DECREASED BAROREFLEX GAIN DURING PREGNANCY: ROLE OF ANGIOTENSIN II, NITRIC OXIDE AND INSULIN SENSITIVITY

By

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CERTIFICATE OF APPROVAL

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Appendix

ABBREVIATIONS

ACTH	AdrenoCorticoTrophic Hormone
ANOVA	ANalysis Of VAriance
BP_{50}	Blood Pressure at the midpoint of a baroreflex curve
cDNA	copy DeoxyriboNucleic Acid
CVLM	Caudal VentroLateral Medulla
DLU	Digital Light Unit
ENaC	Endothelial Sodium Channel
eNOS	endothelial Nitric Oxide Synthase
GABA	γ-AminoButyric Acid
iNOS	inducible Nitric Oxide Synthase
iv	intravenous
kg	kilogram
L-NNA	Nω-Nitro-L-Arginine (nitric oxide synthase blocker)
mA	milliAmps
mg	milligrams
min	minute
ml	milliliter
mmHg	millimeter Mercury

mmHg	millimeter Mercury
mRNA	messenger RiboNucleic Acid
mU	milliUnit
ng	nanogram
NMDA	N-Methyl-D-Aspartate
nNOS	neuronal Nitric Oxide Synthase
NOS	Nitric Oxide Synthase
NTS	Nucleus of the Solitary Tract
pg	picogram
PVN	ParaVentricular Nucleus of the hypothalamus
RNA	RiboNucleic Acid
RVLM	Rostral VentroLateral Medulla
sc	subcutaneous
µl	micoliter

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ABSTRACT

Pregnancy is associated with impaired baroreflex gain but the mechanism is unclear. Nitric oxide and angiotensin II increase during pregnancy and can decrease baroreflex gain. Therefore, I first hypothesized that the decreased baroreflex gain of pregnancy is due to an action of nitric oxide that is dependent on angiotensin II. To test this hypothesis, baroreflex gain was measured in rabbits before and after blockade of nitric oxide production, with and without concomitant blockade of AT_1 angiotensin II receptors, in non-pregnant and near term pregnant rabbits. Neuronal nitric oxide synthase mRNA and protein levels were also measured in brain regions involved in cardiovascular regulation in non-pregnant and pregnant rabbits. Blockade of nitric oxide production, with or without AT_1 receptor blockade, had no effect on baroreflex gain in either pregnant or non-pregnant rabbits. In addition, nNOS levels were unaltered in the cardiovascular brain regions of pregnant compared to non-pregnant rabbits. Other than increases in nitric oxide and angiotensin II, pregnancy is also associated with reduced insulin sensitivity, which may be linked to reduced baroreflex gain in a number of conditions. Insulin can act in the brain to increase baroreflex gain, and insulin resistance appears to decrease the transport of insulin into the brain. Therefore, I hypothesized that insulin resistance drives the impairment in baroreflex gain though reductions in brain insulin concentrations. To test this hypothesis I determined if, 1) insulin sensitivity and baroreflex gain were correlated in non-pregnant

and near term pregnant rabbits, 2) the time-courses for the decreases in baroreflex gain and insulin sensitivity throughout pregnancy matched, 3) treatment of rabbits throughout pregnancy with the insulin sensitizing drug rosiglitazone, prevented the decrease in both insulin sensitivity and baroreflex gain, and 4) brain insulin concentrations were reduced in near term pregnant rabbits. Insulin sensitivity and baroreflex gain were well correlated in non-pregnant and near term pregnant rabbits (r^2 = 0.59). In addition, baroreflex gain and insulin sensitivity fell at the same time-point during gestation (week three of a four-week pregnancy). Treatment with rosiglitazone throughout pregnancy almost completely reverses the decrease in insulin sensitivity and, to the same extent, the reduction in baroreflex gain. The concentration of insulin in the cerebrospinal fluid was reduced in near term pregnant animals. In conclusion, the decrease in baroreflex gain during pregnancy does not appear to involve nitric oxide or angiotensin II, but may instead be due to the insulin resistance that develops and the reduced brain insulin concentrations.

CHAPTER 1

INTRODUCTION

PREGNANCY AND THE CARDIOVASCULAR SYSTEM

Pregnancy is associated with a number of cardiovascular alterations in multiple mammalian species (Hart et al., 1985; Heesch and Rogers, 1995; Brooks et al., 1995; Monga and Creasy, 1999; Thornburg et al., 2000). Since my work has used rabbits in attempts to understand mechanisms in humans, I will focus on these two species in this section.

One cardiovascular alteration that occurs during pregnancy is an increase in blood volume. This expansion is progressive in nature, reaching its peak in the human in the middle of the third trimester (Hytten and Paintin, 1963), and appears to match the needs of the fetus (Morton, 1992). Near term, rabbits also show an increase in blood volume (Brooks et al., 1998).

While blood volume seems to increase in parallel to the needs of the fetus, other changes occur before the fetus places any real demand on the mother. One such change is the decrease in systemic vascular resistance. In women this reduction occurs by five weeks of gestation, and resistance continues to fall until the twentieth week of

pregnancy. After the twentieth week, systemic vascular resistance remains relatively constant, or increases slightly until delivery (Robson et al., 1989). A similar trend occurs in rabbits, in which systemic vascular resistance is reduced by the second third of pregnancy and remains decreased near-term (Nuwayhid, 1979; Brooks et al., 2001a; Clow et al., 2003).

Heart rate and stroke volume rise during pregnancy and mirror the fall in systemic vascular resistance. In women, by the fifth week of pregnancy, heart rate is elevated and continues to rise until the thirty-second week when it remains stable until the end of gestation (Robson et al., 1989). Similarly, stroke volume initially increases by eight weeks of pregnancy, rising until the twentieth week, and remains elevated until term (Robson et al., 1989). Since cardiac output is a function of heart rate and stroke volume, this parameter must also increase during pregnancy. Indeed, cardiac output rises continuously from the non-pregnant state until twenty-four weeks of gestation, when it then remains elevated until delivery (Robson et al., 1989). Anesthetized rabbits exhibit a similar pattern of changes in cardiac output with a significant expansion by the second third of pregnancy; however, by the last third of pregnancy, cardiac output returns to non-pregnant values (Nuwayhid, 1979). This is not the case in conscious rabbits however, in which cardiac output is increased in near-term pregnant rabbits compared to non-pregnant values (Brooks et al., 2001a; Clow et al., 2003). Conscious rabbits also develop tachycardia by the third week of a four week pregnancy, which persists until term (Quesnell and Brooks, 1997).

The net effect of pregnancy on arterial blood pressure is a function of its effects on systemic vascular resistance and cardiac output. The reduction in systemic vascular

resistance tends to decrease blood pressure, and the elevation in cardiac output tends to elevate blood pressure. The net effect of these alterations in women is slight hypotension during the first twenty weeks of pregnancy, followed by a minor rise in blood pressure until arterial pressure is slightly elevated over non-pregnant values just before delivery (Robson et al., 1989). Rabbits, on the other hand, develop hypotension near term but do not exhibit this alteration at mid-gestation (Brooks et al., 1998).

Pregnancy also induces anatomical changes in the cardiovascular system. The heart remodels such that end diastolic volume increases (Katz et al., 1978) without a change in filling pressure (Hart et al., 1985). Ejection fraction remains constant (Katz et al., 1978); thus, the larger heart size underlies the pregnancy-induced stroke volume augmentation.

Pregnancy also alters autonomic reflexes. The cardiopulmonary reflex, which induces sympathoinhibition in response to atrial stretch, is attenuated during pregnancy (Hines and Mifflin, 1995). Finally, pregnancy is associated with a decrease in the sensitivity, or gain, of the arterial baroreceptor reflex. This decrease in gain has been seen in humans (Blake et al., 2000; Greenwood et al., 2001) and rabbits (Humphreys and Joels, 1974; Quesnell and Brooks, 1997; O'Hagan and Casey, 1998), as well as goats (Olsson et al., 1987), dogs (Brooks and Keil, 1994), rats (Masilamani and Heesch, 1997), and sheep (Lumbers and Yu, 1999). The cause for this decrease in baroreflex gain is unclear and is the focus of this dissertation. So now we turn to a description of the baroreceptor reflex.

BARORECEPTOR REFLEX

The baroreceptor reflex is crucial to the maintenance of perfusion pressure in the face of cardiovascular challenges. It acts as a negative feedback system in which increases in arterial pressure are counteracted by decreasing sympathetic activity, increasing parasympathetic activity, and decreasing the release of vasoconstricting hormones (i.e., vasopressin and renin). These changes result in reduced vascular resistance and cardiac output (heart rate), thereby decreasing arterial pressure toward control values. This reflex works to elevate sympathetic activity, inhibit parasympathetic activity, and stimulate vasoconstricting hormone release when arterial pressure is reduced. Baroreflex gain is a measure of the effectiveness of this reflex to prevent changes in pressure [for reviews see (Sunagawa et al., 2001; Chapleau and Abboud, 2001; Stauss, 2002)].

Figure 1 illustrates the circuitry of the baroreflex. Specialized neuronal receptors in the adventitia of the aortic arch, carotid sinus and root of the right subclavian artery sense changes in arterial pressure (Chapleau and Abboud, 2001). Recent evidence suggests that pressure transduction is mediated by the epithelial sodium (Na^+) channel (ENaC) subunits β and γ present on the afferent nerve endings. Responses of cultured baroreceptor neurons to mechanical stimulation is blocked upon administration of the ENaC blocker amiloride (Snitsarev et al., 2002). In addition, administration of another ENaC blocker, benzamil, into the isolated carotid sinus of rabbits blunts the decrease in systemic arterial pressure and blocks the increase in

Figure 1

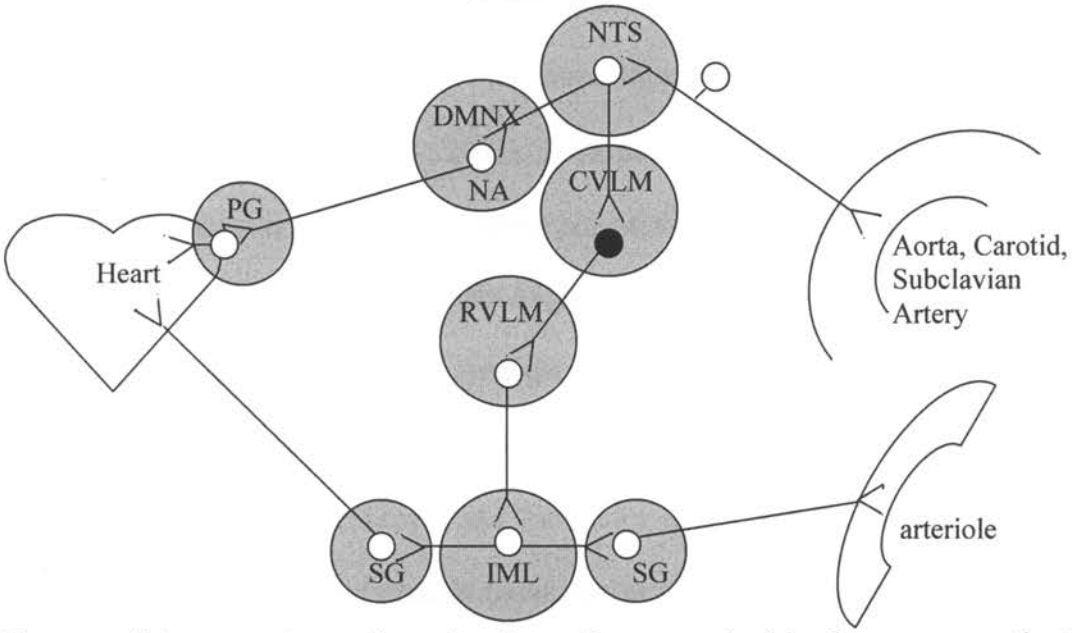


Diagram of baroreceptor reflex circuitry. Increases in blood pressure activate baroreceptors in the walls of the carotid sinus, aortic arch and root of the right subclavian artery. This leads to the stimulation of NTS neurons, which in turn stimulate parasympathetic preganglionic neurons in the DMNX and the nucleus ambiguus (NA), and inhibitory neurons in the CVLM. Activation of the parasympathetic preganglionic neurons leads to activation of the postganglionic neurons in the parasympathetic ganglia (PG) and decreases in heart rate. The CVLM responds to the stimulation from NTS neurons by inhibiting neurons in the RVLM, resulting in a decreased firing of the preganglionic sympathetic neurons in the intermediolateral cell column of the spinal cord (IML) and thus the postganglionic neurons in the sympathetic ganglia (SG). Heart rate is then further reduced and cardiac contractility and systemic vascular resistance fall. These effects combine to lower blood pressure toward control values. Black = inhibitory, White = stimulatory

carotid sinus nerve activity associated with rises in lumen pressure in the sinus (Drummond et al., 1998). ENaCs form a Na^+, and potentially calcium (Ca^{++}), channel that opens in response to stretch of the membrane, thus creating a Na^+ current which depolarizes the cell (Chapleau et al., 2001). If the depolarization is strong enough to reach the spike initiating zone, then an action potential will be fired (Chapleau et al., 2001) conveying the signal to the nucleus of the solitary tract (NTS) in the medulla where the baroreceptor afferents first synapse.

The NTS receives input from baroreceptors, chemoreceptors, and cardiopulmonary receptors (Palkovits and Zaborszky, 1977), as well as inputs from respiratory and gastrointestinal systems (Andresen et al., 2001). Baroreceptor afferents synapse in the dorsomedial caudal portion of this nucleus and use glutamate as their primary neurotransmitter. Glutamate acts predominately on non-N-methyl-D-aspartate (NMDA) receptors on the second-order neurons in the NTS, since blockade of these receptors decreases the response of neurons receiving a monosynaptic input from baroreceptor afferents to stimulation of baroreceptor afferents in the aortic nerve (Zhang and Mifflin, 1998). Blockade of NMDA receptors was ineffective (Zhang and Mifflin, 1998). The normal activity of barosensitive neurons in the NTS receiving polysynaptic baroreceptor afferent input was, however, dependent on both NMDA and non-NMDA receptors, since blockade of either receptor resulted in a decreased response to aortic nerve stimulation (Zhang and Mifflin, 1998). Thus, both NMDA and non-NMDA receptors are important for the overall transduction of baroreflex information in the NTS, since blockade of either receptor results in attenuation of the ability of increases in blood pressure to decrease heart rate (reduced baroreflex gain) (Ohta and Talman,

1994). In addition, the combined blockade of both types of receptors results in a greater attenuation of baroreflex-mediated bradycardia than blockade of either receptor alone (Ohta and Talman, 1994). These data, taken together, suggest that polysynaptic connections within the NTS are important for baroreflex integrity.

However, glutamate acting through ionotropic receptors cannot account for all of the baroreceptor neurotransmission in the NTS. While combined blockade of NMDA and non-NMDA receptors in the NTS results in a failure of the baroreflex to decrease heart rate, it interferes with baroreflex-evoked vasodilation by only about 50% (Machado, 2001). This suggests that NTS neurons involved in parasympathetic control, which is predominately responsible for decreasing heart rate from control levels in the rat (Stornetta et al., 1987), receive input from baroreceptors through glutamate acting at iontropic receptors, whereas NTS neurons involved in sympathetic control, which controls blood vessel constriction (Ferrari et al., 1996; Willis, 1998), must also receive excitatory input from another receptor.

One candidate for this receptor is the metabotropic glutamate receptor. As expected, since the baroreflex-mediated decrease in heart rate is predominately through the parasympathetic nervous system in the rat, blockade of metabotropic receptors in the NTS does not affect baroreflex-mediated bradycardia (Antunes and Machado, 2003). However, activation of metabotropic glutamate receptors in this nucleus at rest decreases renal (Matsumura et al., 1999) and lumbar (Foley et al., 1998) sympathetic nerve activity, suggesting that this receptor may play a role in the baroreflex-mediated sympathoinhibition.

Barosensitive neurons in the NTS then send projections to the nucleus ambiguus, dorsal motor nucleus of the vagus (DMNX) and the caudal ventrolateral medulla (CVLM) (Ross et al., 1985). The nucleus ambiguus and DMNX contain cardiac vagal preganglionic neurons (Cheng et al., 1999; Cheng and Powley, 2000) which are activatived by increases in blood pressure (Okada and Miura, 1997). However, of these regions, only the nucleus ambiguus is necessary for the baroreflex regulation of heart rate. Lesions of the DMNX do not affect the function of the baroreflex control of heart rate (Cheng et al., 2002) but lesioning the nucleus ambiguus almost completely prevents the bradycardia to increases in blood pressure (Cheng et al., 2004).

Vagal preganglionic neurons in the nucleus ambiguus are part of the parasympathetic nervous system and are responsible for lowering heart rate in response to elevations in arterial pressure. NTS neurons release glutamate to activate both NMDA and non-NMDA receptors in the nucleus ambiguus (Neff et al., 1998), thus stimulating the preganglionic neurons in this region. These neurons then project to the heart where they synapse on postganglionic parasympathetic neurons. As with all preganglionic neurons, the nucleus ambiguus neurons release acetylcholine in the ganglia which stimulates the postganglionic neuron via activation of nicotinic receptors. Stimulation of the vagal postganglionic neuron causes it to release acetylcholine on the pacemaker cells in the sinoatrial (SA) node of the heart. The acetylcholine then acts on muscurinic M_2 receptors on the cells of the SA node to slow heart rate (Willis, 1998).

As mentioned earlier, second-order baroreflex neurons in the NTS also send projections to the CVLM. Here neurons from the NTS release glutamate to activate

NMDA glutamate receptors on γ-aminobutyric acidergic (GABAergic) CVLM neurons (Gordon, 1987; Agarwal et al., 1990). These CVLM neurons then project to the sympathetic premotor cells of the rostral ventrolateral medulla (RVLM) (Dampney, 1994). Stimulation of the CVLM by the NTS causes the CVLM nerve terminals in the RVLM to release GABA, thus decreasing the firing of RVLM neurons.

The sympathetic premotor neurons of the RVLM project to the intermediolateral cell column of the spinal cord where they synapse on sympathetic preganglionic neurons (Ross et al., 1984; Dampney et al., 1987). Here, RVLM neurons release glutamate when stimulated, which may (Sundaram and Sapru, 1991; Bazil and Gordon, 1993) or may not (Morrison et al., 1989) act on NMDA receptors on the preganglionic cell to cause its activation. The preganglionic cell then projects to the pre- or para-vertebral sympathetic ganglia where it synapses with postganglionic cells. As with the parasympathetic nervous system, the sympathetic preganglionic neuron releases acetylcholine to activate ganglionic nicotinic receptors, and thus stimulate the postganglionic cell. The postganglionic cells project to the pacemaker and myocardial cells in the heart and to the smooth muscle of the vasculature. In the heart sympathetic postganglionic neurons release norepinephrine, which acts on β_1-adrenergic receptors on sino-atrial nodal cells to increase heart rate and on the myocytes to enhance contractility. In the vasculature, postganglionic cells also release norepinephrine which acts on α_1-adrenergic receptors to cause vasoconstriction [for a review of the events from stimulation of the preganglionic cell downwards see (Willis, 1998)].

Other nuclei not in the direct baroreflex pathway are involved in baroreflex function. One such nucleus is the paraventricular nucleus of the hypothalamus (PVN)

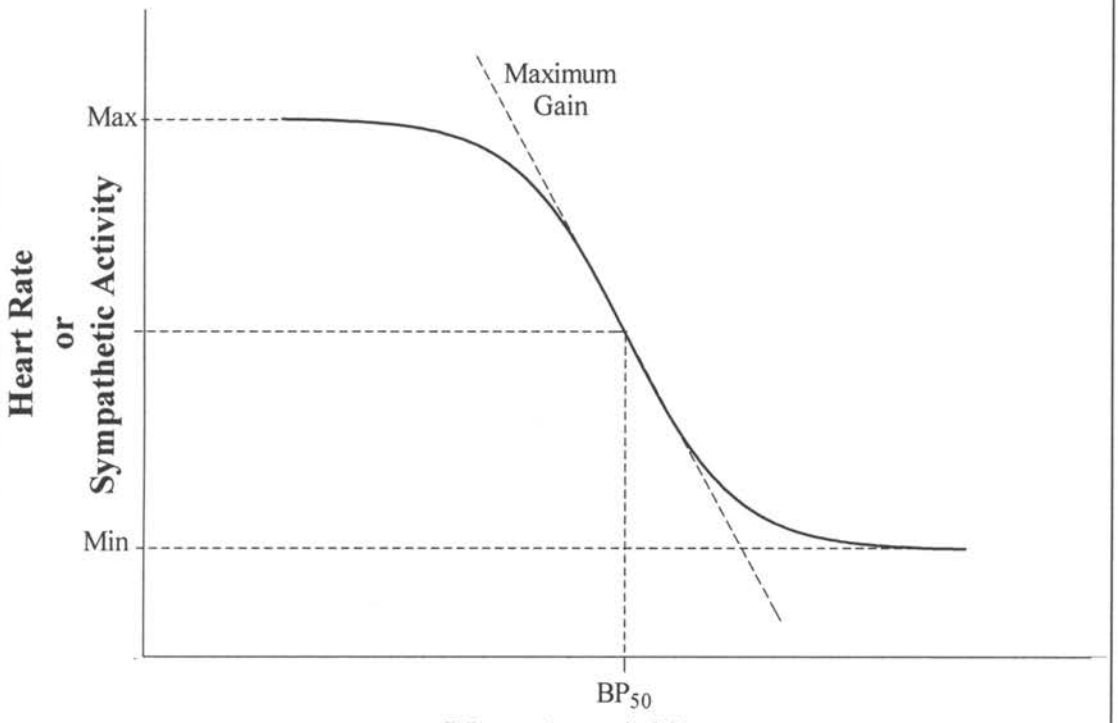
which receives input from the NTS (Sawchenko and Swanson, 1982) and RVLM (Cunningham, Jr. et al., 1990), and can be inhibited by baroreceptor activation (Lovick and Coote, 1988). The PVN sends projections to the intermediolateral cell column of the spinal cord, RVLM, NTS, DMNX and nucleus ambiguous, where it could exert effects on the baroreflex (Dampney, 1994). Indeed, blockade of the PVN increases the gain of the baroreflex control of lumbar sympathetic nerve activity (Patel and Schmid, 1988). In addition, electrical stimulation of the PVN results in inhibition of the gain of the baroreflex control of heart rate (Chen et al., 1996a). Therefore, the PVN appears to play a role in modulating baroreflex gain.

Another nucleus involved in baroreflex control is the area postrema. This nucleus is a circumventricular organ (a brain region lacking a blood-brain-barrier) that allows it to sense and respond to changes in circulating hormones (Ganong, 2000). It receives major input from the PVN and also a minor projection from the NTS (Shapiro and Miselis, 1985). Through its efferent connections to the NTS, DMNX, and RVLM (Shapiro and Miselis, 1985), the area postrema can influence baroreflex control.

The baroreflex can be studied by examining the changes in heart rate or sympathetic nerve activity (postganglionic sympathetic nerves) in response to alterations in arterial pressure. Baroreflex relationships are quantified by plotting heart rate or nerve activity against changes in mean arterial pressure and fitting this relationship to a sigmoidal curve. As illustrated in Figure 2, a number of parameters can be measured from this sigmoidal fit, such as the baroreflex-induced maximum and minimum heart rates or sympathetic activity, heart rate or sympathetic nerve activity range, the mean arterial pressure at the point midway between the maximum and

minimum heart rate or nerve activity (BP_{50}), and the maximum change in heart rate or sympathetic activity for a unit change in mean arterial pressure (gain).

Figure 2



Hypothetical baroreflex curve illustrating maximum heart rate or sympathetic activity (Max), minimum heart rate (Min), BP_{50}, and maximum gain.

PREGNANCY AND THE BAROREFLEX

Pregnancy is associated with impaired baroreflex gain (Humphreys and Joels, 1974; Quesnell and Brooks, 1997; O'Hagan and Casey, 1998; Blake et al., 2000; Greenwood et al., 2001). In addition, in rabbits, pregnancy increases the minimum

heart rate, causing a decrease in heart rate range (Brooks et al., 1997; Quesnell and Brooks, 1997; Brooks et al., 2001b; Brooks et al., 2002); renal sympathetic nerve activity range is also reduced (Masilamani and Heesch, 1997). The BP_{50} decreases, resulting in a shift of the baroreflex curve to the left, so that at any given mean arterial pressure, heart rate is lower during pregnancy (Brooks et al., 1997; Quesnell and Brooks, 1997; Brooks et al., 2001b; Brooks et al., 2002).

The impaired baroreflex gain is of special importance during pregnancy. Due to the decrease in gain, and thus impaired ability to maintain blood pressure in the face of challenges, pregnant women are more prone to orthostatic hypotension (Easterling et al., 1988), and animals, and potentially humans, are less tolerant of hemorrhage (Brooks et al., 1998). By understanding the cause of the decreased baroreflex gain, potential new treatments could be developed. This is especially important since 17% of human maternal mortality in the United States between 1990 and 1999 was related to hemorrhage (Chang et al., 2003).

Although the cause of the decreased baroreflex gain of pregnancy is not entirely known, a number of clues have been gathered. Brooks et al. (Brooks et al., 1997) found that the reduced ability to increase heart rate in response to falls in pressure (reduced baroreflex gain) was mediated by impaired sympathetic control, since blockade of β-adrenergic, but not muscurinic cholinergic, receptors abolished the difference in gain between non-pregnant and pregnant rabbits. In contrast, decreases in parasympathetic control appear to be responsible for the increase in minimum heart rate during pregnancy.

The location of the alteration has also, to a small extent, been studied. Alterations at the level of the heart do not appear to be responsible for the decreased gain of the baroreflex control of heart rate since the hearts of pregnant rabbits respond equally well to isoproterenol (a β-adrenergic agonist) as the hearts of non-pregnant rabbits (Brooks et al., 1997).

The baroreceptors themselves provide an additional potential site of impairment. Hines (Hines and Mifflin, 1995) found that the aortic depressor nerve, which contains baroreceptor afferents, adapts to changes in arterial pressure (resets) more rapidly in anesthetized pregnant rats compared to non-pregnant rats. For example, if blood pressure is increased, then activity of the aortic depressor nerve also increases. With time, however, as the nerve adapts to the new pressure, its activity is reduced (Andresen and Yang, 1989; Chapleau et al., 1989). Hines found that while nerves from virgin rats did not typically adapt within ten minutes, nerves from pregnant rats did. If the relationship between mean arterial pressure and aortic depressor nerve activity was examined one minute after blood pressure was altered by infusing different doses of either phenylephrine (an α-adrenergic agonist) to increase pressure, or sodium nitroprusside (a nitric oxide donor) to decrease pressure, then no differences between non-pregnant and pregnant rats existed. However, if the relationship was examined ten minutes after the start of phenylephrine or sodium nitroprusside infusion, then the slope or gain was reduced in the pregnant animals. Hines concluded that the rapid resetting of the aortic depressor nerve, resulting in a decrease in the gain of the aortic depressor nerve to changes in arterial pressure, could underlie the decrease in baroreflex gain. Thus, the aortic depressor nerve may be the site of impairment. However, Brooks et al.

(Brooks et al., 2002) found reductions in baroreflex gain in conscious rabbits during pregnancy even when changes in heart rate were measured within the first three minutes of alterations in blood pressure. Presumably, this would not allow sufficient time for the aortic depressor nerve to adapt. The measurement of a reduced gain of the baroreflex control of heart rate at a time when the aortic depressor nerve responds similarly in pregnant and non-pregnant animals (Laiprasert et al., 2001) suggest that the impairment in baroreflex control of heart rate is not at the level of the aortic depressor nerve. In addition, baroreflex resetting was similar in pregnant and non-pregnant conscious rabbits (Brooks et al., 2002).

Thus, enhanced baroreceptor resetting does not appear to be a major contibutor to pregnancy-induced reflex impairment. Based on these studies it appears that the alteration in baroreflex gain occurs within the central nervous system. Curtis et al. found pregnancy reduced the activation (Fos expression) of RVLM neurons following decreases in blood pressure (Curtis et al., 1999), suggesting that the impairment may include this region. What has remained elusive, however, is what factor(s) during pregnancy cause(s) the decrease in gain. Multiple hormone systems change during pregnancy that may contribute. My work has focused on three of these systems: angiotensin II, nitric oxide, and insulin resistance. The evidence supporting the role these substances play in baroreflex gain during pregnancy will be clarified in the following sections. In addition, the role of allopregnanolone in the decreased baroreflex gain of pregnancy will be discussed, as this neurosteroid has been extensivly investigated in this context.

Allopregnanolone

Allopregnanolone, also called 3α-hydroxydihydroprogesterone, 3α-hydroxy-5α-pregnan-20-one, and tetrahydroprogesterone, is synthesized from progesterone, which is elevated during pregnancy (Concas et al., 1998; Mellon et al., 2001). Progesterone is converted to 5α-dihydroprogeserone by the enzymes 5α-reductase I and II. From there it is converted to allopregnanolone by 3α hydroxysteroid dehydrogenase III (Mellon et al., 2001). These enzymes are present in the brain (Mellon et al., 2001) where allopregnanolone acts as a potent positive modulator of $GABA_A$ receptors (Paul and Purdy, 1992).

Masilamani and Heesch (Masilamani and Heesch, 1997) found that exogenous systemic administration of this progesterone metabolite to virgin rats decreases the gain of the baroreflex control of renal sympathetic nerve activity (RSNA), similar to the effect of pregnancy. However, we cannot be sure that allopregnanolone is fully responsible for the decrease in baroreflex gain. No specific antagonist for the allopregnanolone binding site on GABA receptors has been identified (Laiprasert et al., 1998). Therefore, it is unknown whether the endogenous metabolite exerts the same effect in the pregnant animal to decrease baroreflex gain. In addition, baroreflex gain reaches its lowest level, in rabbits (Quesnell and Brooks, 1997), and probably also rats (Heesch, personal communication), just before delivery. However, levels of allopregnanolone increase in the cerebral cortex and plasma until nineteen days of gestation, (Masilamani and Heesch, 1997) but by twenty-one days, levels decrease to non-pregnant values (Concas et al., 1998). In rats, baroreflex gain is decreased at

twenty-one days of gestation. This suggests that allopregnanolone is not solely responsible for the decrease in baroreflex gain near term, implying that other factors are involved.

Angiotensin II

Angiotensin II is a circulating hormone produced by a multi-step process in which the substrate, angiotensinogen (produced in the liver), is cleaved at its amino end by the enzyme, renin (produced in the kidney), to create a ten amino acid peptide, angiotensin I. This peptide is inactive and must be cleaved by a second enzyme, angiotensin converting enzyme, into its active form, angiotensin II. This is accomplished by cleaving off two amino acids from the carboxy side to yield an eight amino acid peptide. Angiotensin II can bind to multiple receptor subtypes, including the AT_1 and the AT_2 receptor. Most of angiotensin II's known actions (which, in general, tend to increase blood pressure) are mediated through the AT_1 receptor [for reviews see (Nicholls et al., 2001; Carey and Siragy, 2003)].

In addition to the circulating renin-angiotensin system, local production of angiotensin II is present in a number of tissues, including the brain. Angiotensinogen, renin, angiotensin converting enzyme, angiotensin I and angiotensin II have all been found in the brain (Ferguson and Washburn, 1998), but exactly how angiotensin II is produced in the brain is unknown. The problem is that, while neurons contain angiotensin II, no one cell in the brain contains all of the components of the renin-angiotensin system necessary to make it (Ferguson and Washburn, 1998; Saavedra, 2005). This has led to the proposal that angiotensin II is formed in the brain via multi-

cell interactions (Ferguson and Washburn, 1998) or that different enzymes are used in the brain to produce angiotensin II (Saavedra, 2005).

The presence of systemic and central angiotensin II production allows for this peptide to act at numerous brain sites to alter cardiovascular control. The area postrema, PVN, NTS, and intermediolateral cell column of the spinal cord all contain angiotensinergic neurons and fibers (Lind et al., 1985). In addition, AT_1 receptors have been found in the circumventricular organs (including the area postrema, the subfornical organ, and the organum vasculosum of the lamina terminalis), PVN, NTS, DMNX, CVLM and RVLM (Mendelsohn et al., 1988; Allen et al., 1988a; Allen et al., 1988b; Song et al., 1992). Let us now look at what is known about angiotensin II's actions on autonomic control at these various levels.

Systemic. Systemically, angiotensin II acutely and chronically increases blood pressure and the activity of some sympathetic nerves (Brooks and Osborn, 1995; Li et al., 1996; Fink, 1997). The effect of chronic angiotensin II appears to be through activation of circumventricular organs. The area postrema may be involved in the renal sympathoexcitation as lesions of this brain nucleus prevent the response (Cox and Bishop, 1991). Acutely and chronically, angiotensin II infusion also enhances resetting of the baroreflex (Matsukawa and Reid, 1990; Brooks et al., 1993; Sanderford and Bishop, 2002), and reduces the baroreflex maximum for renal sympathetic nerve activity (Sanderford and Bishop, 2002), again dependent on the area postrema (Matsukawa and Reid, 1990; Sanderford and Bishop, 2002).

In normal animals, systemic angiotensin does not affect baroreflex gain (Matsukawa and Reid, 1990; Murakami et al., 1996; Murakami et al., 1997; Liu et al.,

1999; Sanderford and Bishop, 2002). However, systemic blockade of angiotensin II AT_1 receptors increases gain in rabbits with chronic heart failure (DiBona et al., 1995; Murakami et al., 1996; Murakami et al., 1997; Liu et al., 1999). This, too, is dependent on the area postrema, as lesioning of this area prevents the effect (Liu et al., 1999).

Central. Angiotensin II can also elicit cardiovascular effects when administered into the brain. Angiotensin II given into the lateral or third ventricle of the brain increases blood pressure in cats (Severs et al., 1966) and rats (Hoffman and Phillips, 1976). The rabbit, however, does not respond with increases in blood pressure to angiotensin II in the lateral or third ventricle (Head and Williams, 1992), but does to administration in the fourth ventricle (Head et al., 1988; Elghozi and Head, 1990). Chronic angiotensin II administration into the fourth ventricle decreases baroreflex gain (Gaudet et al., 2000); however, acute central administration has no effect on gain (Dorward and Rudd, 1991). The action of angiotensin II in the third ventricle may be through the PVN, whereas, effects of angiotensin II in the fourth ventricle may be through the area postrema, RVLM, CVLM or NTS.

PVN. In the PVN, angiotensin II acts through AT_1 receptors to increase blood pressure (Bains et al., 1992), partly by stimulating vasopressin release (Shoji et al., 1989). Besides projections to other brain regions involved in cardiovascular regulation, the PVN contains magnocellular neurons that project to and release vasopressin from the posterior pituitary (Gardiner and Bennett, 1986). Since vasopressin induces vasoconstriction (Gardiner and Bennett, 1986), this may be one mechanism by which angiotensin II acts in the PVN to increase pressure. Angiotensin II in this area also

enhances sympathetic nerve activity (Li et al., 2005b), providing another way in which to elevate blood pressure.

RVLM. In RVLM neurons, application of angiotensin II inhibits potassium currents, leading to neuronal depolarization (Li and Guyenet, 1996) and excitation (Chan et al., 1991; Li and Guyenet, 1995), followed by increases in arterial blood pressure, heart rate, and renal sympathetic nerve activity (Allen et al., 1988b; Sasaki and Dampney, 1990; Hirooka et al., 1997). AT_1 receptors mediate these actions since blockade of these receptors prevents the effects (Li and Guyenet, 1995; Li and Guyenet, 1996; Hirooka et al., 1997). In normal animals, blockade of AT_1 or AT_2 receptors in the RVLM has little effect on basal blood pressure, heart rate, renal sympathetic nerve activity, or baroreflex gain (Saigusa and Head, 1993; Hirooka et al., 1997), suggesting that angiotensin II is not acting in this region at rest. However, RVLM activity increases in sodium depletion (Dibona and Jones, 2002) and hypertension (Ito et al., 2002) because of increased stimulation of AT_1 receptors in this region. The source of this peptide may be the PVN. Indeed, the hypotensive effect of PVN inhibition can be prevented by prior blockade of AT_1 receptors in the RVLM of hypertensive rats (Ito et al., 2002), and the hypertensive and sympathoexcitatory effects of PVN stimulation can be partially blocked by inhibition of AT_1 receptors in the RVLM (Tagawa and Dampney, 1999).

CVLM. Angiotensin II can also affect CVLM neurons. Application of angiotensin II into the CVLM decreases arterial pressure and renal sympathetic nerve activity (Sasaki and Dampney, 1990). This is consistent with activation of GABAergic

RVLM-projecting neurons by angiotensin II, presumably by inhibiting potassium currents leading to neuronal depolarization (Li and Guyenet, 1996).

NTS. In the NTS, angiotensin II can either increase or decrease pressure, depending on the dose used (Rettig et al., 1986). At the lower doses, angiotensin II reduces pressure (Diz et al., 1984), again consistent with activation of this region (which would stimulate CVLM and inhibit RVLM). At higher doses, angiotensin II elevates pressure (Casto and Phillips, 1984), and the mechanism behind this may involve nitric oxide stimulation of GABA release (Paton et al., 2001a), as does its ability to inhibit baroreflex gain in this area (Casto and Phillips, 1986; Paton et al., 2001a). At rest, angiotensin II in the NTS does not exert much of an effect on the tonic level of blood pressure and heart rate, since blockade of AT_1 or AT_2 receptors in this region does not affect blood pressure or heart rate in the basal condition (Matsumura et al., 1998b). However, endogenous angiotensin II in this region is involved in baroreflex control, where it decreases baroreflex gain (Matsumura et al., 1998b).

Angiotensin II and pregnancy. Pregnancy is associated with increases in renin and angiotensin II levels (Skinner et al., 1972; Brooks and Keil, 1994; Brooks et al., 1998), and systemic blockade of angiotensin converting enzyme has been shown to increase baroreflex gain in pregnant sheep (Lee and Lumbers, 1981). This finding is variable, however. Administration of the angiotensin converting enzyme inhibitor augmented gain in only three out of four sheep. In addition, central or systemic blockade of AT_1 receptors in pregnant rabbits (O'Hagan et al., 2001; Brooks et al., 2001b) does not improve baroreflex gain. While these conflicting studies may reflect

species differences, it is clear that, in rabbits, angiotensin II alone working through AT_1 receptors is not responsible for the impaired baroreflex gain of pregnancy.

Nitric oxide

Nitric oxide is a gaseous signaling molecule and neurotransmitter [for reviews see (Moncada et al., 1991; Bredt and Snyder, 1994; Andrew and Mayer, 1999)]. It is produced by a group of enzymes called nitric oxide synthases. There are three isoforms of these enzymes, classified by where they were first localized. Endothelial (eNOS) and neuronal (nNOS) NOS are constitutively expressed, whereas inducible NOS (iNOS) is only expressed after induction by cytokines or other factors. The NOS enzymes contain a calmodulin-binding domain that allows for regulation by calcium. Endothelial and neuronal NOS enzymes are activated by increases in intracellular calcium concentrations but iNOS activity is independent of calcium. All of these enzymes produce nitric oxide by catalyzing a reaction in which the amino acid L-arginine is first converted to N^G-hydroxy-L-arginine and then to L-citrulline and nitric oxide.

Nitric oxide was first recognized as a vasodilatator. It fact, before it was identified as nitric oxide it was known as endothelium-derived relaxation factor. Increases in intracellular calcium in endothelial cells, from activation of muscarinic receptors by acetylcholine for example, lead to enhanced production of nitric oxide by eNOS. Nitric oxide diffuses to the smooth muscle cell where it activates soluble guanylate cyclase causing the formation of cyclic guanosine-3',5'-monophosphate (cGMP). Cyclic GMP activates a cGMP-dependent protein kinase, causing protein

phosphorylation, a decrease in intracellular calcium concentration, and vasodilation [for review see (Harrison and Cai, 2003)].

In neurons, nitric oxide also acts through cGMP and protein phosphorylation. Stimulation of NMDA glutamate receptors increases nitric oxide production in neurons (Wood et al., 1990; Marcoli et al., 1997; Maura et al., 2000; Fedele et al., 2001). These receptors act as calcium channels, and increase intracellular concentration of calcium upon their activation, thus increasing nitric oxide production by nNOS. In turn, in the hippocampus, and presumably in other brain regions as well, low concentrations of nitric oxide inhibit glutamate release from neurons, whereas high concentrations stimulate release (Segieth et al., 1995). Nitric oxide has the same concentration-dependent effect on GABA release (Getting et al., 1996). These actions of nitric oxide to concentration-dependently alter the release of both stimulatory and inhibitory amino acids, in addition to its other functions, can help explain the pleiotropic actions of this substance.

Nitric oxide is produced in and has effects in many brain nuclei and regions important in cardiovascular control (Patel et al., 1996; Iwase et al., 1998). Endogenous nitric oxide can exert effects at the level of the baroreceptor, the preganglionic neuron, and in multiple brain regions.

Baroreceptors. Endogenous nitric oxide does not affect the activity or gain of the baroreceptors (Matsuda et al., 1995; Zanzinger et al., 1996). Supraphysiological levels of nitric oxide do decrease activity and baroreflex gain (Matsuda et al., 1995; Zanzinger et al., 1996), but this effect is independent of the cGMP pathway (Matsuda et

al., 1995). Normal physiological levels of endogenous nitric oxide may be involved in attenuating the ability of these receptors to reset (da Silva et al., 1994).

Preganglionic neuron. In untreated anesthetized animals nitric oxide acts at the preganglionic neuron to elevate renal sympathetic nerve activity (Hakim et al., 1995; Yang et al., 2004). However, when the PVN is stimulated or glutamate is administered into the spinal cord, nitric oxide's effects are reversed, and it attenuates the increase in renal sympathetic nerve activity (Yang et al., 2004). This action is through stimulation of glycine release, since blockade of this neurotransmitter prevented the inhibition (Yang et al., 2004). A possible scenario is that under basal conditions nitric oxide levels are relatively low in the intermediolateral cell column of the spinal cord, and nitric oxide acts to inhibit tonic glycine release, similarly to its effect on GABA release (Getting et al., 1996). PVN activation stimulates glutamate release into the intermediolateral cell column of the spinal cord. Glutamate then activates preganglionic renal sympathetic neurons but also stimulates nitric oxide production through NMDA receptors (Wood et al., 1990; Marcoli et al., 1997; Maura et al., 2000; Fedele et al., 2001). Now in higher concentrations, the nitric oxide stimulates glycine release, also similar to its effects on GABA release (Getting et al., 1996).

Brain. Nitric oxide also affects cardiovascular regulation though actions in the brain, where its overall effect in the resting state is to decrease renal sympathetic nerve activity (Togashi et al., 1992; Zanzinger et al., 1997; Matsumura et al., 1998a; Tandai-Hiruma et al., 2005), blood pressure (Togashi et al., 1992; el Karib et al., 1993; Matsumura et al., 1998a; Chikada et al., 2000), and heart rate (Chikada et al., 2000). In addition, nitric oxide counteracts the effects of stimulation, reducing the changes in

blood pressure, heart rate, cardiac output, and renal sympathetic nerve activity in response to central administration of glutamate (Zanzinger et al., 1997), stimulation of the greater sciatic (Zanzinger et al., 1997) or the left ventral ansa (Ma et al., 2005) nerve, and baroreflex activation (Matsumura et al., 1998a). Central blockade of endogenous nitric oxide production increases, and nitric oxide donors reduce, the gain of baroreflex control of renal sympathetic nerve activity and heart rate in rabbits (Matsumura et al., 1998a), suggesting that the net effect of endogenous nitric oxide in the brain is to decrease baroreflex gain.

These overall effects of nitric oxide in the brain are the results of its combined effects in all the brain regions controlling cardiovascular functions. Nitric oxide's effects in some of these regions have been studied, as described below.

NTS. Nitric oxide's actions in the NTS are highly variable, causing increases, decreases and no changes in blood pressure, heart rate and renal sympathetic nerve activity (Harada et al., 1993; Tseng et al., 1996; Hines and Mifflin, 1997; Pontieri et al., 1998; Matsumura et al., 1998c; Lin et al., 1999; Wu et al., 2002). In addition, nitric oxide can enhance (Lo et al., 1996; Talman and Dragon, 2004; Dias et al., 2005), inhibit (Paton et al., 2001b; Smith et al., 2005), or produce no effect on (Harada et al., 1993; Zanzinger et al., 1995a; Pontieri et al., 1998) baroreflex gain. These divergent effects probably reflect differing levels of nitric oxide. Blockade of nitric oxide in a control state, for instance, decreases (Lo et al., 1996; Talman and Dragon, 2004; Dias et al., 2005) or does not change (Harada et al., 1993; Zanzinger et al., 1995a; Pontieri et al., 1998; Paton et al., 2001b) baroreflex gain, suggesting that, if anything, endogenous nitric oxide acts to increase gain at rest. This may be through inhibition of GABA

release (Getting et al., 1996), or stimulation of glutamate (Dias et al., 2005). However, when nitric oxide levels are elevated by either infusion of nitric oxide donors, or by administration of angiotensin II into the NTS, nitric oxide decreases baroreflex gain (Paton et al., 2001b). Paton and colleagues (Paton et al., 2001a) suggest that nitric oxide exerts this influence by stimulation of GABA release.

CVLM. Much less is known about the role of nitric oxide in the CVLM. In this region, nitric oxide increases basal blood pressure and renal sympathetic nerve activity (Shapoval et al., 1991). It also augments the increase in blood pressure and heart rate with muscle contraction (Ishide et al., 2000). This effect is probably through increases in glutamate release (Ishide et al., 2000). Nitric oxide in this area does not affect baroreflex gain (Zanzinger et al., 1995a).

RVLM. In the RVLM, nitric oxide also exerts divergent actions. As in the NTS, it can increase, decrease, and cause no change in mean arterial pressure, heart rate and sympathetic nerve activity (Shapoval et al., 1991; Zanzinger et al., 1995b; Hirooka et al., 1996; Tseng et al., 1996; Wang et al., 2003; Kimura et al., 2005). Its effects on baroreflex gain are more uniform, however, with studies reporting increases in the gain of baroreflex control of heart rate (Mayorov, 2005) and no change in the gain of sympathetic nerve activity control (Zanzinger et al., 1995b; Mayorov, 2005).

PVN. Finally, in the PVN, nitric oxide decreases blood pressure, heart rate, and renal and splenic sympathetic nerve activity (Zhang et al., 1997; Zhang and Patel, 1998; Kenney et al., 2003). This action is through increasing GABA transmission, thus reducing neuronal activity, in this nucleus (Zhang and Patel, 1998). Nitric oxide's effect on baroreflex gain in this region has not been studied, but since PVN activation

inhibits baroreflex gain (Chen et al., 1996a), and nitric oxide reduces PVN activity (Zhang and Patel, 1998), nitric oxide might be expected to increase gain.

Systemic. The effect of systemic administration of NOS inhibitors would allow for the determination of the net effect of endogenous nitric oxide production on sympathetic control in all regions. The net effect of nitric oxide is to either decrease (Kumagai et al., 1994; Hirai et al., 1995; Nishida et al., 2001) or cause no change in (Liu et al., 1996; Liu et al., 1998) renal sympathetic nerve activity independent of changes in blood pressure. On the other hand, its effect on the lumbar sympathetic nerve is to increase activity (Hirai et al., 1995), and it causes no change in the activity of cardiac (Yabe et al., 1998), cutaneous (Habler et al., 1997) or muscle (Hansen et al., 1994) sympathetic nerves. This illustrates the ability of nitric oxide to exert differing effects on different sympathetic nerves.

Besides its role in tonic sympathetic regulation, nitric oxide functions globally in the modulation of the baroreflex. Many studies have found that endogenous nitric oxide decreases baroreflex gain (Minami et al., 1995b; Liu et al., 1996; Kumagai et al., 1997; Brady et al., 2002), but other studies have found no effect on baroreflex gain (Du et al., 1992; Jimbo et al., 1994; Castellano et al., 1995; Miyano et al., 1997; Hogan et al., 1999; Fujisawa et al., 1999b; Lacchini et al., 2001b). These differing effects of nitric oxide probably depend on its basal levels. So the net effect of endogenous nitric oxide is the sum of its stimulatory and inhibitory effects in different regions, which is dependent on its concentration within those regions. In the basal, unstressed state nitric oxide appears to exert no net effect on baroreflex gain (see Appendix). However, if the

balance of nitric oxide production is altered, then nitric oxide can decrease baroreflex gain.

Nitric oxide and pregnancy. Systemic nitric oxide levels are elevated during pregnancy (Kopp et al., 1977; Conrad et al., 1993; Weiner et al., 1994a; Weiner et al., 1994b), perhaps through increases in estradiol (Weiner et al., 1994b). Moreover, a limited number of studies have also demonstrated alterations in brain nitric oxide production during pregnancy. Neuronal NOS levels are elevated in the whole hypothalamus of pregnant rats (Xu et al., 1996), but nitric oxide activity is decreased in the magnocellular region of the PVN, which is responsible for oxytocin and vasopressin production and release (Okere and Higuchi, 1996). Increases in brain nitric oxide may be responsible for the decreased sensitivity of the cardiopulmonary reflex during pregnancy (Tam and Kaufman, 2002). Since alterations in nitric oxide balance, such that levels are increased, appear to decrease arterial baroreflex gain (see Appendix), and since nitric oxide is elevated during pregnancy, this gaseous neurotransmitter may be involved in the pregnancy-induced impairment of arterial baroreflex gain as well.

Nitric oxide/angiotensin II interactions

One factor that may alter nitric oxide balance is angiotensin II since, in different brain regions, angiotensin II can stimulate nitric oxide release (Paton et al., 2001b; Li et al., 2005b). In turn nitric oxide can inhibit the actions of angiotensin II (Li et al., 2005b). Because angiotensin II can stimulate nitric oxide production, it can alter the balance of inhibitory and excitatory effects of nitric oxide. Indeed, nitric oxide in the control state does not affect renal sympathetic nerve activity (Liu et al., 1998).

However, if angiotensin II levels are elevated, which in itself does not affect nerve activity, then blockade of nitric oxide inhibits activity (Liu et al., 1998). The elevations in angiotensin II during pregnancy may work the same way, altering the balance of nitric oxide, such that this factor now decreases baroreflex gain.

Insulin sensitivity

Insulin sensitivity is defined as the ability of insulin to activate its receptor to exert its effect, usually to drive the uptake of glucose from the blood stream into myocytes and adipocytes (Cousins, 1991; Egan, 2003; Gerozissis, 2004). Plasma insulin concentrations normally increase following elevations in blood glucose levels. The insulin then binds to its receptor causing autophosphorylation of the receptor on tyrosine residues. This creates a binding site on the receptor for the docking of insulin receptor substrate (IRS) proteins, of which there are several. IRS-1 and IRS-2 are involved in the glucose uptake role of insulin receptor activation. Upon docking of these proteins to the receptor they become phosphorylated, also on tyrosine residues. Tyrosine phosphorylated IRS proteins provide docking sites for phosphoinositide 3-kinase, activating this protein. Once activated, phosphoinositide 3-kinase phosphorylates phosphatidylinositol 4,5-bisphosphate to create phosphatidylinositol 3,4,5-trisphosphate. This leads to a series of incompletely understood events involving phosphoinositide-dependent protein kinase, protein kinase C, Akt, and AS160 and eventually, insertion of GLUT-4 glucose transporters into the cell membrane and glucose uptake by the cell [for a review of these events see (Bjornholm and Zierath, 2005)].

Insulin resistance, or reduced insulin sensitivity, is associated with decreased insulin receptor function; insulin's ability to cause tyrosine phosphorylation of the insulin receptor, and IRS proteins, is reduced (Schmitz-Peiffer, 2000). The effect is a decrease in the insertion of GLUT-4 transporters and reduced glucose uptake for a given plasma concentration of insulin (Bjornholm and Zierath, 2005).

Pregnancy and insulin sensitivity. Pregnancy is associated with decreases in insulin sensitivity (Cousins, 1991; Gilbert et al., 1991; Ciampelli et al., 1998). A number of hormones increase during pregnancy that might be responsible for this. Glucocorticoids, estrogen, progesterone, and placental lactogen rise progressively during pregnancy (Cousins, 1991). Evidence for the involvement of these hormones comes from Ryan and Enns (Ryan and Enns, 1988) who found that placental lactogen, prolactin, progesterone, and cortisol each induce insulin resistance in cultured adipocytes. In addition, in vivo administration of estrogen and progesterone (Batista et al., 2005), prolactin (Reis et al., 1997), cortisol (Andrews and Walker, 1999), or human placental lactogen (Beck and Daughaday, 1967) induces insulin resistance. Progesterone (Uryszek et al., 1993), prolactin (Fortun-Lamothe et al., 1996), and glucocorticoids (Guillaume-Gentil et al., 1993) can all increase plasma free fatty acid levels, which, as described below, may be part of their mechanism of action to reduce insulin sensitivity during pregnancy.

Plasma levels of free fatty acids increase during pregnancy (Gilbert et al., 1993) and infusion of triglycerides to raise free fatty acid levels results in the development of insulin resistance in non-pregnant rabbits (Gilbert et al., 1991) and early pregnant humans (Sivan et al., 1998). In addition, reducing free fatty acid levels during

pregnancy improves insulin sensitivity (Gilbert et al., 1993). This suggests that free fatty acids play a major role in pregnancy-induced insulin resistance. Pregnancy is also associated with an increase in tumor necrosis factor-α (Melczer et al., 2002b), and the levels of this cytokine are strongly correlated with the decrease in insulin sensitivity during pregnancy (Kirwan et al., 2002; Melczer et al., 2002a), suggesting a role for this substance as well.

Both free fatty acids and tumor necrosis factor-α can stimulate serine phosphorylation of the insulin receptor and IRS proteins (Jiang and Zhang, 2005). Phosphorylation of these proteins on serine residues results in their inactivation, causing a decrease in phosphoinositol 3-kinase activity and, therefore, reduced glucose uptake (Roden, 2004). Obese and diabetic humans have increased serine phosphorylation on insulin signaling proteins (Jiang and Zhang, 2005) and this is presumably true in pregnancy as well.

Insulin sensitivity and baroreflex gain. Many other conditions associated with decreases in baroreflex gain are also associated with decreases in insulin sensitivity such as obesity (Grassi et al., 1995; Ferrannini et al., 1997), hypertension (Grassi, 2004; Frontoni et al., 2005), chronic heart failure (Chen et al., 1992; Coats et al., 2000), type II diabetes mellitus (Pikkujamsa et al., 1998; Frontoni et al., 2005), and aging (Laitinen et al., 1998; Ryan, 2000). Moreover, rats fed a high fructose diet, a relatively specific model of insulin resistance not associated with increased body fat or moderate or severe hypertension, have a decreased baroreflex gain (Miller et al., 1999). In addition, acute administration of metformin (an insulin sensitizing drug) into the lateral ventricle augments baroreflex function in spontaneously hypertensive rats (Petersen and DiBona,

1996). Therefore, in most conditions in which baroreflex gain is reduced there is also a decrease in insulin sensitivity. Thus, it is possible that insulin resistance is responsible for the baroreflex impairment of pregnancy as well.

Insulin in the brain. Insulin increases baroreflex gain when applied to the dorsal surface of the medulla (Man et al., 1998) or when administered into the lateral ventricles (Okada and Bunag, 1994). However, in other conditions of insulin resistance, such as dogs fed a high fat diet, Zucker fatty rats and humans with Alzheimer's disease (Kuusisto et al., 1997), transport of systemic insulin into the cerebrospinal fluid is decreased (Stein et al., 1987; Craft et al., 1998; Kaiyala et al., 2000). It is unknown if the same is true of pregnancy. In addition to reductions in insulin transport into the brain and brain insulin concentrations, neuronal insulin signaling also appears to be suppressed in insulin resistant states (De Souza et al., 2005), thus decreasing the effectiveness of the insulin that does reach the brain.

It is unknown why decreases in insulin sensitivity are associated with reductions in insulin transport into the brain or even exactly how insulin is transported into the brain. Insulin is either not made centrally or is made in very low quantities (Banks, 2004). The majority of central insulin is from pancreatic origin (Banks, 2004); however, insulin is a large polypeptide that cannot pass through the blood-brain-barrier unassisted (Banks, 2004). It has been proposed that insulin gains access to the brain via a receptor-mediated process in which insulin binds to its receptor on the capillary wall, and this complex is internalized by endocytosis (Gerozissis, 2003; Woods et al., 2003; Banks, 2004). The insulin/insulin receptor complex is then expelled by exocytosis on the brain side of the endothelial cell (Gerozissis, 2003; Woods et al., 2003; Banks,

2004). Such a transport process has been demonstrated in cultured aortic endothelial cells in which insulin transport across the endothelial cell is blocked by insulin receptor antibodies (King and Johnson, 1985). Since the endothelial cells of the blood-brain-barrier also contain insulin receptors and can transport insulin intact from the blood to the brain (Gerozissis, 2003; Woods et al., 2003; Banks, 2004), this is a likely means by which insulin gains access to the brain.

Rosiglitazone. Insulin sensitivity can be increased by treatment with thiazolidinediones such as rosiglitazone. Rosiglitazone activates the peroxisome proliferator-activated receptor gamma (PPARγ) which is a ligand-activated transcription factor that regulates lipid metabolism (Vasudevan and Balasubramanyam, 2004). Exactly how PPARγ activation increases insulin sensitivity is not completely understood but may involve reductions in free fatty acid levels or suppression of tumor necrosis factor-α release (Vasudevan and Balasubramanyam, 2004), which, as mentioned above, increase the serine phosphorylation of the insulin receptor and IRS proteins (Jiang and Zhang, 2005).

Rosiglitazone has effects other than increasing insulin sensitivity. Thiazolidinediones can also reduce leptin levels and raise adiponectin levels, both of which may also be involved in the insulin-sensitizing effect of these drugs (Giannini et al., 2004). Other effects include reducing levels of other inflammatory cytokines (interleukins, geletinase B, matrix metaloprotease and scavenger receptor A), reduced intracellular superoxide radical generation, as well as increased nitric oxide production, decreased angiotensin II AT_1 receptor expression and blockade of calcium channels in vasular smooth muscle cells (Giannini et al., 2004).

HYPOTHESIS

Pregnancy decreases baroreflex gain, but the cause is unknown. The purpose of my thesis work has been to identify the factor(s) that induce baroreflex impairment during pregnancy.

Nitric oxide and angiotensin II decrease baroreflex gain during pregnancy

Nitric oxide is increased during pregnancy, and the net effect of nitric oxide appears to be to lower baroreflex gain. Moreover, this action may depend on angiotensin II. Therefore, I hypothesized that the pregnancy-induced increase in nitric oxide causes the decrease in baroreflex gain and that this action requires angiotensin II (Figure 3). Furthermore, I hypothesized that nitric oxide's main site of action to cause this impairment is the brain. To test these hypotheses I determined the following: 1) Does blockade of nitric oxide production during pregnancy increase baroreflex gain in pregnant but not non-pregnant rabbits? 2) Does prior blockade of AT_1 angiotensin II receptors prevent this effect of inhibition of nitric oxide production? 3) Are nNOS mRNA and protein levels altered in the PVN, NTS, CVLM and/or RVLM of pregnant rabbits?

Figure 3

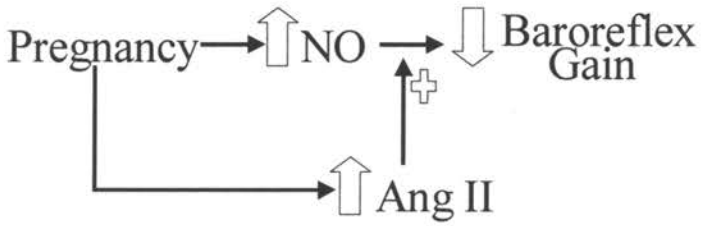


Diagram of hypothesis: Pregnancy decreases baroreflex gain by increasing the activity of nitric oxide, and this is facilitated by angiotensin II.

Insulin resistance decreases baroreflex gain during pregnancy

Pregnancy decreases insulin sensitivity, and many conditions associated with reduced insulin sensitivity also exhibit a decrease in baroreflex gain. Therefore, I hypothesized that the pregnancy-induced decline in insulin sensitivity results in a decrease in brain insulin concentrations leading to the decrease in baroreflex gain. To test this hypothesis I determined the following: 1) Are insulin sensitivity and baroreflex gain correlated in non-pregnant and near-term pregnant rabbits? 2) Does the time course for the decrease in insulin sensitivity match the time-course for impairment of baroreflex gain during pregnancy? 3) Does prevention of insulin resistance through treatment with rosiglitazone throughout pregnancy also prevent the reduction in baroreflex gain? 4) Are insulin concentrations reduced in the cerebrospinal fluid of pregnant rabbits?

ORIGINALITY AND ASSIGNATION OF CREDIT

The ideas and hypotheses presented in this dissertation were developed by me with the assistance of my advisor, Dr. Virginia Brooks. Most of the work was performed by me with a few exceptions, here noted. The assays to measure nNOS mRNA and protein levels were conducted by Dr. Dongmei Liu in Dr. Irving Zucker's laboratory at the University of Nebraska. Measurement of plasma and cerebrospinal fluid levels of insulin were performed by Mr. Terrence Chu in Dr. David Hess' laboratory at the Endocrine Services Laboratory of the Oregon National Primate Research Center. I trained Dr. Mee-Young Chung to perform some of the baroreflex and insulin sensitivity experiments as well as some of the surgeries to implant vascular catheters. In addition, together we modified the technique for implantation of cisterna magna cannulae for use in our laboratory.

CHAPTER 2

ROLES OF NITRIC OXIDE AND ANGIOTENSIN II IN THE IMPAIRED BAROREFLEX GAIN OF PREGNANCY

ABSTRACT

I investigated potential mechanisms underlying the decrease in baroreceptor reflex gain during pregnancy. Decreases in central nitric oxide or angiotensin II can, alone or in combination, decrease baroreflex gain, and these neurotransmitters are increased during pregnancy. Therefore, the present study tested the hypothesis that nitric oxide contributes to the impaired baroreflex gain of pregnancy and that this action is enhanced by angiotensin II. To test these hypotheses I quantified baroreflex control of heart rate in non-pregnant and pregnant conscious rabbits using gradual inflation of an occluder around the inferior thoracic vena cava to decrease pressure and infusion of increasing doses of phenylephrine to increase pressure. Baroreflex gain was determined before and after: 1) blockade of nitric oxide synthase (NOS) with Nω-nitro-L-arginine (20 mg/kg), 2) blockade of the angiotensin II AT_1 receptor with L158-809 (5μg/kg/min), 3) infusion of angiotensin II (1ng/kg/min non-pregnant, 1.6-4 ng/kg/min pregnant), 4) combined blockade of angiotensin II AT_1 receptors and NOS, and 5) combined infusion of angiotensin II and blockade of NOS. To determine if neuronal

NOS (nNOS) levels were altered in brain regions involved in cardiovascular control during pregnancy, nNOS mRNA and protein levels were measured in the paraventricular nucleus of the hypothalamus, nucleus of the solitary tract, caudal ventrolateral medulla and rostral ventrolateral medulla in pregnant and non-pregnant rabbits. The decrease in baroreflex gain observed in pregnant rabbits was not reversed by NOS blockade, angiotensin II blockade, or combined blockade. Angiotensin II infusion, with or without NOS blockade, also did not affect baroreflex gain in pregnant or non-pregnant rabbits. In addition, nNOS mRNA and protein levels in cardiovascular brain regions remained unaltered during pregnancy. Therefore, I conclude 1) that nitric oxide is not responsible for the decrease in baroreflex gain during pregnancy and 2) that angiotensin II and nitric oxide do not interact to decrease baroreflex gain in conscious rabbits.

INTRODUCTION

Pregnancy is associated with a decrease in baroreflex gain [see (Heesch and Rogers, 1995; Brooks et al., 1995) for reviews], which results in a reduced ability to maintain blood pressure in response to challenges. Therefore, it may underlie the increased incidence of orthostatic hypotension in pregnant women (Easterling et al., 1988) and the decreased tolerance of pregnant animals to hemorrhage (Brooks et al., 1998). Indeed, 17% of human maternal mortality in the United States between 1990 and 1999 was related to hemorrhage (Chang et al., 2003). Despite this clear clinical significance, the mechanism for the suppression of baroreflex gain remains unclear.

One potential contributor is nitric oxide. Indirect evidence to support this possibility is that nitric oxide, at least from the endothelial isoform of nitric oxide synthase (eNOS), is increased in pregnancy [for reviews see (McLaughlin and Conrad, 1995; Sladek et al., 1997; Weiner and Thompson, 1997)]. Moreover, in conscious animals, the net effect of nitric oxide may to be to decrease baroreflex gain, as systemic (Liu et al., 1996) and central (Matsumura et al., 1998a) nitric oxide synthase (NOS) blockade increases gain. Therefore, in the current study, I determined if acute systemic blockade of the NOS enzyme increases baroreflex gain in conscious pregnant rabbits, to test the hypothesis that during pregnancy nitric oxide decreases baroreflex gain.

Previous studies have shown that mRNA levels of the neuronal isoform of the NOS enzyme (nNOS) are normally found in high levels in multiple brain regions important in cardiovascular control, including the paraventricular nucleus of the hypothalamus (PVN), nucleus of the solitary tract (NTS) (Iwase et al., 1998), caudal ventrolateral medulla (CVLM) and rostral ventrolateral medulla (RVLM) (Patel et al., 1996). The effect of nitric oxide on baroreflex function in these regions varies. For example, nitric oxide acts in the RVLM to increase baroreflex gain (Mayorov, 2005). In the NTS, basal levels of endogenous nitric oxide enhance baroreflex gain (Lin et al., 1999; Talman and Dragon, 2004; Dias et al., 2005) by facilitation of glutamate transmission (Dias et al., 2005), or have no effect on baroreflex gain (Harada et al., 1993; Zanzinger et al., 1995a; Lo et al., 1996; Pontieri et al., 1998; Paton et al., 2001b; Talman and Dragon, 2004; Dias et al., 2005); however, if nitric oxide levels increase by either administration of nitric oxide donors or angiotensin II, then nitric oxide inhibits gain (Paton et al., 2001b) via stimulation of γ-amino-butyric acid (GABA) release

(Paton et al., 2001a). In the PVN, nitric oxide has the potential to increase gain. PVN activation inhibits baroreflex gain (Chen et al., 1996a), and nitric oxide reduces PVN activity (Zhang and Patel, 1998); however, the actions of nitric oxide in the PVN on baroreflex gain have not been directly studied. These studies suggest that pregnancy could decrease baroreflex gain by differentially altering NOS activity in specific brain regions. To test this hypothesis, we determined whether the mRNA and protein levels of nNOS in the NTS, RVLM, CVLM and PVN were altered in pregnant compared to non-pregnant rabbits. If alterations in the balance of nitric oxide production in the brain are involved in the decreased baroreflex gain of pregnancy, then nNOS levels may be increased in some brain regions while decreased in others during pregnancy.

Recent studies have indicated that the effects of nitric oxide in setting the basal sympathetic tone and baroreflex control depend on an interaction with angiotensin II (Kumagai et al., 1993; Liu et al., 1998; Paton et al., 2001b; Latchford and Ferguson, 2003; McKeogh et al., 2004). For example, Kumagai et al. (Kumagai et al., 1993) found that, in spontaneously hypertensive rats, blockade of either angiotensin II AT_1 receptors or the NOS enzyme alone increased the gain of baroreflex control of heart rate and renal sympathetic nerve activity. However, the effects of combined AT_1 receptor and NOS blockade were not additive and, in the case of the baroreflex control of renal sympathetic nerve activity, they occluded each other, suggesting interdependence.

Pregnancy is associated with elevated angiotensin II levels (Skinner et al., 1972; Brooks and Keil, 1994; Brooks et al., 1998), but whether angiotensin II interacts with nitric oxide in the control of baroreflex gain during pregnancy is unknown. Therefore, a third aim of this study was to test the hypothesis that nitric oxide's ability to decrease

baroreflex gain during pregnancy is enhanced by angiotensin II. To test this hypothesis I determined the effect of NOS blockade following AT_1 receptor blockade. If angiotensin II enhances the nitric oxide-induced decrease in baroreflex gain, then, following AT_1 receptor blockade, NOS blockade should not increase baroreflex gain to the same extent as blockade of NOS alone.

METHODS

All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of Oregon Health & Science University.

Female New Zealand White rabbits (Western Oregon Rabbit; Philomath, OR) weighing 4.0 ± 0.1 kg (non-pregnant, n=18) were used for these experiments. The rabbits were received when they were fourteen weeks old, and allowed a minimum of six days to acclimate before surgery.

Surgical preparation

Surgery was performed to implant non-occluding abdominal aortic and vena caval catheters as previously described (Gronan et al., 1983) for the measurement of mean arterial pressure and heart rate, and the infusion of drugs, respectively. Briefly, the animals were initially anesthetized with a cocktail containing ketamine (58.8 mg/kg), xylazine (5.9 mg/kg), and acepromazine (1.2 mg/kg) administered subcutaneously (s.c.). The rabbits were then intubated, placed on a respirator, and ventilated with 100% oxygen throughout the surgery. A surgical plane of anesthesia

was maintained by administration of ketamine intravenously as needed. A midline abdominal incision was made in all rabbits and polyethylene catheters with silastic tips were implanted in the abdominal aorta (one) and vena cava (two). The catheters were tunneled subcutaneously from the abdominal cavity and exited at the nape of the neck.

As this study sought to determine the role of nitric oxide on the baroreflex, use of a nitric oxide donor, such as nitroprusside, to decrease mean arterial pressure could pose a confound. Therefore, after a minimum two-week recovery period, a second surgery was performed to implant a vena caval occluder to decrease blood pressure in tests of baroreflex function. Rabbits were initially anesthetized with ketamine (250mg, s.c.), intubated, placed on a respirator and ventilated with 100% oxygen; a surgical plane of anesthesia was maintained with isoflurane (2%). An occluder was implanted around the thoracic inferior vena cava via a right thoracotomy through the fifth intercostal space. The end of the occluder also exited at the nape of the neck, and the incision was closed in layers. The end of the occluder and catheters were protected in a plastic pillbox sutured to the rabbits' skin.

The rabbits were given an intramuscular injection of enrofloxacin (22.7 mg) just before each surgery and an intravenous (i.v.) injection of this antibiotic for four days following surgery (22.7 mg/day). The animals were also injected with buprenorphine hydrochloride (0.09 mg s.c.) two to three hours after surgery, and again the next day to relieve pain. The catheters were flushed immediately after surgery, and then three times weekly with sterile 0.9% saline, and filled with heparin (1,000 U/ml) to maintain patency. Animals were allowed at least two weeks for recovery from the thoracotomy

before any experiments were performed. During this time the rabbits were conditioned to the 37x17x21 cm black Plexiglas box used during experiments.

Baroreflex curve generation

On the experimental day, the rabbits were placed in the box and allowed approximately thirty minutes to acclimate. Mean arterial pressure and heart rate were measured continuously via the aortic catheter using a Statham pressure transducer, a Grass tachometer, Grass polygraph and a Biopac (Santa Barbara, CA) MP100 data acquisition and analysis system. To determine the baroreflex relationship between mean arterial pressure and heart rate, and to ensure activation of both the sympathetic and parasympathetic nervous systems (Coleman, 1980), a slow ramp method of altering mean arterial pressure was performed. A slow inflation of the occluder around the vena cava was used to lower arterial pressure until heart rate reached maximum values. Mean arterial pressure was lowered at a rate of 0.47 ± 0.02 mmHg/second, and this part of the baroreflex curve was generated in 64 ± 2 seconds. Infusion of increasing doses of phenylephrine (0.5, 1, 2, 4, and 8 μg/kg/min in 5% dextrose in water vehicle, i.v.) was used to increase mean arterial pressure, increasing the dose every fifteen seconds until mean arterial pressure was increased by twenty mmHg over basal (Brooks et al., 2002). This took 59 ± 2 seconds so that the entire curve was generated in 123 ± 3 seconds. More than one occluder inflation was performed in each experiment, and at least ten minutes was allowed between inflations to allow mean arterial pressure and heart rate to return to basal conditions.

Effects of NOS blockade

To test the hypothesis that the decreased gain of pregnancy is due to the actions of nitric oxide, I determined if NOS blockade increases gain in pregnant rabbits. Baroreflex curves were generated before and at least ninety minutes (105-134 minutes) after a bolus i.v. injection of the non-specific NOS inhibitor Nω-nitro-L-arginine (L-NNA, 20 mg/kg dissolved in 10 ml of 0.5% sodium carbonate in water then adjusted to pH 7.4) in six rabbits. This dose produces maximal increases in mean arterial pressure and heart rate (Brooks et al., 2001a). Following this experiment, rabbits were mated and the effect of L-NNA on baroreflex function was studied again at the end of gestation (day thirty; term is thirty-one days).

Effects of NOS and AT_1 angiotensin II receptor blockade

The purpose of this protocol was to determine if the actions of nitric oxide require angiotensin II. Animals were studied in either the non-pregnant (n=8) or pregnant (n=6) states. After a control baroreflex curve was generated, a continuous i.v. infusion of the AT_1 angiotensin II receptor antagonist, L158-809 (5μg/kg/min in 5% dextrose in water vehicle), was begun. This dose was sufficient to prevent increases in arterial pressure with administration of 100 ng of angiotensin II in three non-pregnant and three pregnant rabbits in this study. Before L158-809, this dose of angiotensin II increased mean arterial pressure by 20 ± 3 mmHg. Thirty to sixty minutes after the start of the L158-809 infusion, a second baroreflex curve was generated and then L-NNA (20 mg/kg, iv) was administered. Finally, after 105-135 minutes a third baroreflex curve was generated. All pregnant animals in this protocol were studied at day thirty of their

second pregnancy, and one of the non-pregnant animals was studied two weeks following delivery of her second litter.

Effects of NOS blockade during angiotensin II infusion

Since NOS blockade suppresses renin release (Gardes et al., 1992), angiotensin II levels may be suppressed in rabbits receiving L-NNA. If the actions of nitric oxide require angiotensin II, this suppression could confound the interpretation of the effects of blockade of NOS alone. Therefore, it was determined if NOS blockade increases baroreflex gain during an infusion of angiotensin II. Rabbits (n=4) were studied in the non-pregnant state and during their first pregnancy (at day thirty of gestation). After a control baroreflex curve was generated, a continuous i.v. infusion of angiotensin II was begun. For non-pregnant rabbits, angiotensin II was infused at a rate of 1 ng/kg/min (in 5% dextose in water vehicle), which increased mean arterial pressure by about five mmHg. During pregnancy, angiotensin II levels are higher (Brooks et al., 1998) than in non-pregnant animals, and more angiotensin II is required to increase blood pressure. Again angiotensin II was infused at a dose sufficient to increase pressure by about 5 mmHg (1.6 to 4 ng/kg/min).

Levels of nNOS protein and mRNA in brain regions involved in cardiovascular control

This protocol was employed to determine if alterations in the balance of nNOS expression in different cardiovascular brain nuclei might be involved in the decreased baroreflex gain of pregnancy. For these experiments, rabbits (n=4) at day thirty of

gestation and age matched non-pregnant rabbits were euthanized with 1ml of Euthasol (390 mg pentobarbital, 50 mg phenytoin; i.v.), and their brains were removed, quickly frozen in -80°C 2-methylbutane, and stored at -80°C until shipment to the University of Nebraska, where the assays were performed.

The PVN, NTS, CVLM and RVLM sections were punched out of 600 μm thick slices with a 15-gauge needle stub using the Palkovits technique (Palkovits and Brownstein, 1983). The punched tissue was put in 0.5 ml of Tri-Reagent and homogenized. Total RNA and proteins in the homogenate were extracted according to the Tri-Reagent manufacturer's instructions.

Quantification of mRNA levels. Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) assays were performed to assess relative mRNA levels. RNA was isolated followed by a reverse transcription reaction for forty minutes at 38°C in the presence of 1.5 μM random hexamers and 200 units of Moloney murine leukemia virus reverse transcriptase. Each 1.5 μl aliquot of the reverse transcriptase product was used for nNOS cDNA amplification. The following polymerase chain reaction primers were used: nNOS, 5'-GATCGCTGACCGTATGCAG-3' (sense); 5'-GTCGTACTCCTGCTTGGTG-3' (antisense); and β-actin, 5'-GGGAAATCGTGCGTGACATT-3' (sense); 5'-CGGATGTCAACGTCACACTT-3' (antisense). The polymerase chain reaction mixture contained 0.7 μM primers, dNTP, bovine serum albumin and one unit of Taq DNA polymerase. β-Actin was coamplified with each receptor cDNA as an internal control. After four minutes of denaturing at 94°C, the amplification was performed at 94°C for one minute, at 46°C for one minute,

and at 72°C for one minute for thirty cycles. The products of this reaction (7 μl) were fractionated in a 1% agarose gel and transferred to a Nytran membrane. A southern blot was then performed using 5'-[^{32}P]-antisense deoxyoligonucleotides (5'-GTCGTACTCCTGCTTGGTG-3' for nNOS, 5'-CCGCCGATCCACAC-3' for β-actin). The radioactive signal emitted from the cDNA was quantified by phosphor imaging, and the data is expressed as digital light units per unit time (DLU) for nNOS mRNA relative to DLU for β-actin mRNA.

Quantification of protein levels. The protein extract obtained above was used for Western blot analysis of nNOS. The protein concentration was measured using a protein assay kit (BCA kit, Pierce). The protein sample was mixed with an equal volume of 2x 4% sodium dodecyl lauryl sulfate sample buffer. The sample was boiled for five minutes, and then loaded onto a 7.5% sodium dodecyl lauryl sulfate-polyacrylamide gel for electrophoresis at 40 mA/gel for fifty minutes. The fractionated proteins on the gel were electrophoretically transferred onto a polyvinylidene diflouoride membrane at 300 mA for ninety minutes. The membrane was incubated with 5% milk-Tris-buffered saline-Tween 20 solution for thirty minutes at room temperature, then with primary antibody (BD Transduction Laboratories, 1:1,000) at 4°C overnight. After being washed three times, the membrane was incubated with secondary antibody (peroxidase conjugated, Pierce, 1:5,000) for thirty minutes at room temperature. After being washed three times, the membrane was treated with enhanced chemiluminescence reagent for five minutes and detected by exposing a film. The bands on the developed film were visualized and analyzed using UVP BioImaging

Systems. The light signal emitted was quantified and the values normalized to the β-tubulin protein band to determine specific changes in nNOS levels.

Baroreflex curve analysis

The data for the baroreflex curves were collected at 200 Hz and processed using a Biopac (Santa Barbara, CA) MP100 data acquisition and analysis system. Raw data were grouped into one-second bins from which mean values were obtained. Since more than one curve was generated using the vena caval occluder, curves were selected that were free from movement artifact, and exhibited the best sigmoidal fit. Often the basal mean arterial pressures and heart rates before the pressor (phenylephrine) part of the reflex curve were different from the depressor (occluder) part. When this occurred, to avoid erroneous measurements of baroreflex gain, half of the pressure difference was added to all pressure values in the segment with the lower basal pressure and half the difference was subtracted from the pressures in the segment with the higher pressure, so that the two segments were aligned, as previously described (Dorward et al., 1985; Brooks et al., 2002). The same was done for heart rate. Figure 4A illustrates representative curves for one rabbit and shows that pregnancy decreases gain.

The sigmoidal baroreflex relationships between mean arterial pressure and heart rate generated in each experiment were fitted and compared using the Boltzmann sigmoidal equation [heart rate=A+B/1+ $e^{(C\text{-mean arterial pressure})/D}$, where A equals the minimum heart rate, B equals the heart rate range, C equals the mean arterial pressure at the midpoint between the minimum and maximum heart rate (or BP_{50}), and D is the slope coefficient]. Maximum gain was calculated by dividing the heart rate

range by four times the slope coefficient (Brooks et al., 1997; Quesnell and Brooks, 1997; Brooks et al., 2001b; Brooks et al., 2002). Due to the exponential nature of changes in baroreflex gain and therefore the high variability in this parameter, the log of the maximum gain was used for statistical analysis.

Statistics

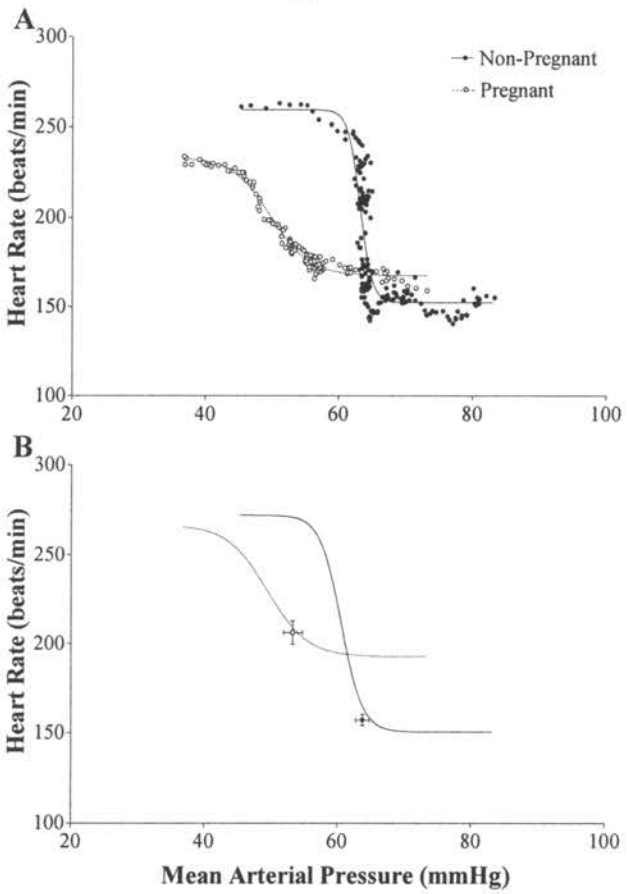
Basal mean arterial pressures, heart rates and curve fitting parameters were compared between groups using ANOVA for repeated measures and the post-hoc Bonferroni test. Differences in mRNA and protein levels of nNOS in different brain nuclei and basal mean arterial pressures, heart rates and curve fitting parameters between non-pregnant and pregnant rabbits were assessed using Student's t-test. In the figures, the sigmoidal curves derived from the averaged parameters are shown along with basal points ± mean standard error.

RESULTS

Effects of pregnancy

As previously reported (Brooks et al., 1995), pregnancy decreased basal mean arterial pressure and the operating pressure for the baroreflex (decreased BP_{50}), decreased baroreflex gain and increased baroreflex-induced minimum and basal heart rates (Figure 4, Tables 1-3).

Figure 4



Pregnancy decreases baroreflex gain. A) Representative curve from one rabbit in the non-pregnant and pregnant states. B) Mean heart rate baroreflex curves from rabbits in the non-pregnant (n=18) and pregnant (n=16) states. Pregnancy had no effect on baroreflex-induced maximum heart rate (non-pregnant 272 ± 4 bpm, pregnant 267 ± 7 bpm) but increased baroreflex-induced minimum heart rate from 150 ± 3 to 193 ± 6 bpm and basal heart rate from 157 ± 3 to 206 ± 7 bpm ($p<0.05$). In addition, pregnancy decreased BP_{50} from 61 ± 1 to 50 ± 1 mmHg, basal mean arterial pressure from 64 ± 1 to 53 ± 1 mmHg, and maximum baroreflex gain from 23.3 ± 3.6 to 7.1 ± 0.9 bpm/mmHg ($p<0.05$).

Effects of NOS blockade

Blockade of NOS did not increase baroreflex gain in either pregnant or non-pregnant rabbits (Table 1, Figure 5). In both states L-NNA increased basal mean arterial pressure and the operating pressure for the baroreflex, and decreased baroreflex-induced minimum and basal heart rate (Table 1, Figure 5). In the pregnant animals, L-NNA also decreased baroreflex-induced maximum heart rate (Table 1, Figure 5B).

Table 1

Baroreflex and basal parameters before and after NOS blockade in non-pregnant and pregnant rabbits.

	Non-Pregnant		Pregnant	
	Control	L-NNA	Control	L-NNA
Maximum Heart Rate (bpm)	278 ± 7	266 ± 10	281 ± 4	257 ± 6$^{\#}$
Minimum Heart Rate (bpm)	157 ± 5	110 ± 8$^{\#}$	204 ± 5*	138 ± 7* $^{\#}$
BP_{50} (mmHg)	61 ± 2	70 ± 4$^{\#}$	50 ± 3*	66 ± 2$^{\#}$
Maximum Gain (bpm/mmHg)	14.2 ± 2.3	16.1 ± 1.4	7.7 ± 1.9*	8.3 ± 2.5*
Basal Mean Arterial Pressure (mmHg)	64 ± 2	78 ± 5$^{\#}$	54 ± 3*	72 ± 3$^{\#}$
Basal heart rate (bpm)	168 ± 4	119 ± 3$^{\#}$	217 ± 7*	164 ± 13* $^{\#}$

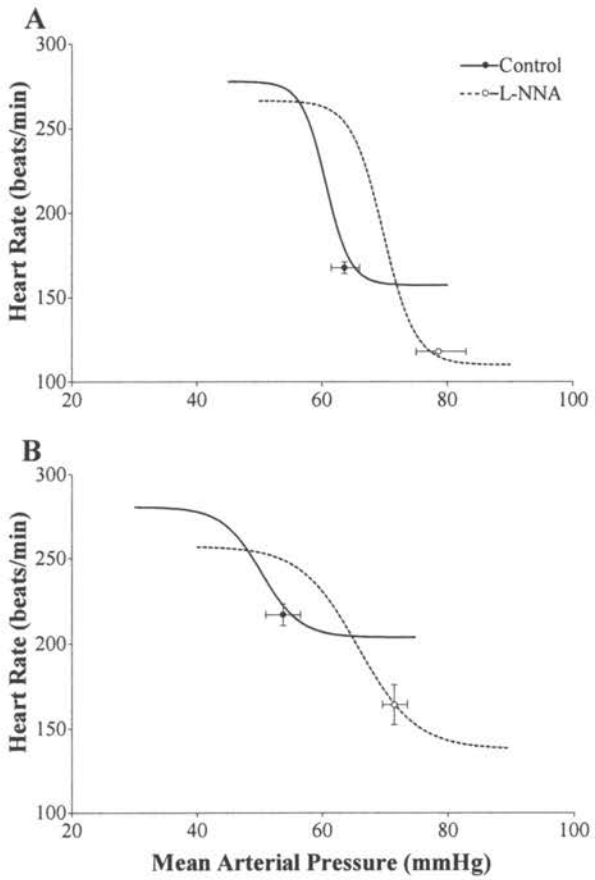
*$p<0.05$ different from corresponding non-pregnant.

$^{\#}p<0.05$ different from corresponding control

Effects of AT_1 angiotensin II receptor and NOS blockade

Treatment with L158-809, an inhibitor of AT_1 receptors, alone or in combination with NOS blockade did not alter baroreflex gain in either pregnant or non-pregnant rabbits (Table 2, Figure 6). Blockade of AT_1 receptors decreased basal blood pressure and the operating pressures of the baroreflex in both non-pregnant and

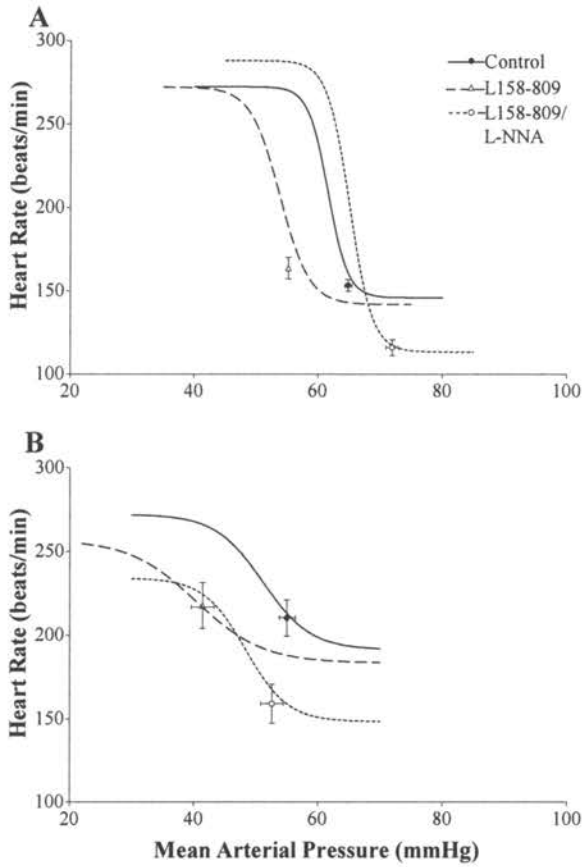
Figure 5



Blockade of NOS does not affect baroreflex gain. Mean heart rate baroreflex curves before and after blockade of NOS with L-NNA in the same non-pregnant (A) and pregnant (B) rabbits. Points represent basal blood pressures and heart rates ± standard error mean (n=6).

pregnant rabbits (Table 2, Figure 6). In addition, with the combined blockade of both AT_1 receptors and NOS, baroreflex-induced minimum and basal heart rates decreased in non-pregnant and pregnant rabbits (Table 2) similar to the effect of NOS blockade

Figure 6



Blockade of AT_1 angiotensin II receptors does not alter the effect of NOS blockade on baroreflex gain. Mean heart rate baroreflex curves in unblocked, AT_1 angiotensin II receptor blocked, and AT_1 angiotensin II receptor / NOS blocked non-pregnant (A, n=8) and pregnant (B, n=6) rabbits. AT_1 angiotensin II receptors were blocked with L158-809 and NOS was blocked with L-NNA. Points represent basal blood pressures and heart rates ± standard error mean.

alone. Also similar to the effect of NOS blockade alone, the combined blockade reduced the baroreflex-induced maximum heart rate in pregnant but not non-pregnant animals (Table 2, Figure 6). No significant differences in any of these parameters existed between rabbits treated with L-NNA alone or treated with both L-NNA and L158-809.

A significant difference ($p<0.05$), however, was noted in basal mean arterial pressure and BP_{50} between pregnant rabbits treated with L-NNA alone and those treated with both L-NNA and L158-809 (Table 2, Figure 6B). Blockade of NOS during L158-809 administration increased basal mean arterial pressure above levels in non-pregnant and pregnant rabbits treated with the AT_1 receptor antagonist alone. However, this increase was reduced in pregnant animals (11 ± 0.4 mmHg pregnant, compared to 16 ± 2 mmHg nonpregnant; $p<0.05$) such that basal mean arterial pressure after combined blockade did not differ from the control state in pregnant rabbits, whereas in non-pregnant rabbits combined blockade increased basal mean arterial pressure above control values.

Table 2

Baroreflex and basal parameters before and after blockade of angiotensin II AT_1 receptors, with and without NOS blockade, in non-pregnant and pregnant rabbits.

	Non-Pregnant			Pregnant		
	Control	L158-809	L158-809/ L-NNA	Control	L158-809	L158-809/ L-NNA
Maximum Heart Rate (bpm)	272 ± 8	272 ± 8	288 ± 6	267 ± 14	254 ± 9	239 ± 10* #
Minimum Heart Rate (bpm)	146 ± 4	142 ± 5	113 ± 5#	186 ± 10*	176 ± 10*	148 ± 8* #
BP_{50} (mmHg)	62 ± 1	54 ± 2#	65 ± 2	50 ± 2*	41 ± 3* #	49 ± 4*
Maximum Gain (bpm/mmHg)	22.2 ± 3.7	15.3 ± 3.1	26.6 ± 3.7	6.5 ± 1.5*	4.7 ± 1.0*	11.7 ± 4.6*
Basal Mean Arterial Pressure (mmHg)	65 ± 1	55 ± 1#	72 ± 1#	54 ± 2*	42 ± 3* #	53 ± 3*
Basal Heart Rate (bpm)	153 ± 4	164 ± 7	118 ± 4#	204 ± 12*	212 ± 14*	160 ± 10* #

*p<0.05 different from corresponding non-pregnant.

#p<0.05 different from corresponding control

Effects of NOS blockade during angiotensin II infusion

Infusion of angiotensin II did not substantially alter the effects of NOS blockade (Table 3, Figure 7). Angiotensin II slightly increased basal mean arterial pressure and the operating pressures of the baroreflex in both non-pregnant and pregnant rabbits, but this reached significance only when the animals were pregnant (Table 3, Figure 7). With the combined administration of angiotensin II and L-NNA, basal mean arterial pressure rose, baroreflex-induced minimum and basal heart rates fell and the baroreflex

operated at higher pressures in both non-pregnant and pregnant rabbits, similar to the effect of L-NNA alone (Table 3, Figure 7). Importantly, baroreflex gain was unaffected (Table 3, Figure 7).

Table 3

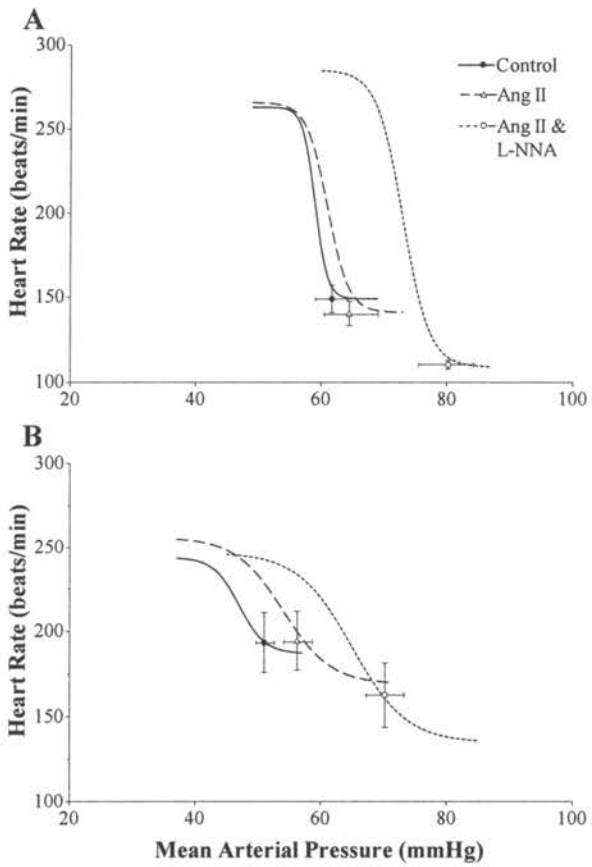
Baroreflex and basal parameters before and after angiotensin II infusion, with or without NOS blockade, in non-pregnant and pregnant rabbits.

	Non-Pregnant			Pregnant		
	Control	Ang	Ang/ L-NNA	Control	Ang	Ang/ L-NNA
Maximum Heart rate (bpm)	263 ± 8	266 ± 6	285 ± 5	244 ± 12	256 ± 7	247 ± 13*
Minimum Heart rate (bpm)	149 ± 10	141 ± 7	109 ± 4#	187 ± 22*	169 ± 21	135 ± 7* #
BP_{50} (mmHg)	59 ± 3	61 ± 3	73 ± 4#	47 ± 1*	54 ± 2* #	65 ± 5* #
Maximum Gain (bpm/mmHg)	37.3 ± 14.1	19.1 ± 2.8	25.5 ± 8.8	7.2 ± 1.7*	5.7 ± 1.0*	8.4 ± 2.4*
Basal Mean Arterial Pressure (mmHg)	62 ± 2	65 ± 4	80 ± 5#	51 ± 2*	56 ± 3* #	70 ± 3* #
Basal Heart Rate (bpm)	149 ± 9	141 ± 8	110 ± 3#	194 ± 20*	195 ± 20*	163 ± 22* #

*$p<0.05$ different from corresponding non-pregnant.

#$p<0.05$ different from corresponding control

Figure 7



Angiotensin II infusion does not alter the effect of NOS blockade on baroreflex gain. Mean heart rate baroreflex curves in control, angiotensin II infused, and angiotensin II infused / NOS blocked non-pregnant (A) and pregnant (B) rabbits. NOS was blocked with L-NNA. Points represent basal blood pressures and heart rates ± standard error mean (n=4).

Levels of nNOS protein and mRNA in cardiovascular centers of the brain

When compared to values in paired (n=4) non-pregnant rabbits (Table 4) neuronal NOS mRNA was not altered by pregnancy in the NTS (129 ± 25% of non-

pregnant), the CVLM (74 ± 6% of non-pregnant), the RVLM (98 ± 17% of non-pregnant), or the PVN (148 ± 46% of non-pregnant). The protein levels of nNOS were also unaltered by pregnancy in the NTS (154 ± 69% of non-pregnant), the CVLM (101 ± 10% of non-pregnant), the RVLM (122 ± 54% of non-pregnant), or the PVN (112 ± 47% of non-pregnant).

Table 4

Levels of nNOS mRNA and protein in cardiovascular regulating centers in the brains of non-pregnant and pregnant rabbits.

	mRNA (nNOS/β-actin)		Protein (nNOS/tubulin)	
	Non-Pregnant	Pregnant	Non-Pregnant	Pregnant
NTS	0.10 ± 0.02	0.12 ± 0.02	1.62 ± 0.33	2.21 ± 0.72
CVLM	0.12 ± 0.03	0.10 ± 0.03	1.75 ± 0.49	1.70 ± 0.43
RVLM	0.13 ± 0.02	0.13 ± 0.04	1.81 ± 0.38	1.74 ± 0.58
PVN	0.10 ± 0.03	0.13 ± 0.02	1.97 ± 0.65	2.05 ± 0.82

DISCUSSION

This study tested the hypothesis that nitric oxide contributes to the decreased baroreflex gain of pregnancy, and that angiotensin II modulates this action. The novel findings of this study are that 1) blockade of nitric oxide with or without AT_1 receptor blockade or angiotensin II infusion does not alter baroreflex gain in pregnant or non-pregnant rabbits, 2) NOS blockade decreases baroreflex-induced maximum heart rate only in pregnant rabbits, 3) in pregnant rabbits after blockade of AT_1 receptors, the

increase in mean arterial pressure following NOS blockade is reduced, and 4) pregnancy does not alter nNOS levels in the PVN, NTS, CVLM or RVLM of rabbits. Based on these findings I conclude that 1) neither nitric oxide nor angiotensin II, alone or in combination, contributes to the impaired baroreflex gain of pregnant rabbits, 2) alterations in nNOS levels in the PVN, NTS, CVLM or RVLM are not responsible for the decrease in baroreflex gain during pregnancy in rabbits, 3) in pregnant rabbits only, nitric oxide supports baroreflex-induced maximum heart rate and 4) in the pregnant rabbit, nitric oxide exerts part of its vasodilatory actions through inhibition of AT_1 receptors.

As in previous reports, near the end of pregnancy in rabbits, basal mean arterial pressure is decreased (due to reduced vascular resistance) and basal heart rate is increased [see (Heesch and Rogers, 1995; Brooks et al., 1995) for reviews]. In response to the fall in blood pressure, the baroreflex curves shift to the left (BP_{50} is reduced), presumably due to baroreflex resetting (Chapleau et al., 1989). This resetting allows the animal to better defend the new basal blood pressure, and it is likely due to adaptation of the peripheral baroreceptors as well as alterations in central neurons involved in baroreflex control (Chapleau et al., 1989). In addition, baroreflex gain is reduced, and although it has been intensely studied, the cause for this fall in gain has remained elusive.

Because nitric oxide increases during pregnancy (McLaughlin and Conrad, 1995; Sladek et al., 1997; Weiner and Thompson, 1997) and can inhibit baroreflex gain (Liu et al., 1996; Matsumura et al., 1998a), I hypothesized that this hormone is involved in the decreased gain of pregnancy. Similar to previous reports in male rabbits and non-

pregnant and pregnant female rabbits (Liu et al., 1996; Brooks et al., 2001a), NOS blockade increased basal mean arterial pressure and the operating pressure for the baroreflex, and decreased basal and baroreflex-induced minimum heart rate, indicating that endogenous nitric oxide is vasodilatory and supports basal and baroreflex-induced minimum heart rates. A novel finding is that in pregnant but not in non-pregnant rabbits, NOS blockade decreased the baroreflex-induced maximum heart rate, suggesting that nitric oxide acts during pregnancy to prevent decreases in baroreflex-induced maximum heart rate. Therefore, one role for the elevated nitric oxide levels during pregnancy is to avert a further reduced ability to respond to hypotensive challenges.

However, contrary to my hypothesis and the work of Liu et al. (Liu et al., 1996) in conscious male rabbits, this study found no effect of systemic NOS blockade on baroreflex gain in either non-pregnant or pregnant rabbits. This lack of effect is similar to that observed by Chiu and Reid (Chiu and Reid, 1995) also in conscious rabbits. Conflicting results have also been observed in rats in which systemic NOS blockade can either increase baroreflex gain (Minami et al., 1995a; Brady et al., 2002), or cause no change in this parameter (Fujisawa et al., 1999a; Lacchini et al., 2001a). The cause for the discrepancies between studies is unclear, but it may relate to the stress state or the tonic nitric oxide levels of the animal. In a recent study (Daubert and Brooks, 2004b; Appendix), we found that acute psychological stress decreases baroreflex gain through a nitric oxide-mediated mechanism. Similarly, at low levels, nitric oxide either increases or does not affect baroreflex gain in the NTS (Harada et al., 1993; Zanzinger et al., 1995a; Lo et al., 1996; Pontieri et al., 1998; Paton et al., 2001b; Talman and Dragon,

2004; Dias et al., 2005); however, when nitric oxide levels are elevated by increasing angiotensin II (Paton et al., 2003), nitric oxide decreases baroreflex gain (Paton et al., 2001b), suggesting that the actions of nitric oxide are dependant of the levels of angiotensin II. In unstressed animals with low basal nitric oxide and angiotensin II levels, endogenous nitric oxide does not appear to contribute to baroreflex gain.

Because nitric oxide interacts with angiotensin II in the control of baroreflex gain (Kumagai et al., 1993; Paton et al., 2001b), I hypothesized that the nitric oxide-mediated decrease in baroreflex gain during pregnancy would be dependent on the elevations in angiotensin II. However, pretreatment with an AT_1 receptor blocker did not alter the subsequent effect of NOS blockade on gain in either pregnant or non-pregnant rabbits. Moreover, infusion of angiotensin II to maintain elevated levels also did not influence the effects of NOS blockade. Collectively these data suggest that an interaction between angiotensin II and nitric oxide is not important in the gain of baroreflex control of heart rate in normal and pregnant conscious rabbits.

I also observed that AT_1 receptor blockade alone did not affect baroreflex gain in non-pregnant or pregnant rabbits, in agreement with previous studies (O'Hagan et al., 2001; Brooks et al., 2001b). The only effect of AT_1 receptor blockade was to decrease basal mean arterial pressure and the operating pressure of the baroreflex in both non-pregnant and pregnant animals, suggesting that endogenous angiotensin II supports mean arterial pressure and increases the operating pressure of the baroreflex through the AT_1 receptor.

After AT_1 receptor blockade, the ability of NOS blockade to increase mean arterial pressure was reduced in pregnant rabbits compared to non-pregnant rabbits.

This effect is not surprising since nitric oxide has been shown to act during pregnancy to attenuate the pressor effects of angiotensin II (Molnar and Hertelendy, 1992; Tresham et al., 1996). Angiotensin II, which is increased during pregnancy (Skinner et al., 1972; Brooks and Keil, 1994; Brooks et al., 1998), can stimulate nitric oxide production in vascular endothelial cells (Pueyo et al., 1998; Patzak et al., 2004; Patzak et al., 2005). Subsequently, nitric oxide counteracts the effects of angiotensin II: it limits the vasoconstrictive actions of angiotensin II by causing vasodilation (Boulanger et al., 1995; Patzak et al., 2005). Therefore one way in which L-NNA increases mean arterial pressure during pregnancy is by increasing the pressor effects of angiotensin II. When angiotensin II is no longer exerting a pressor effect, then NOS blockade is not as effective at increasing mean arterial pressure.

Consistent with my finding that NOS blockade did not alter baroreflex gain in either non-pregnant or pregnant rabbits, we found no significant difference in either gene expression or protein levels of nNOS in tissue punches of the PVN, NTS, CVLM or RVLM between rabbits in these two states. This is in contrast to previous studies which demonstrated elevations in nNOS levels in the whole hypothalamus of pregnant rats (Xu et al., 1996) and both decreases (Okere and Higuchi, 1996) and increases (Woodside and Amir, 1996) in nitric oxide activity in the magnocellular region of the PVN, which is responsible for oxytocin and vasopressin production and release. In addition, Woodside and Amir (Woodside and Amir, 1996) found higher levels of nitric oxide activity in the parvocellular region of the PVN of the pregnant rat. Thus, my failure to detect significant differences may be due to opposing changes within the same nucleus (Okere and Higuchi, 1996; Woodside and Amir, 1996). In addition, other

isoforms of the NOS enzyme (eNOS or inducible NOS) may be differentially regulated during pregnancy which could explain the differences between the results of this study and previous reports (Okere and Higuchi, 1996; Woodside and Amir, 1996). Therefore, while it remains possible that opposing changes in NOS and nitric oxide are induced by pregnancy, I conclude that net increases in nitric oxide production, and regional changes in nNOS levels in the PVN, NTS, CVLM or RVLM, are not responsible for the decreased baroreflex gain.

In summary, near term pregnant rabbits exhibit decreases in basal mean arterial pressure and baroreflex gain, increases in basal heart rate and baroreflex-induced minimum heart rate, and lower baroreflex operating pressures. Nitric oxide is without effect on baroreflex gain in either the non-pregnant and pregnant rabbit. Neither blockade of angiotensin II AT_1 receptors nor angiotensin II infusion significantly altered the responses to NOS blockade. Consistent with these results, we found that levels of nNOS mRNA and protein are also unaltered by pregnancy in the PVN, NTS, CVLM and RVLM. Taken together these results suggest that the decrease in baroreflex gain during pregnancy is not due to the net effects of nitric oxide, changes in nNOS levels in the regions of the brain involved in cardiovascular control, or an interaction between nitric oxide and angiotensin II.

CHAPTER 3

THE ROLE OF INSULIN RESISTANCE IN THE REDUCED BAROREFLEX GAIN OF PREGNANCY

ABSTRACT

Pregnancy depresses baroreflex gain, but the mechanism has not been identified. Insulin resistance, which is associated with reduced transport of insulin into the brain, is a consistent feature of many pathophysiological conditions associated with impaired baroreflex gain, including pregnancy. Therefore, I tested the novel hypothesis that the pregnancy-induced impairment in baroreflex gain is due to insulin resistance and reduced brain insulin. Here I report that insulin sensitivity and baroreflex gain are strongly correlated in non-pregnant and term pregnant rabbits (r^2=0.59). The decrease in insulin sensitivity and in baroreflex gain exhibited similar time-courses throughout pregnancy, reaching significantly lower levels at three weeks of gestation and remaining reduced at four weeks (term is thirty-one days). Treatment of rabbits with the insulin-sensitizing drug rosiglitazone throughout pregnancy almost completely normalized baroreflex gain. Finally, pregnancy significantly lowered cerebrospinal fluid insulin concentrations. These data are the first to identify insulin resistance as a mechanism

underlying pregnancy-induced baroreflex impairment and suggest, for the first time in any condition, that decreased brain insulin concentrations are the link between reductions in peripheral insulin resistance and baroreflex gain.

INTRODUCTION

The cardiovascular system undergoes considerable transformation during pregnancy [for reviews, see (Hart et al., 1985; Heesch and Rogers, 1995; Brooks et al., 1995; Monga and Creasy, 1999; Thornburg et al., 2000)]. Many of these adaptations, such as the increases in blood volume and cardiac output, are clearly beneficial for the fetus. However, the progressive baroreflex impairment that accompanies pregnancy (Quesnell and Brooks, 1997) poses significant risk for the mother. Decreased baroreflex gain may, by reducing the ability to maintain blood pressure in the face of challenges, underlie the increased incidence of orthostatic hypotension in pregnant women (Easterling et al., 1988), as well as the decreased tolerance of pregnant animals to hemorrhage (Brooks et al., 1998). Importantly, 17% of human maternal mortality in the United States between 1990 and 1999 was related to hemorrhage (Chang et al., 2003). Despite clear clinical significance, the mechanism for the suppression of baroreflex gain remains unclear.

Multiple conditions other than pregnancy are associated with decreases in baroreflex gain, including obesity (Grassi et al., 1995), hypertension (Grassi, 2004), congestive heart failure (Chen et al., 1992), type II diabetes mellitus (Pikkujamsa et al., 1998), and aging (Laitinen et al., 1998). Pregnancy, as well as of each of these pathophysiological states, is characterized by a reduction in insulin sensitivity

(Ferrannini et al., 1997; Ciampelli et al., 1998; Coats et al., 2000; Ryan, 2000; Frontoni et al., 2005). Interestingly, a link between insulin resistance and suppressed baroreflex gain is supported by the finding that weight loss in obese subjects improves baroreflex control of muscle sympathetic nerve activity as it increases insulin sensitivity (Grassi et al., 1998). However, whether insulin resistance contributes to baroreflex impairment in pregnancy is unknown. Moreover, the mechanism by which reductions in peripheral insulin sensitivity could decrease baroreflex gain has not been identified in any condition.

Previous research indicates that the mechanism underlying the depression of baroreflex gain during pregnancy is located within the central nervous system (Brooks et al., 1997; Laiprasert et al., 2001). Moreover, in the case of two insulin resistant states, obesity (Ferrannini et al., 1997) and Alzheimer's disease (Kuusisto et al., 1997), the transport of insulin into the brain is reduced, resulting in decreased brain insulin concentrations (Stein et al., 1987; Craft et al., 1998; Kaiyala et al., 2000). Because insulin can act in the brain to enhance baroreflex gain (Okada and Bunag, 1994; Man et al., 1998), decreased insulin concentrations may underlie an impairment in baroreflex gain. However, whether declining brain insulin levels is a critical determinant of the decreased baroreflex gain during pregnancy, or any condition, is unknown.

Therefore, I sought to establish an association between decreased insulin sensitivity and reduced baroreflex gain during pregnancy and to test the hypothesis that a reduction in brain insulin concentration could contribute to the impaired baroreflex gain during pregnancy. In these studies I determined: 1) if the suppression of baroreflex gain during pregnancy correlates with the reduction in insulin sensitivity, 2) if the time-

course for the decline in baroreflex gain matches the time-course for development of insulin resistance during pregnancy, 3) if normalization of insulin sensitivity during pregnancy by administration of the insulin sensitizing drug, rosiglitazone, restores baroreflex gain, and 4) if pregnancy is associated with reduced cerebrospinal fluid insulin concentrations.

METHODS

Female New Zealand White rabbits (Western Oregon Rabbit; Philomath, OR) weighing 3.8 ± 0.1 kg (non-pregnant, n=28) were used for these experiments. The rabbits were received when they were fourteen weeks old and were allowed a minimum of five days to acclimate to the new environment before any surgery was performed. All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee.

Surgical preparation

Surgery was performed to implant non-occluding abdominal aortic and vena caval catheters as previously described (Gronan et al., 1983) for the measurement of mean arterial pressure and heart rate and the infusion of drugs, respectively. Briefly, the animals were initially anesthetized with a cocktail containing ketamine (58.8 mg/kg), xylazine (5.9 mg/kg), and acepromazine (1.2 mg/kg) administered subcutaneously (s.c.). The rabbits were then intubated and ventilated with 100% oxygen throughout the surgery. A surgical plane of anesthesia was maintained by

administration of ketamine intravenously (i.v.) as needed. A midline abdominal incision was made and polyethylene catheters with silastic tips were implanted non-occlusively in the abdominal aorta (one) and vena cava (two). The catheters were tunneled subcutaneously from the abdominal cavity, and exited at the nape of the neck. The catheters were flushed immediately after surgery, and then three times weekly with sterile 0.9% saline, and filled with heparin (1,000 U/ml) to maintain patency. Rabbits were allowed at least two weeks to recover from surgery before any experiments were performed. During this time the rabbits were conditioned to the black Plexiglas box used during experiments.

In order to determine if insulin levels in the cerebrospinal fluid are decreased during pregnancy, I performed surgery to implant cannulae into the cisterna magna for the collection of cerebrospinal fluid using a technique modified from Vistelle et al. (Vistelle et al., 1994). Animals were sedated with 150 mg ketamine, further anesthetized with isoflurane (5% in oxygen), and intubated. Rabbits were then maintained on 2% isoflurane in oxygen throughout the surgery. With the rabbit's head immobilized in a stereotaxic frame, a midsagittal incision was made. The dorsal surface of the parietal, intraparietal, and occipital bones was cleared of muscle and periosteum. Using dental burrs, a hole was made in the occipital bone. A small incision was made in the dura mater and polytetrafluorethylene Teflon® tubing (cannula, 0.022" inner diameter, 0.010" wall thickness, Zeus Industrial Products, Orangeburg, SC) was inserted a distance of fourteen millimeters from the surface of the dura mater so that the distal end lay in the cisterna magna. A bluntly cut twenty-three-gauge needle was glued into the end of the cannula, and a two centimeter length, one centimeter diameter open

plastic cylinder (made from the distal end of a 3cc syringe) was then placed around the cannula with the needle hub to help protect and secure the cannula. A screw was placed in the skull and dental cement filled the cylinder, closed the hole in the skull, and secured the cannula to the skull. The muscle and skin were then sutured closed around the plastic cylinder. A stopcock plug screwed into the needle hub capped the cannula. To maintain patency, 500 to 1000 μl of cerebrospinal fluid were collected daily from the cannula and the cannula was filled with the antibiotic enrofloxacin (7.6 mg/ml). No samples were used for assay until a least one week after surgery and until at least two days after the cerebrospinal fluid was clear (not clouded with blood). Microscopic analysis from three rabbits at this time demonstrated zero red blood cells per ten microliters of cerebrospinal fluid.

For the vascular catheter surgery rabbits were given an intramuscular injection of enrofloxacin (22.7 mg) just before the surgery and an i.v. injection of this antibiotic for the four days following surgery (22.7 mg/day). For the cisterna magna cannula surgery the rabbits were given s.c. injections of chloramphenicol (100 mg) just before the surgery and the following day to prevent infection. All animals also received buprenorphine hydrochloride (0.09 mg, s.c.) two to three hours after each surgery, and again the following day, to minimize pain.

Measurement of baroreflex function

On the experimental day, the rabbits were placed in the experimental box and allowed approximately thirty minutes to acclimate. Mean arterial pressure and heart rate were measured continuously via the aortic catheter using a Statham pressure

transducer, a Grass tachometer, and Grass polygraph. To determine the baroreflex relationship between blood pressure and heart rate, arterial pressure was first lowered by intravenous infusion of increasing doses of nitroprusside (1.5, 3, 6, 12, 24, 48, 63 μg/kg/min, nonpregnant; 0.8, 1.5, 3, 6, 12, 24, 48, 63 μg/kg[Error! Bookmark not defined.]/min, pregnant; 37.5 μg/kg/ml in 5% dextrose in water vehicle). After a thirty to sixty minute rest period, arterial pressure was then raised by intravenous infusion of increasing doses of phenylephrine (0.5, 1, 2, 4 μg/kg/min; 12.5 μg/kg/ml in 5% dextrose in water vehicle). Doses of nitroprusside and phenylephrine were increased by increasing the flow rate. Each dose was infused until blood pressure and HR stabilized, about two to eight minutes; usually about five to ten milliliters of fluid were infused for each nitroprusside or phenylephrine segment of the reflex curve.

Measurement of insulin sensitivity

On the experimental day, after a twelve to eighteen hour fast, the rabbits were placed in the experimental box and allowed approximately thirty minutes to settle. Insulin sensitivity was then determined using the hyperinsulinemic-euglycemic method, as described by DeFronzo et al. (DeFronzo et al., 1979), usually the day before or the day after measurement of baroreflex function. Heparin (1000 U/ml) was infused into the arterial catheter at a rate of one milliliter per hour to maintain patency. Five blood samples were collected from the arterial catheter over at least thirty minutes. Blood was either spun down and the plasma analyzed on a Beckman Glucose Analyzer II, or the blood was analyzed on a Freestyle Flash handheld blood glucose monitor. Insulin sensitivity was the same (within 1%) in nine animals whether the Beckman or the

Freestyle was used. The highest and the lowest values for plasma or blood glucose were dropped and the range of euglycemia was determined from the remaining three values. A priming dose of insulin in normal saline (21 mU/kg total) was then infused into a venous catheter over ten minutes followed by a constant infusion of insulin (1.2 mU/kg/min with a flow rate of 21 µl/min). Dextrose (95 mg/kg/ml in water) was infused in the other venous catheter starting at a rate of 2 mg/kg/min four minutes after the start of the priming insulin dose. After the initial insulin dose, the dextrose infusion was increased to 3 mg/kg/min. Plasma or blood glucose values were then measured every ten to fifteen minutes and the dextrose infusion rate was altered until plasma and/or blood glucose values where back in the euglycemic range. Two to three samples were collected over the next twenty to forty-five minutes to ensure that the euglycemia was at steady-state. Steady-state euglycemia was usually reached within three to four hours of the start of the insulin infusion.

Measurements of insulin concentrations

After a twelve to eighteen hour fast, three to six milliliters of heparized blood (less than six units of heparin per ml of blood) was collected from the ear artery. The blood was then spun down and the plasma was collected, frozen, and stored at –20°C until assayed. Cerebrospinal fluid was also collected after a twelve to eighteen hour fast from the cisterna magna cannula and frozen at -20°C. Because of the low concentration of insulin in the cerebrospinal fluid (sometimes below 0.02 ng/ml) these samples were concentrated before assay. To do this, cerebrospinal fluid collected from the same rabbit over four to six (non-pregnant) or two (pregnant) adjacent days (1ml/day) was

pooled. This pooled cerebrospinal fluid was then concentrated using Centricon Ultracel YM-3 Centrifugal Filter Devices (Millipore, Bedford, MA). After concentrating, the samples were again frozen at -20°C and stored until shipment to the Oregon National Primate Research Center, where the assay was performed.

Insulin levels were quantified using the Linco Research sensitive rat radioimmunoassay kit, which uses a guinea pig anti-rat insulin antibody and can specifically detect rat, pig, sheep, hamster, and mouse insulin with 100% recovery. Validation of the assay for rabbit plasma included tests for parallelism; halving the sample volume reduced the measured insulin level by 53%. In addition, recovery of rat insulin spiked into samples was 85% in plasma and 98% in cerebrospinal fluid. Non-pregnant and pregnant plasma and cerebrospinal fluid samples from the same rabbit were assayed at the same time and the intra- and inter-assay coefficients averaged 8.4%.

Protocols

Protocol 1) Are insulin sensitivity and baroreflex gain correlated? Insulin sensitivity and baroreflex function were measured in twenty-one non-pregnant and fourteen near term pregnant rabbits (day twenty-nine to thirty of pregnancy), and the linear relationship between these variables was determined.

Protocol 2) Does the time-course for the decrease in baroreflex gain during pregnancy match the time-course for the decrease in insulin sensitivity? In five rabbits, insulin sensitivity and baroreflex gain were measured before pregnancy and at days fifteen and sixteen (two weeks), twenty-two and twenty-three (three weeks), and

twenty-nine and thirty (four weeks) of gestation, to determine at which point baroreflex gain and insulin sensitivity significantly decrease.

Protocol 3) Does preventing the decrease in insulin sensitivity during pregnancy prevent the decrease in baroreflex gain? Insulin sensitivity and baroreflex gain were determined in five rabbits when they were non-pregnant, at days twenty-nine and thirty during a control first pregnancy, and at days twenty-nine and thirty of a second pregnancy during which the rabbits were continuously treated with the insulin-sensitizing drug, rosiglitazone. Rosiglitazone (14 mg/day) was administered orally once daily in five milliliters of yogurt. Four rabbits were also given five ml of yogurt without rosiglitazone daily during their first pregnancy to determine if yogurt alone had any effects on insulin sensitivity or baroreflex gain.

Five rabbits were studied to determine the effects of rosiglitazone in the non-pregnant state. In all of these rabbits, insulin sensitivity and baroreflex gain were first measured in the non-pregnant control state. Two of the animals remained virgins, were treated for twenty-nine to thirty days with rosiglitazone, and then insulin sensitivity and baroreflex gain were again tested. One of the rabbits began receiving rosiglitazone treatment nine days after delivery of her first litter and insulin sensitivity and baroreflex gain where again measured at the end of the twenty-nine to thirty days of treatment. Two of the rabbits were given rosiglitazone throughout their second pregnancy and remained on rosiglitazone for two weeks after delivery. There were no differences in responses between rabbits so these data were combined.

Protocol 4) Do cerebrospinal fluid insulin concentrations decrease during pregnancy? Cerebrospinal fluid (n = 7) and plasma insulin concentrations (n = 6) were

measured in the same animals in the non-pregnant and near term pregnant states to determine if pregnancy decreases brain insulin concentrations.

Data analysis

The sigmoidal baroreflex relationships between mean arterial pressure and heart rate generated in each experiment were fitted and compared using the Boltzmann sigmoidal equation [Heart Rate=A+B/1+ $e^{(C\text{-mean arterial pressure})/D}$, where A equals the minimum heart rate, B equals the heart rate range, C equals the mean arterial pressure at the midpoint between the minimum and maximum heart rate (or BP_{50}), and D is the slope coefficient]. Maximum gain was calculated by dividing the heart rate range by four times the slope coefficient. Because of the exponential nature of baroreflex gain, the log of this parameter was used for linear regression analyses.

Basal mean arterial pressure and heart rate, curve fitting parameters, insulin sensitivity, and cerebrospinal fluid and plasma insulin concentrations were compared between groups using the Student's t-test in protocol 1, a one-way ANOVA for repeated measures and the post hoc Bonferroni correction test in protocols 2 and 3 (non-pregnant, control pregnant, rosiglitazone-treated pregnant), and a paired t-test in protocol 3 (non-pregnant, rosiglitazone-treated non-pregnant) and 4. A least-squares linear regression was used to determine the correlation coefficient between baroreflex gain and insulin sensitivity in protocols 1 and 2. All data are reported as mean ± standard error mean.

RESULTS

Protocol 1) Effects of pregnancy on the baroreflex and insulin sensitivity

My first approach to test the hypothesis that the decrease in baroreflex gain during pregnancy is mediated by the decrease in insulin sensitivity was to determine if these two parameters are correlated in non-pregnant and twenty-nine to thirty day pregnant rabbits (gestation is thirty-one days). Both baroreflex gain (Figure 8A, Table 5) and insulin sensitivity (Table 5), were decreased during pregnancy and these two parameters were correlated in non-pregnant and near term pregnant rabbits (Figure 8B, r^2=0.59). In addition, as previously described (Brooks et al., 1997; Quesnell and Brooks, 1997; Brooks et al., 2001b; Brooks et al., 2002), pregnancy was characterized by decreased basal mean arterial pressure and baroreflex maximum heart rate,

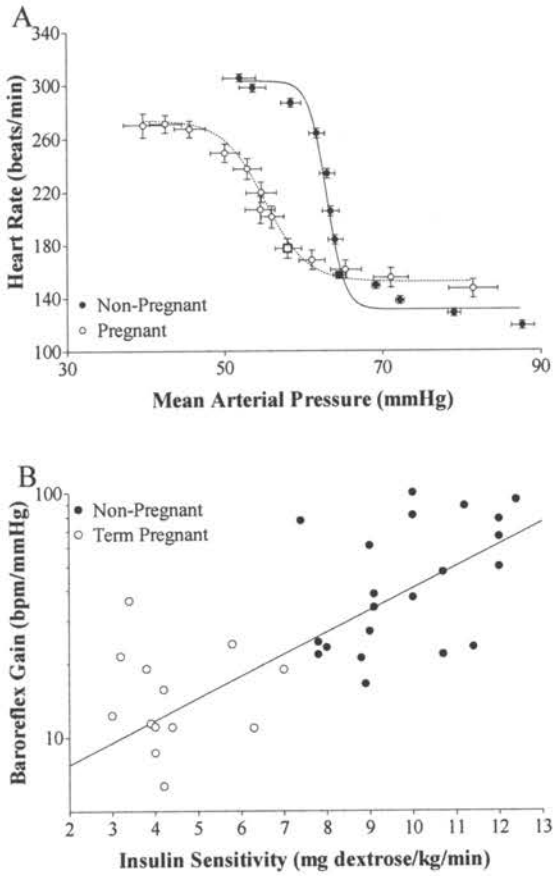
Table 5

Baroreflex and basal parameters and insulin sensitivity in non-pregnant and near term pregnant rabbits.

	Non-Pregnant (n=21)	Pregnant (n=14)
Basal Mean Arterial Pressure (mmHg)	65 ± 1	57 ±1 *
Basal Heart Rate (bpm)	157 ± 2	183 ± 7 *
Maximum Heart Rate (bpm)	303 ± 4	276 ± 6 *
Minimum Heart Rate (bpm)	131 ± 3	158 ± 6 *
BP_{50} (mmHg)	63 ± 1	54 ± 1 *
Maximum Gain (bpm/mmHg)	50 ± 1	15 ± 2 *
Insulin Sensitivity (mg dextrose/kg/min)	9.9 ± 0.3	4.5 ± 0.3 *

* Significantly different from Non-Pregnant ($p<0.05$)

Figure 8



Pregnancy decreases baroreflex gain, which is correlated to insulin sensitivity. A) Mean baroreflex curves from non-pregnant (n=21) and twenty-nine to thirty day pregnant rabbits (n=14) from protocol 1. Squares represent basal mean arterial pressure and heart rate. B) Correlation between insulin sensitivity and baroreflex gain in the same rabbits from A. $r^2 = 0.59$.

increased basal and baroreflex minimum heart rate, and a resetting of the baroreflex curve to lower pressure (reduced the BP_{50}, Figure 8A, Table 5).

Protocol 2) Time-course

If the fall in baroreflex gain is associated with a decrease in insulin sensitivity, then the changes in these two variables during pregnancy should exhibit similar time-courses. Baroreflex gain was decreased significantly at three and four weeks of gestation (Figure 9A, Table 6). Insulin sensitivity fell at the same time (Figure 9A, Table 6), and these changes were correlated (Figure 9B, $r^2 = 0.38$). Linear regression analysis of insulin sensitivity and baroreflex gain in individual animals at the four time points yielded correlations coefficients (r^2) ranging from 0.43-0.86 (mean = 0.65 ± 0.8). Other baroreflex and basal cardiovascular parameters were not significantly altered until four weeks of gestation, at which time changes were generally similar to those observed in protocol 1 (Table 6).

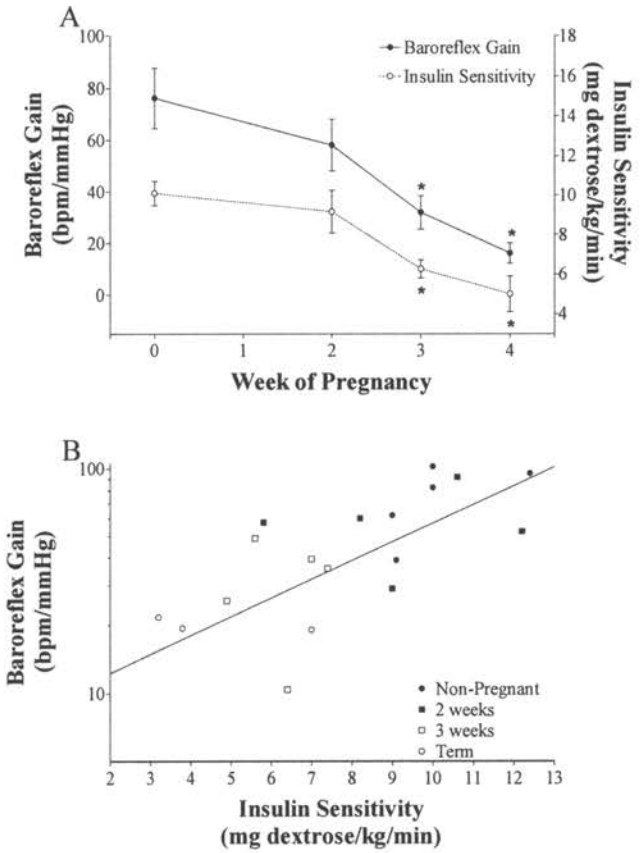
Table 6

Baroreflex and basal parameters and insulin sensitivity in non-pregnant, and two-week, three-week, and four-week pregnant rabbits.

	Non-Pregnant	2 Weeks Pregnant	3 Weeks Pregnant	4 Weeks Pregnant
Basal Mean Arterial Pressure (mmHg)	65 ± 2	64 ± 3	63 ± 2	55 ± 2 *
Basal Heart Rate (bpm)	157 ± 5	160 ± 5	162 ± 12	183 ± 14 *
Maximum Heart Rate (bpm)	298 ± 4	284 ± 10	294 ± 14	282 ± 20
Minimum Heart Rate (bpm)	135 ± 5	135 ± 11	137 ± 12	158 ± 15
BP_{50} (mmHg)	64 ± 2	63 ± 3	61 ± 2	53 ± 2 *
Maximum Gain (bpm/mmHg)	76 ± 12	58 ± 10	32 ± 7 *	16 ± 4 *
Insulin Sensitivity (mg dextrose/kg/min)	10.1 ± 0.6	9.2 ± 1.1	6.3 ± 0.5 *	5.0 ± 0.9 *

* Significantly different from Non-Pregnant ($p<0.05$)

Figure 9



Baroreflex gain and insulin sensitivity decrease with similar time-courses. A) Time-course of changes in baroreflex gain and insulin sensitivity throughout pregnancy in rabbits (n=5). * Signifies significant decrease ($p<0.05$) compared to non-pregnant (week 0). B) Correlation between insulin sensitivity and baroreflex gain in rabbits from A. $r^2 = 0.38$.

Protocol 3) Effects of rosiglitazone treatment

If the decrease in insulin sensitivity is coupled to the impaired baroreflex gain during pregnancy, then improving insulin sensitivity should normalize baroreflex gain. Treatment of rabbits with the insulin-sensitizing drug, rosiglitazone, throughout pregnancy increased insulin sensitivity compared to untreated pregnant animals (Figure 10, Table 7); however, rosiglitazone did not completely reverse the pregnancy-induced decrease in insulin sensitivity (Figure 10, Table 7). Rosiglitazone similarly increased baroreflex gain in pregnant rabbits compared to untreated pregnant animals, but gain still remained below the values of non-pregnant rabbits (Figure 10, Table 7). Rosiglitazone exerted no other effects on basal or baroreflex-mediated cardiovascular

Table 7

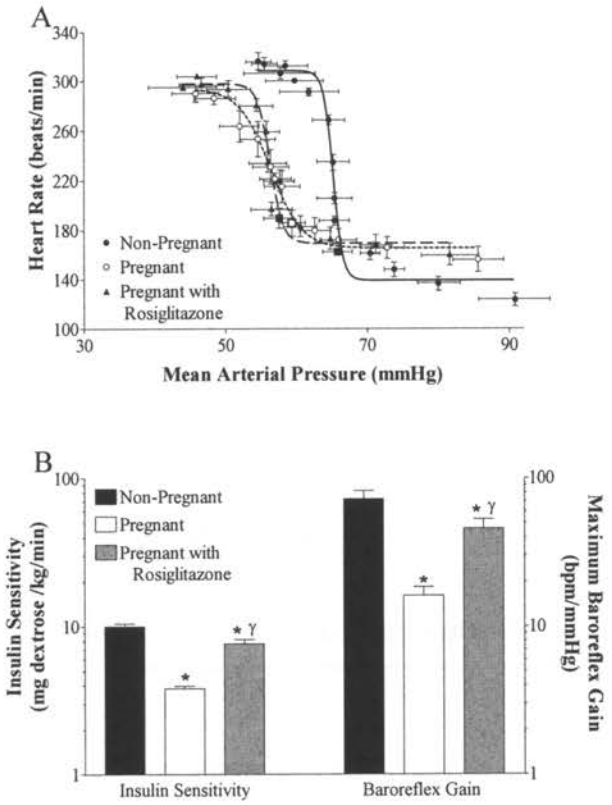
Baroreflex and basal parameters and insulin sensitivity in rabbits in the non-pregnant, control pregnant and rosiglitazone-treated pregnant states.

	Non-Pregnant	Pregnant Control	Pregnant Rosiglitazone-Treated
Basal Mean Arterial Pressure (mmHg)	66 ± 2	57 ± 2 *	58 ± 2 *
Basal Heart Rate (bpm)	162 ± 2	204 ± 12 *	192 ± 9 *
Maximum Heart Rate (bpm)	308 ± 5	298 ± 8	298 ± 4
Minimum Heart Rate (bpm)	139 ± 5	176 ± 6 *	170 ± 6 *
BP_{50} (mmHg)	65 ± 2	54 ± 2 *	56 ± 2 *
Maximum Gain (bpm/mmHg)	68 ± 9	15 ± 3 *	42 ± 7 * $^{\gamma}$
Insulin Sensitivity (mg dextrose/kg/min)	9.8 ± 0.4	3.7 + 0.2 *	7.6 ± 0.5 * $^{\gamma}$

* Significantly different from Non-Pregnant ($p<0.05$)

γ Significantly different from Pregnant Control ($p<0.05$)

Figure 10



Rosiglitazone improves insulin sensitivity and baroreflex gain during pregnancy. A) Mean baroreflex curves from rabbits (n=5) in the non-pregnant, untreated pregnant and rosiglitazone-treated pregnant states. Squares represent basal blood pressure and heart rate. B) Bar graph illustrating insulin sensitivity and baroreflex gain from the groups in A. * indicates significantly different ($p<0.05$) from non-pregnant. $^{\gamma}$ indicates significantly different ($p<0.05$) from untreated pregnant.

function during pregnancy (Figure 10, Table 7). In non-pregnant rabbits, rosiglitazone did not alter baroreflex gain or insulin sensitivity (Figure 11, Table 8).

Table 8

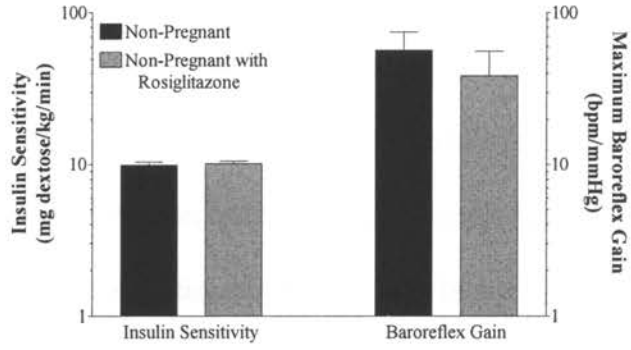
Baroreflex and basal parameters and insulin sensitivity in non-pregnant rabbits before and after treatment with rosiglitazone.

	Non-Pregnant Control	Non-Pregnant Rosiglitazone-Treated
Basal Mean Arterial Pressure (mmHg)	63 ± 2	60 ± 2
Basal Heart Rate (bpm)	158 ± 4	173 ± 17
Maximum Heart Rate (bpm)	296 ± 10	279 ± 12 *
Minimum Heart Rate (bpm)	132 ± 9	150 ± 14
BP_{50} (mmHg)	62 ± 1	58 ± 2
Maximum Gain (bpm/mmHg)	57 ± 20	39 ± 20
Insulin Sensitivity (mg dextrose/kg/min)	9.7 ± 0.6	10.2 ± 0.4

* Significantly different from Non-Pregnant Control

The yogurt vehicle used to administer rosiglitazone also had no effect on baroreflex function or insulin sensitivity. Rabbits receiving yogurt during pregnancy exhibited a decrease ($p<0.05$) in baroreflex gain (from 77 ± 20 to 21 ± 7 bpm/mmHg) and insulin sensitivity (from 10.4 ± 0.8 to 5.1 ± 1.0 mg dextrose/kg/min, n=4) similarly to rabbits receiving no vehicle (from 45 ± 9 to 13 ± 2 bpm/mmHg, and from 9.7 ± 0.6 to 4.3 ± 0.3 mg dextrose/kg/min, $p<0.05$; n=10).

Figure 11



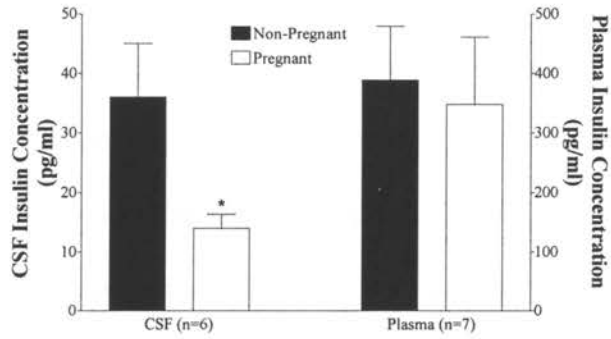
Rosiglitazone has no effect on insulin sensitivity or baroreflex gain in non-pregnant rabbits. Bar graph representing insulin sensitivity (n=5) and baroreflex gain (n=4) in untreated non-pregnant rabbits and rosiglitazone-treated non-pregnant rabbits.

All values for untreated pregnant rabbits in this study were from a first pregnancy and all values for rosiglitazone-treated pregnant rabbits were from a second pregnancy. Therefore, the possibility that the increases in baroreflex gain and insulin sensitivity were a confound of multiple pregnancies should be considered. However, in a previous study (Brooks et al., 2002) baroreflex gain was reduced to a similar level during a second pregnancy (12 ± 3 bpm/mmHg, n=5) as during a first pregnancy (13 ± 1 bpm/mmHg, n=3) compared to nonpregnant values (24 ± 3 and 19 ± 3, respectively). Also, in one rabbit in the present study, insulin sensitivity was reduced from 10.0 mg dextrose/kg/min to 4.0 mg dextrose/kg/min during a first pregnancy. During a second pregnancy insulin sensitivity was decreased similarly to 3.6 mg dextrose/kg/min.

Protocol 4) Effect of pregnancy on cerebrospinal fluid insulin concentrations

To provide support for the hypothesis that the decrease in brain insulin concentrations contributes to the decrease in baroreflex gain, we determined if cerebrospinal fluid insulin concentrations decrease during pregnancy. Insulin levels in plasma were unaltered during pregnancy (Figure 12) similar to previous reports in the rabbit (Gilbert et al., 1993). However, pregnancy significantly ($p<0.05$) decreased cerebrospinal fluid insulin concentrations from 36 ± 9 to 14 ± 2 pg/ml (Figure 12).

Figure 12



Pregnancy decreases concentrations of insulin in the cerebrospinal fluid. Bar graph representing the concentrations of insulin in the cerebrospinal fluid (n=6) and plasma (n=7) of rabbits in the non-pregnant and pregnant states. * signifies significantly different ($p<0.05$) from non-pregnant.

DISCUSSION

The purpose of this study was to test the association between the decrease in baroreflex gain during pregnancy and the decreased insulin sensitivity. The new

findings are that: 1) baroreflex gain and insulin sensitivity are positively correlated in non-pregnant and pregnant rabbits, 2) the decline in baroreflex gain exhibits the same time-course as the decrease in insulin sensitivity, 3) treatment of rabbits with the insulin-sensitizing drug rosiglitazone throughout pregnancy improves both insulin sensitivity and baroreflex gain, and 4) pregnancy is associated with reduced cerebrospinal fluid insulin concentrations. These data support a link between the reduced baroreflex gain and decreased insulin sensitivity and brain insulin concentrations during pregnancy.

It has long been known that pregnancy impairs baroreflex gain (Humphreys and Joels, 1974; Brooks et al., 1995; Heesch and Foley, 2001), and significant research has been directed at uncovering the mechanism. Our laboratory and others have ruled out a role for angiotensin II (O'Hagan et al., 2001; Brooks et al., 2001b; Daubert and Brooks, 2004a; Chapter 2) and nitric oxide (Daubert and Brooks, 2004a; Chapter 2). Masilamani and Heesch (Masilamani and Heesch, 1997) suggest that the pregnancy-induced decrease in baroreflex gain is due to increases in a metabolite of progesterone, allopregnanolone. They reported that infusion of allopregnanolone in virgin rats decreased baroreflex gain similar to pregnancy (Masilamani and Heesch, 1997). However, because it is not possible to block the actions of this metabolite and maintain pregnancy, it remains uncertain if endogenous allopregnanolone is responsible for the pregnancy-induced decrease in baroreflex gain.

Pregnancy is associated with a progressive decline in insulin sensitivity (Ciampelli et al., 1998), which may be involved in the impaired baroreflex gain. Insulin resistance develops secondary to the actions of several hormones elevated during

pregnancy including glucocorticoids, estrogen, progesterone, placental lactogen and tumor necrosis factor α (Cousins, 1991; Kirwan et al., 2002; Melczer et al., 2002a). These hormones may induce insulin resistance by increasing free fatty acid levels (Guillaume-Gentil et al., 1993; Uryszek et al., 1993; Fortun-Lamothe et al., 1996), which can inhibit insulin signaling by inducing serine phosphorylation of the insulin receptor and insulin receptor substrate proteins (Roden, 2004; Jiang and Zhang, 2005). Based on the association between insulin sensitivity and baroreflex gain in numerous other conditions (Grassi et al., 1995; Ferrannini et al., 1997; Ciampelli et al., 1998; Coats et al., 2000; Ryan, 2000; Frontoni et al., 2005), I hypothesized that the decrease in baroreflex gain during pregnancy is linked to the decrease in insulin sensitivity.

In support of this hypothesis, I found that decreases in both insulin sensitivity and baroreflex gain were correlated during pregnancy (Figure 8B), similar to the reduction in insulin sensitivity and baroreflex gain that occurs in obese humans that is reversed by weight loss (Grassi et al., 1998). I also showed, for the first time in any condition, that the time-course for the decrease in baroreflex gain matches the decrease in insulin sensitivity (Figure 9). These two results are in accordance with the hypothesis that the decrease in insulin sensitivity drives the decrease in baroreflex gain. However, these correlations, while tight, do not prove a cause and effect. Therefore, I next tested whether preventing the decrease in insulin sensitivity during pregnancy prevents the decrease in baroreflex gain.

In the current study, rosiglitazone almost completely prevented the decrease in insulin sensitivity with pregnancy, and to a similar extent, the reduction in baroreflex gain. These data strongly implicate insulin resistance as the cause of the baroreflex

impairment. Interestingly, rosiglitazone did not reverse the effects of pregnancy on other aspects of baroreflex function such as the increase in minimum heart rate (Table 3, Figure 10A) (Brooks et al., 1995). We have previously demonstrated that the decrease in baroreflex gain during pregnancy is mediated by reductions in the sympathetic component of heart rate control whereas the increase in minimum heart rate is due to decreases in parasympathetic control of heart rate (Brooks et al., 1997). This implies that the decline in insulin sensitivity is associated with greater impairment of sympathetic baroreflex control with less change in the parasympathetic nervous system.

Insulin resistant states, such as dogs fed a high fat diet, Zucker fatty rats and humans with Alzheimer's disease (Kuusisto et al., 1997), are characterized by reductions in insulin transport into the brain and in brain insulin concentrations (Stein et al., 1987; Craft et al., 1998; Kaiyala et al., 2000). In addition, neuronal insulin signaling may be suppressed (De Souza et al., 2005), thus decreasing the effectiveness of the insulin that does reach the brain. Insulin increases baroreflex gain when applied to the dorsal surface of the medulla (Man et al., 1998) or when administered into the lateral ventricles (Okada and Bunag, 1994). Together with my findings that cerebrospinal fluid insulin concentrations are decreased during pregnancy, these results support the hypothesis that the pregnancy-induced decrease in brain insulin concentration contributes to the decrease in baroreflex gain.

The basis for a link between decreased insulin sensitivity and reductions in insulin transport into the brain is unclear. Insulin must be transported into the brain since it is either not made centrally or is made in very low quantities (Banks, 2004). The majority of insulin in the brain is of pancreatic origin (Banks, 2004); however,

insulin is a large polypeptide that cannot pass through the blood-brain-barrier unassisted (Banks, 2004). It has been proposed that insulin gains access to the brain via a receptor-mediated process in which insulin binds to its receptor on the capillary wall, and this complex is internalized by endocytosis (Gerozissis, 2003; Woods et al., 2003; Banks, 2004). The insulin/insulin receptor complex is then expelled by exocytosis on the brain side of the endothelial cell (Gerozissis, 2003; Woods et al., 2003; Banks, 2004). Such a transport process has been demonstrated in cultured aortic endothelial cells in which insulin transport across the endothelial cell is blocked by insulin receptors antibodies (King and Johnson, 1985). Since the endothelial cells of the blood-brain-barrier also contain insulin receptors and can transport insulin intact from the blood to the brain (Gerozissis, 2003; Woods et al., 2003; Banks, 2004), this is a likely means by which insulin gains access to the brain.

It is also not known where insulin might be acting in the brain to increase baroreflex gain. Insulin receptors are concentrated in several brain regions important in the control of the autonomic nervous system, including the nucleus of the solitary tract (NTS), the paraventricular nucleus of the hypothalamus (PVN), and the ventrolateral medulla (Hill et al., 1986; Werther et al., 1987). Insulin hyperpolarizes neurons, thereby decreasing their activity (Fadool et al., 2000; O'Malley et al., 2003; O'Malley and Harvey, 2004). Since inactivation of the PVN enhances baroreflex gain (Chen et al., 1996b), insulin may act in the PVN to increase gain. Alternatively, insulin may suppress PVN activity indirectly, by binding to receptors in the arcuate nucleus, since this hypothalamic site contains high levels of insulin receptors and projects to the PVN (Silverman et al., 1981; Hill et al., 1986; Werther et al., 1987). Application of insulin

onto the dorsal medulla results in enhancement of baroreflex gain (Man et al., 1998), suggesting that insulin might act in the NTS to exert this effect. However, microinjection of insulin into the NTS decreases the activity of barosensitive neurons (Ruggeri et al., 2001) and decreases baroreflex gain (McKernan and Calaresu, 1996), suggesting a possible role for other nuclei in the brain stem (rostral and/or caudal ventrolateral medulla).

In summary, the results of this study support an association between decreased insulin sensitivity, reduced brain insulin concentrations and the decrease in baroreflex gain during pregnancy. It is tempting to speculate that this association may also underlie the decreased gain observed in a number of other conditions in which insulin sensitivity is reduced.

CHAPTER 4

DISCUSSION

The purpose of my research has been to determine what factors contribute to the pregnancy-induced impairment of baroreflex gain. To this end I have discovered that the decrease is not due to nitric oxide, alone or in conjunction with angiotensin II, but may instead be related to the insulin resistance that develops with pregnancy.

Hyperinsulinemia in the absence of hypoglycemia (ie, when blood glucose levels are clamped) induces increases in lumbar sympathetic nerve activity (Muntzel et al., 1994b), possibly through actions in the forebrain (Muntzel et al., 1994a). Prior blockade of angiotensin II production (Muntzel et al., 2001b) or NOS activity (Muntzel et al., 2001a) prevents this response, suggesting that insulin acts through angiotensin II and nitric oxide to increase sympathetic activity. However, while I found that the pregnancy-associated decreases in insulin sensitivity and brain insulin concentrations were linked to alterations in baroreflex sensitivity, angiotensin II AT_1 receptor blockade or inhibition of nitric oxide production did not improve baroreflex gain. Therefore, angiotensin II and nitric oxide do not appear to contribute to insulin's actions on baroreflex gain, at least not during pregnancy.

Is it really the reduction in insulin sensitivity that drives the impaired baroreflex gain of pregnancy and is the reduction in brain insulin concentrations required for this effect? The evidence presented in this dissertation is strong but correlative; thus, a

direct link between decreased brain insulin concentrations and impaired baroreflex gain has not been established. Indeed, baroreflex gain decreases in parallel to changes in insulin sensitivity, and rosiglitazone treatment increases both insulin sensitivity and baroreflex gain; however, some other factor may be causing the decrease in both of these parameters.

Decreases in insulin sensitivity are always associated with other changes. The most specific model of insulin resistance in animals is the fructose-fed model (Miller et al., 1999), which is associated with mild hypertension, hyperinsulinemia, cardiac hypertrophy, decreased adiponectin and nitric oxide bioavailability, as well as increased free fatty acids, angiotensin II activity, leptin, and TNFα (Togashi et al., 2002; Miatello et al., 2004; Li et al., 2005a; Lee et al., 2006). Many of these changes are also seen in Zucker fatty rats (Van Zwieten et al., 1996; onso-Galicia et al., 1996; Galisteo et al., 2005) and human type II diabetics (Reusch, 2002; Deinum and Chaturvedi, 2002; Matsuzawa, 2005). Pregnancy too, is associated with a number of these alterations (Skinner et al., 1972; Clapp, III and Kiess, 2000; Kirwan et al., 2002; Sivan and Boden, 2003; Fuglsang et al., 2006). These alterations may be a result of insulin resistance, they may be the cause of the resistance, or both. Three different ways of inducing insulin resistance (high fructose, obesity, pregnancy) decrease adiponectin and increase free fatty acids, angiotensin II activity, and TNFα, suggesting that reduced insulin sensitivity drives these alterations, directly or indirectly. Some of these factors may, in turn, feed-forward to worsen insulin resistance (Gilbert et al., 1991; Togashi et al., 2002; Gual et al., 2005).

Similarly, rosiglitazone is not a specific drug. Besides its effect to reverse insulin resistance, it also reverses many of the other alterations associated with decreased insulin sensitivity listed above (Giannini et al., 2004). Therefore, based on the results of the experiments performed in the studies described in this dissertation, it is not at this time possible to rule out a role for leptin, TNFα, adiponectin or free fatty acids.

In order to more definitively prove a role for insulin resistance in the impaired baroreflex gain of pregnancy, more experiments are needed. There is no specific way to increase insulin sensitivity since no drug decreases insulin resistance without other effects; however, if insulin sensitivity is increased in a way distinct from the mechanism employed by rosiglitazone, this would strengthen the claim. A stronger case could be made if aerobic exercise during pregnancy, for instance, prevented the decrease in baroreflex gain in parallel with preventing insulin resistance. Chronic aerobic exercise increases insulin sensitivity through mechanisms that are not completely understood. It may involve local effects to increase insulin sensitivity in exercised myocytes (Roberts and Barnard, 2005), but this cannot account for all the effects of exercise. Increases in circulating factors also appear to play a part (Dela et al., 1995; Roberts and Barnard, 2005). Exercise decreases free fatty acid levels, leptin and TNFα (Sato, 2000; Clapp, III and Kiess, 2000; Roberts and Barnard, 2005), which may be part of how it improves insulin sensitivity. Unfortunately, these factors are also altered by rosiglitazone, making exercise a less than ideal way to determine if increasing insulin sensitivity via another mechanism also increases baroreflex gain.

To more definitively prove that reduced brain insulin concentrations are responsible for the decrease in baroreflex gain with pregnancy, brain insulin concentrations following rosiglitazone treatment during pregnancy should be measured. Rosiglitazone increased baroreflex gain, but it is unknown if this was through improvements in the transport of insulin into the brain. An increase in brain insulin concentrations with drug treatment would provide more evidence for a role of brain insulin in the impaired baroreflex gain of pregnancy.

Administration of insulin intracerebroventricularly during pregnancy would also provide more information about the role of brain insulin in baroreflex control during this state. If my hypothesis is true then intracerebroventricular insulin infusion should improve gain during pregnancy. In addition, mice who lack the neuronal insulin receptor (Bruning et al., 2000) should exhibit a reduced baroreflex gain in the non-pregnant state and see no further impairment in gain with pregnancy. Alternatively, antisense oligonucleotides directed against the brain insulin receptor (Obici et al., 2002) could be used not only to determine if decreases in brain insulin receptor activation drive the reduced baroreflex gain during pregnancy, but also to help localize the site of this alteration in the brain.

If reductions in brain insulin concentrations are not involved in the decreased gain of pregnancy then it would appear that some factor closely related to insulin sensitivity can be implicated. Such factors include TNFα, free fatty acids, and leptin. Free fatty acids can decrease baroreflex gain (Gadegbeku et al., 2002) but this may be through alterations in insulin sensitivity (Gilbert et al., 1993). Leptin is probably not

involved since infusion of this protein does not impair baroreflex gain (Hausberg et al., 2002).

Although the data presented here are correlative, and I have not ruled out a role for other factors, these results are the first to show a temporal relationship between insulin sensitivity and baroreflex gain in any condition. It is also the first to show that rosiglitazone treatment can increase baroreflex gain, presumably through increases in insulin sensitivity. If insulin sensitivity drives the decrease in baroreflex gain during pregnancy, it may play a role in other pathophysiological conditions as well.

SUMMARY AND CONCLUSIONS

In summary, I tested the following hypotheses: 1) Increased nitric oxide activity during pregnancy inhibits baroreflex gain and this action is dependent on angiotensin II. 2) Pregnancy decreases baroreflex gain by altering the balance of nNOS in different brain regions. 3) Insulin resistance during pregnancy produces falls in brain insulin concentrations leading to decreased baroreflex gain. My major novel findings were that 1) blockade of nitric oxide production and/or AT_1 angiotensin II receptors does not improve baroreflex gain in pregnant or non-pregnant rabbits, 2) maintenance of constant angiotensin II levels does not change the effect of NOS blockade, 3) nNOS levels are not altered in the PVN, RVLM, CVLM, or NTS during pregnancy, 4) baroreflex gain and insulin sensitivity are correlated in non-pregnant and term-pregnant rabbits, 5) insulin sensitivity and baroreflex gain decrease at the same time-point during pregnancy, 6) treatment of rabbits with rosiglitazone throughout pregnancy almost completely prevents insulin resistance and impaired baroreflex gain, and 7) pregnancy

decreases brain insulin concentrations. Based on these findings, I conclude that pregnancy may decrease baroreflex gain by inducing insulin resistance and reducing brain insulin concentrations independent of changes in angiotensin II and nitric oxide.

REFERENCES

Agarwal, S. K., A. J. Gelsema, and F. R. Calaresu, 1990, Inhibition of rostral VLM by baroreceptor activation is relayed through caudal VLM: Am.J.Physiol, v. 258, no. 5 Pt 2, p. R1271-R1278.

Allen, A. M., S. Y. Chai, J. Clevers, M. J. McKinley, G. Paxinos, and F. A. Mendelsohn, 1988a, Localization and characterization of angiotensin II receptor binding and angiotensin converting enzyme in the human medulla oblongata: J.Comp Neurol., v. 269, no. 2, p. 249-264.

Allen, A. M., R. A. Dampney, and F. A. Mendelsohn, 1988b, Angiotensin receptor binding and pressor effects in cat subretrofacial nucleus: Am.J.Physiol, v. 255, no. 5 Pt 2, p. H1011-H1017.

Andresen, M. C., M. W. Doyle, Y. H. Jin, and T. W. Bailey, 2001, Cellular mechanisms of baroreceptor integration at the nucleus tractus solitarius: Ann.N.Y.Acad.Sci., v. 940, p. 132-141.

Andresen, M. C., and M. Yang, 1989, Arterial baroreceptor resetting: contributions of chronic and acute processes: Clin.Exp.Pharmacol.Physiol Suppl, v. 15, p. 19-30.

Andrew, P. J., and B. Mayer, 1999, Enzymatic function of nitric oxide synthases: Cardiovasc.Res., v. 43, no. 3, p. 521-531.

Andrews, R. C., and B. R. Walker, 1999, Glucocorticoids and insulin resistance: old hormones, new targets: Clin.Sci.(Lond), v. 96, no. 5, p. 513-523.

Antunes, V. R., and B. H. Machado, 2003, Antagonism of glutamatergic metabotropic receptors in the NTS of awake rats does not affect the gain of the baroreflex: Auton.Neurosci., v. 103, no. 1-2, p. 65-71.

Bains, J. S., A. Potyok, and A. V. Ferguson, 1992, Angiotensin II actions in paraventricular nucleus: functional evidence for neurotransmitter role in efferents originating in subfornical organ: Brain Res., v. 599, no. 2, p. 223-229.

Banks, W. A., 2004, The source of cerebral insulin: Eur.J.Pharmacol., v. 490, no. 1-3, p. 5-12.

Batista, M. R., M. S. Smith, W. L. Snead, C. C. Connolly, D. B. Lacy, and M. C. Moore, 2005, Chronic estradiol and progesterone treatment in conscious dogs: effects on insulin sensitivity and response to hypoglycemia: Am.J.Physiol Regul.Integr.Comp Physiol, v. 289, no. 4, p. R1064-R1073.

Bazil, M. K., and F. J. Gordon, 1993, Sympathoexcitation from the rostral ventrolateral medulla is mediated by spinal NMDA receptors: Brain Res.Bull., v. 31, no. 3-4, p. 273-278.

Beck, P., and W. H. Daughaday, 1967, Human placental lactogen: studies of its acute metabolic effects and disposition in normal man: J.Clin.Invest, v. 46, no. 1, p. 103-110.

Bjornholm, M., and J. R. Zierath, 2005, Insulin signal transduction in human skeletal muscle: identifying the defects in Type II diabetes: Biochem.Soc.Trans., v. 33, no. Pt 2, p. 354-357.

Blake, M. J., A. Martin, B. N. Manktelow, C. Armstrong, A. W. Halligan, R. B. Panerai, and J. F. Potter, 2000, Changes in baroreceptor sensitivity for heart rate during normotensive pregnancy and the puerperium: Clin.Sci.(Lond), v. 98, no. 3, p. 259-268.

Boulanger, C. M., L. Caputo, and B. I. Levy, 1995, Endothelial AT1-mediated release of nitric oxide decreases angiotensin II contractions in rat carotid artery: Hypertension, v. 26, no. 5, p. 752-757.

Brady, F., Y. S. Bakhle, and C. Bell, 2002, Evaluation of the involvement of nitric oxide and substance P in reducing baroreflex gain in the genetically hypertensive (GH) rat: Acta Physiol Hung., v. 89, no. 4, p. 451-461.

Bredt, D. S., and S. H. Snyder, 1994, Nitric oxide: a physiologic messenger molecule: Annu.Rev.Biochem., v. 63, p. 175-195.

Brooks, V. L., K. A. Clow, and K. P. O'Hagan, 2002, Pregnancy and acute baroreflex resetting in conscious rabbits: American Journal of Physiology-Regulatory Integrative and Comparative Physiology, v. 283, no. 2, p. R429-R440.

Brooks, V. L., K. A. Clow, L. S. Welch, and G. D. Giraud, 2001a, Does nitric oxide contribute to the basal vasodilation of pregnancy in conscious rabbits?: Am.J.Physiol Regul.Integr.Comp Physiol, v. 281, no. 5, p. R1624-R1632.

Brooks, V. L., K. R. Ell, and R. M. Wright, 1993, Pressure-independent baroreflex resetting produced by chronic infusion of angiotensin II in rabbits: Am.J.Physiol, v. 265, no. 4 Pt 2, p. H1275-H1282.

Brooks, V. L., C. M. Kane, and D. M. Van Winkle, 1997, Altered heart rate baroreflex during pregnancy: role of sympathetic and parasympathetic nervous systems: Am.J.Physiol, v. 273, no. 3 Pt 2, p. R960-R966.

Brooks, V. L., and L. C. Keil, 1994, Changes in the baroreflex during pregnancy in conscious dogs: heart rate and hormonal responses: Endocrinology, v. 135, no. 5, p. 1894-1901.

Brooks, V. L., and J. W. Osborn, 1995, Hormonal-sympathetic interactions in long-term regulation of arterial pressure: an hypothesis: Am.J.Physiol, v. 268, no. 6 Pt 2, p. R1343-R1358.

Brooks, V. L., R. R. Quesnell, S. R. Cumbee, and V. S. Bishop, 1995, Pregnancy attenuates activity of the baroreceptor reflex: Clin.Exp.Pharmacol.Physiol, v. 22, no. 2, p. 152-156.

Brooks, V. L., R. R. Quesnell, C. M. Kane, and L. C. Keil, 1998, Hemodynamic and hormonal responses to hemorrhage in conscious rabbits at mid- and late gestation: Am.J.Physiol, v. 275, no. 4 Pt 2, p. R1082-R1090.

Brooks, V. L., L. S. Welch, and C. M. Kane, 2001b, Role of angiotensin II in altered baroreflex function of conscious rabbits during late pregnancy: American Journal of Obstetrics and Gynecology, v. 184, no. 3, p. 476-482.

Bruning, J. C. et al., 2000, Role of brain insulin receptor in control of body weight and reproduction: Science, v. 289, no. 5487, p. 2122-2125.

Carey, R. M., and H. M. Siragy, 2003, Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation: Endocr.Rev., v. 24, no. 3, p. 261-271.

Castellano, M. et al., 1995, Relationship between sympathetic nervous system activity, baroreflex and cardiovascular effects after acute nitric oxide synthesis inhibition in humans: J.Hypertens., v. 13, no. 10, p. 1153-1161.

Casto, R., and M. I. Phillips, 1984, Cardiovascular actions of microinjections of angiotensin II in the brain stem of rats: Am.J.Physiol, v. 246, no. 5 Pt 2, p. R811-R816.

Casto, R., and M. I. Phillips, 1986, Angiotensin II attenuates baroreflexes at nucleus tractus solitarius of rats: Am.J.Physiol, v. 250, no. 2 Pt 2, p. R193-R198.

Chan, R. K., Y. S. Chan, and T. M. Wong, 1991, Responses of cardiovascular neurons in the rostral ventrolateral medulla of the normotensive Wistar Kyoto and spontaneously hypertensive rats to iontophoretic application of angiotensin II: Brain Res., v. 556, no. 1, p. 145-150.

Chang, J., L. D. Elam-Evans, C. J. Berg, J. Herndon, L. Flowers, K. A. Seed, and C. J. Syverson, 2003, Pregnancy-related mortality surveillance--united states, 1991-1999: Morbidity and Mortality Weekly Report Surveillance Summaries, v. 52, no. SS-2, p. 1-8.

Chapleau, M. W., and F. M. Abboud, 2001, Neuro-cardiovascular regulation: from molecules to man. Introduction: Ann.N.Y.Acad.Sci., v. 940, p. xiii-xxii.

Chapleau, M. W., G. Hajduczok, and F. M. Abboud, 1989, Peripheral and central mechanisms of baroreflex resetting: Clin.Exp.Pharmacol.Physiol Suppl, v. 15, p. 31-43.

Chapleau, M. W., Z. Li, S. S. Meyrelles, X. Ma, and F. M. Abboud, 2001, Mechanisms determining sensitivity of baroreceptor afferents in health and disease: Ann.N.Y.Acad.Sci., v. 940, p. 1-19.

Chen, J. S., W. Wang, K. G. Cornish, and I. H. Zucker, 1992, Baro- and ventricular reflexes in conscious dogs subjected to chronic tachycardia: Am.J.Physiol, v. 263, no. 4 Pt 2, p. H1084-H1089.

Chen, Y. L., S. H. Chan, and J. Y. Chan, 1996a, Participation of galanin in baroreflex inhibition of heart rate by hypothalamic PVN in rat: Am.J.Physiol, v. 271, no. 5 Pt 2, p. H1823-H1828.

Chen, Y. L., S. H. Chan, and J. Y. Chan, 1996b, Participation of galanin in baroreflex inhibition of heart rate by hypothalamic PVN in rat: Am.J.Physiol, v. 271, no. 5 Pt 2, p. H1823-H1828.

Cheng, Z., S. Z. Guo, A. J. Lipton, and D. Gozal, 2002, Domoic acid lesions in nucleus of the solitary tract: time-dependent recovery of hypoxic ventilatory response and peripheral afferent axonal plasticity: J.Neurosci., v. 22, no. 8, p. 3215-3226.

Cheng, Z., and T. L. Powley, 2000, Nucleus ambiguus projections to cardiac ganglia of rat atria: an anterograde tracing study: J.Comp Neurol., v. 424, no. 4, p. 588-606.

Cheng, Z., T. L. Powley, J. S. Schwaber, and F. J. Doyle, III, 1999, Projections of the dorsal motor nucleus of the vagus to cardiac ganglia of rat atria: an anterograde tracing study: J.Comp Neurol., v. 410, no. 2, p. 320-341.

Cheng, Z., H. Zhang, J. Yu, R. D. Wurster, and D. Gozal, 2004, Attenuation of baroreflex sensitivity after domoic acid lesion of the nucleus ambiguus of rats: J.Appl.Physiol, v. 96, no. 3, p. 1137-1145.

Chikada, N. et al., 2000, Distribution of c-fos mRNA in the brain following intracerebroventricular injection of nitric oxide (NO)-releasing compounds: possible

role of NO in central cardiovascular regulation: J.Neuroendocrinol., v. 12, no. 11, p. 1112-1123.

Chiu, T., and I. A. Reid, 1995, Effect of inhibition of nitric oxide synthesis on the cardiovascular and endocrine responses to hemorrhage in conscious rabbits: Hypertens.Res., v. 18, no. 1, p. 55-61.

Ciampelli, M., A. Lanzone, and A. Caruso, 1998, Insulin in obstetrics: a main parameter in the management of pregnancy: Hum.Reprod.Update., v. 4, no. 6, p. 904-914.

Clapp, J. F., III, and W. Kiess, 2000, Effects of pregnancy and exercise on concentrations of the metabolic markers tumor necrosis factor alpha and leptin: Am.J.Obstet.Gynecol., v. 182, no. 2, p. 300-306.

Clow, K. A., G. D. Giraud, B. E. Ogden, and V. L. Brooks, 2003, Pregnancy alters hemodynamic responses to hemorrhage in conscious rabbits: Am.J.Physiol Heart Circ.Physiol, v. 284, no. 4, p. H1110-H1118.

Coats, A. J., S. D. Anker, and S. Anker, 2000, Insulin resistance in chronic heart failure: J.Cardiovasc.Pharmacol., v. 35, no. 7 Suppl 4, p. S9-14.

Coleman, T. G., 1980, Arterial baroreflex control of heart rate in the conscious rat: Am.J.Physiol, v. 238, no. 4, p. H515-H520.

Concas, A., M. C. Mostallino, P. Porcu, P. Follesa, M. L. Barbaccia, M. Trabucchi, R. H. Purdy, P. Grisenti, and G. Biggio, 1998, Role of brain allopregnanolone in the plasticity of gamma-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery: Proc.Natl.Acad.Sci.U.S.A, v. 95, no. 22, p. 13284-13289.

Conrad, K. P., G. M. Joffe, H. Kruszyna, R. Kruszyna, L. G. Rochelle, R. P. Smith, J. E. Chavez, and M. D. Mosher, 1993, Identification of increased nitric oxide biosynthesis during pregnancy in rats: FASEB J., v. 7, no. 6, p. 566-571.

Cousins, L., 1991, Insulin sensitivity in pregnancy: Diabetes, v. 40 Suppl 2, p. 39-43.

Cox, B. F., and V. S. Bishop, 1991, Neural and humoral mechanisms of angiotensin-dependent hypertension: Am.J.Physiol, v. 261, no. 4 Pt 2, p. H1284-H1291.

Craft, S., E. Peskind, M. W. Schwartz, G. D. Schellenberg, M. Raskind, and D. Porte, Jr., 1998, Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype: Neurology, v. 50, no. 1, p. 164-168.

Cunningham, E. T., Jr., M. C. Bohn, and P. E. Sawchenko, 1990, Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat: J.Comp Neurol., v. 292, no. 4, p. 651-667.

Curtis, K. S., J. T. Cunningham, and C. M. Heesch, 1999, Fos expression in brain stem nuclei of pregnant rats after hydralazine-induced hypotension: Am.J.Physiol, v. 277, no. 2 Pt 2, p. R532-R540.

da Silva, S. V., S. da, V, G. Ballejo, M. C. Salgado, and H. C. Salgado, 1994, Blockers of the L-arginine-nitric oxide-cyclic GMP pathway facilitate baroreceptor resetting: Hypertension, v. 23, no. 1 Suppl, p. I60-I63.

Dampney, R. A., 1994, Functional organization of central pathways regulating the cardiovascular system: Physiol Rev., v. 74, no. 2, p. 323-364.

Dampney, R. A., J. Czachurski, K. Dembowsky, A. K. Goodchild, and H. Seller, 1987, Afferent connections and spinal projections of the pressor region in the rostral ventrolateral medulla of the cat: J.Auton.Nerv.Syst., v. 20, no. 1, p. 73-86.

Daubert, D. L., and V. L. Brooks, 2004b, Stress decreases baroreflex gain (BRG) through increases in nitric oxide (NO): FASEB J., v. 18, no. 4, p. A294.

Daubert, D. L., and V. L. Brooks, 2004a, Role of angiotensin II (AngII) and nitric oxide (NO) in the decreased baroreflex gain of pregnancy: FASEB J., v. 18, no. 5, p. A1078.

De Souza, C. T., E. P. Araujo, S. Bordin, R. Ashimine, R. L. Zollner, A. C. Boschero, M. J. Saad, and L. A. Velloso, 2005, Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus: Endocrinology, v. 146, no. 10, p. 4192-4199.

DeFronzo, R. A., J. D. Tobin, and R. Andres, 1979, Glucose clamp technique: a method for quantifying insulin secretion and resistance: Am.J.Physiol, v. 237, no. 3, p. E214-E223.

Deinum, J., and N. Chaturvedi, 2002, The Renin-Angiotensin system and vascular disease in diabetes: Semin.Vasc.Med., v. 2, no. 2, p. 149-156.

Dela, F., J. J. Larsen, K. J. Mikines, T. Ploug, L. N. Petersen, and H. Galbo, 1995, Insulin-stimulated muscle glucose clearance in patients with NIDDM. Effects of one-legged physical training: Diabetes, v. 44, no. 9, p. 1010-1020.

Dias, A. C., M. Vitela, E. Colombari, and S. W. Mifflin, 2005, Nitric oxide modulation of glutamatergic, baroreflex, and cardiopulmonary transmission in the nucleus of the solitary tract: Am.J.Physiol Heart Circ.Physiol, v. 288, no. 1, p. H256-H262.

Dibona, G. F., and S. Y. Jones, 2002, Effect of dietary sodium intake on central angiotensinergic pathways: Auton.Neurosci., v. 98, no. 1-2, p. 17-19.

DiBona, G. F., S. Y. Jones, and V. L. Brooks, 1995, ANG II receptor blockade and arterial baroreflex regulation of renal nerve activity in cardiac failure: Am.J.Physiol, v. 269, no. 5 Pt 2, p. R1189-R1196.

Diz, D. I., K. L. Barnes, and C. M. Ferrario, 1984, Hypotensive actions of microinjections of angiotensin II into the dorsal motor nucleus of the vagus: J.Hypertens.Suppl, v. 2, no. 3, p. S53-S56.

Dorward, P. K., W. Riedel, S. L. Burke, J. Gipps, and P. I. Korner, 1985, The renal sympathetic baroreflex in the rabbit. Arterial and cardiac baroreceptor influences, resetting, and effect of anesthesia: Circ.Res., v. 57, no. 4, p. 618-633.

Dorward, P. K., and C. D. Rudd, 1991, Influence of brain renin-angiotensin system on renal sympathetic and cardiac baroreflexes in conscious rabbits: Am.J.Physiol, v. 260, no. 3 Pt 2, p. H770-H778.

Drummond, H. A., M. P. Price, M. J. Welsh, and F. M. Abboud, 1998, A molecular component of the arterial baroreceptor mechanotransducer: Neuron, v. 21, no. 6, p. 1435-1441.

Du, Z. Y., G. J. Dusting, and O. L. Woodman, 1992, Baroreceptor reflexes and vascular reactivity during inhibition of nitric oxide synthesis in conscious rabbits: Eur.J.Pharmacol., v. 214, no. 1, p. 21-26.

Easterling, T. R., B. C. Schmucker, and T. J. Benedetti, 1988, The hemodynamic effects of orthostatic stress during pregnancy: Obstet.Gynecol., v. 72, no. 4, p. 550-552.

Egan, B. M., 2003, Insulin resistance and the sympathetic nervous system: Curr.Hypertens.Rep., v. 5, no. 3, p. 247-254.

el Karib, A. O., J. Sheng, A. L. Betz, and R. L. Malvin, 1993, The central effects of a nitric oxide synthase inhibitor (N omega-nitro-L-arginine) on blood pressure and plasma renin: Clin.Exp.Hypertens., v. 15, no. 5, p. 819-832.

Elghozi, J. L., and G. A. Head, 1990, Spinal noradrenergic pathways and pressor responses to central angiotensin II: Am.J.Physiol, v. 258, no. 1 Pt 2, p. H240-H246.

Fadool, D. A., K. Tucker, J. J. Phillips, and J. A. Simmen, 2000, Brain insulin receptor causes activity-dependent current suppression in the olfactory bulb through multiple phosphorylation of Kv1.3: Journal of Neurophysiology, v. 83, no. 4, p. 2332-2348.

Fedele, E., M. Marchi, and M. Raiteri, 2001, In vivo NO/cGMP signalling in the hippocampus: Neurochem.Res., v. 26, no. 8-9, p. 1069-1078.

Ferguson, A. V., and D. L. Washburn, 1998, Angiotensin II: a peptidergic neurotransmitter in central autonomic pathways: Prog.Neurobiol., v. 54, no. 2, p. 169-192.

Ferrannini, E., A. Natali, P. Bell, P. Cavallo-Perin, N. Lalic, and G. Mingrone, 1997, Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR): J.Clin.Invest, v. 100, no. 5, p. 1166-1173.

Ferrari, A. U., C. Franzelli, A. Daffonchio, S. Perlini, and M. Dirienzo, 1996, Sympathovagal interplay in the control of overall blood pressure variability in unanesthetized rats: Am.J.Physiol, v. 270, no. 6 Pt 2, p. H2143-H2148.

Fink, G. D., 1997, Long-term sympatho-excitatory effect of angiotensin II: a mechanism of spontaneous and renovascular hypertension: Clin.Exp.Pharmacol.Physiol, v. 24, no. 1, p. 91-95.

Foley, C. M., J. A. Moffitt, M. Hay, and E. M. Hasser, 1998, Glutamate in the nucleus of the solitary tract activates both ionotropic and metabotropic glutamate receptors: Am.J.Physiol, v. 275, no. 6 Pt 2, p. R1858-R1866.

Fortun-Lamothe, L., D. Langin, and M. Lafontan, 1996, Influence of prolactin on in vivo and in vitro lipolysis in rabbits: Comp Biochem.Physiol C.Pharmacol.Toxicol.Endocrinol., v. 115, no. 2, p. 141-147.

Frontoni, S., D. Bracaglia, and F. Gigli, 2005, Relationship between autonomic dysfunction, insulin resistance and hypertension, in diabetes: Nutr.Metab Cardiovasc.Dis., v. 15, no. 6, p. 441-449.

Fuglsang, J., C. Skjaerbaek, J. Frystyk, A. Flyvbjerg, and P. Ovesen, 2006, A longitudinal study of serum adiponectin during normal pregnancy: BJOG., v. 113, no. 1, p. 110-113.

Fujisawa, Y., N. Mori, K. Yube, H. Miyanaka, A. Miyatake, and Y. Abe, 1999b, Role of nitric oxide in regulation of renal sympathetic nerve activity during hemorrhage in conscious rats: Am.J.Physiol, v. 277, no. 1 Pt 2, p. H8-14.

Fujisawa, Y., N. Mori, K. Yube, H. Miyanaka, A. Miyatake, and Y. Abe, 1999a, Role of nitric oxide in regulation of renal sympathetic nerve activity during hemorrhage in conscious rats: Am.J.Physiol, v. 277, no. 1 Pt 2, p. H8-14.

Gadegbeku, C. A., A. Dhandayuthapani, Z. E. Sadler, and B. M. Egan, 2002, Raising lipids acutely reduces baroreflex sensitivity: Am.J.Hypertens., v. 15, no. 6, p. 479-485.

Galisteo, M., M. Sanchez, R. Vera, M. Gonzalez, A. Anguera, J. Duarte, and A. Zarzuelo, 2005, A diet supplemented with husks of Plantago ovata reduces the development of endothelial dysfunction, hypertension, and obesity by affecting adiponectin and TNF-alpha in obese Zucker rats: J.Nutr., v. 135, no. 10, p. 2399-2404.

Ganong, W. F., 2000, Circumventricular organs: definition and role in the regulation of endocrine and autonomic function: Clin.Exp.Pharmacol.Physiol, v. 27, no. 5-6, p. 422-427.

Gardes, J., J. M. Poux, M. F. Gonzalez, F. Alhenc-Gelas, and J. Menard, 1992, Decreased renin release and constant kallikrein secretion after injection of L-NAME in isolated perfused rat kidney: Life Sci., v. 50, no. 14, p. 987-993.

Gardiner, S. M., and T. Bennett, 1986, Endogenous vasopressin and baroreflex mechanisms: Brain Res., v. 396, no. 4, p. 317-334.

Gaudet, E., S. J. Godwin, and G. A. Head, 2000, Effects of central infusion of ANG II and losartan on the cardiac baroreflex in rabbits: Am.J.Physiol Heart Circ.Physiol, v. 278, no. 2, p. H558-H566.

Gerozissis, K., 2003, Brain insulin: Regulation, mechanisms of action, and functions (vol 23, pg 1, 2003): Cellular and Molecular Neurobiology, v. 23, no. 4-5, p. 873-874.

Gerozissis, K., 2004, Brain insulin and feeding: a bi-directional communication: Eur.J.Pharmacol., v. 490, no. 1-3, p. 59-70.

Getting, S. J., J. Segieth, S. Ahmad, C. S. Biggs, and P. S. Whitton, 1996, Biphasic modulation of GABA release by nitric oxide in the hippocampus of freely moving rats in vivo: Brain Res., v. 717, no. 1-2, p. 196-199.

Giannini, S., M. Serio, and A. Galli, 2004, Pleiotropic effects of thiazolidinediones: taking a look beyond antidiabetic activity: J.Endocrinol.Invest, v. 27, no. 10, p. 982-991.

Gilbert, M., S. Basile, A. Baudelin, and M. C. Pere, 1993, Lowering plasma free fatty acid levels improves insulin action in conscious pregnant rabbits: Am.J.Physiol, v. 264, no. 4 Pt 1, p. E576-E582.

Gilbert, M., M. C. Pere, A. Baudelin, and F. C. Battaglia, 1991, Role of free fatty acids in hepatic insulin resistance during late pregnancy in conscious rabbits: Am.J.Physiol, v. 260, no. 6 Pt 1, p. E938-E945.

Gordon, F. J., 1987, Aortic baroreceptor reflexes are mediated by NMDA receptors in caudal ventrolateral medulla: Am.J.Physiol, v. 252, no. 3 Pt 2, p. R628-R633.

Grassi, G., 2004, Sympathetic and baroreflex function in hypertension: implications for current and new drugs: Curr.Pharm.Des, v. 10, no. 29, p. 3579-3589.

Grassi, G. et al., 1995, Sympathetic activation in obese normotensive subjects: Hypertension, v. 25, no. 4 Pt 1, p. 560-563.

Grassi, G., G. Seravalle, M. Colombo, G. Bolla, B. M. Cattaneo, F. Cavagnini, and G. Mancia, 1998, Body weight reduction, sympathetic nerve traffic, and arterial baroreflex in obese normotensive humans: Circulation, v. 97, no. 20, p. 2037-2042.

Greenwood, J. P., E. M. Scott, J. B. Stoker, J. J. Walker, and D. A. Mary, 2001, Sympathetic neural mechanisms in normal and hypertensive pregnancy in humans: Circulation, v. 104, no. 18, p. 2200-2204.

Gronan, R. J., J. C. Schadt, and D. H. York, 1983, Routine, direct measurement of aortic pressure in the conscious rabbit: Physiol Behav., v. 30, no. 5, p. 719-722.

Gual, P., Y. Le Marchand-Brustel, and J. F. Tanti, 2005, Positive and negative regulation of insulin signaling through IRS-1 phosphorylation: Biochimie, v. 87, no. 1, p. 99-109.

Guillaume-Gentil, C., F. ssimacopoulos-Jeannet, and B. Jeanrenaud, 1993, Involvement of non-esterified fatty acid oxidation in glucocorticoid-induced peripheral insulin resistance in vivo in rats: Diabetologia, v. 36, no. 10, p. 899-906.

Habler, H. J., G. Wasner, T. Bartsch, and W. Janig, 1997, Responses of rat postganglionic sympathetic vasoconstrictor neurons following blockade of nitric oxide synthesis in vivo: Neuroscience, v. 77, no. 3, p. 899-909.

Hakim, M. A., Y. Hirooka, M. J. Coleman, M. R. Bennett, and R. A. Dampney, 1995, Evidence for a critical role of nitric oxide in the tonic excitation of rabbit renal sympathetic preganglionic neurones: J.Physiol (Lond), v. 482 (Pt 2), p. 401-407.

Hansen, J., T. N. Jacobsen, and R. G. Victor, 1994, Is nitric oxide involved in the tonic inhibition of central sympathetic outflow in humans?: Hypertension, v. 24, no. 4, p. 439-444.

Harada, S., S. Tokunaga, M. Momohara, H. Masaki, T. Tagawa, T. Imaizumi, and A. Takeshita, 1993, Inhibition of nitric oxide formation in the nucleus tractus solitarius increases renal sympathetic nerve activity in rabbits: Circ.Res., v. 72, no. 3, p. 511-516.

Harrison, D. G., and H. Cai, 2003, Endothelial control of vasomotion and nitric oxide production: Cardiol.Clin., v. 21, no. 3, p. 289-302.

Hart, M. V., J. D. Hosenpud, A. R. Hohimer, and M. J. Morton, 1985, Hemodynamics during pregnancy and sex steroid administration in guinea pigs: Am.J.Physiol, v. 249, no. 2 Pt 2, p. R179-R185.

Hausberg, M., D. A. Morgan, M. A. Chapleau, W. I. Sivitz, A. L. Mark, and W. G. Haynes, 2002, Differential modulation of leptin-induced sympathoexcitation by baroreflex activation: J.Hypertens., v. 20, no. 8, p. 1633-1641.

Head, G. A., J. L. Elghozi, and P. I. Korner, 1988, Baroreflex modulation of central angiotensin II pressor responses in conscious rabbits: J.Hypertens.Suppl, v. 6, no. 4, p. S505-S507.

Head, G. A., and N. S. Williams, 1992, Hemodynamic effects of central angiotensin I, II, and III in conscious rabbits: Am.J.Physiol, v. 263, no. 4 Pt 2, p. R845-R851.

Heesch, C. M., and C. M. Foley, 2001, CNS effects of ovarian hormones and metabolites on neural control of circulation: Ann.N.Y.Acad.Sci., v. 940, p. 348-360.

Heesch, C. M., and R. C. Rogers, 1995, Effects of pregnancy and progesterone metabolites on regulation of sympathetic outflow: Clin.Exp.Pharmacol.Physiol, v. 22, no. 2, p. 136-142.

Hill, J. M., M. A. Lesniak, C. B. Pert, and J. Roth, 1986, Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas: Neuroscience, v. 17, no. 4, p. 1127-1138.

Hines, T., and S. W. Mifflin, 1997, Nitric oxide mediation of cardiac receptor reflex responses in the pregnant rat: Am.J.Obstet.Gynecol., v. 177, no. 6, p. 1502-1508.

Hines, T., and S. W. Mifflin, 1995, Gestational effects on volume-sensitive cardiopulmonary receptor reflexes in the rat: Am.J.Physiol, v. 268, no. 3 Pt 2, p. R736-R743.

Hirai, T., T. I. Musch, D. A. Morgan, K. C. Kregel, D. E. Claassen, J. G. Pickar, S. J. Lewis, and M. J. Kenney, 1995, Differential sympathetic nerve responses to nitric oxide synthase inhibition in anesthetized rats: Am.J.Physiol, v. 269, no. 4 Pt 2, p. R807-R813.

Hirooka, Y., J. W. Polson, and R. A. Dampney, 1996, Pressor and sympathoexcitatory effects of nitric oxide in the rostral ventrolateral medulla: J.Hypertens., v. 14, no. 11, p. 1317-1324.

Hirooka, Y., P. D. Potts, and R. A. Dampney, 1997, Role of angiotensin II receptor subtypes in mediating the sympathoexcitatory effects of exogenous and endogenous angiotensin peptides in the rostral ventrolateral medulla of the rabbit: Brain Res., v. 772, no. 1-2, p. 107-114.

Hoffman, W. E., and M. I. Phillips, 1976, Regional study of cerebral ventricle sensitive sites to angiotensin II: Brain Res., v. 110, no. 2, p. 313-330.

Hogan, N., A. Kardos, D. J. Paterson, and B. Casadei, 1999, Effect of exogenous nitric oxide on baroreflex function in humans: Am.J.Physiol, v. 277, no. 1 Pt 2, p. H221-H227.

Humphreys, P. W., and N. Joels, 1974, The carotid sinus baroreceptor reflex in the pregnant rabbit: J.Physiol, v. 239, no. 1, p. 89-102.

Hytten, F. E., and D. B. Paintin, 1963, Increase in plasma volume during normal pregnancy: J.Obstet.Gynaecol.Br.Emp., v. 70, p. 402-407.

Ishide, T., Y. Hara, T. J. Maher, and A. Ally, 2000, Glutamate neurotransmission and nitric oxide interaction within the ventrolateral medulla during cardiovascular responses to muscle contraction: Brain Res., v. 874, no. 2, p. 107-115.

Ito, S., K. Komatsu, K. Tsukamoto, K. Kanmatsuse, and A. F. Sved, 2002, Ventrolateral medulla AT1 receptors support blood pressure in hypertensive rats: Hypertension, v. 40, no. 4, p. 552-559.

Iwase, K., K. Iyama, K. Akagi, S. Yano, K. Fukunaga, E. Miyamoto, M. Mori, and M. Takiguchi, 1998, Precise distribution of neuronal nitric oxide synthase mRNA in the rat brain revealed by non-radioisotopic in situ hybridization: Brain Res.Mol.Brain Res., v. 53, no. 1-2, p. 1-12.

Jiang, G., and B. B. Zhang, 2005, Modulation of insulin signalling by insulin sensitizers: Biochem.Soc.Trans., v. 33, no. Pt 2, p. 358-361.

Jimbo, M., H. Suzuki, M. Ichikawa, K. Kumagai, M. Nishizawa, and T. Saruta, 1994, Role of nitric oxide in regulation of baroreceptor reflex: J.Auton.Nerv.Syst., v. 50, no. 2, p. 209-219.

Kaiyala, K. J., R. L. Prigeon, S. E. Kahn, S. C. Woods, and M. W. Schwartz, 2000, Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs: Diabetes, v. 49, no. 9, p. 1525-1533.

Katz, R., J. S. Karliner, and R. Resnik, 1978, Effects of a natural volume overload state (pregnancy) on left ventricular performance in normal human subjects: Circulation, v. 58, no. 3 Pt 1, p. 434-441.

Kenney, M. J., M. L. Weiss, T. Mendes, Y. Wang, and R. J. Fels, 2003, Role of paraventricular nucleus in regulation of sympathetic nerve frequency components: Am.J.Physiol Heart Circ.Physiol, v. 284, no. 5, p. H1710-H1720.

Kimura, Y., Y. Hirooka, Y. Sagara, K. Ito, T. Kishi, H. Shimokawa, A. Takeshita, and K. Sunagawa, 2005, Overexpression of inducible nitric oxide synthase in rostral ventrolateral medulla causes hypertension and sympathoexcitation via an increase in oxidative stress: Circ.Res., v. 96, no. 2, p. 252-260.

King, G. L., and S. M. Johnson, 1985, Receptor-mediated transport of insulin across endothelial cells: Science, v. 227, no. 4694, p. 1583-1586.

Kirwan, J. P., M. S. Hauguel-De, J. Lepercq, J. C. Challier, L. Huston-Presley, J. E. Friedman, S. C. Kalhan, and P. M. Catalano, 2002, TNF-alpha is a predictor of insulin resistance in human pregnancy: Diabetes, v. 51, no. 7, p. 2207-2213.

Kopp, L., G. Paradiz, and J. R. Tucci, 1977, Urinary excretion of cyclic 3',5'-adenosine monophosphate and cyclic 3',5'-guanosine monophosphate during and after pregnancy: J.Clin.Endocrinol.Metab, v. 44, no. 3, p. 590-594.

Kumagai, H., D. B. Averill, M. C. Khosla, and C. M. Ferrario, 1993, Role of nitric oxide and angiotensin II in the regulation of sympathetic nerve activity in spontaneously hypertensive rats: Hypertension, v. 21, no. 4, p. 476-484.

Kumagai, H., H. Suzuki, M. Ichikawa, M. Nishizawa, N. Oshima, and T. Saruta, 1997, Interaction between endothelin and nitric oxide in sympathetic nerve modulation in hypertensive rats: Hypertens.Res., v. 20, no. 1, p. 35-42.

Kumagai, K., H. Suzuki, M. Ichikawa, M. Jimbo, M. Murakami, M. Ryuzaki, and T. Saruta, 1994, Nitric oxide increases renal blood flow by interacting with the sympathetic nervous system: Hypertension, v. 24, no. 2, p. 220-226.

Kuusisto, J. et al., 1997, Association between features of the insulin resistance syndrome and Alzheimer's disease independently of apolipoprotein E4 phenotype: cross sectional population based study: BMJ, v. 315, no. 7115, p. 1045-1049.

Lacchini, S., E. L. Ferlin, R. S. Moraes, J. P. Ribeiro, and M. C. Irigoyen, 2001b, Contribution of nitric oxide to arterial pressure and heart rate variability in rats submitted to high-sodium intake: Hypertension, v. 38, no. 3, p. 326-331.

Lacchini, S., E. L. Ferlin, R. S. Moraes, J. P. Ribeiro, and M. C. Irigoyen, 2001a, Contribution of nitric oxide to arterial pressure and heart rate variability in rats submitted to high-sodium intake: Hypertension, v. 38, no. 3, p. 326-331.

Laiprasert, J. D., R. L. Hamlin, and C. M. Heesch, 2001, Afferent baroreceptor discharge in pregnant rats: American Journal of Physiology-Heart and Circulatory Physiology, v. 281, no. 6, p. H2456-H2462.

Laiprasert, J. D., R. C. Rogers, and C. M. Heesch, 1998, Neurosteroid modulation of arterial baroreflex-sensitive neurons in rat rostral ventrolateral medulla: Am.J.Physiol, v. 274, no. 4 Pt 2, p. R903-R911.

Laitinen, T., J. Hartikainen, E. Vanninen, L. Niskanen, G. Geelen, and E. Lansimies, 1998, Age and gender dependency of baroreflex sensitivity in healthy subjects: J.Appl.Physiol, v. 84, no. 2, p. 576-583.

Latchford, K. J., and A. V. Ferguson, 2003, Angiotensin II activates a nitric-oxide-driven inhibitory feedback in the rat paraventricular nucleus: J.Neurophysiol., v. 89, no. 3, p. 1238-1244.

Lee, W. B., and E. R. Lumbers, 1981, Angiotensin and the cardiac baroreflex response to phenylephrine: Clin.Exp.Pharmacol.Physiol, v. 8, no. 2, p. 109-117.

Lee, Y. C., Y. H. Ko, Y. P. Hsu, and L. T. Ho, 2006, Plasma leptin response to oral glucose tolerance and fasting/re-feeding tests in rats with fructose-induced metabolic derangements: Life Sci..

Li, Q., W. E. Dale, E. M. Hasser, and E. H. Blaine, 1996, Acute and chronic angiotensin hypertension: neural and nonneural components, time course, and dose dependency: Am.J.Physiol, v. 271, no. 1 Pt 2, p. R200-R207.

Li, R. W., T. D. Douglas, G. K. Maiyoh, K. Adeli, and A. G. Theriault, 2005a, Green tea leaf extract improves lipid and glucose homeostasis in a fructose-fed insulin-resistant hamster model: J.Ethnopharmacol..

Li, Y. F., W. Wang, W. G. Mayhan, and K. P. Patel, 2005b, Angiotensin-mediated increase in renal sympathetic nerve discharge within the pvn: role of nitric oxide: Am.J.Physiol Regul.Integr.Comp Physiol.

Li, Y. W., and P. G. Guyenet, 1995, Neuronal excitation by angiotensin II in the rostral ventrolateral medulla of the rat in vitro: Am.J.Physiol, v. 268, no. 1 Pt 2, p. R272-R277.

Li, Y. W., and P. G. Guyenet, 1996, Angiotensin II decreases a resting K+ conductance in rat bulbospinal neurons of the C1 area: Circ.Res., v. 78, no. 2, p. 274-282.

Lin, H. C., F. J. Wan, and C. J. Tseng, 1999, Modulation of cardiovascular effects produced by nitric oxide and ionotropic glutamate receptor interaction in the nucleus tractus solitarii of rats: Neuropharmacology, v. 38, no. 7, p. 935-941.

Lind, R. W., L. W. Swanson, and D. Ganten, 1985, Organization of angiotensin II immunoreactive cells and fibers in the rat central nervous system. An immunohistochemical study: Neuroendocrinology, v. 40, no. 1, p. 2-24.

Liu, J. L., H. Murakami, M. Sanderford, V. S. Bishop, and I. H. Zucker, 1999, ANG II and baroreflex function in rabbits with CHF and lesions of the area postrema: Am.J.Physiol, v. 277, no. 1 Pt 2, p. H342-H350.

Liu, J. L., H. Murakami, and I. H. Zucker, 1996, Effects of NO on baroreflex control of heart rate and renal nerve activity in conscious rabbits: Am.J.Physiol, v. 270, no. 6 Pt 2, p. R1361-R1370.

Liu, J. L., H. Murakami, and I. H. Zucker, 1998, Angiotensin II-nitric oxide interaction on sympathetic outflow in conscious rabbits: Circ.Res., v. 82, no. 4, p. 496-502.

Lo, W. J., H. W. Liu, H. C. Lin, L. P. Ger, C. S. Tung, and C. J. Tseng, 1996, Modulatory effects of nitric oxide on baroreflex activation in the brainstem nuclei of rats: Chin J.Physiol, v. 39, no. 1, p. 57-62.

Lovick, T. A., and J. H. Coote, 1988, Electrophysiological properties of paraventriculo-spinal neurones in the rat: Brain Res., v. 454, no. 1-2, p. 123-130.

Lumbers, E. R., and Z. Y. Yu, 1999, A method for determining baroreflex-mediated sympathetic and parasympathetic control of the heart in pregnant and non-pregnant sheep: J.Physiol, v. 515 (Pt 2), p. 555-566.

Ma, R., G. Q. Zhu, and W. Wang, 2005, Interaction of central Ang II and NO on the cardiac sympathetic afferent reflex in dogs: Auton.Neurosci., v. 118, no. 1-2, p. 51-60.

Machado, B. H., 2001, Neurotransmission of the cardiovascular reflexes in the nucleus tractus solitarii of awake rats: Ann.N.Y.Acad.Sci., v. 940, p. 179-196.

Man, H. Y., T. Erclik, L. E. Becker, and Y. T. Wang, 1998, Modulation of baroreflex sensitivity by the state of protein tyrosine phosphorylation in the brainstem of the rat: Brain Res., v. 792, no. 1, p. 141-148.

Marcoli, M., G. Maura, M. Tortarolo, and M. Raiteri, 1997, Serotonin inhibition of the NMDA receptor/nitric oxide/cyclic GMP pathway in rat cerebellum: involvement of 5-hydroxytryptamine2C receptors: J.Neurochem., v. 69, no. 1, p. 427-430.

Masilamani, S., and C. M. Heesch, 1997, Effects of pregnancy and progesterone metabolites on arterial baroreflex in conscious rats: Am.J.Physiol, v. 272, no. 3 Pt 2, p. R924-R934.

Matsuda, T., J. N. Bates, S. J. Lewis, F. M. Abboud, and M. W. Chapleau, 1995, Modulation of baroreceptor activity by nitric oxide and S-nitrosocysteine: Circ.Res., v. 76, no. 3, p. 426-433.

Matsukawa, S., and I. A. Reid, 1990, Role of the area postrema in the modulation of the baroreflex control of heart rate by angiotensin II: Circ.Res., v. 67, no. 6, p. 1462-1473.

Matsumura, K., I. Abe, T. Tsuchihashi, and M. Fujishima, 1998a, Central nitric oxide attenuates the baroreceptor reflex in conscious rabbits: Am.J.Physiol, v. 274, no. 4 Pt 2, p. R1142-R1149.

Matsumura, K., D. B. Averill, and C. M. Ferrario, 1998b, Angiotensin II acts at AT(1) receptors in the nucleus of the solitary tract to attenuate the baroreceptor reflex: American Journal of Physiology-Regulatory Integrative and Comparative Physiology, v. 44, no. 5, p. R1611-R1619.

Matsumura, K., T. Tsuchihashi, S. Kagiyama, I. Abe, and M. Fujishima, 1998c, Role of nitric oxide in the nucleus of the solitary tract of rats: Brain Res., v. 798, no. 1-2, p. 232-238.

Matsumura, K., T. Tsuchihashi, S. Kagiyama, I. Abe, and M. Fujishima, 1999, Subtypes of metabotropic glutamate receptors in the nucleus of the solitary tract of rats: Brain Res., v. 842, no. 2, p. 461-468.

Matsuzawa, Y., 2005, Adipocytokines and metabolic syndrome: Semin.Vasc.Med., v. 5, no. 1, p. 34-39.

Maura, G., M. Marcoli, O. Pepicelli, C. Rosu, C. Viola, and M. Raiteri, 2000, Serotonin inhibition of the NMDA receptor/nitric oxide/cyclic GMP pathway in human neocortex slices: involvement of 5-HT(2C) and 5-HT(1A) receptors: Br.J.Pharmacol., v. 130, no. 8, p. 1853-1858.

Mayorov, D. N., 2005, Selective Sensitization by Nitric Oxide of Sympathetic Baroreflex in Rostral Ventrolateral Medulla of Conscious Rabbits: Hypertension.

McKeogh, D. F., T. L. O'Donaughy, and V. L. Brooks, 2004, NO and endogenous angiotensin II interact in the generation of renal sympathetic nerve activity in conscious rats: Am.J.Physiol Heart Circ.Physiol, v. 286, no. 4, p. H1258-H1265.

McKernan, A. M., and F. R. Calaresu, 1996, Insulin microinjection into the nucleus tractus solitarii of the rat attenuates the baroreceptor reflex: J.Auton.Nerv.Syst., v. 61, no. 2, p. 128-138.

McLaughlin, M. K., and K. P. Conrad, 1995, Nitric oxide biosynthesis during pregnancy: implications for circulatory changes: Clin.Exp.Pharmacol.Physiol, v. 22, no. 2, p. 164-171.

Melczer, Z., F. Banhidy, S. Csomor, M. Kovacs, P. Siklos, G. Winkler, and K. Cseh, 2002a, Role of tumour necrosis factor-alpha in insulin resistance during normal pregnancy: Eur.J.Obstet.Gynecol.Reprod.Biol., v. 105, no. 1, p. 7-10.

Melczer, Z., F. Banhidy, S. Csomor, M. Kovacs, P. Siklos, G. Winkler, and K. Cseh, 2002b, Role of tumour necrosis factor-alpha in insulin resistance during normal pregnancy: Eur.J.Obstet.Gynecol.Reprod.Biol., v. 105, no. 1, p. 7-10.

Mellon, S. H., L. D. Griffin, and N. A. Compagnone, 2001, Biosynthesis and action of neurosteroids: Brain Res.Brain Res.Rev., v. 37, no. 1-3, p. 3-12.

Mendelsohn, F. A., A. M. Allen, J. Clevers, D. A. Denton, E. Tarjan, and M. J. McKinley, 1988, Localization of angiotensin II receptor binding in rabbit brain by in vitro autoradiography: J.Comp Neurol., v. 270, no. 3, p. 372-384.

Miatello, R., M. Cruzado, and N. Risler, 2004, Mechanisms of cardiovascular changes in an experimental model of syndrome X and pharmacological intervention on the renin-angiotensin-system: Curr.Vasc.Pharmacol., v. 2, no. 4, p. 371-377.

Miller, A. W., J. J. Sims, A. Canavan, T. Hsu, and M. R. Ujhelyi, 1999, Impaired vagal reflex activity in insulin-resistant rats: J.Cardiovasc.Pharmacol., v. 33, no. 5, p. 698-702.

Minami, N., Y. Imai, J. Hashimoto, and K. Abe, 1995b, The role of nitric oxide in the baroreceptor-cardiac reflex in conscious Wistar rats: Am.J.Physiol, v. 269, no. 3 Pt 2, p. H851-H855.

Minami, N., Y. Imai, J. Hashimoto, and K. Abe, 1995a, The role of nitric oxide in the baroreceptor-cardiac reflex in conscious Wistar rats: Am.J.Physiol, v. 269, no. 3 Pt 2, p. H851-H855.

Miyano, H., T. Kawada, T. Shishido, T. Sato, M. Sugimachi, J. Alexander, Jr., and K. Sunagawa, 1997, Inhibition of NO synthesis minimally affects the dynamic baroreflex regulation of sympathetic nerve activity: Am.J.Physiol, v. 272, no. 5 Pt 2, p. H2446-H2452.

Molnar, M., and F. Hertelendy, 1992, N omega-nitro-L-arginine, an inhibitor of nitric oxide synthesis, increases blood pressure in rats and reverses the pregnancy-induced refractoriness to vasopressor agents: Am.J.Obstet.Gynecol., v. 166, no. 5, p. 1560-1567.

Moncada, S., R. M. Palmer, and E. A. Higgs, 1991, Nitric oxide: physiology, pathophysiology, and pharmacology: Pharmacol.Rev., v. 43, no. 2, p. 109-142.

Monga, M., and R. K. Creasy, 1999, Cardiovascular and renal adaptation to pregnancy, p. 758-766.

Morrison, S. F., P. Ernsberger, T. A. Milner, J. Callaway, A. Gong, and D. J. Reis, 1989, A glutamate mechanism in the intermediolateral nucleus mediates sympathoexcitatory responses to stimulation of the rostral ventrolateral medulla: Prog.Brain Res., v. 81, p. 159-169.

Morton, M. J., 1992, Cardiovascular Adaptation to Pregnancy, in P Ramwell, G Rubanyi, and E Schillinger eds., Sex Steroids and the Cardiovascular System.: New York, NY, Springer-Verlag,Schering Foundation Workshop, p. 53-64.

Muntzel, M., T. Beltz, A. L. Mark, and A. K. Johnson, 1994a, Anteroventral third ventricle lesions abolish lumbar sympathetic responses to insulin: Hypertension, v. 23, no. 6 Pt 2, p. 1059-1062.

Muntzel, M. S., H. Malena, and T. Drueke, 2001a, Inhibition of nitric oxide synthesis attenuates insulin-mediated sympathetic activation in rats: J.Hypertens., v. 19, no. 9, p. 1625-1631.

Muntzel, M. S., J. Morales, and A. Akinsefunmi, 2001b, Converting enzyme inhibition with captopril abolishes sympathoexcitation to euglycemic hyperinsulinemia in rats: Am.J.Hypertens., v. 14, no. 6 Pt 1, p. 592-598.

Muntzel, M. S., D. A. Morgan, A. L. Mark, and A. K. Johnson, 1994b, Intracerebroventricular insulin produces nonuniform regional increases in sympathetic nerve activity: Am.J.Physiol, v. 267, no. 5 Pt 2, p. R1350-R1355.

Murakami, H., J. L. Liu, and I. H. Zucker, 1996, Blockade of AT1 receptors enhances baroreflex control of heart rate in conscious rabbits with heart failure: Am.J.Physiol, v. 271, no. 1 Pt 2, p. R303-R309.

Murakami, H., J. L. Liu, and I. H. Zucker, 1997, Angiotensin II blockade [corrected] enhances baroreflex control of sympathetic outflow in heart failure: Hypertension, v. 29, no. 2, p. 564-569.

Neff, R. A., M. Mihalevich, and D. Mendelowitz, 1998, Stimulation of NTS activates NMDA and non-NMDA receptors in rat cardiac vagal neurons in the nucleus ambiguus: Brain Res., v. 792, no. 2, p. 277-282.

Nicholls, M. G., J. I. Robertson, and T. Inagami, 2001, The renin-angiotensin system in the twenty-first century: Blood Press, v. 10, no. 5-6, p. 327-343.

Nishida, Y., Q. H. Chen, M. Tandai-Hiruma, S. Terada, and J. Horiuchi, 2001, Neuronal nitric oxide strongly suppresses sympathetic outflow in high-salt Dahl rats: J.Hypertens., v. 19, no. 3 Pt 2, p. 627-634.

Nuwayhid, B., 1979, Hemodynamic changes during pregnancy in the rabbit: Am.J.Obstet.Gynecol., v. 135, no. 5, p. 590-596.

O'Hagan, K. P., and S. M. Casey, 1998, Arterial baroreflex during pregnancy and renal sympathetic nerve activity during parturition in rabbits: Am.J.Physiol, v. 274, no. 5 Pt 2, p. H1635-H1642.

O'Hagan, K. P., K. A. Skogg, and J. B. Stevenson, 2001, AT1 receptor block does not affect arterial baroreflex during pregnancy in rabbits: Am.J.Physiol Heart Circ.Physiol, v. 280, no. 5, p. H1996-H2005.

O'Malley, D., and J. Harvey, 2004, Insulin activates native and recombinant large conductance Ca(2+)-activated potassium channels via a mitogen-activated protein kinase-dependent process: Mol.Pharmacol., v. 65, no. 6, p. 1352-1363.

O'Malley, D., L. J. Shanley, and J. Harvey, 2003, Insulin inhibits rat hippocampal neurones via activation of ATP-sensitive K+ and large conductance Ca2+-activated K+ channels: Neuropharmacology, v. 44, no. 7, p. 855-863.

Obici, S., Z. Feng, G. Karkanias, D. G. Baskin, and L. Rossetti, 2002, Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats: Nat.Neurosci., v. 5, no. 6, p. 566-572.

Ohta, H., and W. T. Talman, 1994, Both NMDA and non-NMDA receptors in the NTS participate in the baroreceptor reflex in rats: Am.J.Physiol, v. 267, no. 4 Pt 2, p. R1065-R1070.

Okada, J., and M. Miura, 1997, Barosensitive cardioinhibitory neurons in the medulla: comparison of FosB/ChAT-positive neurons with CT-HRP-labeled neurons: J.Auton.Nerv.Syst., v. 64, no. 2-3, p. 85-90.

Okada, M., and R. D. Bunag, 1994, Insulin acts centrally to enhance reflex tachycardia in conscious rats: Am.J.Physiol, v. 266, no. 2 Pt 2, p. R481-R486.

Okere, C. O., and T. Higuchi, 1996, Down-regulation of endogenous nitric oxide synthase in late-pregnancy and parturition in the rat hypothalamic magnocellular neurons and neurohypophysis: Neurosci.Lett., v. 220, no. 2, p. 133-136.

Olsson, K., N. E. Anden, K. Johansson, and U. Thornstrom, 1987, Effects of acute haemorrhagic hypotension during pregnancy and lactation in conscious goats: Acta Physiol Scand., v. 129, no. 4, p. 479-487.

onso-Galicia, M., M. W. Brands, D. H. Zappe, and J. E. Hall, 1996, Hypertension in obese Zucker rats. Role of angiotensin II and adrenergic activity: Hypertension, v. 28, no. 6, p. 1047-1054.

Palkovits, M., and M. Brownstein, 1983, Microdissection of brain areas by the punch technique, in AC Cuello ed., Brain Microdissection Techniques: Chichester, John Wiley and Sons Ltd,Methods in the neurosiences series, p. 1-36.

Palkovits, M., and L. Zaborszky, 1977, Neuroanatomy of central cardiovascular control. Nucleus tractus solitarii: afferent and efferent neuronal connections in relation to the baroreceptor reflex arc: Prog.Brain Res., v. 47, p. 9-34.

Patel, K. P., and P. G. Schmid, 1988, Role of paraventricular nucleus (PVH) in baroreflex-mediated changes in lumbar sympathetic nerve activity and heart rate: J.Auton.Nerv.Syst., v. 22, no. 3, p. 211-219.

Patel, K. P., K. Zhang, I. H. Zucker, and T. L. Krukoff, 1996, Decreased gene expression of neuronal nitric oxide synthase in hypothalamus and brainstem of rats in heart failure: Brain Res., v. 734, no. 1-2, p. 109-115.

Paton, J. F., P. Boscan, D. Murphy, and S. Kasparov, 2001a, Unravelling mechanisms of action of angiotensin II on cardiorespiratory function using in vivo gene transfer: Acta Physiol Scand., v. 173, no. 1, p. 127-137.

Paton, J. F., J. Deuchars, Z. Ahmad, L. F. Wong, D. Murphy, and S. Kasparov, 2001b, Adenoviral vector demonstrates that angiotensin II-induced depression of the cardiac baroreflex is mediated by endothelial nitric oxide synthase in the nucleus tractus solitarii of the rat: J.Physiol, v. 531, no. Pt 2, p. 445-458.

Paton, J. F., H. Waki, and S. Kasparov, 2003, In vivo gene transfer to dissect neuronal mechanisms regulating cardiorespiratory function: Can.J.Physiol Pharmacol., v. 81, no. 4, p. 311-316.

Patzak, A., E. Lai, P. B. Persson, and A. E. Persson, 2005, Angiotensin II-nitric oxide interaction in glomerular arterioles: Clin.Exp.Pharmacol.Physiol, v. 32, no. 5-6, p. 410-414.

Patzak, A., E. Y. Lai, R. Mrowka, A. Steege, P. B. Persson, and A. E. Persson, 2004, AT1 receptors mediate angiotensin II-induced release of nitric oxide in afferent arterioles: Kidney Int., v. 66, no. 5, p. 1949-1958.

Paul, S. M., and R. H. Purdy, 1992, Neuroactive steroids: FASEB J., v. 6, no. 6, p. 2311-2322.

Petersen, J. S., and G. F. DiBona, 1996, Acute sympathoinhibitory actions of metformin in spontaneously hypertensive rats: Hypertension, v. 27, no. 3 Pt 2, p. 619-625.

Pikkujamsa, S. M., H. V. Huikuri, K. E. Airaksinen, A. O. Rantala, H. Kauma, M. Lilja, M. J. Savolainen, and Y. A. Kesaniemi, 1998, Heart rate variability and baroreflex

sensitivity in hypertensive subjects with and without metabolic features of insulin resistance syndrome: Am.J.Hypertens., v. 11, no. 5, p. 523-531.

Pontieri, V., M. K. Venezuela, C. Scavone, and L. C. Michelini, 1998, Role of endogenous nitric oxide in the nucleus tratus solitarii on baroreflex control of heart rate in spontaneously hypertensive rats: J.Hypertens., v. 16, no. 12 Pt 2, p. 1993-1999.

Pueyo, M. E., J. F. Arnal, J. Rami, and J. B. Michel, 1998, Angiotensin II stimulates the production of NO and peroxynitrite in endothelial cells: Am.J.Physiol, v. 274, no. 1 Pt 1, p. C214-C220.

Quesnell, R. R., and V. L. Brooks, 1997, Alterations in the baroreflex occur late in pregnancy in conscious rabbits: Am.J.Obstet.Gynecol., v. 176, no. 3, p. 692-694.

Reis, F. M., A. M. Reis, and C. C. Coimbra, 1997, Effects of hyperprolactinaemia on glucose tolerance and insulin release in male and female rats: J.Endocrinol., v. 153, no. 3, p. 423-428.

Rettig, R., D. P. Healy, and M. P. Printz, 1986, Cardiovascular effects of microinjections of angiotensin II into the nucleus tractus solitarii: Brain Res., v. 364, no. 2, p. 233-240.

Reusch, J. E., 2002, Current concepts in insulin resistance, type 2 diabetes mellitus, and the metabolic syndrome: Am.J.Cardiol., v. 90, no. 5A, p. 19G-26G.

Roberts, C. K., and R. J. Barnard, 2005, Effects of exercise and diet on chronic disease: J.Appl.Physiol, v. 98, no. 1, p. 3-30.

Robson, S. C., S. Hunter, R. J. Boys, and W. Dunlop, 1989, Serial study of factors influencing changes in cardiac output during human pregnancy: Am.J.Physiol, v. 256, no. 4 Pt 2, p. H1060-H1065.

Roden, M., 2004, How free fatty acids inhibit glucose utilization in human skeletal muscle: News Physiol Sci., v. 19, p. 92-96.

Ross, C. A., D. A. Ruggiero, T. H. Joh, D. H. Park, and D. J. Reis, 1984, Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C1 adrenaline neurons: J.Comp Neurol., v. 228, no. 2, p. 168-185.

Ross, C. A., D. A. Ruggiero, and D. J. Reis, 1985, Projections from the nucleus tractus solitarii to the rostral ventrolateral medulla: J.Comp Neurol., v. 242, no. 4, p. 511-534.

Ruggeri, P., C. Molinari, A. Brunori, C. E. Cogo, D. A. Mary, V. Picchio, and G. Vacca, 2001, The direct effect of insulin on barosensitive neurones in the nucleus tractus solitarii of rats: Neuroreport, v. 12, no. 17, p. 3719-3722.

Ryan, A. S., 2000, Insulin resistance with aging: effects of diet and exercise: Sports Med., v. 30, no. 5, p. 327-346.

Ryan, E. A., and L. Enns, 1988, Role of gestational hormones in the induction of insulin resistance: J.Clin.Endocrinol.Metab, v. 67, no. 2, p. 341-347.

Saavedra, J. M., 2005, Brain angiotensin II: new developments, unanswered questions and therapeutic opportunities: Cell Mol.Neurobiol., v. 25, no. 3-4, p. 485-512.

Saigusa, T., and G. A. Head, 1993, Renal sympathetic baroreflex effects of angiotensin II infusions into the rostral ventrolateral medulla of the rabbit: Clin.Exp.Pharmacol.Physiol, v. 20, no. 5, p. 351-354.

Sanderford, M. G., and V. S. Bishop, 2002, Central mechanisms of acute ANG II modulation of arterial baroreflex control of renal sympathetic nerve activity: Am.J.Physiol Heart Circ.Physiol, v. 282, no. 5, p. H1592-H1602.

Sasaki, S., and R. A. Dampney, 1990, Tonic cardiovascular effects of angiotensin II in the ventrolateral medulla: Hypertension, v. 15, no. 3, p. 274-283.

Sato, Y., 2000, Diabetes and life-styles: role of physical exercise for primary prevention: Br.J.Nutr., v. 84 Suppl 2, p. S187-S190.

Sawchenko, P. E., and L. W. Swanson, 1982, The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat: Brain Res., v. 257, no. 3, p. 275-325.

Schmitz-Peiffer, C., 2000, Signalling aspects of insulin resistance in skeletal muscle: mechanisms induced by lipid oversupply: Cell Signal., v. 12, no. 9-10, p. 583-594.

Segieth, J., S. J. Getting, C. S. Biggs, and P. S. Whitton, 1995, Nitric oxide regulates excitatory amino acid release in a biphasic manner in freely moving rats: Neurosci.Lett., v. 200, no. 2, p. 101-104.

Severs, W. B., A. E. Daniels, H. H. Smookler, W. J. Kinnard, and J. P. Buckley, 1966, Interrelationship between angiotensin II and the sympathetic nervous system: J.Pharmacol.Exp.Ther., v. 153, no. 3, p. 530-537.

Shapiro, R. E., and R. R. Miselis, 1985, The central neural connections of the area postrema of the rat: J.Comp Neurol., v. 234, no. 3, p. 344-364.

Shapoval, L. N., V. F. Sagach, and L. S. Pobegailo, 1991, Nitric oxide influences ventrolateral medullary mechanisms of vasomotor control in the cat: Neurosci.Lett., v. 132, no. 1, p. 47-50.

Shoji, M., L. Share, and J. T. Crofton, 1989, Effect on vasopressin release of microinjection of angiotensin II into the paraventricular nucleus of conscious rats: Neuroendocrinology, v. 50, no. 3, p. 327-333.

Silverman, A. J., D. L. Hoffman, and E. A. Zimmerman, 1981, The descending afferent connections of the paraventricular nucleus of the hypothalamus (PVN): Brain Res.Bull., v. 6, no. 1, p. 47-61.

Sivan, E., and G. Boden, 2003, Free fatty acids, insulin resistance, and pregnancy: Curr.Diab.Rep., v. 3, no. 4, p. 319-322.

Sivan, E., C. J. Homko, P. G. Whittaker, E. A. Reece, X. Chen, and G. Boden, 1998, Free fatty acids and insulin resistance during pregnancy: J.Clin.Endocrinol.Metab, v. 83, no. 7, p. 2338-2342.

Skinner, S. L., E. R. Lumbers, and E. M. Symonds, 1972, Analysis of changes in the renin-angiotensin system during pregnancy: Clin.Sci., v. 42, no. 4, p. 479-488.

Sladek, S. M., R. R. Magness, and K. P. Conrad, 1997, Nitric oxide and pregnancy: Am.J.Physiol, v. 272, no. 2 Pt 2, p. R441-R463.

Smith, S. A., J. H. Mitchell, and J. Li, 2005, Independent modification of baroreceptor and exercise pressor reflex function by nitric oxide in nucleus tractus solitarius: Am.J.Physiol Heart Circ.Physiol, v. 288, no. 5, p. H2068-H2076.

Snitsarev, V., C. A. Whiteis, F. M. Abboud, and M. W. Chapleau, 2002, Mechanosensory transduction of vagal and baroreceptor afferents revealed by study of isolated nodose neurons in culture: Auton.Neurosci., v. 98, no. 1-2, p. 59-63.

Song, K., A. M. Allen, G. Paxinos, and F. A. Mendelsohn, 1992, Mapping of angiotensin II receptor subtype heterogeneity in rat brain: J.Comp Neurol., v. 316, no. 4, p. 467-484.

Stauss, H. M., 2002, Baroreceptor reflex function: Am.J.Physiol Regul.Integr.Comp Physiol, v. 283, no. 2, p. R284-R286.

Stein, L. J., D. M. Dorsa, D. G. Baskin, D. P. Figlewicz, D. Porte, Jr., and S. C. Woods, 1987, Reduced effect of experimental peripheral hyperinsulinemia to elevate cerebrospinal fluid insulin concentrations of obese Zucker rats: Endocrinology, v. 121, no. 5, p. 1611-1615.

Stornetta, R. L., P. G. Guyenet, and R. C. McCarty, 1987, Autonomic nervous system control of heart rate during baroreceptor activation in conscious and anesthetized rats: J.Auton.Nerv.Syst., v. 20, no. 2, p. 121-127.

Sunagawa, K., T. Sato, and T. Kawada, 2001, Integrative sympathetic baroreflex regulation of arterial pressure: Ann.N.Y.Acad.Sci., v. 940, p. 314-323.

Sundaram, K., and H. Sapru, 1991, NMDA receptors in the intermediolateral column of the spinal cord mediate sympathoexcitatory cardiac responses elicited from the ventrolateral medullary pressor area: Brain Res., v. 544, no. 1, p. 33-41.

Tagawa, T., and R. A. Dampney, 1999, AT(1) receptors mediate excitatory inputs to rostral ventrolateral medulla pressor neurons from hypothalamus: Hypertension, v. 34, no. 6, p. 1301-1307.

Talman, W. T., and D. N. Dragon, 2004, Transmission of arterial baroreflex signals depends on neuronal nitric oxide synthase: Hypertension, v. 43, no. 4, p. 820-824.

Tam, S. L., and S. Kaufman, 2002, NOS inhibition restores renal responses to atrial distension during pregnancy: Am.J.Physiol Regul.Integr.Comp Physiol, v. 282, no. 5, p. R1364-R1367.

Tandai-Hiruma, M., J. Horiuchi, H. Sakamoto, T. Kemuriyama, H. Hirakawa, and Y. Nishida, 2005, Brain neuronal nitric oxide synthase neuron-mediated sympathoinhibition is enhanced in hypertensive Dahl rats: J.Hypertens., v. 23, no. 4, p. 825-834.

Thornburg, K. L., S. L. Jacobson, G. D. Giraud, and M. J. Morton, 2000, Hemodynamic changes in pregnancy: Seminars in Perinatology, v. 24, no. 1, p. 11-14.

Togashi, H., I. Sakuma, M. Yoshioka, T. Kobayashi, H. Yasuda, A. Kitabatake, H. Saito, S. S. Gross, and R. Levi, 1992, A central nervous system action of nitric oxide in blood pressure regulation: J.Pharmacol.Exp.Ther., v. 262, no. 1, p. 343-347.

Togashi, N., N. Ura, K. Higashiura, H. Murakami, and K. Shimamoto, 2002, Effect of TNF-alpha--converting enzyme inhibitor on insulin resistance in fructose-fed rats: Hypertension, v. 39, no. 2 Pt 2, p. 578-580.

Tresham, J. J., P. Boutros, E. M. Wintour, and C. N. May, 1996, Role of nitric oxide in the attenuated pressor responses of pregnant or sodium-deplete sheep: J.Cardiovasc.Pharmacol., v. 27, no. 6, p. 908-915.

Tseng, C. J., H. Y. Liu, H. C. Lin, L. P. Ger, C. S. Tung, and M. H. Yen, 1996, Cardiovascular effects of nitric oxide in the brain stem nuclei of rats: Hypertension, v. 27, no. 1, p. 36-42.

Uryszek, W., J. Paluszak, E. Uryszek, Z. Soszynska, and M. Kaminska, 1993, [Effect of progesterone on lipolysis in blood and adipose tissue of the female rat]: Endokrynol.Pol., v. 44, no. 2, p. 195-203.

Van Zwieten, P. A., K. L. Kam, A. J. Pijl, M. G. Hendriks, O. H. Beenen, and M. Pfaffendorf, 1996, Hypertensive diabetic rats in pharmacological studies: Pharmacol.Res., v. 33, no. 2, p. 95-105.

Vasudevan, A. R., and A. Balasubramanyam, 2004, Thiazolidinediones: a review of their mechanisms of insulin sensitization, therapeutic potential, clinical efficacy, and tolerability: Diabetes Technol.Ther., v. 6, no. 6, p. 850-863.

Vistelle, R., R. Jaussaud, T. Trenque, and M. Wiczewski, 1994, Rapid and simple cannulation technique for repeated sampling of cerebrospinal fluid in the conscious rabbit: Lab Anim Sci., v. 44, no. 4, p. 362-364.

Wang, Y., K. P. Patel, K. G. Cornish, K. M. Channon, and I. H. Zucker, 2003, nNOS gene transfer to RVLM improves baroreflex function in rats with chronic heart failure: Am.J.Physiol Heart Circ.Physiol, v. 285, no. 4, p. H1660-H1667.

Weiner, C. P., R. G. Knowles, and S. Moncada, 1994a, Induction of nitric oxide synthases early in pregnancy: Am.J.Obstet.Gynecol., v. 171, no. 3, p. 838-843.

Weiner, C. P., I. Lizasoain, S. A. Baylis, R. G. Knowles, I. G. Charles, and S. Moncada, 1994b, Induction of calcium-dependent nitric oxide synthases by sex hormones: Proc.Natl.Acad.Sci.U.S.A, v. 91, no. 11, p. 5212-5216.

Weiner, C. P., and L. P. Thompson, 1997, Nitric oxide and pregnancy: Semin.Perinatol., v. 21, no. 5, p. 367-380.

Werther, G. A., A. Hogg, B. J. Oldfield, M. J. McKinley, R. Figdor, A. M. Allen, and F. A. Mendelsohn, 1987, Localization and characterization of insulin receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry: Endocrinology, v. 121, no. 4, p. 1562-1570.

Willis, W. D., 1998, The autonomic nervous system and its central control, in RM Berne and MN Levy eds., Physiology: Portland, OR, Mosby, p. 233-248.

Wood, P. L., M. R. Emmett, T. S. Rao, J. Cler, S. Mick, and S. Iyengar, 1990, Inhibition of nitric oxide synthase blocks N-methyl-D-aspartate-, quisqualate-, kainate-, harmaline-, and pentylenetetrazole-dependent increases in cerebellar cyclic GMP in vivo: J.Neurochem., v. 55, no. 1, p. 346-348.

Woods, S. C., R. J. Seeley, D. G. Baskin, and M. W. Schwartz, 2003, Insulin and the blood-brain barrier: Curr.Pharm.Des, v. 9, no. 10, p. 795-800.

Woodside, B., and S. Amir, 1996, Reproductive state changes NADPH-diaphorase staining in the paraventricular and supraoptic nuclei of female rats: Brain Res., v. 739, no. 1-2, p. 339-342.

Wu, W. C., Y. Wang, L. S. Kao, F. I. Tang, and C. Y. Chai, 2002, Nitric oxide reduces blood pressure in the nucleus tractus solitarius: a real time electrochemical study: Brain Res.Bull., v. 57, no. 2, p. 171-177.

Xu, D. L., P. Y. Martin, J. St.John, P. Tsai, S. N. Summer, M. Ohara, J. K. Kim, and R. W. Schrier, 1996, Upregulation of endothelial and neuronal constitutive nitric oxide synthase in pregnant rats: Am.J.Physiol, v. 271, no. 6 Pt 2, p. R1739-R1745.

Yabe, M., K. Nishikawa, T. Terai, H. Yukioka, and M. Fujimori, 1998, The effects of intrinsic nitric oxide on cardiac neural regulation in cats: Anesth.Analg., v. 86, no. 6, p. 1194-1200.

Yang, Z., L. Smith, and J. H. Coote, 2004, Paraventricular nucleus activation of renal sympathetic neurones is synaptically depressed by nitric oxide and glycine acting at a spinal level: Neuroscience, v. 124, no. 2, p. 421-428.

Zanzinger, J., J. Czachurski, and H. Seller, 1995b, Inhibition of basal and reflex-mediated sympathetic activity in the RVLM by nitric oxide: Am.J.Physiol, v. 268, no. 4 Pt 2, p. R958-R962.

Zanzinger, J., J. Czachurski, and H. Seller, 1995a, Effects of nitric oxide on sympathetic baroreflex transmission in the nucleus tractus solitarii and caudal ventrolateral medulla in cats: Neurosci.Lett., v. 197, no. 3, p. 199-202.

Zanzinger, J., J. Czachurski, and H. Seller, 1996, Lack of nitric oxide sensitivity of carotid sinus baroreceptors activated by normal blood pressure stimuli in cats: Neurosci.Lett., v. 208, no. 2, p. 121-124.

Zanzinger, J., J. Czachurski, and H. Seller, 1997, Neuronal nitric oxide reduces sympathetic excitability by modulation of central glutamate effects in pigs: Circ.Res., v. 80, no. 4, p. 565-571.

Zhang, J., and S. W. Mifflin, 1998, Differential roles for NMDA and non-NMDA receptor subtypes in baroreceptor afferent integration in the nucleus of the solitary tract of the rat: J.Physiol, v. 511 (Pt 3), p. 733-745.

Zhang, K., W. G. Mayhan, and K. P. Patel, 1997, Nitric oxide within the paraventricular nucleus mediates changes in renal sympathetic nerve activity: Am.J.Physiol, v. 273, no. 3 Pt 2, p. R864-R872.

Zhang, K., and K. P. Patel, 1998, Effect of nitric oxide within the paraventricular nucleus on renal sympathetic nerve discharge: role of GABA: Am.J.Physiol, v. 275, no. 3 Pt 2, p. R728-R734.

APPENDIX

NITRIC OXIDE IMPAIRS BAROREFLEX GAIN DURING ACUTE PSYCHOLOGICAL STRESS

ABSTRACT

Psychological stress suppresses baroreflex function, but the mechanism has not been fully elucidated. Nitric oxide and glucocorticoids increase during stress and have been shown to suppress reflex gain in unstressed animals. Therefore, the purpose of this study was to test the hypothesis that stress, caused by exposure to a novel environment, decreases baroreflex gain in rabbits through the actions of nitric oxide to increase corticosterone release. Baroreflex control of heart rate and plasma corticosterone levels were quantified before and after blockade of nitric oxide synthase (NOS) with Nω-nitro-L-arginine (20mg/kg, i.v.) in conscious rabbits exposed to a novel environment and the same rabbits once they had been conditioned to the environment. Stress significantly reduced baroreflex gain from –23.4 ± 2 to –12.2 ± 1.6 beats per min (bpm)/mmHg ($p<0.05$) and increased plasma corticosterone levels from 5.4 ± 0.7 to 15.5 ± 5.0 ng/ml ($p<0.05$). NOS blockade increased gain in stressed animals (to -27.2 ±

5.4 bpm/mmHg, $p<0.05$) but did not alter gain in unstressed rabbits (-26.8 ± 4.9 bpm/mmHg), such that gain was equalized between the two states. NOS blockade increased plasma corticosterone levels in unstressed animals (to 14.3 ± 2.1 ng/ml, $p<0.05$) but failed to significantly alter levels in stressed rabbits (14.0 ± 3.9 ng/ml). In conclusion, psychological stress may act via nitric oxide, independently of increases in corticosterone, to decrease baroreflex gain.

INTRODUCTION

Psychological stress can exert profound effects on the cardiovascular system, causing increases in sympathetic activity, blood pressure, and heart rate [for review see (Perna et al., 1997)] and decreases in the sensitivity or gain of the baroreflex (Steptoe et al., 1993; Steptoe et al., 1996; Fauvel et al., 2000; Mezzacappa et al., 2001). Persistent psychological stress may be a contributing factor to hypertension and heart failure (Esch et al., 2002); acute stress is linked with myocardial infarction (Gullette et al., 1997; Stalnikowicz and Tsafrir, 2002). Interestingly, these diseases are also associated with decreases in baroreflex gain (Atherton et al., 1999; Cat et al., 2001), and, in the case of myocardial infarction, a decrease in baroreflex gain is linked to an increased death rate (Billman et al., 1982; Schwartz et al., 1988). Importantly, improving baroreflex gain leads to decreased mortality in these patients (La Rovere et al., 2002). Despite the potential deleterious effects of stress-induced decreases in baroreflex gain, relatively little is known about the mechanism.

Indirect evidence suggests that nitric oxide, a gaseous neurotransmitter, may be involved. Endogenous nitric oxide can act in the brain to modulate central control of

the cardiovascular system [see (Krukoff, 1999) for review] and can decrease baroreflex gain (Liu et al., 1996; Murakami et al., 1998; Paton et al., 2001). Acute stress increases the activity of neurons containing nitric oxide synthase (NOS) activity in brain regions involved in cardiovascular regulation (Hatakeyama et al., 1996; Krukoff and Khalili, 1997). In addition, in rats, acute stress increases nitric oxide production in the brain stem and hypothalamus, in particular the paraventricular nucleus (PVN) (Kawa et al., 2002; Cherney et al., 2003). A positive correlation exists between the increase in nitric oxide in the PVN and the increase in blood pressure during stress (Kawa et al., 2002).

In addition to central actions, stress-induced nitric oxide may also act peripherally to alter the cardiovascular system. Nitric oxide production is increased in the adrenal cortex during stress (Tsuchiya et al., 1997) and can stimulate the release of corticosterone from isolated rat adrenals (Rettori et al., 2003; Mohn et al., 2005). Since glucocorticoids can decrease baroreflex gain (Scheuer and Bechtold, 2002), nitric oxide may exert it effects in part through increased glucocorticoid production. Despite these data, the role of nitric oxide synthesis in the decrease in reflex gain caused by stress has not been previously investigated.

Therefore, we tested the hypothesis in conscious rabbits that acute stress caused by exposure to a novel environment decreases baroreflex gain via a nitric oxide-mediated mechanism and that part of this mechanism involves increased plasma corticosterone levels. To test this hypothesis, we determined if rabbits exhibited a lower baroreflex gain on the first day they were placed in a testing box (stressed), compared to their baroreflex gain after conditioning to the box (unstressed). We also

determined if systemic blockade of NOS in stressed rabbits decreased plasma corticosterone levels as it restored baroreflex gain.

METHODS

All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Oregon Health & Science University Institutional Animal Care and Use Committee.

Female (n = 10) New Zealand White rabbits (Western Oregon Rabbit; Philomath, OR) weighing 3.7 ± 0.1 kg on the first experimental day were used for these experiments. The rabbits were received when they were fourteen weeks old, and allowed a minimum of six days to acclimate to the laboratory environment before surgery.

Surgical preparation

Surgery was performed to implant non-occluding abdominal aortic and vena caval catheters as previously described (Gronan et al., 1983). Briefly, the animals were initially anesthetized with a cocktail containing ketamine (58.8 mg/kg), xylazine (5.9 mg/kg), and acepromazine (1.2 mg/kg) administered subcutaneously. They were intubated, placed on a respirator, and ventilated with 100% oxygen throughout the surgery. A surgical plane of anesthesia was maintained with ketamine (10mg/ml) that was administered intravenously as needed. A midline abdominal incision was made in all rabbits and polyethylene catheters with Silastic tips were implanted in the abdominal

aorta (one) and vena cava (two). The catheters were tunneled subcutaneously from the abdominal cavity, and exited at the nape of the neck.

After a minimum 2-wk recovery period, a second surgery was performed to implant a vena caval occluder. Rabbits were initially anesthetized with ketamine (250mg, s.c.), intubated, placed on a respirator and ventilated with 100% oxygen; a surgical plane of anesthesia was maintained with isoflurane (2%). An occluder was implanted around the thoracic inferior vena cava via a right thoracotomy through the fifth intercostal space. The distal end of the occluder again exited at the nape of the neck, and the incision was closed in layers.

The ends of the occluder and catheters were protected in a plastic pillbox, which was sutured to the rabbits' skin. The rabbits were given an intramuscular injection of enrofloxacin (22.7 mg) just before each surgery and intravenous injections for the four days following surgery. The animals also received buprenorphine hydrochloride (0.09 mg s.c.) two to three hours after surgery, and again the next day to minimize pain. The catheters were flushed immediately after surgery and then three times weekly with the use of sterile 0.9% saline, and filled with heparin (1,000 U/ml) to maintain patency.

Baroreflex curve generation

On the experimental day, the rabbits were placed in a 37x17x21 cm black Plexiglas box, with screened openings to prevent overheating, and were allowed approximately thirty minutes to settle. Mean arterial pressure and heart were measured continuously via the aortic catheter using a Statham pressure transducer, a Grass tachometer, Grass polygraph and a Biopac (Santa Barbara, CA) MP100 data acquisition

and analysis system. To determine the baroreflex relationship between mean arterial pressure and heart rate, and to ensure steady-state changes in both the sympathetic and parasympathetic nervous system (Coleman, 1980), a slow ramp method of altering mean arterial pressure was performed. Arterial pressure was lowered via inflation of the occluder around the vena cava until heart rate reached maximum values. Mean arterial pressure was lowered at a rate of 0.51 ± 0.07 mmHg/second, and this part of the baroreflex curve was generated in 57 ± 5 seconds. Mean arterial pressure was raised by infusing increasing doses of phenylephrine (0.5, 1, 2, 4, and 8 μg/kg/min), increasing the dose every fifteen seconds until mean arterial pressure was increased by twenty mmHg over basal (Brooks et al., 2002). This took 59 ± 4 seconds. Therefore, the entire curve was generated in 116 ± 7 seconds. More than one occluder inflation was performed in each experiment and at least ten minutes was allowed between each inflation to allow mean arterial pressure and heart rate to return to basal conditions.

Protocol

In order to eliminate the physical stress of surgery as a contributing factor, two weeks were allowed after the final surgery before first exposure to the experimental Plexiglas box. In most rabbits, this first exposure to a novel environment was stressful as indicated by a heart rate higher than 170 bpm. Only rabbits that exhibited this high heart rate were considered stressed. Baroreflex curves were then generated in five rabbits before and 105-120 minutes after a bolus intravenous administration of the non-specific NOS inhibitor Nω-nitro-L-arginine (L-NNA, 20 mg/kg).

Following this experiment, rabbits were conditioned to the environment of the experimental box by placing them in the box for two to five hours a day for at least five days. Some rabbits required longer conditioning periods of up to four weeks. Rabbits were considered conditioned and unstressed when heart rate was less than 170 bpm. At this point the experiment was repeated, and baroreflex curves were again generated before and 107-144 min after the administration of L-NNA.

Plasma corticosterone measurements

To determine if blockade of nitric oxide production returns plasma corticosterone levels to normal during stress blood samples were collected from five additional rabbits for plasma corticosterone measurements. Samples (3 ml) were drawn from the arterial catheter while the animals were resting quietly before and 120 minutes after L-NNA on the first day they were placed in the experimental box. Samples were also collected after they had been conditioned to the box. Again, conditioning lasted one to four weeks, until resting heart rate was less than 170 bpm. Blood was replaced with an equal volume of sterile isotonic saline. Samples were placed into chilled heparinized tubes and centrifuged at 4°C. Plasma was stored at -20°C until assayed. Corticosterone levels were determined by the Endocrine Services Laboratory of the Oregon National Primate Research Center by radioimmunoassay. Fifty μl of rabbit serum was first diluted in 950 μl of redistilled ethanol. The samples were centrifuged to pellet precipitated proteins, and 50 and 100 μl of ethanol aliquots were dried in 13 x 100 mm assay tubes using room air. Radioimmunoassay of these reconstituted samples

used a corticosterone antisera as described previously (Gruenewald et al., 1992). Solvent blanks were routinely less than 5 pg/tube and the intra- and inter-assay coefficients of variation did not exceed 9%.

Data analysis

Data for the baroreflex curves were collected at 200 Hz and processed using a Biopac (Santa Barbara, CA) MP100 data acquisition and analysis system. Raw data were grouped into one-second bins from which mean values were obtained. Since more than one curve was generated using the vena caval occluder, curves that were free from movement artifact and exhibited the best sigmoidal fit were selected for further analysis (see figure insets for representative curves). Often the basal mean arterial pressures and heart rates before the pressor (phenylephrine) part of the reflex curve were slightly different from the depressor (occluder) part. When this occurred, to avoid erroneous measurements of baroreflex gain, half of the pressure difference was added to all pressure values in the segment with the lower basal pressure and half the difference was subtracted from the pressures in the segment with the higher pressure, so that the two segments were aligned, as previously described (Dorward et al., 1985). The same was done for heart rate.

The sigmoidal baroreflex relationships between mean arterial pressure and heart rate generated in each experiment were fitted and compared using the Boltzmann sigmoidal equation (heart rate=A+B/1+ $e^{(C\text{-mean arterial pressure})/D}$, where A equals the baroreflex-induced minimum heart rate, B equals the baroreflex-induced heart rate range, C equals the mean arterial pressure at the midpoint between the baroreflex-

induced minimum and maximum heart rate, and D is the slope coefficient). Maximum gain was calculated by dividing the heart rate range by four times the slope coefficient. Because of the exponential nature of baroreflex gain and the high variability of plasma corticosterone levels in stressed animals, the log of these parameters were used for statistical analysis.

Basal mean arterial pressures, heart rates, curve fitting parameters, and plasma corticosterone levels were compared between groups using ANOVA for repeated measures and the post-hoc Bonferroni test. All data are reported as mean ± standard error mean. In the figures, the sigmoidal curves derived from the averaged parameters are shown along with basal points ± standard error mean.

RESULTS

Effects of stress

Animals exposed to the novel environment exhibited a higher basal mean arterial pressure (73 ± 4 mmHg, $p<0.05$), heart rate (185 ± 6 bpm, $p<0.05$) and plasma corticosterone levels (15.5 ± 5.0 ng/ml, $p<0.05$) compared to after conditioning (65 ± 2 mmHg, 159 ± 3 bpm, 5.4 ± 0.7 ng/ml). Baroreflex gain (Table 1, Figure 1A) was suppressed in the rabbits when they were first exposed to the experimental environment, compared to after conditioning. Stress also increased the midpoint of the baroreflex curve (BP_{50}) and the minimum heart rate (Table 1, Figure 1A).

Table 1

Effects of stress and L-NNA on logistic parameters

	Unstressed		Stressed	
	Control	L-NNA	Control	L-NNA
Maximum Gain (bpm/mmHg)	-23.4 ± 2.6	-26.8 ± 4.9	-12.2 ±1.6*	-27.2 ± 5.4#
BP_{50} (mmHg)	61.6 ± 5.9	72.5 ± 2.9#	65.9 ± 2.3*	73.9 ± 2.0#
Maximum Heart Rate (bpm)	274 ± 5	302 ± 7	294±9	290 ± 11
Minimum Heart Rate (bpm)	152 ± 3	113 ± 3#	175 ± 3*	117 ± 2#

*$p<0.05$, unstressed versus stressed

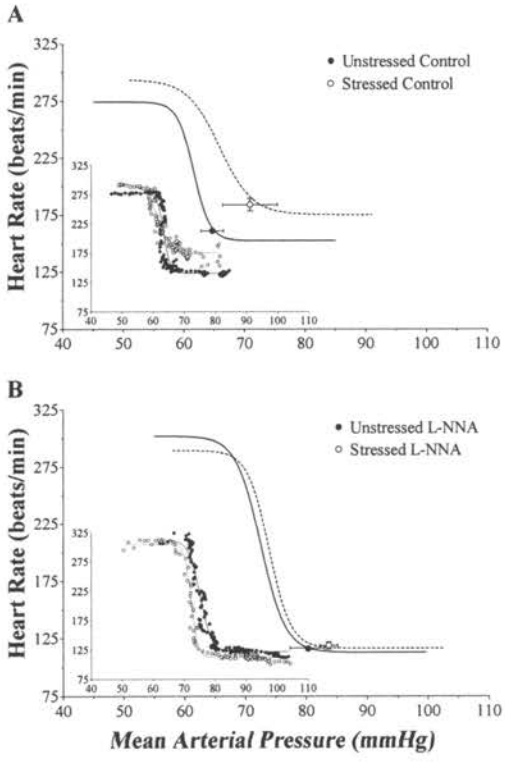
#$p<0.05$, before versus after L-NNA

Effects of NOS blockade on the baroreflex control of heart rate and on corticosterone levels

The effect of L-NNA on baroreflex control of heart rate was examined in the same rabbits in the stressed and unstressed state. L-NNA increased basal mean arterial pressure ($p<0.05$) and decreased basal heart rate ($p<0.05$) in animals in both the stressed (mean arterial pressure to 84 ± 2 mmHg; heart rate to 118 ± 3 bpm) and unstressed states (mean arterial pressure to 80 ± 3 mmHg; heart rate to 116 ± 3 bpm). After L-NNA, basal mean arterial pressure and heart rate were not different between the stressed and unstressed states (Figure 1B).

Blockade of NOS in stressed animals increased baroreflex gain (Table 1, Figure 2B); however, there was no effect of NOS blockade on baroreflex gain in unstressed animals (Table 1, Figure 2A). In animals in both the unstressed and stressed states,

Figure 1

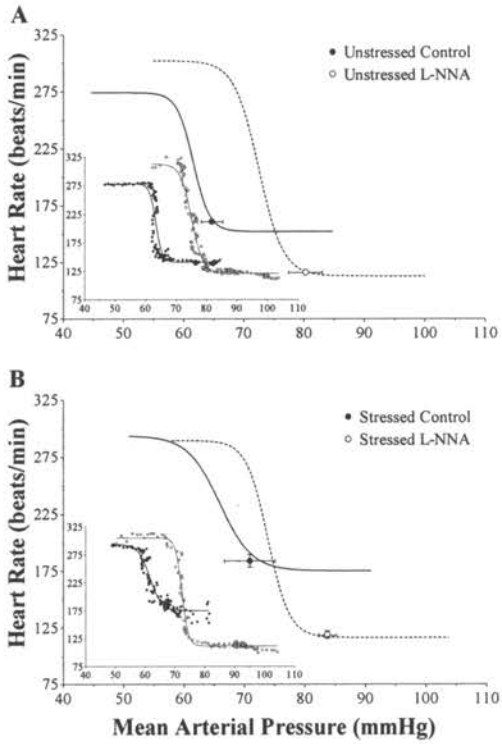


Acute psychological stress decreases baroreflex gain in rabbits which is ameliorated by nitric oxide synthase blockade. Effect of stress on the mean (n=5) baroreflex control of heart rate in rabbits before (A) and after (B) blockade of nitric oxide synthase (L-NNA). Inset demonstrates representative baroreflex curves from one rabbit.

NOS blockade decreased the minimum heart rate and increased the BP_{50} (Table, Figure 2). After L-NNA, baroreflex curves were superimposable, and baroreflex parameters in the stressed and unstressed states were not different (Table 1, Figure 1B). Blockade of NOS did not decrease corticosterone levels in stressed animals (14.0 ± 3.9 ng/ml). L-

NNA administration in unstressed rabbits increased plasma corticosterone levels (to 14.3 ± 2.1 ng/ml, $p<0.05$).

Figure 2



Nitric oxide blockade increases baroreflex gain in stressed rabbits, with no effect on gain in unstressed animals. Effect of nitric oxide synthase blockade (L-NNA) on the mean (n=5) baroreflex control of heart rate in rabbits in the unstressed (A) and stressed (B) states (n=5). Same curves from Figure 1. Inset demonstrates representative baroreflex curves from one rabbit.

DISCUSSION

The purpose of this study was to investigate the role of nitric oxide in the decrease in baroreflex gain during psychological stress. The novel findings are 1) acute psychological stress decreases gain in rabbits, 2) this decrease is completely reversed by blockade of NOS, and 3) blockade of NOS increases corticosterone levels in unstressed but not stressed rabbits. These data support the hypothesis that stress decreases baroreflex gain through actions of nitric oxide, but that these actions are independent of changes in corticosterone release.

Previous studies have shown that exposure to a novel environment induces stress in rats as demonstrated by an increased mean arterial pressure and heart rate (van Den et al., 2002), as well as increased plasma corticosterone levels (Marquez et al., 2005). Rabbits exhibit similar responses, including increases in mean arterial pressure, heart rate and plasma corticosterone levels on the first day they were placed in the experimental box as compared to when they had been conditioned. In addition, we observed that this stress was associated with depressed baroreflex gain.

Several previous studies have investigated the effects of acute stress on baroreflex gain in humans (Steptoe et al., 1993; Sawada, 1993; Steptoe et al., 1996; Al Kubati et al., 1997), rats (Hatton et al., 1997; Porter, 2000) and rabbits (Schadt and Hasser, 1998). While many of the human studies demonstrated a decrease in baroreflex gain with stress, studies in rats and rabbits usually reported no change or an increase in baroreflex gain. The discrepancy between the present study and the previous study of Schadt et al. (Schadt and Hasser, 1998), who found no effect of stress on baroreflex gain in rabbits, may be due to differences in the type of stress employed. The earlier

study examined the effects of airjet and shaker stress, which may evoke different emotional responses than that of exposure to a novel environment. Sawada et al. (Sawada, 1993) found that various types of stress differentially altered baroreflex gain in humans. Exposure of their subjects to intermittent pink noise or the cold pressor test caused a decrease in gain, whereas isometric handgrip and mental arithmetic did not affect gain.

Another difference between the current study and previous studies in rabbits and rats was the method used to generate baroreflex curves. Schadt et al. (Schadt and Hasser, 1998) and Hatton et. al. (Hatton et al., 1997) quantified heart rate responses to rapid changes in blood pressure produced by bolus injections of phenylephrine and nitroprusside. Rapid pressure changes may not allow enough time for the sympathetic nervous system to completely respond, so that primarily vagal-mediated alterations in heart rate are observed (Coleman, 1980). In the current study, occluder inflations and phenylephrine infusions were purposely performed slowly to allow complete sympathetic activation and withdrawal.

The major purpose of the present study was to determine the mechanism by which stress decreases baroreflex gain. Previous studies have shown that NOS activity can decrease baroreflex gain (Minami et al., 1995; Liu et al., 1996; Kumagai et al., 1997; Paton et al., 2001; Brady et al., 2002), but this study is the first to demonstrate that nitric oxide underlies impaired baroreflex function during stress. In fact, after NOS blockade, baroreflex curves of animals in the stressed and unstressed state were superimposable, suggesting that stress-induced increases in NOS activity may be entirely responsible for the decrease in baroreflex gain.

One potential site of action of nitric oxide is the adrenal cortex. Immobilization stress increases NOS mRNA and protein levels as well as NOS enzyme activity in the adrenal cortex of rats (Tsuchiya et al., 1997). Moreover, nitric oxide contributes to increased glucocorticoid release in response to adrenocorticotrophic hormone (ACTH) (Mohn et al., 2005), which is increased by stress. Scheuer et al. (Scheuer and Mifflin, 2001; Scheuer and Bechtold, 2002) report that administration of corticosterone in rats decreases baroreflex gain. In addition, blockade of cortisol production in humans before exposure to a stressful stimulus prevented the decrease in baroreflex gain normally observed (Broadley et al., 2005). Therefore, we hypothesized that blockade of NOS could improve gain in stressed animals in part by decreasing adrenal glucocorticoid production.

The current study did not support this hypothesis, however. While stress increased corticosterone levels, blockade of NOS did not reverse this response. Moreover, NOS blockade increased plasma corticosterone levels in unstressed animals. These paradoxical findings likely reflect the complex interactions between nitric oxide, ACTH and adrenal glucocorticoid production in vivo. In mice, systemic blockade of NOS also increased plasma ACTH and corticosterone levels (Giordano et al., 1996), demonstrating that nitric oxide tonically suppresses basal ACTH/glucocorticoid release. On the other hand, nitric oxide has been reported to both enhance and inhibit ACTH responses to stress [for reviews, see (Riedel, 2000; Rivier, 2001)]. It has been suggested that the ultimate response is determined by the balance between opposing actions of nitric oxide, which in turn are influenced by the type and intensity of stress (Rivier, 2001). Because NOS blockade did not alter plasma corticosterone levels in

rabbits exposed to a novel environment, it appears there is little net effect of nitric oxide on these divergent pathways.

Nitric oxide synthase inhibitors such as L-NNA can cross the blood-brain-barrier to inhibit NOS in the brain (Traystman et al., 1995; Rivier, 2001). Therefore, L-NNA may increase baroreflex gain during stress by blocking the effects of nitric oxide in the brain. Both the PVN (Chen et al., 1996; Hwang et al., 1998; Jiang et al., 2005) and nucleus of the solitary tract (NTS) (Machado, 2001) are known to influence gain. Moreover, NOS-containing neurons in both brain regions are activated following stress (Krukoff and Khalili, 1997). Stress increases PVN nitric oxide production and NOS mRNA levels (Calza et al., 1993; Kishimoto et al., 1996; Kawa et al., 2002; Cherney et al., 2003), and nitric oxide can act in the NTS (Paton et al., 2001; Waki et al., 2003; Smith et al., 2005) to decrease baroreflex gain. Thus, either or both of these brain regions may be involved, but further experiments are required to test this hypothesis.

In conclusion, exposure of rabbits to a novel environment decreases baroreflex gain, which is mediated by actions of nitric oxide independent of changes in corticosterone levels.

Perspectives

Cardiovascular disease is the leading cause of morbidity and mortality in developed countries (Lopez, 1993), and psychological stress is a known risk factor for cardiovascular disease (Frasure-Smith, 1991; Rosengren et al., 1991), but the mechanism for this link is not clear. Acute stress can increase sympathetic nerve activity, blood pressure, and heart rate (Perna et al., 1997) thus increasing the load on

the heart and perhaps contributing to cardiovascular disease. Changes in baroreflex sensitivity may also play a role in the pathogenesis of this disease since a decrease in baroreflex sensitivity has been suggested as a predictive factor in the mortality following a myocardial infarction (Billman et al., 1982; Schwartz et al., 1988). This study provides a possible mechanism for the decreased baroreflex gain during an acute psychological stress. Stress is often an unavoidable fact of life and understanding the mechanisms behind the deleterious effects of psychological stress can lead to possible therapeutic interventions to reduce morbidity and mortality.

REFERENCES

Al Kubati, M. A., B. Fiser, and J. Siegelova, 1997, Baroreflex sensitivity during psychological stress: Physiol Res., v. 46, no. 1, p. 27-33.

Atherton, J. J., M. P. Frenneaux, and H. L. Thomson, 1999, The role of diastolic ventricular interaction in abnormal cardiac baroreflex function in chronic heart failure: Aust.N.Z.J.Med., v. 29, no. 3, p. 428-432.

Billman, G. E., P. J. Schwartz, and H. L. Stone, 1982, Baroreceptor reflex control of heart rate: a predictor of sudden cardiac death: Circulation, v. 66, no. 4, p. 874-880.

Brady, F., Y. S. Bakhle, and C. Bell, 2002, Evaluation of the involvement of nitric oxide and substance P in reducing baroreflex gain in the genetically hypertensive (GH) rat: Acta Physiol Hung., v. 89, no. 4, p. 451-461.

Broadley, A. J., A. Korszun, E. Abdelaal, V. Moskvina, C. J. Jones, G. B. Nash, C. Ray, J. Deanfield, and M. P. Frenneaux, 2005, Inhibition of cortisol production with metyrapone prevents mental stress-induced endothelial dysfunction and baroreflex impairment: J.Am.Coll.Cardiol., v. 46, no. 2, p. 344-350.

Brooks, V. L., K. A. Clow, and K. P. O'Hagan, 2002, Pregnancy and acute baroreflex resetting in conscious rabbits: American Journal of Physiology-Regulatory Integrative and Comparative Physiology, v. 283, no. 2, p. R429-R440.

Calza, L., L. Giardino, and S. Ceccatelli, 1993, NOS mRNA in the paraventricular nucleus of young and old rats after immobilization stress: Neuroreport, v. 4, no. 6, p. 627-630.

Cat, G. G., F. Veglio, F. Rabbia, A. Milan, T. Grosso, and L. Chiandussi, 2001, Baroreflex sensitivity in secondary hypertension: Clin.Exp.Hypertens., v. 23, no. 1-2, p. 89-99.

Chen, Y. L., S. H. Chan, and J. Y. Chan, 1996, Participation of galanin in baroreflex inhibition of heart rate by hypothalamic PVN in rat: Am.J.Physiol, v. 271, no. 5 Pt 2, p. H1823-H1828.

Cherney, A., H. Edgell, and T. L. Krukoff, 2003, NO mediates effects of estrogen on central regulation of blood pressure in restrained, ovariectomized rats: American Journal of Physiology-Regulatory Integrative and Comparative Physiology, v. 285, no. 4, p. R842-R849.

Coleman, T. G., 1980, Arterial baroreflex control of heart rate in the conscious rat: Am.J.Physiol, v. 238, no. 4, p. H515-H520.

Dorward, P. K., W. Riedel, S. L. Burke, J. Gipps, and P. I. Korner, 1985, The renal sympathetic baroreflex in the rabbit. Arterial and cardiac baroreceptor influences, resetting, and effect of anesthesia: Circ.Res., v. 57, no. 4, p. 618-633.

Esch, T., G. B. Stefano, G. L. Fricchione, and H. Benson, 2002, Stress in cardiovascular diseases: Med.Sci.Monit., v. 8, no. 5, p. RA93-RA101.

Fauvel, J. P., C. Cerutti, P. Quelin, M. Laville, M. P. Gustin, C. Z. Paultre, and M. Ducher, 2000, Mental stress-induced increase in blood pressure is not related to baroreflex sensitivity in middle-aged healthy men: Hypertension, v. 35, no. 4, p. 887-891.

Frasure-Smith, N., 1991, In-hospital symptoms of psychological stress as predictors of long-term outcome after acute myocardial infarction in men: Am.J.Cardiol., v. 67, no. 2, p. 121-127.

Giordano, M., M. Vermeulen, A. S. Trevani, G. Dran, G. Andonegui, and J. R. Geffner, 1996, Nitric oxide synthase inhibitors enhance plasma levels of corticosterone and ACTH: Acta Physiol Scand., v. 157, no. 2, p. 259-264.

Gronan, R. J., J. C. Schadt, and D. H. York, 1983, Routine, direct measurement of aortic pressure in the conscious rabbit: Physiol Behav., v. 30, no. 5, p. 719-722.

Gruenewald, D. A., D. L. Hess, C. W. Wilkinson, and A. M. Matsumoto, 1992, Excessive testicular progesterone secretion in aged male Fischer 344 rats: a potential cause of age-related gonadotropin suppression and confounding variable in aging studies: J.Gerontol., v. 47, no. 5, p. B164-B170.

Gullette, E. C., J. A. Blumenthal, M. Babyak, W. Jiang, R. A. Waugh, D. J. Frid, C. M. O'Connor, J. J. Morris, and D. S. Krantz, 1997, Effects of mental stress on myocardial ischemia during daily life: JAMA, v. 277, no. 19, p. 1521-1526.

Hatakeyama, S., Y. Kawai, T. Ueyama, and E. Senba, 1996, Nitric oxide synthase-containing magnocellular neurons of the rat hypothalamus synthesize oxytocin and vasopressin and express Fos following stress stimuli: J.Chem.Neuroanat., v. 11, no. 4, p. 243-256.

Hatton, D. C., V. Brooks, Y. Qi, and D. A. McCarron, 1997, Cardiovascular response to stress: baroreflex resetting and hemodynamics: Am.J.Physiol, v. 272, no. 5 Pt 2, p. R1588-R1594.

Hwang, K. R., S. H. Chan, and J. Y. Chan, 1998, Noradrenergic neurotransmission at PVN in locus ceruleus-induced baroreflex suppression in rats: Am.J.Physiol, v. 274, no. 4 Pt 2, p. H1284-H1292.

Jiang, H. Y., Q. H. Jin, Y. J. Li, D. Y. Xu, Y. Z. Jin, and X. J. Jin, 2005, [Effect of microinjection of atrial natriuretic peptide into the paraventricular nucleus on baroreflex sensitivity in conscious rats.]: Sheng Li Xue.Bao., v. 57, no. 2, p. 175-180.

Kawa, T. et al., 2002, The role of the hypothalamic nitric oxide in the pressor responses elicited by acute environmental stress in awake rats: Life Sciences, v. 71, no. 12, p. 1429-1438.

Kishimoto, J., T. Tsuchiya, P. C. Emson, and Y. Nakayama, 1996, Immobilization-induced stress activates neuronal nitric oxide synthase (nNOS) mRNA and protein in hypothalamic-pituitary-adrenal axis in rats: Brain Res., v. 720, no. 1-2, p. 159-171.

Krukoff, T. L., 1999, Central actions of nitric oxide in regulation of autonomic functions: Brain Res.Brain Res.Rev., v. 30, no. 1, p. 52-65.

Krukoff, T. L., and P. Khalili, 1997, Stress-induced activation of nitric oxide-producing neurons in the rat brain: J.Comp Neurol., v. 377, no. 4, p. 509-519.

Kumagai, H., H. Suzuki, M. Ichikawa, M. Nishizawa, N. Oshima, and T. Saruta, 1997, Interaction between endothelin and nitric oxide in sympathetic nerve modulation in hypertensive rats: Hypertens.Res., v. 20, no. 1, p. 35-42.

La Rovere, M. T., C. Bersano, M. Gnemmi, G. Specchia, and P. J. Schwartz, 2002, Exercise-induced increase in baroreflex sensitivity predicts improved prognosis after myocardial infarction: Circulation, v. 106, no. 8, p. 945-949.

Liu, J. L., H. Murakami, and I. H. Zucker, 1996, Effects of NO on baroreflex control of heart rate and renal nerve activity in conscious rabbits: Am.J.Physiol, v. 270, no. 6 Pt 2, p. R1361-R1370.

Lopez, A. D., 1993, Assessing the burden of mortality from cardiovascular diseases: World Health Stat.Q., v. 46, no. 2, p. 91-96.

Machado, B. H., 2001, Neurotransmission of the cardiovascular reflexes in the nucleus tractus solitarii of awake rats: Ann.N.Y.Acad.Sci., v. 940, p. 179-196.

Marquez, C., R. Nadal, and A. Armario, 2005, Influence of reactivity to novelty and anxiety on hypothalamic-pituitary-adrenal and prolactin responses to two different novel environments in adult male rats: Behav.Brain Res..

Mezzacappa, E. S., R. M. Kelsey, E. S. Katkin, and R. P. Sloan, 2001, Vagal rebound and recovery from psychological stress: Psychosom.Med., v. 63, no. 4, p. 650-657.

Minami, N., Y. Imai, J. Hashimoto, and K. Abe, 1995, The role of nitric oxide in the baroreceptor-cardiac reflex in conscious Wistar rats: Am.J.Physiol, v. 269, no. 3 Pt 2, p. H851-H855.

Mohn, C. E., J. Fernandez-Solari, A. De Laurentiis, J. P. Prestifilippo, C. C. de la, R. Funk, S. R. Bornstein, S. M. McCann, and V. Rettori, 2005, The rapid release of corticosterone from the adrenal induced by ACTH is mediated by nitric oxide acting by prostaglandin E2: Proc.Natl.Acad.Sci.U.S.A, v. 102, no. 17, p. 6213-6218.

Murakami, H., J. L. Liu, H. Yoneyama, Y. Nishida, K. Okada, H. Kosaka, H. Morita, and I. H. Zucker, 1998, Blockade of neuronal nitric oxide synthase alters the baroreflex control of heart rate in the rabbit: Am.J.Physiol, v. 274, no. 1 Pt 2, p. R181-R186.

Paton, J. F., J. Deuchars, Z. Ahmad, L. F. Wong, D. Murphy, and S. Kasparov, 2001, Adenoviral vector demonstrates that angiotensin II-induced depression of the cardiac baroreflex is mediated by endothelial nitric oxide synthase in the nucleus tractus solitarii of the rat: J.Physiol, v. 531, no. Pt 2, p. 445-458.

Perna, F. M., N. Schneiderman, and A. LaPerriere, 1997, Psychological stress, exercise and immunity: International Journal of Sports Medicine, v. 18, p. S78-S83.

Porter, J. P., 2000, Contribution of central ANG II to acute stress-induced changes in baroreflex function in young rats: Am.J.Physiol Regul.Integr.Comp Physiol, v. 279, no. 4, p. R1386-R1391.

Rettori, V., C. Mohn, C. Scorticati, P. Vissio, M. Cella, M. Farina, A. Franchi, and S. M. McCann, 2003, Effect of neurogenic stress and ethanol on nitric oxide synthase and cyclooxygenase activities in rat adrenals: Ann.N.Y.Acad.Sci., v. 992, p. 86-98.

Riedel, W., 2000, Role of nitric oxide in the control of the hypothalamic-pituitary-adrenocortical axis: Z.Rheumatol., v. 59 Suppl 2, p. II/36-II/42.

Rivier, C., 2001, Role of gaseous neurotransmitters in the hypothalamic-pituitary-adrenal axis: Ann.N.Y.Acad.Sci., v. 933, p. 254-264.

Rosengren, A., G. Tibblin, and L. Wilhelmsen, 1991, Self-perceived psychological stress and incidence of coronary artery disease in middle-aged men: Am.J.Cardiol., v. 68, no. 11, p. 1171-1175.

Sawada, Y., 1993, Reproducible increases in blood pressure during intermittent noise exposure: underlying haemodynamic mechanisms specific to passive coping: Eur.J.Appl.Physiol Occup.Physiol, v. 67, no. 4, p. 367-374.

Schadt, J. C., and E. M. Hasser, 1998, Hemodynamic effects of acute stressors in the conscious rabbit: Am.J.Physiol, v. 274, no. 3 Pt 2, p. R814-R821.

Scheuer, D. A., and A. G. Bechtold, 2002, Glucocorticoids modulate baroreflex control of heart rate in conscious normotensive rats: Am.J.Physiol Regul.Integr.Comp Physiol, v. 282, no. 2, p. R475-R483.

Scheuer, D. A., and S. W. Mifflin, 2001, Glucocorticoids modulate baroreflex control of renal sympathetic nerve activity: Am.J.Physiol Regul.Integr.Comp Physiol, v. 280, no. 5, p. R1440-R1449.

Schwartz, P. J., E. Vanoli, M. Stramba-Badiale, G. M. De Ferrari, G. E. Billman, and R. D. Foreman, 1988, Autonomic mechanisms and sudden death. New insights from analysis of baroreceptor reflexes in conscious dogs with and without a myocardial infarction: Circulation, v. 78, no. 4, p. 969-979.

Smith, S. A., J. H. Mitchell, and J. Li, 2005, Independent modification of baroreceptor and exercise pressor reflex function by nitric oxide in nucleus tractus solitarius: Am.J.Physiol Heart Circ.Physiol, v. 288, no. 5, p. H2068-H2076.

Stalnikowicz, R., and A. Tsafrir, 2002, Acute psychosocial stress and cardiovascular events: Am.J.Emerg.Med., v. 20, no. 5, p. 488-491.

Steptoe, A., G. Fieldman, O. Evans, and L. Perry, 1996, Cardiovascular risk and responsivity to mental stress: the influence of age, gender and risk factors: J.Cardiovasc.Risk, v. 3, no. 1, p. 83-93.

Steptoe, A., G. Fieldman, O. Evans, and L. Perry, 1993, Control Over Work Pace, Job Strain and Cardiovascular-Responses in Middle-Aged Men: Journal of Hypertension, v. 11, no. 7, p. 751-759.

Traystman, R. J., L. E. Moore, M. A. Helfaer, S. Davis, K. Banasiak, M. Williams, and P. D. Hurn, 1995, Nitro-L-arginine analogues. Dose- and time-related nitric oxide synthase inhibition in brain: Stroke, v. 26, no. 5, p. 864-869.

Tsuchiya, T., J. Kishimoto, J. Koyama, and T. Ozawa, 1997, Modulatory effect of L-NAME, a specific nitric oxide synthase (NOS) inhibitor, on stress-induced changes in plasma adrenocorticotropic hormone (ACTH) and corticosterone levels in rats: physiological significance of stress-induced NOS activation in hypothalamic-pituitary-adrenal axis: Brain Res., v. 776, no. 1-2, p. 68-74.

van Den, B. M., S. A. van Acker, M. F. Fluttert, and E. R. de Kloet, 2002, Involvement of corticosterone in cardiovascular responses to an open-field novelty stressor in freely moving rats: Physiol Behav., v. 75, no. 1-2, p. 207-215.

Waki, H., S. Kasparov, L. F. Wong, D. Murphy, T. Shimizu, and J. F. Paton, 2003, Chronic inhibition of endothelial nitric oxide synthase activity in nucleus tractus solitarii enhances baroreceptor reflex in conscious rats: J.Physiol, v. 546, no. Pt 1, p. 233-242.