Published in final edited form as:

Hypertension. 2015 December ; 66(6): 1191–1198. doi:10.1161/HYPERTENSIONAHA.115.06045.

The hypothalamic paraventricular and arcuate nuclei contribute to elevated sympathetic nerve activity in pregnant rats: roles of Neuropeptide Y and α -melanocyte stimulating hormone

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Abstract

Pregnancy increases sympathetic nerve activity, but the mechanisms are unknown. Here, we investigated the contributions of the hypothalamic paraventricular and arcuate nuclei in α -chloralose-anesthetized pregnant and nonpregnant rats. Baseline arterial pressure was lower, and heart rate, lumbar sympathetic activity and splanchnic sympathetic nerve activity were higher in pregnant compared to nonpregnant rats. Inhibition of the paraventricular nucleus via bilateral muscimol nanoinjections decreased arterial pressure more in pregnant than nonpregnant rats, and decreased heart rate and lumbar sympathetic nerve activity only in pregnant rats. Similarly, following arcuate muscimol nanoinjections, the decreases in arterial pressure, heart rate, and lumbar, renal, and splanchnic sympathetic nerve activities, were greater in pregnant than nonpregnant rats. Major arcuate neuronal groups that project to the paraventricular nucleus express inhibitory Neuropeptide Y and excitatory α -melanocyte stimulating hormone. Inhibition of paraventricular melanocortin 3/4 receptors with SHU9119 also decreased arterial pressure, heart rate, and lumbar sympathetic nerve activity in pregnant, but not nonpregnant rats. Conversely, paraventricular nucleus Neuropeptide Y expression was reduced in pregnant animals, and while blockade of paraventricular Neuropeptide Y Y1 receptors increased arterial pressure, heart rate, and lumbar sympathetic activity in nonpregnant rats, it had no effects in pregnant rats. Yet, the sympathoinhibitory, depressor and bradycardic effects of Neuropeptide Y nanoinjections were similar between groups. In conclusion, the paraventricular and arcuate nuclei contribute to increased basal sympathetic nerve activity during pregnancy, likely due in part to decreased tonic Neuropeptide Y inhibition and increased tonic α -melanocyte stimulating hormone excitation of presympathetic neurons in the paraventricular nucleus.

Keywords

paraventricular nucleus; arcuate nucleus; pregnancy; POMC; NPY

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DISCLOSURES.

None.

INTRODUCTION

Normal pregnancy profoundly alters fluid balance and arterial pressure (AP) regulation. Blood volume and cardiac output increase, but AP falls due to decreases in systemic vascular resistance.¹ To partially counteract primary vasodilation and hypotension, pregnancy increases SNA to several organs, including the heart,²⁻⁴ adrenal,⁵ kidney,⁶ and skeletal muscle.⁷⁻⁹ In contrast, in women with preeclampsia (PE), blood volume expansion is blunted and hypertension is produced,¹⁰ with further increases in SNA.^{7, 8, 11} PE is a complication of considerable medical importance; it occurs in 6-10% of pregnant women and ranks among the top causes of maternal death.¹⁰ It is therefore imperative to understand the mechanisms that contribute, including the elevations in SNA. However, before we can understand PE, we first need to identify the mechanisms by which normal pregnancy increases SNA, since PE may exaggerate these same mechanisms. Remarkably, very little information is currently available.

The rostral ventrolateral medulla (RVLM) is a major source of basal sympathetic tone in normal individuals, yet GABAergic inhibition of RVLM presympathetic neurons is increased during pregnancy,¹² at variance with a major role. In contrast, while the paraventricular nucleus of the hypothalamus (PVN) normally contributes little to basal sympathetic tone, indirect evidence suggests that the PVN may underlie basal sympathoexcitation in pregnancy. More specifically, sympathoinhibitory influences in the PVN, including the considerable tonic GABAergic restraint^{13, 14} and the expression and activity of neuronal nitric oxide synthase (nNOS),^{15, 16} are decreased. However, whether this decreased inhibition translates into higher tonic support of basal SNA is unknown. Therefore, the first purpose of the present experiments was to test the hypothesis that the PVN is a source of elevated basal SNA drive, by determining if PVN inhibition, via bilateral nanoinjection of muscimol, decreases lumbar SNA (LSNA) and AP more in P compared to nonpregnant (NP) anesthetized rats.

Another tonic inhibitor of PVN presympathetic neurons is Neuropeptide Y (NPY),¹⁷ and PVN NPY expression is reduced during pregnancy, at least in mice;¹⁸ however, whether tonic NPY inhibition of SNA decreases during pregnancy, like GABA, is unknown. Therefore, we used immunohistochemistry (ihc) to quantify PVN NPY expression in P and NP rats and tested if blockade of PVN type 1 NPY receptors (NPY1 $\times$) increases LSNA less in P compared to NP rats. Because inhibitory NPY inputs converge with excitatory α -melanocyte-stimulating hormone (α -MSH) inputs onto PVN presympathetic neurons,¹⁷ we next tested if blockade of melanocortin type 3/4 receptors (MC3/4R) decreases SNA in P rats. Lastly, because a major source of both NPY and α -MSH inputs into the PVN is the arcuate nucleus (ArcN), we tested if the ArcN also contributes to increased basal SNA, by determining the effects of ArcN muscimol in P and NP rats.

METHODS

An expanded Methods section is available in the Online Supplement at <http://hyper.ahajournals.org>.

Animals

Experiments were performed using female virgin or pregnant Sprague-Dawley rats. For pregnant animals, the presence of sperm was designated pregnancy *day 0*, and experiments were performed on pregnancy day 20. All procedures were conducted in accordance with the National Institutes of Health's *Guide for the Health and Use of Laboratory Animals* and were approved by the Institutional (Oregon Health & Science University) Animal Care and Use Committee.

Surgery

Anesthesia was induced and maintained with 2–5% isoflurane in 100% oxygen. The rat was then surgically prepared with a tracheal tube, femoral arterial and venous catheters, and stainless steel electrodes around the lumbar, splanchnic or renal sympathetic nerves, and for PVN or ArcN nanoinjections, as previously described.^{17, 19} After surgery, isoflurane anesthesia was slowly transitioned to a continuous intravenous infusion of α -chloralose. Virgin rats received a $50 \text{ mg} \cdot \text{kg}^{-1}$ loading dose over 30 min followed by a $25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ maintenance dose; pregnant rats received a dose equivalent to the weight of a virgin rat at a similar age. After the α -chloralose loading dose, rats were allowed to stabilize for 60 min before experimentation.

PVN and ArcN nanoinjections.²⁰

All nanoinjections (60 nl for PVN and 30 nl for ArcN) were made bilaterally, with ~2 min between injections, and each injection was conducted over approximately 5–10 s using a pressure injection system. The following drugs and chemicals were used: Muscimol (1 mM/L), SHU9119 [blocks MC3/4R; 0.5 mM/L in artificial cerebrospinal fluid (aCSF) with 10% DMSO], BIBO 3304 (blocks NPY Y1R; NPY1 $\times$; 1 mM/L), and NPY (0.1 mM/L). In some animals, prior to nanoinjection of drugs, aCSF was injected into the ArcN or PVN as vehicle control.

Immunocytochemistry

Paired P and NP rats were deeply anesthetized and perfused with 4% paraformaldehyde. The brains were removed, sectioned, processed for immunohistochemical (ihc) detection of NPY as previously described.^{17, 20} The sections were imaged in pairs, and staining was quantified using ImageJ.

Data analysis

All data are presented as means $\pm$ SEM. Maximal responses (30 sec) to PVN and ArcN nanoinjections were compared using 2- and 3-way repeated measures ANOVA. PVN NPY fiber densities were compared between P and NP rats using a t-test. All data are presented as means $\pm$ SEM. P values < 0.05 were considered statistically significant.

RESULTS

Baseline values

Pregnancy decreased AP and increased HR and LSNA (Figure S1). In the limited numbers of rats tested, we also observed increased splanchnic SNA (SSNA); however, renal SNA (RSNA) was not significantly different between P and NP rats (Figure S1).

Blockade of the PVN (Figure 1)

Bilateral PVN nanoinjections of muscimol significantly decreased ($P<0.05$) MAP, HR, and LSNA in P rats, but decreased ($P<0.05$) only MAP and HR in NP rats; these variables began to fall immediately and reached a nadir 7 ± 1 min after initiating the nanoinjections in both groups. aCSF had no effects in either group. The decreases in MAP, HR, and LSNA in P rats were greater than in NP rats and also greater compared to the effects of aCSF. Collectively, these data indicate that the PVN supports elevated LSNA during pregnancy in rats.

PVN NPY

As depicted in representative PVN sections in Figure 2, NPY-ir was lower in P compared to NP rats, as in mice.¹⁸ In 4 experiments, the intensity of staining in P rats was $61\pm 8\%$ of NP rats ($P<0.05$). To test if the reduced NPY expression is functionally significant, we determined the effects of acute NPY1 $\times$ in NP and P animals. NPY1 $\times$ increased ($P<0.05$; maximum reached at 9.3 ± 0.5 min) MAP, HR, and LSNA in NP rats more than in pregnant rats ($P<0.05$; Figure 3). Indeed, this treatment was ineffective in P rats. Nevertheless, the sympathoinhibitory, bradycardic, and depressor responses (all $P<0.05$) to PVN NPY injections were similar between groups (Figure 4).

PVN MC3/4R (Figure 5)

We have shown that PVN NPY inputs directly inhibit presympathetic neurons also activated by α -MSH.¹⁷ Therefore, because pregnancy reduced tonic PVN NPY inhibition of SNA, we next determined if elevated basal SNA is mediated in part by increased MC3/4R drive of PVN neurons. As shown previously,^{21, 22} PVN nanoinjection of SHU9119 had no effects in NP rats. In contrast, PVN SHU9119 significantly decreased ($P<0.05$) MAP, HR, and LSNA in P rats, reaching a nadir in 5 ± 1 min. Thus, the decreases in MAP, HR and LSNA in P rats were greater than in NP rats. These data support the hypothesis that elevated LSNA is supported at least in part by PVN MC3/4R.

Blockade of the ArcN (Figure 6)

PVN NPY inputs originate largely from the ArcN and brainstem,^{23, 24} and α -MSH inputs primarily arise from the ArcN,²⁵ suggesting that the ArcN contributes to SNA support during pregnancy. Therefore, we quantified the effects of acute ArcN blockade. A key feature of this protocol is that we studied not only LSNA, but also RSNA and SSNA, given the diverse effects of the ArcN on reproduction, energy balance, and AP regulation, as well as the limited information regarding ArcN control of SNA. While ArcN aCSF had no significant effects, ArcN muscimol injections decreased ($P<0.05$) MAP in both NP and P rats, with the maximum response occurring after 6.1 ± 0.4 min in P rats and 6.5 ± 0.4 min in

NP rats. However, the depressor response was significantly greater in P rats. ArcN muscimol also decreased ($P<0.05$) HR in both groups, but the nadir was delayed compared to the decreases in MAP (P: 8.5 ± 0.4 min; NP: 6.9 ± 0.6 min), and the responses were not significantly different between groups. On the other hand, ArcN muscimol decreased ($P<0.05$) LSNA, SSNA, and RSNA in P rats more than either ArcN muscimol injections in NP rats or aCSF injections in either group, and the falls occurred simultaneously with the decreases in MAP. Collectively, these data indicate that the ArcN supports SNA and MAP during pregnancy.

Histological verification of injection sites

Figures S2 and S3 summarize the location of PVN and ArcN injection sites.

DISCUSSION

The purpose of the present study was to test if the PVN contributes to increased basal SNA in pregnant rats and to begin to investigate PVN inputs that drive this elevated sympathetic tone. We confirm that pregnancy decreases MAP and profoundly elevates basal SNA and HR. Our major new findings are: 1) Acute PVN inhibition decreases MAP, HR and LSNA more in P compared to NP rats; 2) PVN NPY expression is reduced in P rats, blockade of PVN NPY1R increases MAP, HR, and LSNA in NP, but not P rats, yet responses to PVN NPY are preserved during pregnancy; 3) blockade of PVN MC3/4R decreases MAP, HR, and LSNA more in P compared to NP rats; and 4) acute ArcN inhibition decreases MAP, LSNA, RSNA, and SSNA more in P compared to NP rats. Collectively, these data indicate that both the PVN and the ArcN contribute to increased basal SNA, likely in part due to decreased tonic PVN NPY inhibition and also to increased tonic PVN α -MSH stimulation.

Previous studies have documented increased SNA to multiple organs during pregnancy,²⁻⁹ and we show for the first time that SSNA is also markedly elevated. Increased RSNA has been detected in one study⁶ but not others;^{26, 27} we also did not observe such an elevation. This inconsistency may be explained by methodological differences and also the difficulty in detecting small between group differences in SNA due to variations in experimental preparation. In support, we found that acute ArcN inhibition produced a small fall in RSNA, as it did LSNA and SSNA, suggesting that at least ArcN drive of RSNA is increased in P rats.

A key finding was that acute PVN inhibition profoundly decreases MAP, HR, and LSNA in P more than in NP rats, indicating that the PVN supports elevated SNA and HR during pregnancy. A major contributor to this increased SNA may be reduced tonic inhibition of PVN presympathetic neurons. In addition to the previously reported decreases in GABAergic^{13, 14} and nNOS-mediated¹⁵ SNA suppression, our data suggest that tonic PVN NPY inhibition of SNA is also lessened. First, similar to the mouse,¹⁸ we found that PVN NPY expression is decreased in pregnant rats. Second, PVN NPY-induced sympathoinhibition was preserved, but the normal sympathoexcitatory response to PVN NPY Y1R blockade was abolished in pregnant rats. The failure of NPY1 $\times$ to increase SNA in pregnant rats cannot be because the elevated basal SNA has reached a maximal level, since baroreceptor unloading^{12, 14} and PVN nanoinjection of the GABA_A antagonist,

bicuculline,^{13, 14} can both increase SNA further in pregnant rats. Nevertheless, our ihc data appear to conflict with studies showing that the expression of NPY [and the co-expressed agouti-related peptide (AgRP)] in the ArcN, one major source of PVN NPY inputs, is increased or unchanged during late pregnancy,²⁸⁻³⁰ and that PVN NPY Y1R may be elevated.¹⁸ In late pregnant women, CSF concentrations of AgRP also rise.³¹ However, ArcN and PVN NPY regulate multiple modalities, including food intake, which is increased during pregnancy in association with the elevations in the orexigenic peptides, NPY and AgRP.^{28, 30} Thus, the lack of correlation between whole ArcN NPY expression and the ability of PVN NPY to inhibit SNA during pregnancy may be explained by a differential regulation of ArcN neurons that influence SNA versus those that modulate, for example, energy balance. In support, it has been reported that changes in ArcN NPY/AgRP and proopiomelanocortin (POMC) expression during pregnancy vary within the nucleus.^{30, 32} Moreover, the altered ArcN regulation of SNA versus energy balance is divergent in another state, obesity.³³ Alternatively, because the PVN also receives a significant NPY projection from the brainstem,²³ it may be that a decrease in this component largely accounts for the decreases in PVN NPY expression and tonic sympathetic inhibition. Future experiments are required to delineate the source of NPY inputs that are diminished during pregnancy. Regardless, collectively the data support the hypothesis that reduced tonic NPY inhibition of PVN presympathetic neurons supports elevated SNA during pregnancy.

The current study also identified one source of excitatory support of PVN presympathetic neurons during pregnancy, α -MSH. The ArcN is the primary if not the sole source of PVN α -MSH inputs.²⁵ However, many studies have reported unchanged or decreased ArcN POMC expression during late pregnancy.^{30, 34-36} Therefore, in parallel to the results with NPY, it may be that ArcN POMC expression is not relevant to its sympathoexcitatory actions in PVN. Alternatively, because PVN SHU9119 blocks the excitatory effects of PVN NPY1 $\times$ ¹⁷ and withdrawal of tonic NPY inhibition is required to unmask α -MSH excitation,³⁷ the decrease in tonic NPY inhibition may be sufficient to explain the increased α -MSH drive of PVN presympathetic neurons during pregnancy.

Because pregnancy decreased tonic NPY inhibition and increased α -MSH excitation in PVN, and the ArcN is a major source of NPY and α -MSH inputs into PVN, we next tested if the ArcN, like the PVN, contributes to increased basal SNA. Indeed, inhibition of the ArcN decreased MAP, SSNA, LSNA, and RSNA considerably more in P than in NP rats, suggesting that the excitatory influences from the ArcN, likely mediated in part by α -MSH, predominate. To our knowledge, this is the first demonstration that the ArcN is capable of driving increased basal SNA in any physiological or pathophysiological state. We did not investigate the factors that mediate the changes in ArcN activity during pregnancy, but several candidates immerge. First, insulin and leptin are increased in P women and rats, and both hormones are sympathoexcitatory at least in part by binding to receptors in the ArcN and triggering a pathway that includes the PVN.^{20, 21, 38, 39} Second, angiotensin II, via actions at AT1 receptors, supports AP and RSNA during pregnancy,⁴⁰⁻⁴² and stimulation of ArcN AT1 receptors increases SNA.⁴³ Future experiments are required to test these possibilities. Nevertheless, it is noteworthy that while basal raw LSNA and SSNA were more than double in P compared to NP rats, complete PVN inhibition or blockade of PVN MC3/4R reduced LSNA by about only 20%, and ArcN muscimol only decreased LSNA,

SSNA, and RSNA by slightly more than 10%. Thus, it would appear that other brain regions are also involved in setting elevated basal SNA tone during pregnancy.

One limitation of the present work is that experiments were performed in rats under anesthesia, which can influence autonomic control of the cardiovascular system. However, MAP and HR levels measured in the pregnant and virgin rats used in the present study were essentially identical to values obtained using telemetry in conscious rats.⁴⁴ In addition, the effect of pregnancy to impair baroreflex control of heart rate is similar in conscious^{44, 45} compared to anesthetized¹⁴ animals, as is the pregnancy-induced elevation in basal SNA. Finally, recent studies reveal that the influence of neurotransmitters and neuromodulators such as GABA and nitric oxide in the PVN on MAP and HR is similar in conscious and anesthetized rats.⁴⁶ Thus, the impact of anesthesia may be minimal.

Perspectives

While pregnancy clearly increases basal sympathetic tone, preeclampsia increases it even more (for review, see¹¹). However, the mechanisms are unknown. One hypothesis is that the factors that mediate increased SNA during normal pregnancy contribute to this further elevation. Indeed, preeclampsia is associated with even greater increases in insulin and leptin, and the balance of pressor versus depressor components of the renin-angiotensin system is shifted towards the hypertension arm (for reviews, see⁴⁷⁻⁴⁹). If true, then before we can identify the cause of sympathetic hyperactivity with hypertensive pregnancy, the mechanisms underlying increased SNA in normal pregnancy must first be established. The present studies, identifying key roles for the PVN and ArcN, are one step towards this goal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

The authors are grateful for the technical assistance provided by Dr. Baoxin Li and Cole Streiff.

SOURCES OF FUNDING.

This study was supported in part by a National Institutes of Health grant HL088552, a Grant-in-Aid from the American Heart Association (12GRNT11550018), the Medical Research Foundation of Oregon, and by a NIH National Center for Advancing Translational Sciences grant UL1TR000128.

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NOVELTY AND SIGNIFICANCE

What is new?

- Inhibition of the PVN with muscimol decreases LSNA, MAP and HR in pregnant but not nonpregnant rats.
- Blockade of PVN MC3/4R decreases LSNA, HR, and MAP in pregnant but not nonpregnant rats.
- PVN NPY expression is reduced in pregnant rats, blockade of PVN NPY Y1 receptors fails to increase LSNA, HR, and MAP in pregnant rats, unlike nonpregnant animals, yet the decreases in LSNA, HR, and MAP following PVN NPY injections are preserved during pregnancy.
- Inhibition of the ArcN with muscimol decreases LSNA, SSNA, RSNA, and MAP more in pregnant compared to nonpregnant rats.

What is relevant?

- Our partial identification of the neurocircuitry through which normal pregnancy increases SNA may contribute towards an understanding of the mechanisms by which preeclampsia increases SNA even more, since preeclampsia may exaggerate the very same processes that elevate SNA during normal pregnancy.
- We show for the first time in any physiological or pathophysiological state that a decrease in tonic PVN NPY inhibition of SNA contributes to basal sympathoexcitation, which may lead to novel pharmacological approaches to treat excess SNA in other conditions like hypertension.
- These are the first data implicating the ArcN in increased basal SNA in any physiological or pathophysiological state, which may open the door to a new therapeutic arena.

Summary

The PVN and the ArcN contribute to increased basal SNA during normal pregnancy, at least in part due to decreased tonic PVN NPY inhibition and also increased stimulation of PVN MC3/4R.

1.

Acute inhibition of the PVN with bilateral nanoinjections of muscimol (Musc) decreases MAP, HR, and LSNA more in late pregnant (P; n=7) compared to nonpregnant (NP; n=7) rats and compared to aCSF nanoinjections in P (n=5) and NP (n=7) rats. The left and middle panels illustrate representative experiments. The right panel shows group data. Double arrows indicate time of injections. Triangles above the integrated LSNA tracings indicate times at which 5 sec sections of raw LSNA, shown below, were obtained. *: P<0.05 compared to all other groups.

2.
Representative images showing PVN NPY-ir is less in pregnant (P) compared to nonpregnant (NP) rats. Scale bar: 100 μ m.

3.

Bilateral nanoinjections of the NPY Y1R antagonist BIBO3304 (NPY1x) into the PVN increases MAP, HR, and LSNA in nonpregnant (NP; n=8) but not late pregnant (P; n=5) rats. The left and middle panels illustrate representative experiments. The right panel shows group data. Double arrows indicate time of injections. Triangles above the integrated LSNA tracings indicate times at which 5 sec sections of raw LSNA, shown below, were obtained. *: P<0.05, P compared to NP rats.

4.

Bilateral PVN nanoinjections of NPY decreases MAP, HR, and LSNA similarly in nonpregnant (NP; n=5) and late pregnant (P; n=4) rats. The left and middle panels illustrate representative experiments. The right panel shows group data. Double arrows indicate time of injections. Triangles above the integrated LSNA tracings indicate times at which 5 sec sections of raw LSNA, shown below, were obtained.

5.

Bilateral nano-injections of the MC3/4R antagonist SHU9119 (SHU) into the PVN decreases MAP, HR, and LSNA in pregnant (P; n=5) but not nonpregnant (NP; n=7) rats. The left and middle panels illustrate representative experiments. The right panel shows group data. Double arrows indicate time of injections. Triangles above the integrated LSNA tracings indicate times at which 5 sec sections of raw LSNA, shown below, were obtained. *: $P < 0.05$, P compared to NP rats.

6.

Acute inhibition of the ArcN with bilateral nano-injections of muscimol (musc) decreases MAP (n=13, NP; n=13, P), HR (n=13, NP; n=13, P), LSNA (n=4, NP; n=5, P), SSNA (n=5, NP; n=4, P), and RSNA (n=4, NP; n=4, P) more in late pregnant (P) compared to nonpregnant (NP) rats and compared to aCSF nano-injections in NP (LSNA: n=4; SSNA: n=4; RSNA: n=4) and P (LSNA: n=4; SSNA: n=4; RSNA: n=4) rats. The left and middle panels illustrate representative experiments. The right panel shows group data. Double arrows indicate time of injections. Triangles above the integrated SNA tracings indicate times at which 5 sec sections of raw SNA, shown directly below, were obtained. *: P<0.05 compared to all other groups.