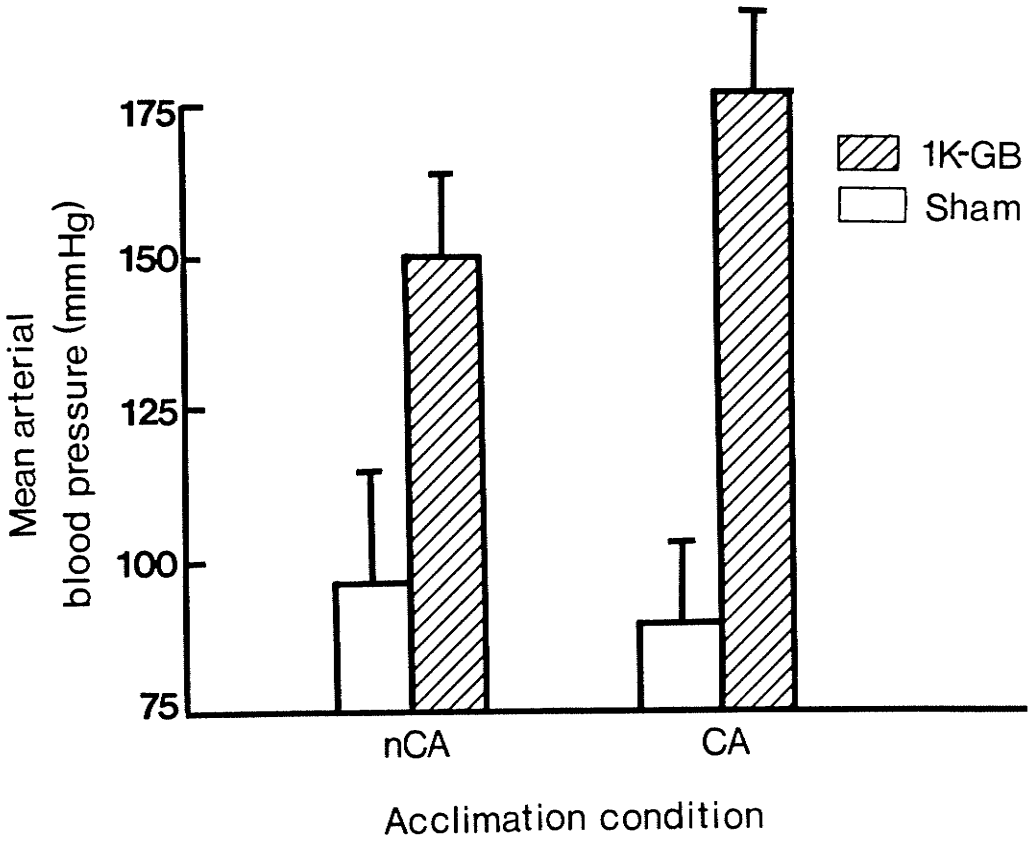
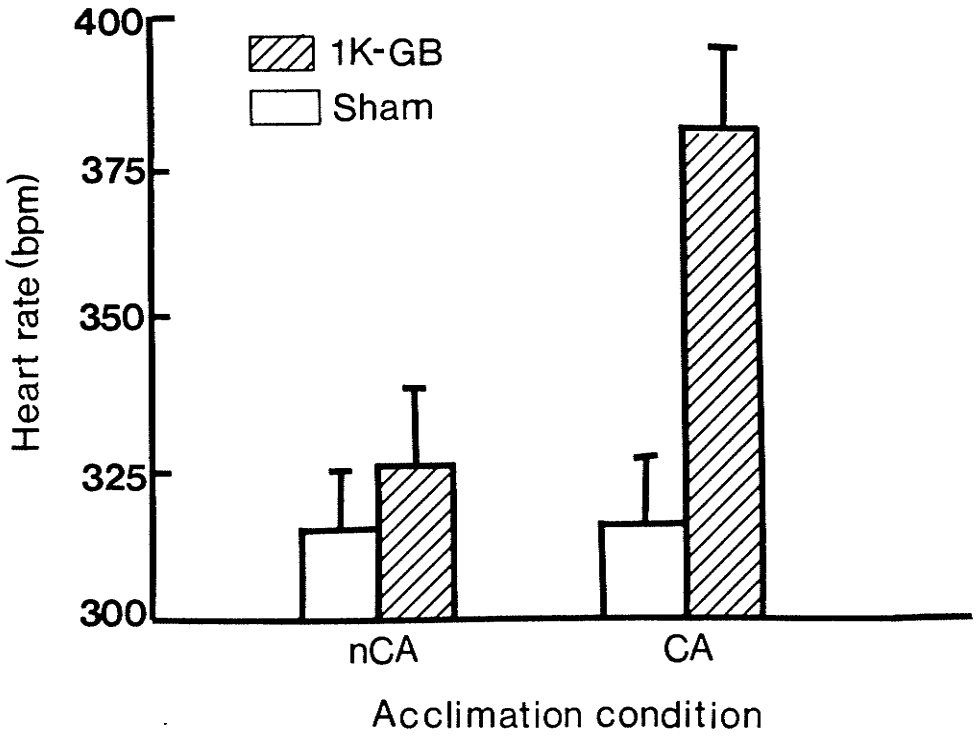

175
150
125
100
75
Mean arterial
blood pressure (mmHg)
1K-GB
Sham
nCA
CA
Acclimation condition

Figure 37. Mean (±SEM) heart rate (bpm) as a function of the blood pressure and acclimation status of the rats.


400
375
350
325
300
Heart rate (bpm)
1K-GB
Sham
nCA
CA
Acclimation condition

Appendix A

Calibration Procedure for the Expired Gas Analyzers

Two types of calibration procedures were routinely undertaken. Weekly calibration was conducted with calibrated Linde gases for both the O_2 and CO_2 analyzers to assess the range and linearity of the responses. The O_2 analyzer was calibrated at a low (15.7% O_2) and high (21.2% O_2) end with the Linde gases to establish the proper range of sensitivity and with room air (20.94% O_2) to approximate linearity. The CO_2 analyzer was calibrated at a low (0% CO_2 by room air passage through ascarite) and high (4.12% CO_2 with the Linde gas) end to set the range and with room air (0.03% CO_2) to approximate linearity. Daily calibration was conducted prior to, and following, the testing of each animal. For this purpose, room air (20.94% O_2 and 0.03% CO_2) was used. Accordingly, prior to placing the animal in the metabolic chamber, the gas analyzers were calibrated to 20.94 and 0.03 for $\%O_2$ and $\%CO_2$, respectively. The animal was then placed in the metabolic chamber to equilibrate for 40 min. At this point a barometric pressure value was obtained and used for calculation of the first baseline value and for all subsequent values obtained at 10-min intervals for that specific animal. When the 2.5 hr testing session was completed the animal was removed from the metabolic chamber and room air was again passed through the analyzers for a minimum of 30-min. At the end of this 30-min period the % O_2 and % CO_2

displayed on the analyzers were recorded and later incorporated in a correction factor to adjust for drift. Both gas analyzers were then recalibrated with room air and the next animal was started.

Any factor contributing to drift in the expired gas analyzers, including changes in barometric pressure, was accounted for at the end of the 2.5 hr test session for each animal. To this end, a correction factor for drift was incorporated into the calculation of expired gases for each animal if the % O_2 and % CO_2, following the 30-min post-test period with room air, differed from 20.94 and 0.03, respectively. For instance, if after 30-min with room air the % O_2 was recorded as 20.74 instead of 20.94 a correction factor was determined by taking the difference between the two values, in this case 0.2, and dividing it by ten, the number of 10-min intervals interpolated between the start of baseline and the final expired gas recording. In general, this correction factor varied from between 0.05% to 0.15% of the atmospheric O_2 concentration. The resultant value, in this instance 0.02, was then added to the first baseline recording for % O_2 concentration in expired air. To the second O_2 recording, 0.04 was added, to the third, 0.06, and so on until 0.2 was added to the final O_2 recording. These corrected values, for the % O_2 concentration in expired air were then incorporated into the formula used to determine oxygen consumption. An identical procedure was followed when correcting the % CO_2 concentration in the expired air for drift.

Appendix B

Formulae used in the determination of carbon dioxide production and oxygen consumption

1. Calculation of carbon dioxide production (VCO_2):

$$VCO_2 = \frac{V_{ATP}}{60} \times \frac{P_b}{760} \times \frac{273}{273 + T\ ^{o}C} \times \frac{(LB2 - 0.03)}{100}$$

body weight (g)

2. Calculation of oxygen consumption (VO_2):

$$VO_2 = \frac{V_{ATP}}{60} \times \frac{P_b}{760} \times \frac{273}{273 + T\ ^{o}C} \times \frac{(20.94 - OM11)}{100}$$

body weight (g)

3. Calculation of respiratory quotient (RQ):

$$RQ = \frac{VCO_2}{VO_2}$$

Where: V_{ATP} is the rate of air flow through the metabolic chamber in ml/h.

P_b is the barometric pressure in mm Hg.

LB2 is the percentage of carbon dioxide production as expressed by the Beckman medical gas analyzer (Model LB2).

OM11 is the percentage of oxygen consumption as expressed by the Beckman medical gas analyzer (Model OM11).

Appendix C

The Influence of 1K-GB Hypertension and Depiling on Thermophysiological and Myocardial Responses to Combined Isoproterenol and Phenylephrine Infusions in Cold Acclimated Rats

The distribution of cutenaous blood flow may serve an important thermoregulatory role, that of regulating tissue conductance or the amount of heat dissipated from the animal to its environment (Raman et al., 1983). When an animal is rendered hypertensive, an elevation in peripheral vascular resistance is often evident (Bralet et al., 1973). Such is the case with one-kidney Goldblatt (1K-GB) renovascular hypertension, where a 22% increase in cutaneous vascular resistance is apparent. A chronic rise in peripheral resistance may impair the thermolytic ability of an endotherm and hence may render the animal less tolerant to warm ambient temperatures, as reported by Fregly (1954).

However, in endotherms with pelage, the thermoregulatory significance of the vasculature may be questioned due to the insulating properties of fur (Richards, 1973). If pelage buffers the thermolytic ability of the vasculature then it may negate any differentiating effect of hypertension on thermoregulatory processes. One way to uncover the thermal effects of hypertension is to depile the animals prior to thermoregulatory testing. The present study assessed the consequences of 1K-GB hypertension and depiling on thermophysiological and myocardial responses to adrenergic stimulation in cold acclimated rats.

Method

Twenty four male, Sprague Dawley rats initially weighing 70-90 g were used. Two days after arrival, twelve of the animals were shved except for the facial and anogenital regions while under light pentobarbitone anesthesia (Sodium Pentobarbitone, 50 mg/Kg, i.p.), whereas the remaining animals had their pelage left intact. Shaving was repeated every 4 days for the duration of the experiment, whereas the unshaved animals were only lightly anesthetized every 4 days. All animals were maintained at room temperature until complete recovery from the anesthesia.

Five days after arrival, all animals began 21 days of cold acclimation. The shaved animals were cold acclimated at 13 ^{o}C (D. O. Foster, June, 1986, personal communication), whereas the unshaved animals were maintained at 6 ^{o}C. Both cold acclimation and the surgical procedures were performed in an identical manner to that described in the method section of this dissertation.

On the 10th day of cold acclimation, all animals had a right nephrectomy performed and on the 14th day of acclimation the animals were randomly assigned to either the 1K-GB hypertensive group or to the Sham normotensive condition. The 1K-GB animals underwent a left renal adtery stenosis, whereas the Sham animals had a left laparotomy performed. Accordingly, the experimental groups consisted of the 1K-GB-shaved ($\underline{n}$=6), the 1K-GB-unshaved ($\underline{n}$=6), the Sham-shaved ($\underline{n}$=6), and the Sham-unshaved ($\underline{n}$=6) animals. Starting of the 16th day of cold acclimation, all animals were restraint adapted as described previously and following the last day of cold acclimation, had their right jugular vein catheterized. Following a 48 hr recovery period

the animals underwent metabolic testing.

Metabolic testing occurred in an identical manner to that described for Experiment 1, except that the only drug administered was combined isoproterenol ((-1)-isoproterenol hydrochloride, 3 ug/Kg/min) and phenylephrine (l-phenylephrine hydrochloride, 10 ug/Kg/min). During testing, oxygen consumption, carbon dioxide production, rectal temperature, and heart rate were monitored every 10 min, both during the 40 min baseline and the 50 min infusion period. Following completion of testing the animals were sacrificed with an overdose of Sodium Pentobarbital (60 mg/Kg) via the jugular catheter.

Baseline data were analyzed using 2 x 2 x 4 (BP status x Pelage status x Time) mixed effects ANOVAs with repeated measures on the last factor. Infusion data were analyzed using 2 x 2 x 5 (BP status x Pelage status x Time) mixed effects ANOVAs with repeated measures on the last factor. Significance was set at $p<.05$.

Results

Baseline: Metabolic rate

Both oxygen consumption (VO_2), $F(1,20)=11.87$, $p<.0026$, and carbon dioxide production (VCO_2), $F(1,20)=27.14$, $p<.0001$, were higher for the 1K-GB animals relative to the Sham controls (2.3 vs. 1.9 $ml/O_2/g/hr$) and (2.0 vs. 1.6 $mlCO_2/g/hr$).

Baseline: Rectal temperature

A Pelage status x Time interaction, $F(3,60)=5.62$, $p<.0018$, revealed that the unshaved animals maintained a stable rectal temperature of approximately 37.4 ^{o}C, whereas the rectal temperature of the shaved animals declined from 37.3 ^{o}C to

37.0 °C. A BP status x Pelage status interaction, $F(1,20)=4.57$, $p<.045$, revealed that for the Sham animals, rectal temperature did not differ between the shaved and the unshaved groups, whereas for the 1K-GB animals the unshaved group had a higher rectal temperature relative to the shaved condition, $F(1,20)=26.13$, $p<.0001$, (37.9 ° vs. 37.0 °C).

Baseline: Heart rate

The 1K-GB animals had a higher heart rate relative to the Sham group, $F(1,20)=4.90$, $p<.039$, (436 vs. 392 bpm).

Combined isoproterenol and phenylephrine administration: Metabolic rate

Both VO_2, $F(3,60)=10.95$, $p<.0001$, and VCO_2, $F(3,60)=10.30$, $p<.0001$, increased over time (3.3 to 3.8 $mlO_2/g/hr$) and (2.7 to 3.2 $mlCO_2/g/hr$).

Combined isoproterenol and phenylephrine administration: Rectal temperature

Rectal temperature, $F(3,60)=73.22$, $p<.0001$, increased over time from 37.3 ° to 38.8 °C. A BP status x Pelage status interaction, $F(1,20)=4.69$, $p<.043$, revealed that for the Sham animals, rectal temperature did not differ between the shaved and the unshaved animals, whereas for the 1K-GB group, the unshaved group had a higher rectal temperature relative to the shaved condition, $F(1,20)=20.92$, $p<.0001$, (39.5 ° vs. 38.4 °C).

Combined isoproterenol and phenylephrine administration: Heart rate

Heart rate increased over time, $F(3,60)=14.57$, $p<.0001$ and this rise was dependent on the BP status of the animals, $F(3,60)=3.93$, $p<.013$. By the 30th min of infusion, $F(1,20)=5.89$, $p<.025$, the

1K-GB group exhibited an attenuated heart rate relative to the Sham animals.

Discussion

The purpose of this experiment was to assess whether shaving (a) had a deleterious effect on the cold acclimated endotherm's ability to thermoregulate and (b)would unmask a latent thermogenic or thermolytic difference between normotensive and 1K-GB hypertensive rats. Baseline metabolic rate and rectal temperatures were within the normal range of variability reported for cold acclimated animals (Flaim et al., 1977; Foster, 1985). Thus, neither cold acclimation, shaving, nor the surgical manipulations rendered the animals incabable of maintaining an adequate metabolic rate and rectal temperature.

Several general features of both the 1K-GB hypertensive and the shaved animals emerged during baseline. For instance, the 1K-GB animals had an elevated baseline metabolic and heart rate. This profile is consistent with their purported state of sympathoexcitation, as assessed by the elevation in cardiac NE turnover (Tanaka et al., 1982) and plasma NE levels (Katholi et al., 1982; Reid et al., 1977). The sympathetic postganglionic release of NE could mediate nonshivering thermogenesis (Depocas et al., 1978) and stimulate cardiac $beta_1$-receptors to increase heart rate (DeJong et al., 1975). During baseline the rectal temperature of the shaved animals dropped by .3 ^{o}C and it did so in the absence of a similar decline in metabolism. Metabolic rate and rectal temperature covary in endotherms (Hart, 1971) and a dissociation of the two under thermoneutral conditions is regarded as an index of a deterioration

in thermoregulatory ability (Poole & Stevensen, 1977). Since in the shaved animal, metabolism and rectal temperature did appear to be somewhat dissociated, their thermoregulatory ability may have been imparied. Moreover, baseline rectal temperature was affected by both the blood pressure and pelage status of the animals. For the unshaved animals, the 1K-GB group had a higher rectal temperature than did the normotensive controls, whereas for the shaved animals, rectal temperature did not differ between the two groups. The increased rectal temperature for the 1K-GB-unshaved animal may reflect its enhanced metabolic rate (Hart, 1971). Alternatively, the increased peripheral resistance characteristic of the 1K-GB rat (Bralet et al., 1973) may compromise tissue conductance (Raman et al., 1983) and promote a higher rectal temperature by impairing thermolysis. When the pelage is removed, the antithermolytic consequences of 1K-GB hypertension may be buffered, thus, rendering the 1K-GB animal thermally indistinguishable from its normotensive counterpart. Thus, in order to uncover the thermal effects of hypertension, shaving is counterindicated.

During the combined administration of isoproterenol and phenylephrine, metabolic rate, rectal temperature, and heart rate increased. The thermogenic response to combined isoproterenol and phenylephrine is consistant with its beta- and $alpha_1$-stimulating action on brown adipose tissue (Foster, 1985; Fregly, 1975). The increased rectal temperature supports findings that beta- and $alpha_1$-stimulation elevates core temperature which is secondary to the thermogenesis (Flaim et al., 1977). The tachycardic repsonse primarily reflects cardiac $beta_1$-receptor stimulation with

isoproterenol (Buckley et al., 1984). This tachycardic response to the drugs was attenuated in the 1K-GB animals, possibly inidicating a reduced beta-receptor responsivity. Moreover, although rectal temperature increased during the administration of isoproterenol and phenylephrine, the blood pressure by pelage interaction observed during baseline was still apparent. During the drug infusions, the unshaved hypertensive animals had a higher rectal temperature than did the normotensive controls, whereas for the shaved animals, rectal temperature did not differ betwee the two groups. Thus, not only did shaving render the 1K-GB animals thermally indistinguishable from the controls, it failed to alter thermogenic responsivity to adrenergic stimulation. With regard to the purpose of this experiment, shaving seemed to (a) dissociate metabolism from rectal temperature, an effect which may indicate impaired thermoregulation, and (b) mask a latent thermolytic difference between 1K-GB hypertensive and normotensive rats. Hence, shaving a cold acclimated endotherm is contraindicated if one wishes to enhance the thermal difference between the hypertensive and normotensive rat.