Invited Review

Pregnancy and the endocrine regulation of the baroreceptor reflex

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ABSTRACT

The purpose of this review is to delineate the general features of endocrine regulation of the baroreceptor reflex, as well as specific contributions during pregnancy. In contrast to the programmed changes in baroreflex function that occur in situations initiated by central command (e.g. exercise or stress), the complex endocrine milieu often associated with physiological and pathophysiological states can influence the central baroreflex neuronal circuitry via multiple sites and mechanisms, thereby producing varied changes in baroreflex function. During pregnancy, baroreflex gain is markedly attenuated, and at least two hormonal mechanisms contribute, each at different brain sites: increased levels of the neurosteroid 3α-OH-DHP, acting in the RVLM, and reduced actions of insulin in the forebrain. 3α-OH-DHP appears to potentiate baroreflex-independent GABAergic inhibition of premotor neurons in the RVLM, which decreases the range of sympathetic nerve activity that can be elicited by changes in arterial pressure. In contrast, reductions in the levels or actions of insulin in the brain blunt baroreflex efferent responses to increments or decrements in arterial pressure. Although plasma levels of angiotensin II are increased in pregnancy, this is not responsible for the reduction in baroreflex gain, though it may contribute to the increased level of sympathetic nerve activity in this condition. How these different hormonal effects are integrated within the brain, as well as possible interactions with additional potential neuromodulators that influence baroreflex function during pregnancy and other physiological and pathophysiological states, remains to be clearly delineated.
INTRODUCTION

Normal pregnancy is characterized by profound changes in fluid and electrolyte balance and blood pressure regulation. Blood volume and cardiac output increase by 30-50%, but arterial pressure falls due to even greater decreases in systemic vascular resistance (120; 138; 189). These hemodynamic alterations are evident early in pregnancy, precede the increases in uterine blood flow, and are sustained until parturition (127; 138; 189). Considerable data implicates increases in relaxin and nitric oxide in the systemic vasodilation induced by pregnancy (20; 127; 128; 177; 196). Additionally, peripheral vascular sensitivity to circulating vasoconstrictors including vasopressin, angiotensin II (AngII), and norepinephrine (NE) is decreased (50; 71; 154).

Unlike the hemodynamic changes, which likely subserve adequate fetal development, an adverse consequence of normal pregnancy is a marked impairment of the arterial baroreceptor reflex. This change was first noted by Humphreys and Joels in an extensive series of studies in anesthetized pregnant rabbits, in which reductions in carotid sinus pressure failed to elicit normal increases in arterial pressure and peripheral vascular resistance (97; 98). Since then, arterial baroreflex impairment has been documented in several species studied in the conscious state, including dogs (23), rats (24; 124), rabbits (21; 97; 162), goats (152), sheep (104; 123) and humans (16; 79; 122; 164; 175; 193). Reflex responses to atrial stretch are also blunted (51; 94; 95; 103), which may contribute to retention of the expanded blood volume. The depressed baroreflex function observed during normal pregnancy appears to worsen in pregnancy-associated diseases such as preeclampsia (69; 129; 137; 175; 195). Interestingly, early gestational measurements of depressed baroreflex sensitivity in conjunction with reduced uterine perfusion have been proposed as a means to predict the ultimate development of this disease (194).
The consequences of depressed baroreflex function are serious. Pregnant women are prone to orthostatic hypotension, due to blunted reflex activation of the sympathetic nervous system and inadequate peripheral vasoconstriction (56; 122; 132). Moreover, in many species, pregnancy interferes with the normal ability to maintain arterial pressure during hemorrhage (26; 36; 99; 152; 186). Hemodynamic studies have revealed that in pregnant animals arterial pressure falls with less blood loss due to both a failure to maintain cardiac output and also to inadequate peripheral vasoconstriction (22; 36). Importantly, 11-22% of maternal mortality is attributed to peripartum hemorrhage (156; 166), a common event during vaginal delivery, C-section and after delivery.

Given the potentially deleterious effects of impaired baroreflex function during pregnancy, considerable effort has been devoted to identifying the mechanisms. Experiments have assessed the site(s) within the baroreflex arc that are functionally depressed. Initial studies documented depressed baroreflex control of multiple efferents, including heart rate (5; 21; 24; 123; 175; 193), renal and muscle sympathetic nerve activity (79; 89; 148; 149) and hormones such as vasopressin and ACTH/glucocorticoids (26; 104). Multiple approaches have been used to assess the effect of pregnancy on reflex control of the autonomic nervous system [for reviews, see (69; 163)], including infusion of vasoactive drugs to generate complete sigmoidal baroreflex relationships between arterial pressure and heart rate or sympathetic nerve activity. Three aspects of these curves are particularly attenuated: the midpoint of the baroreflex function curve relative to MAP or the “setpoint,” the maximal level of heart rate or sympathetic activity achieved when arterial pressure is lowered, and the maximal gain or sensitivity of the most linear segment of the curve (24; 47; 89; 123; 149) (Figure 1). Pharmacological blockade of the sympathetic or parasympathetic nervous systems has revealed that the sympathetic contribution to heart rate baroreflex curves is particularly blunted (21; 123). Additionally, pregnancy-induced decreases in baroreflex function in humans and rats have been successfully detected via measurement of spontaneous baroreflex sensitivity, which identifies brief
spontaneous baroreflex-mediated ramps in which systolic pressure and pulse interval both simultaneously increase or decrease (5; 24; 175; 193). This measurement is restricted to the more linear segment of baroreflex curves close to basal values and highlights in particular the impact of rapid changes in parasympathetic control of the heart, documenting that pregnancy depresses these aspects of baroreflex function as well.

Several studies have determined whether pregnancy impairs specifically the responsiveness of baroreflex afferent pathways. In pregnant rats, the aortic baroreceptor discharge function curve is shifted to the lower prevailing arterial pressure level, suggesting that the parallel shift in baroreflex curves is secondary to pressure-dependent resetting of arterial baroreceptors (92; 115). On the other hand, the sensitivity of arterial baroreceptors to increments in pressure (gain), at least to short-term pressure changes, is preserved (92; 115). In contrast, different from arterial baroreceptor afferent input which is well maintained, in response to atrial stretch, discharge of the high frequency subgroup of cardiac vagal afferent fibers is greatly reduced in pregnant rats (51; 183). Thus, depressed atrial afferent responsiveness may play a role in the blunted volume-regulating reflexes observed during pregnancy.

The preservation of arterial baroreceptor afferent function suggests that the decrease in baroreflex sensitivity that occurs during pregnancy must be due to a large extent to changes within the brain. To determine potential sites within the medullary baroreflex pathway that may be altered, Curtis et. al. (43) evaluated expression of c-fos in response to sustained hypotension in the brainstem of pregnant compared to nonpregnant rats. Although in both groups arterial blood pressure was decreased to a level that would produce maximum baroreflex sympathoexcitation, activation of the rostral ventrolateral medulla (RVLM), a brainstem region important to reflex sympathetic control, was less in pregnant rats, suggesting that RVLM neurons that are normally activated by baroreceptor unloading are less responsive in pregnancy. Similarly, Deng and Kaufman (51) found, also using the c-fos method, that there is
much less activation of neurons in the paraventricular nucleus of the hypothalamus (PVN) in response to atrial distention in pregnant animals as compared to non-pregnant animals. Thus, the function of multiple cardiovascularly relevant brain regions is depressed during pregnancy.

If brain control of arterial pressure is altered during pregnancy, then what causes this change? This has been a challenging question to answer, since the endocrine milieu of pregnancy is complex. Multiple hormones known to influence the cardiovascular system and baroreflex regulation are increased, including steroids such as estrogen and progesterone, pressor hormones such as AngII and aldosterone, ovarian hormones such as relaxin, placental hormones such as corticotrophin-releasing hormone, pituitary hormones such as oxytocin, adipokines such as leptin, and inflammatory factors such as TNF-α and IL-6. Longitudinal studies during pregnancy indicate that the depression of baroreflex function does not correlate temporally with hemodynamic changes (e.g. blood volume, blood pressure, or cardiac output), at least in rats (24; 178) and rabbits (47; 147; 162), suggesting that different factors are involved. Moreover, estrogen is likely not an initiating factor, since levels are not increased significantly in some species such as rabbit and sheep, and since several studies have demonstrated that this steroid enhances arterial baroreflex function via an action in the hindbrain (135; 155; 169). In this review, we shall review evidence for increased actions of 3α-hydroxy-dihydroprogesterone (3α-OH-DHP), and decreased actions of insulin, as contributing factors to CNS changes in baroreflex function associated with pregnancy. Before discussing these factors in detail, we shall begin with a brief description of the central pathways that subserve the arterial baroreflex, followed by an outline of the general features of hormonal regulation of baroreflex function.

Central baroreflex pathways and their modulation by hormones.

The essential features of the central pathways subserving the arterial baroreflex are now well established (46; 81), and are illustrated in Figure 2. Primary baroreceptor afferent fibers
terminate in the nucleus tractus solitarius (NTS), mainly in its dorsomedial portion. Second-order neurons within the NTS, many of which receive direct monosynaptic inputs from primary baroreceptor afferent fibers (7), project to and excite neurons in the caudal ventrolateral medulla (CVLM), which contains a group of interneurons that project to and inhibit sympathetic premotor neurons in the RVLM.

The main transmitter released by primary baroreceptor afferent fibers within the NTS is glutamate, which excites second order NTS neurons primarily via non-NMDA receptors (77). Second-order barosensitive neurons that terminate in the CVLM also release glutamate, which acts on NMDA receptors on the GABAergic inhibitory neurons that project to the RVLM (76). Also, independent of the baroreflex pathway, there are other GABAergic projections from the CVLM to the RVLM that inhibit sympathetic outflow and that may influence reflex responses (170) (Figure 2). The RVLM sympathetic premotor neurons are glutamatergic (184) and directly excite sympathetic pathways to the heart and blood vessels (46; 81) (Figure 2). The RVLM neurons are also a site of convergence of inputs from many other sources, including both peripheral receptors and other brain nuclei (46; 81). In addition, second-order neurons within the NTS directly innervate and excite cardiac vagal preganglionic neurons in the nucleus ambiguus.

Studies of the baroreflex in conscious animals and humans have shown that the reflex is continuously modulated, depending on the prevailing behavioural and physiological conditions. For example, during exercise and psychological stress, baroreflex control of heart rate and vasomotor activity is reset in the short-term such that it continues to regulate both these variables but over a higher operating range of arterial pressure compared to resting conditions (102; 130; 165). Rather than due to adaptation of the baroreceptors themselves, the resetting of the baroreceptor reflex that occurs in association with these relatively short term challenges is a consequence of “central command”, i.e. is often driven by centers in the cortex and other forebrain regions (44). In the case of exercise, feedback from peripheral receptors, particularly from muscle receptors (so-called “ergoreceptors”) also contributes to the acute resetting of the
baroreflex (165). The effect of this resetting is that the baroreflex continues to regulate arterial pressure at all times but at a level that is appropriate for the particular behavioural conditions.

Circulating hormones can also modulate baroreflex function via a variety of brain sites and mechanisms. First, some hormones [e.g. progesterone (191)] cross the blood-brain barrier and therefore can directly access any of the critical neurons. Second, other hormones [e.g. insulin (8)] gain access to the brain via specific transport systems. Third, some hormones [e.g. AngII (70; 192)] act on receptors on neurons in circumventricular organs (CVO) that are located outside the blood-brain barrier. Activation of these neurons can then influence brain function via the projections of the hormone-sensitive CVO neurons to particular target nuclei within the brain. Fourth, some circulating hormones [e.g. AngII or 3α-OH-DHP (61; 72; 160; 190)] are also synthesized de novo in the brain where they serve as local neuromodulators. Finally, some hormones (e.g. AngII again) may act on receptors in cerebral blood vessels, triggering events that lead to the production of neuromodulatory compounds, such as nitric oxide (NO), within the local brain tissue (159).

The central mechanisms that cause changes in the operating characteristics of the baroreflex during pregnancy or other physiological or pathophysiological conditions associated with endocrine changes may therefore be due to effects that occur at one or more of the critical central synapses that subserve the reflex. Figure 2 illustrates some of the different ways that circulating hormones such as AngII could cause such effects, either directly or indirectly. In particular, the NTS has long been regarded as a prime site at which baroreflex modulation can occur (1). It receives direct inputs from the area postrema and subfornical organ (46) and therefore can be influenced by hormones such as AngII that act on AT1 receptors on neurons within these CVOs. There is also a major direct input to the NTS from neurons in the PVN, which itself receives inputs from the subfornical organ (46). Finally, the NTS has been proposed as a site at which circulating AngII stimulates nitric oxide production via endothelial
nitric oxide synthase in blood vessels (159). In the NTS, the increased level of nitric oxide in turn enhances GABA release, impairing baroreflex function (159).

The RVLM also receives synaptic inputs from the PVN (46) and so is also a potential site at which circulating hormones that affect PVN neurons may modulate baroreflex function. Furthermore, there is a direct input to this region from the area postrema (17). Also, as noted above, hormones such as progesterone (or their metabolites) that cross the blood-brain barrier can potentially affect neurons within the CVLM and RVLM, as well as the NTS or in any other brain regions that project to these nuclei. Finally, substances produced within the brain parenchyma, such as AngII or 3α-OH-DHP, may act directly on neurons in several brain nuclei that influence baroreflex function, such as the NTS, the PVN and the RVLM (45; 63; 85).

Since circulating hormones may affect neurons in many different sites, the overall effect on the baroreflex of an increase in the level of a hormone could be the consequence of several different effects operating simultaneously and could vary. For example, increased AngII levels appear to support or increase the arterial pressure level at which the baroreflex operates in some states such as hypertension (78; 88; 108; 109; 185), sodium and water deprivation (18; 54; 199), and pregnancy (27; 149), while in other states including heart failure (53; 143; 144) this action is minimal. Similarly, the effect of AngII to decrease baroreflex gain is revealed only in a subset of states with elevated levels, such as heart failure (53; 144; 145) and some forms of hypertension (96; 108; 109). Since on a cellular level brain AngII is modulatory, it is likely that the presence or absence of other inputs determines the ultimate effect of this neurohormone (45).

In summary, there are several potential mechanisms that could explain the fact that AngII or other hormones can produce different effects on the baroreflex control of heart rate, RSNA or other outputs, depending on the physiological or pathophysiological condition. In pregnancy, it is also very important to consider the effects of chronic changes in hormones, especially those whose levels are greatly altered in this condition. For example, a sustained increase (or
decrease) in the activity of a hormone may result in genomic changes that in turn may lead to a wide range of effects that would not occur acutely, such as changes in the expression of receptors or the synthesis of neurotransmitters or enzymes that affect synaptic transmission.

Apart from the arterial baroreflex, there are other important reflexes that contribute to circulatory control and which are also profoundly altered in pregnancy. In particular, receptors in the right atrium have a critical role in regulating blood volume. Activation of these receptors results in a rather selective reflex inhibition in renal sympathetic nerve activity (121; 200). The central pathways mediating this reflex have not been fully determined, but include a critical synapse in the PVN (121; 200). Based on various observations, it is thought that signals from atrial receptors, mediated via the NTS, excite GABAergic neurons within the PVN that in turn inhibit other PVN neurons that directly or indirectly regulate the renal sympathetic outflow (51; 200). As described above, activation of neurons in the PVN in response to atrial distention is blunted in pregnant as compared to non-pregnant animals (51). Very little information is available, however, on the possible central mechanisms that cause the inhibition of this reflex. Therefore, the following sections of the review will focus primarily on the endocrine factors that modulate the arterial baroreflex during pregnancy.

**Roles of angiotensin II and nitric oxide in baroreflex modulation during pregnancy.**

The levels of circulating AngII and vascular (endothelial) NO are elevated during pregnancy (3; 176; 177; 196) and may impair baroreflex function via a central action (85; 126). Indeed, AngII and/or NO have been implicated in the baroreflex dysfunction associated with hypertension and heart failure (53; 109; 146; 159). Therefore, both substances were considered excellent candidates as potential modulators of baroreflex function during pregnancy. However, neither systemic nor central blockade of AngII AT1 receptors and of NO production, alone or together, improved the gain of arterial baroreflex control of renal sympathetic nerve activity or heart rate (27; 41; 48; 149). These data suggest that a central action of AngII and/or NO is not
responsible for the decrease in baroreflex gain during pregnancy, at least via a rapidly reversible mechanism.

On the other hand, baseline sympathetic tone is increased in pregnancy in both humans and animals (2; 21; 37; 79; 124), and changes in CNS actions of AngII may contribute. Compared to nonpregnant animals, pregnant animals are more dependent on AngII for maintenance of baseline arterial pressure (27; 149). Moreover, acute administration of an AT1 receptor antagonist, either intravenously or intracerebroventricularly, caused a leftward shift in both HR and RSNA baroreflex function curves, effectively reducing SNA at any given arterial pressure level (27; 48; 149). It has been suggested that the elevated baseline sympathetic activity in pregnant rats is a consequence of increased activity of PVN sympathoexcitatory neurons which in turn is due, at least in part, to the increased level of circulating AngII that occurs (112) (Figure 2). Consistent with this hypothesis, chronic infusion of AngII has been shown to cause activation of PVN neurons (49).

It is also possible that reductions in neuronal NOS (nNOS) activity specifically in the PVN could contribute to elevated baseline sympathetic outflow in pregnancy. In the PVN, NO increases GABA release and results in inhibition of autonomic-related parvocellular neurons (119). Decreased nNOS activity, and a consequent reduction in GABA release in the PVN, has been associated with augmented sympathoexcitation in other conditions, such as hypertension and heart failure (157). Therefore, it is interesting that in near term pregnant rats, PVN nNOS expression and activity (91) and baseline GABAergic inhibition (25; 112) are both decreased. However, further study is required to determine the physiological significance of these changes in the PVN in pregnancy.

While an increased level of NO does not appear to be involved in the depression of arterial baroreflex gain in pregnancy, it may contribute to impaired function of volume-dependent atrial reflexes. In pregnant animals, administration of the NO synthase inhibitor, L-NAME, completely restored the appearance of immunoreactive c-fos in both parvo- and magnocellular
regions of the PVN, and the resulting natriuresis, in response to atrial distension (187; 188). These findings emphasize the potential complexity of endocrine control of baroreflex function in a state such as pregnancy.

**Insulin resistance: a contributor to pregnancy-induced decreases in baroreflex gain?**

Multiple conditions besides pregnancy are associated with arterial baroreflex impairment, including heart failure, obesity, hypertension, Alzheimer’s disease, and aging. Intriguingly, a common feature of all these conditions is insulin resistance, which results from blunted intracellular signaling following binding of insulin to its receptor. One consequence can be elevated plasma glucose levels, since a major action of insulin is to stimulate glucose uptake from plasma into skeletal muscle and adipocytes. During pregnancy, the development of insulin resistance is teleologically beneficial, since elevated plasma glucose levels would favor the movement of this nutrient into the placental-fetal compartments.

This common association between arterial baroreflex dysfunction and insulin resistance led to the hypothesis that the two are causally related during pregnancy. In support of this hypothesis, decreases in insulin sensitivity (40; 47; 100; 114; 141; 167) and baroreflex gain (16; 24; 47; 122) exhibit the same time course during gestation in humans, rabbits (Figure 3) and rats. Interestingly, preeclampsia produces further reductions in insulin sensitivity (100; 180), and as indicated above, is associated with further baroreflex impairment. More importantly, when pregnant rabbits were treated throughout gestation with the insulin-sensitizing drug rosiglitazone, insulin sensitivity improved and, to the same extent, baroreflex gain increased, without altering other aspects of the sigmoidal baroreflex curve (47). Collectively, these data implicate insulin resistance as a factor involved in the baroreflex impairment induced by pregnancy.

If pregnancy-induced insulin resistance and baroreflex impairment are mechanistically linked, then what is the link? One possible mechanism involves the actions of insulin in the brain.
Insulin receptors are present in numerous but discrete sites throughout the brain, including regions directly or indirectly involved in central pathways regulating the cardiovascular system: hypothalamus (PVN, arcuate nucleus, supraoptic nucleus, dorsomedial and ventromedial hypothalamus) and brainstem (NTS, the raphe nuclei and nucleus ambiguus) (171; 197). While insulin, a large polypeptide, cannot penetrate the blood-brain-barrier unassisted, a specific and saturable transport system moves insulin from the plasma compartment into brain interstitial fluid via receptor-mediated endocytosis through vascular endothelial cells [for reviews, see (8; 62; 74; 198)]. Kinetic studies suggest that insulin passes from plasma to brain interstitial fluid and then into CSF (198). The rate of passage of insulin varies throughout the brain, with the highest levels recorded in the medulla and hypothalamus (8; 12; 171).

Once plasma insulin enters the brain, it binds to insulin receptors to affect cardiovascular function (Figure 2). It is well established that insulin is sympathoexcitatory, via a central action (117; 142). In addition, acute and chronic increases in brain insulin increase the gain of baroreflex control of both heart rate and sympathetic activity (150; 161). Interestingly, however, the rate of transport of insulin into brain, and therefore brain insulin levels, can be altered; indeed, insulin resistance is associated with impaired transport of insulin into brain (62; 74; 198). For example, adiposity in dogs reduces brain insulin transport (101), and brain insulin levels are decreased in Zucker obese rats (13) and in individuals with Alzheimer’s Disease (8; 74). While the influence of pregnancy on transport of insulin into the brain has not been investigated, recent studies indicate that levels of insulin in cerebrospinal fluid are reduced (47). Because brain insulin supports or enhances baroreflex gain, decreases in brain insulin levels or in its actions could attenuate baroreflex function during pregnancy. In support of this hypothesis, it has been shown that intracerebroventricular infusion of insulin normalizes gain of baroreflex control of heart rate in conscious pregnant rats (6). Yet, similar to the effects of rosiglitazone, increases in brain insulin did not alter the baroreflex in virgin rats nor did it reverse the
depressed maximal level of heart rate in pregnant rats. These results have several important implications. First, brain insulin levels must be sufficiently high in normal conscious animals to support optimal heart rate baroreflex function. Second, pregnancy-induced decreases in brain insulin contribute to the baroreflex impairment. Third, other factors must underlie the reduced ability of pregnant animals to respond to severe hypotension with maximal increases in heart rate or sympathetic activity.

Recent studies have explored the brain sites and mechanisms by which insulin in brain increases baroreflex gain. As described above, insulin receptors are concentrated in many forebrain and hindbrain regions known to influence neural control of the circulation. However, infusion of insulin via the fourth cerebral ventricle failed to alter baroreflex control of heart rate or lumbar sympathetic nerve activity, suggesting that insulin initiates its effect at a forebrain site when infused via the lateral ventricles (161). Among potential hypothalamic sites, the PVN is a likely candidate, since this region is enriched with insulin receptors (171; 197) (Figure 2) and is known to influence baroreflex gain (35; 55; 158). In support of this hypothesis, it was found that blockade of PVN, secondary to PVN microinjection of the GABA\textsubscript{A} agonist, muscimol, reversed the effect of insulin to increase baroreflex gain (Figure 4). Thus, the PVN is involved.

Nevertheless, several questions remain. Is the PVN the site at which insulin initiates its action or merely a relay station? Does the role of PVN change during pregnancy or other insulin resistant states? What downstream pathways and brainstem sites effect the action of forebrain insulin to increase gain?

**Neurosteroid metabolite of progesterone: a contributor to attenuated baroreflex sympathoexcitation in pregnancy?**

The studies discussed above indicate that decreased insulin in the forebrain of pregnant animals, possibly via the PVN, contributes to the decrease in maximum gain of the arterial baroreflex. However, the attenuated ability to increase heart rate or sympathetic nerve activity in
response to a hypotensive challenge appears to be unrelated to changes in insulin levels and may instead involve augmented GABAergic influences in the RVLM. As described above, when arterial pressure was decreased to a level expected to produce maximum sympathoexcitation, activation of the RVLM (but not the NTS or CVLM) was attenuated in term pregnant rats (43), consistent with a role for the RVLM in the attenuated baroreflex sympathoexcitation in pregnancy (Figure 1).

It is important to consider that arterial baroreflex sympathoinhibition during elevated pressure is mediated by increased inhibitory input and activation of GABA_\text{A} receptors in the RVLM, while sympathoexcitation due to baroreceptor unloading (decreased arterial pressure) is mediated by withdrawal of GABAergic tone (disinhibition) (46; 80). Interestingly, arterial baroreflex sympathoinhibitory responses are well maintained or even potentiated in pregnancy (41; 93; 124) (Figure 1) and the major effect of pregnancy on baroreflex range occurs at low arterial blood pressures, where baroreceptor discharge should be minimal. In this regard, the RVLM receives a substantial baroreflex independent GABAergic input, a large portion of which originates in the CVLM (42) (Figure 2), although other GABAergic influences, including GABA interneurons within the RVLM, may contribute (81; 134; 170). Thus, although the effect of pregnancy is manifested as blunted baroreflex mediated increases in heart rate and sympathetic nerve activity, it is possible that mechanisms unrelated to the arterial baroreflex contribute to the attenuated baroreflex sympathoexcitation.

The final sympathetic outflow emanating from the RVLM at any given time is determined by the balance of excitatory and inhibitory inputs to this region (46). Thus, the observation of decreased activation of the RVLM during hypotension in pregnant rats (43) could be due either to decreased baseline excitatory drive to the RVLM, increased baseline baroreflex independent inhibitory drive, or both, in pregnant compared to nonpregnant rats. To test the hypothesis that the RVLM of pregnant rats is under greater baroreflex independent GABAergic inhibition, responses to bilateral blockade of GABA_\text{A} receptors in the RVLM were tested in pregnant and
non-pregnant rats in which the arterial baroreceptors had been surgically denervated. Disinhibition of the RVLM with the GABA$_A$ antagonist, bicuculline, produced greater increases in mean arterial pressure and renal sympathetic nerve activity in pregnant compared to nonpregnant rats (Figure 5) (110). Thus, it appears that pregnancy is associated with enhanced baseline GABAergic inhibition of the RVLM. Prior blockade of AT1 receptors in the RVLM decreased responses to disinhibition, but the difference in responses to bicuculline between pregnant and nonpregnant rats persisted. Thus, while AngII likely contributes to elevated baseline sympathetic outflow in pregnancy (21; 149), within the RVLM, other underlying excitatory influences must contribute to observed differences between pregnant and nonpregnant rats (110).

In regard to the mechanisms for increased GABA$_A$ influence in the RVLM, it is possible that, independent of arterial baroreflex inputs, GABA release in the RVLM is greater in pregnant animals. However, the source of drive to non-baroreflex GABAergic inputs to the RVLM and the potential signal to increase that input during pregnancy is unclear. Another possibility, which would not require increased GABAergic input to the RVLM, would be an increase in positive modulation of GABA$_A$ receptors within the RVLM, so that the effectiveness of existing GABAergic input would be amplified in pregnancy. It is known that, through stereospecific binding to a unique site on the GABA$_A$ receptor complex, the major metabolite of progesterone, 3α-OH-DHP, which is elevated in pregnancy (38; 67; 68), is a potent positive modulator of GABA$_A$ receptor function (151). An autoradiographic study on brain sections from nonpregnant female rats indicated that in vitro exposure to 3α-OH-DHP increases binding of the GABA$_A$ ligand, flunitrazepam, in the brainstem (73). Thus, GABA$_A$ receptors in brain regions involved in control of sympathetic nerve activity are susceptible to modulation by this neurosteroid metabolite of progesterone.

In support of this concept, acute systemic administration of the neurosteroid metabolite of progesterone, 3α-OH-DHP, to nonpregnant rats mimics the effects of pregnancy to suppress arterial baroreflex sympathoexcitation (89; 90; 124), while having no effect on afferent
baroreceptor discharge (115). In all cases, the inactive isomer, 3β-OH-DHP, was without effect. Figure 6 shows effects of microinjection of 3α-OH-DHP into the RVLM, NTS and CVLM of nonpregnant female rats on maximum sympathetic nerve activity achieved in response to baroreceptor unloading. Microinjection of 3α-OH-DHP, but not 3β-OH-DHP (not shown), into the RVLM resulted in a reversible decrease in maximum sympathetic nerve activity, while microinjection into the NTS and CVLM was without effect (63). Thus, similar to the effects of pregnancy on baroreflex mediated activation of brainstem nuclei which are specific to the RVLM (43), effects of the neurosteroid metabolite of progesterone to suppress baroreflex mediated increases in sympathetic nerve activity also appear to be specific to the RVLM. This result is consistent with the concept that 3α-OH-DHP, through positive modulation of GABA_A receptors, may contribute to the increased baseline GABAergic inhibition of the RVLM that is seen in pregnancy (110).

On the other hand, pregnancy is also characterized by well maintained or potentiated baroreflex mediated sympathoinhibition in response to a hypertensive challenge (41; 93; 124) (Figure 1). Since baroreflex mediated inhibition is due to incremental increases in GABAergic input to the RVLM, positive modulation of GABA_A receptors by 3α-OH-DHP at the level of the RVLM would be consistent with increased responsiveness of RVLM neurons to increases in pressure. Indeed, i.v. administration of 3α-OH-DHP (but not 3β-OH-DHP) to nonpregnant female rats decreased the pressure threshold for baroreflex mediated inhibition of single unit neuronal discharge in spinally projecting RVLM neurons (116). Likewise, local application of 3α-OH-DHP (<1 nl, 4 µM) directly onto spinally projecting, arterial pressure sensitive neurons in the RVLM (n = 7) resulted in a decrease in pressure threshold for neuronal inhibition during a hypertensive stimulus. These results suggest that sensitivity of RVLM neurons to endogenously released GABA is increased by 3α-OH-DHP.

As discussed earlier, volume reflexes which are activated by atrial stretch are attenuated by pregnancy. Interestingly, exogenous administration of 3α-OH-DHP to virgin female rats
mimics the effects of pregnancy to attenuate discharge of afferent fibers and to decrease activation of forebrain regions involved in control of blood volume (52; 183). Thus, it appears that 3α-OH-DHP, a neurosteroid metabolite of progesterone, is a likely contributor to alterations that occur in both the arterial baroreflex and volume reflexes in pregnancy.

Sensitivity of GABA_A receptors to modulation by neurosteroid compounds, such as 3α-OH-DHP, is partially dependent on subunit composition of GABA_A receptors which may change during pregnancy. Expression of different GABA_A receptor subunits varies between brain regions and even cell types within a region (133) and can be differentially regulated by numerous factors, including neuronal activity (174), and the ovarian hormones estrogen (39; 172) and progesterone (60; 136; 140; 172; 174). It was previously reported that GABA_A receptors with a higher proportion of α_1 compared to α_2 receptor subunits were most sensitive to neurosteroid modulation (153) and expression of the α_1 subunit is increased in hypothalamic oxytocin neurons in late pregnancy (31; 59). In the RVLM expression of the α_1 subunit is several hundred fold greater than expression of the α_2 subunit, suggesting that GABA_A receptors in this region should be sensitive to neurosteroid modulation (64). However, the α_1:α_2 ratio in the RVLM was not changed by pregnancy. Thus increased levels of 3α-OH-DHP in pregnant rats, rather than changes in α_1 subunit, were proposed as a mechanism contributing to increased GABAergic tone in the RVLM of pregnant rats (64). More recently it has been suggested that, rather than the GABA_A receptor α_1 subunit, the δ subunit, which appears to be crucial for extrasynaptic tonic inhibition (15; 133), might be the most important receptor subunit in conferring high sensitivity to positive modulation by 3α-OH-DHP in native GABA_A receptors (15; 133). Currently the effect of pregnancy on expression of the GABA_A receptor δ subunit in the RVLM is not known.

Although receptor subunit composition is one factor determining sensitivity to 3α-OH-DHP, other factors affecting efficacy of neurosteroids include regulation of local concentrations of 3α-OH-DHP (synthesis and degradation) and post-translational changes such as
phosphorylation, which can affect responses to GABA<sub>A</sub> receptor activation (58; 83; 107; 139). Enzymes responsible for synthesis of 3α-OH-DHP from progesterone, 5α-reductase and 3α-OH steroid oxidoreductase, are present in the medulla (105; 118; 168) and ovarian hormones can regulate 3α-OH-DHP synthesis in brain tissue (131). Importantly, differences in peripheral and CNS levels of 3α-OH-DHP during pregnancy suggest that local regulation determines the effective concentrations of 3α-OH-DHP in a brain region specific manner (14; 38; 168; 173). Preliminary data suggest that messenger RNA for both 5α-reductase and 3α-OH steroid oxidoreductase is increased in the RVLM of term pregnant rats (87) and treatment of near pregnant rats with the 5α-reductase inhibitor, Finasteride, partially restores baroreflex mediated sympathoexcitation (125). Taken together these studies in pregnant rats suggest that increased levels of the major metabolite of progesterone, 3α-OH-DHP, in the RVLM may contribute to attenuated baroreflex mediated sympathoexcitation in pregnancy.

**CONCLUSIONS AND FUTURE DIRECTIONS**

The function of the arterial baroreflex is in continuous flux. In some circumstances, such as stress or exercise, programmed efferent commands that issue from higher centers reset the baroreflex to a higher pressure, a teleologically appropriate response. In contrast, the often complex endocrine milieu associated with acute and chronic physiological and pathophysiological states influences multiple central sites to ultimately produce varied changes in the baroreflex.

During pregnancy, baroreflex gain is markedly attenuated, and at least two hormonal mechanisms have been shown to contribute, each at different brain sites: increased levels of 3α-OH-DHP acting in the RVLM and reduced actions of insulin in the forebrain. Maximal baroreflex gain depends on two parameters, the heart rate or sympathetic nerve activity range (difference between the maximum and minimum heart rate or level of sympathetic nerve
activity) and the width (the arterial pressure range over which the reflex operates) (84). Alterations in both parameters are involved in the decreased gain induced by pregnancy (Figure 1) (19; 24; 124). Interestingly, the decrease in brain insulin appears to primarily increase the range over which arterial pressure is regulated (X-axis, Figure 1), whereas 3α-OH-DHP acts mainly to decrease the sympathetic nerve activity or heart rate range (Y-axis Figure 1), apparently by potentiating baroreflex-independent GABAergic inhibition of premotor neurons in the RVLM. This later effect is reminiscent of other situations in which control of sympathetic outflow is compromised. For example, heart rate range is decreased following pharmacological blockade of cardiac sympathetics (86), and splanchnic sympathetic nerve activity range is reduced following destruction of catecholaminergic RVLM premotor neurons (82). Where and how the forebrain action of insulin and the hindbrain effect of 3α-OH-DHP integrate in the brain to depress baroreflex function is not known, primarily because the neuronal pathways and mechanisms by which forebrain insulin influences brainstem circuitry have not been completely delineated. Nevertheless, the PVN is one site identified in the pathway (Figure 4). Moreover, insulin has recently been shown to increase glutamatergic drive to the RVLM (11), and the vast majority of PVN projections to the RVLM are glutamatergic (182). Therefore, one potential scenario is that the RVLM is the site at which forebrain insulin derived-inputs and brainstem 3α-OH-DHP actions are integrated (Figure 2).

As described earlier, undesirable effects of decreased baroreflex sensitivity in pregnancy include orthostatic hypotension, and more seriously, a decreased ability to compensate for blood loss. Nevertheless, the attenuated ability to rapidly increase sympathetic outflow through the arterial baroreflex could serve a protective role by limiting vasoconstriction in the face of substantially increased blood volume and cardiac output. Furthermore, this adaptation would minimize possible detrimental effects of excessive shunting of blood flow (due to constriction of other beds) to low resistance beds, such as the uterine circulation in pregnancy.
Besides the baroreflex, pregnancy has been shown to suppress other homeostatic reflexes that utilize brain pathways, including fever and ACTH responses induced by systemic inflammation, ACTH released in response to behavioral stress, as well as the appetite-inhibiting actions of leptin (4; 28; 29; 179; 181). These changes are also likely beneficial to the mother and fetus, since they minimize the potentially negative consequences of elevated glucocorticoid concentrations or body temperature and favor increased energy consumption. The depression of parallel brain homeostatic mechanisms leads logically to the question as to whether similar mechanisms and brain pathways are involved. In support of this idea, 3α-OH-DHP has also been shown to be involved in the depressed responses of the hypothalamo-pituitary-adrenal axis to stress (29; 30). Moreover, placental lactogen has been identified as one factor that reduces brain leptin sensitivity in the context of energy balance (4). Placental lactogen (40; 113) and other circulating factors emanating from the placenta and adipose tissue such as progesterone, TNF-α, IL-6, resistin, and free fatty acids (9; 10; 32; 33; 57; 66; 75; 106) decrease insulin sensitivity during pregnancy. Therefore, these factors may also contribute to baroreflex impairment by decreasing brain levels or actions of insulin in brain to support baroreflex gain. Other parallels also may exist; for example, does the increased GABAergic tone in RVLM attenuate other sympathoexcitatory responses mediated by this brain region, such as the sympathoexcitation induced by stress? Future experimental work is required to further explore mechanisms and possible interactions between various neuromodulators that contribute to alterations in baroreflex function in pregnancy and other physiological and pathophysiological states.
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1. Pregnancy impairs baroreflex control of heart rate (top) and renal sympathetic nerve activity (RSNA; bottom) in conscious rats. Arterial pressure was increased and decreased by infusion of increasing doses of phenylephrine or nitroprusside, respectively, while continuously recording arterial pressure, heart rate and RSNA. Sigmoidal relationships between arterial pressure and heart rate or RSNA were determined by fitting a four parameter equation, from which maximal gain was calculated, and parameters were statistically compared between pregnant (P) and nonpregnant (NP) rats (24; 124). In terms of baroreflex control of heart rate (top), pregnancy decreased the maximum level of heart rate, the heart rate range, and the maximal baroreflex gain, and increased the range over which arterial pressure is regulated. Pregnancy also decreased the RSNA range, RSNA maximum, and RSNA minimum (bottom); the slope of RSNA responses to decreases in arterial pressure was also decreased (bottom). The figure was adapted from previously published data (24; 124) with kind permission of the American Physiological Society.

2. Schematic diagram showing the essential pathways that subserve the baroreflex control of the sympathetic outflow to the heart and blood vessels and possible sites of action and mechanisms by which changes in the activity of the hormones angiotensin II (AngII), 3α-hydroxy-dihydroprogesterone (3α-OH-DHP, a metabolite of progesterone), and insulin can alter the baroreflex. Note that in addition to the inhibitory input to RVLM sympathetic premotor neurons from barosensitive neurons in the CVLM, there is a baroreceptor-independent inhibitory input to the RVLM neurons arising from other CVLM neurons that receive excitatory inputs from neurons in unknown brain regions. Abbreviations: CVLM, caudal ventrolateral medulla; NTS, nucleus tractus solitarius; RVLM, rostral ventrolateral medulla; AP, area postrema, PVN,
paraventricular nucleus; SFO, subfornical organ. + indicates an excitatory effect, and – an inhibitory effect.

3. Insulin sensitivity and baroreflex gain decreased in parallel during pregnancy in rabbits, and the changes were well correlated ($r^2=0.65\pm0.08$, n=5). This figure was reprinted from (47) with kind permission of the American Physiological Society.

4. Local blockade of the paraventricular nucleus (PVN) with bilateral microinjection of muscimol reverses the effect of intracerebroventricular (icv) insulin infusion to increase baroreflex gain (n=4). In contrast, in control animals not receiving insulin, bilateral PVN muscimol did not alter baroreflex gain (1.1±0.3 bpm/mmHg, control; 1.1±0.3 bpm/mmHg, icv aCSF or no infusion; 1.2±0.3 bpm/mmHg, +PVN muscimol; n=5; P>0.50). Experiments were performed in urethane-anesthetized male rats using previously published procedures (65; 161). *: P<0.05 compared to the other two treatments, ANOVA for repeated measures and the Newman Keuls post hoc test. Similar results have recently been found in female rats (34).

5: Increased baroreflex independent GABA$_A$ inhibition of the RVLM in pregnant (P) rats. In sinoaortic denervated rats, increases in mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) in response to bilateral blockade of GABA$_A$ receptors (bicuculline, Bic) into the RVLM was greater in P compared to nonpregnant (NP) rats. Prior blockade of AngII AT1 receptors (L158,809; AT1X) in the RVLM attenuated responses to Bic, but differences between NP and P rats persisted. (*, greater than NP; †, main effect of AT1X; p ≤ 0.05). Figure was reprinted from (111) with kind permission of the American Physiological Society.

Figure 6: 3α-OH-DHP in the RVLM of nonpregnant rats limits maximum baroreflex mediated sympathoexcitation. Maximum RSNA due to baroreflex unloading is reversibly attenuated
following microinjection of 3α-OH-DHP (3α) into the RVLM, but not the CVLM or NTS. (C, control; Rec, recovery; *, less than C; †, less than Rec; p ≤ 0.05).
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Figure 1

The graph illustrates the relationship between MAP (mmHg) and Heart Rate (bpm) for two groups: NP (n = 10) and P (n = 8). The solid line represents the NP group, while the dashed line represents the P group. Similarly, the graph below shows the relationship between MAP (mmHg) and RSNA (% baseline) for the same groups. The solid line represents the NP group, and the dashed line represents the P group.
Figure 2
Figure 3

A

Baroreflex Gain (bpm/mmHg)

Week of Pregnancy

B

Baroreflex Gain (bpm/mmHg)

Insulin Sensitivity (mg dextrose/kg/min)

- Non-Pregnant
- 2 weeks
- 3 weeks
- Term

Figure 3
Figure 4
Figure 5

ΔMAP (mm Hg)

ΔRSNA (% base)

Bic 1

Bic 2 (AT1X)

NP (9)
P (6)

†

∗
Figure 6