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Pregnancy decreases GABAergic inhibition of the hypothalamic paraventricular nucleus

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Abstract

Depressor responses to peripheral or central infusion of Angiotensin II type 1 (AT₁) receptor antagonists (AT₁X) are greater in pregnant (P) compared to nonpregnant (NP) animals. AT₁ and ionotropic excitatory amino acid (EAA) receptors contribute to pressor responses to GABA_A receptor blockade with bicuculline (Bic) in the paraventricular nucleus (PVN) of male rats. Therefore, we hypothesized that GABAergic inhibition is decreased and AT₁ receptors play a greater excitatory role in the PVN of P versus NP rats. Unilateral microinjection of Bic was performed before (Bic₁), after AT₁X (Bic₂), and after AT₁X + EAA blockade (kynurenate, Kyn) (Bic₃) in the PVN. Increases in mean arterial pressure (MAP: NP= 20 ± 2; P= 12 ± 2 mmHg), heart rate (HR: NP= 57 ± 6; P= 19 ± 6 beats/min) and renal sympathetic nerve activity (RSNA: NP= 70 ± 9; P= 33 ± 7 %) due to Bic (Bic₁) were attenuated in P rats. Responses to AT₁X and Kyn alone were insignificant in both groups. In NP rats, AT₁X attenuated (+12 ± 4 mmHg), and AT₁X + Kyn further decreased the pressor response to Bic in the PVN (+6 ± 2 mmHg). In P rats AT₁X reduced the pressor response to Bic (+5 ± 1 mmHg), and Kyn had no additional effect (+3 ± 1 mmHg). Effects of PVN Bic to alter the autospectra of RSNA were suppressed by prior AT₁X and Kyn in both groups. Thus, tonic GABAergic inhibition is decreased and the contribution of AT₁ receptors in the PVN may be greater in P rats.

Index Terms

rats; excitatory amino acid receptors; angiotensin AT₁ receptors; renal sympathetic nerve activity; kynurenate; L158,809; bicuculline; PVN

1. Introduction

Physiological changes during normal pregnancy include decreased arterial pressure, tachycardia, increased blood volume, and increased circulating levels of angiotensin II (A II) [1,2]. Term pregnant rats have altered arterial baroreflex function with an attenuated ability to increase sympathetic nerve activity in response to a decrease in arterial blood pressure [3]. The rostral ventrolateral medulla (RVLM) is a major source of resting sympathetic outflow and disinhibition of the RVLM is responsible for pressor and sympathoexcitatory responses due to hypotension. Recently our laboratory demonstrated that tonic baroreflex independent

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GABAergic inhibition of the RVLM is greater in near term pregnant compared to nonpregnant rats [4]. Increased non-baroreflex GABAergic inhibition of the RVLM could contribute to decreased activation of the RVLM in response to hypotension [5], and thus to blunted baroreflex sympathoexcitation in pregnancy [3]. On the other hand, this observation seems counter to evidence that resting sympathetic tone to the heart [6,7] and vasculature [3,8] is maintained or slightly elevated in pregnant animals, including humans. Thus, although increased tonic GABAergic inhibition of RVLM neurons in the baroreflex pathway likely contributes to attenuated baroreflex sympathoexcitation, it cannot explain the elevated resting sympathetic nerve activity in pregnancy. In short, the central nervous system (CNS) mechanisms responsible for maintaining sympathetic tone to the heart and blood vessels during pregnancy are not known.

The paraventricular nucleus of the hypothalamus (PVN) is an integrative center for autonomic and neuroendocrine regulation. A population of parvocellular neurons in the PVN contributes to activation of sympathetic preganglionic neurons directly, via projections to the intermediolateral cell column of the spinal cord (IML), and indirectly through projections to the RVLM [9,10]. Disinhibition of the PVN with the GABA_A antagonist, bicuculline (Bic), increases arterial pressure and sympathetic nerve activity [11–13], demonstrating a functional role of tonic GABAergic inhibition of the PVN. Prior inhibition of RVLM neurons with the long-acting GABA_A agonist, muscimol, reduces the response to disinhibition of the PVN by approximately 50% [11]. The remaining response to Bic in the PVN after inhibition of the RVLM likely is mediated by the PVN to IML pathway [11,14]. Thus, the PVN is an important source of sympathoexcitatory input to both the IML and RVLM.

The contribution of the PVN to maintenance of resting sympathetic nerve activity in an unchallenged physiological state is controversial. Some studies report that inhibition of neuronal activity in the PVN of male rats produces a modest but significant decrease in arterial pressure and renal sympathetic nerve activity (RSNA), suggesting that the PVN contributes to resting sympathetic tone [12,15,16]. In contrast, other studies have not seen significant changes in blood pressure or sympathetic nerve activity following inhibition of neuronal activity in the PVN [17,18]. However, numerous studies indicate that the PVN can contribute substantially to the increased sympathetic nerve discharge that occurs in response to physiological challenges such as hemorrhage [19] or dehydration [20], and in pathophysiological states including hypertension [21,22], heart failure [23,24] and diabetes [25]. In all of these conditions, decreased GABAergic inhibition within the PVN has been proposed as a contributing factor to the elevated sympathetic outflow. In addition, it is worth noting that circulating A II is often elevated in these states and CNS actions of circulating A II include increased arterial pressure and sympathetic nerve activity [20,26].

A complex interaction among excitatory and inhibitory influences within the PVN determines the final output to the IML and RVLM. Although a number of transmitters and peptides have been identified in the PVN, the major excitatory influences appear to be glutamate and angiotensin II [27,28]. GABA is the predominant inhibitory transmitter in the PVN and sympathoinhibition is mediated primarily through GABA_A receptors [22,28].

The current experiments tested the hypothesis that tonic GABAergic inhibition of the PVN is decreased in pregnant rats. In addition, since circulating A II is increased in pregnancy, and sympathoexcitation due to circulating A II is thought to be mediated by activation of pathways involving both AT₁ and ionotropic excitatory amino acid (EAA) receptors within the PVN [27], possible differences in the role of A II and EAA in the PVN between pregnant and nonpregnant rats were evaluated.

2. Methods

2.1 Animals

Experiments were performed in 20 female Sprague-Dawley rats (Harlan, Indianapolis, IN). They were maintained in individual cages in a temperature- and light-dark cycle-controlled room with free access to water and food. Nonpregnant rats 3 to 5 months of age were monitored for at least two consecutive regular 4-day estrous cycles before they were entered into an experiment. Stage of the cycle was determined by daily vaginal smear cytology. To avoid confounding effects of fluctuating hormone levels, experiments in nonpregnant rats were performed in the late estrus or early diestrus stage of the cycle when circulating levels of ovarian hormones (estrogen and progesterone) are consistent and low [29,30]. Timed pregnant Sprague-Dawley rats were purchased from Harlan. Presence of a vaginal plug in the cage was defined as day 1 of pregnancy and experiments in near-term pregnant rats were performed on the morning of day 21 which is 1.5 – 2 days prior to expected delivery [31,32] and is characterized by elevated estrogen and progesterone levels [31,33]. All surgical procedures and experimental protocols were performed according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee at the University of Missouri-Columbia.

2.2 Surgical Instrumentation

Rats were anesthetized with intraperitoneal inactin (100 mg/kg) supplemented (10 mg/kg, iv) as needed to maintain inhibition of the pedal and palpebral reflexes. Rectal temperature was monitored (telethermometer, Yellow Springs Instruments) and maintained at 38–39°C with a water-circulating heating pad beneath the animal. The trachea was cannulated and catheters were implanted in a femoral artery and vein for measurement of arterial pressure and injection of drugs. A renal sympathetic nerve was isolated retroperitoneally, placed on a bipolar platinum electrode, and sealed with dental impression material (Coltene President polyvinylsiloxane). The nerve signal was monitored on a dual-beam oscilloscope (Hitachi VC-6523) and audio amplifier (Winston Electronics Co). A band-pass filter of 30 Hz–3 KHz was used, and the signal was amplified 5,000–10,000X (RPS 107 amplifier, Grass Instruments, Quincy, MA). Nerve activity was converted from an analog to a digital signal with Powerlab Data Acquisition System (ADInstruments, Pty Ltd.) and stored on a computer hard drive along with arterial blood pressure. The raw nerve activity signal was rectified, integrated (time constant = 28 ms), and smoothed using Powerlab software. Electrical noise was defined as the residual signal 30–60 min after euthanasia with Beuthanasia-D solution (97 mg/kg pentobarbital + 12.5 mg/kg phenytoin sodium, i.v.) and was subtracted from all nerve activity values. Nerve activity was standardized as a percent of the baseline value prior to microinjection of drugs into the PVN. Powerlab software was used to determine MAP and HR as described previously [4].

2.3 Drugs and Solutions

Inactin, bicuculline methiodide, kynurenic acid and angiotensin II were purchased from Sigma Chemical Co., (St. Louis, MO). L158,809 was kindly provided by Merck Research Laboratories (Rahway, NJ). Inactin was dissolved in sterile isotonic saline. All other chemicals were dissolved in phosphate buffered saline. Beuthanasia-D solution was purchased from Schering Plough Animal Health (Canada).

2.4 PVN Microinjections

Following catheter and nerve electrode placement, anesthetized rats were positioned prone in a stereotaxic frame (David Kopf). For PVN exposure, a dorsal skin incision was made,

and the skin blunt dissected. The rat's head was positioned horizontally and a small burr hole, centered at midline and 1.8 mm caudal to bregma, was drilled in the skull to provide visualization and access to the PVN for microinjections.

Triple barrel microinjection pipettes were used for microinjections into the PVN (tip diameter ~ 10 μ m per barrel). Depending on the protocol, each of three individual barrels contained one of the following: angiotensin II (Ang II, 6 mM), the GABA_A receptor antagonist bicuculline (Bic, 4 mM), the AT₁ receptor antagonist L158,809 (AT₁X, 10 mM) or the excitatory amino acid (EAA) receptor antagonist kynurenic acid (Kyn, 72 mM). Pipette barrels were connected with silicone tubing to pressure injection modules of a custom-built micropressure control unit. Volume of microinjections was determined by observing movement of the solution meniscus in the pipette barrel with a 175X microscope supplied with a scaled reticule (Rolyn Optics, Germany). The microinjection pipette was lowered vertically into the left PVN using the following stereotaxic coordinates relative to bregma: caudal = 1.6–1.8 mm, lateral = 0.5 mm (left of midline), and ventral = 7.6–7.8 mm from the dura surface. These coordinates are within the range reported by other investigators for the PVN in male rats [22,34,35].

We defined the PVN as the site that produced an increase in MAP $\geq$ 10 mmHg to microinjection of Bic (4 mM, 100 nl) or an increase in MAP $\geq$ 7 mm Hg in response to Ang II (6 mM, 50 nl) depending on the protocol. In all animals, the PVN was identified within two injection trials.

2.5 Control Experiments in Nonpregnant Rats

Preliminary experiments were performed in 8 nonpregnant rats after the PVN was functionally identified as described above. In three of these rats, responses to injection of Ang II (6 mM, 50 nl) into the PVN were determined. Thirty minutes after the first Ang II injection, L158,809 (10 mM, 100 nl) was microinjected into the PVN, and 20 minutes later responses to microinjection of Ang II were tested again. In five other rats responses to three repeated unilateral microinjections of Bic (4 mM, 100 nl) in the PVN were determined to test for effects of time. To approximate the time course of the experimental protocol in which AT₁ and EAA receptor blockade were performed, the second injection was 70–90 minutes after the first and the third Bic injection was 50 minutes later.

2.6 Experimental Protocol: Effects of Pregnancy, AT₁ Receptor Blockade and Ionotropic Glutamate Receptor Blockade on GABA_A Inhibition of the PVN

In six nonpregnant and six pregnant rats, a triple-barrel glass micropipette, filled with Bic, L158,809, and Kyn in separate barrels was used for microinjections into the PVN. The left PVN was functionally identified based on pressor and sympathoexcitatory responses to Bic (4 mM, 100 nl) (Bic₁). Following a 45- to 50-minute recovery period, L158,809 (10 mM, 100 nl) was microinjected into the PVN and, after 20 minutes, Bic injection was repeated (Bic₂). Following a 45- to 50-minute recovery period, AT₁X supplement (10 mM, 50 nl) and Kyn (72 mM, 100 nl) were microinjected into the PVN, and within 3 minutes responses to Bic were tested a final time (Bic₃).

2.7 Post-experiment Verifications

At the end of all experiments, the injection site was marked by microinjection of Chicago Sky Blue dye (2%, 100 nl). Standard histological methods and comparisons with a rat brain atlas [36] were used to verify that microinjection sites were located within the PVN. In pregnant rats number of fetuses was counted. Three to four fetuses were weighed and an average fetal weight was calculated for each pregnant rat to verify normal pregnancy.

2.8 Data Analysis and Statistical Comparison

In addition to previously described calculations for nerve activity, autospectral analysis was performed. For purposes of analysis of autospectra the recorded nerve signal was full-wave rectified and integrated with a time constant of 10 ms and expressed in volts times seconds. The noise level recorded 30–60 minutes after euthanasia was subtracted from nerve activity values. Fast Fourier transformation was used to convert RSNA from the time domain to the frequency domain (Powerlab software off-line analysis). Autospectra of 15–18 contiguous fields of 5 seconds duration were computed over a frequency band of 0–15 Hz [34,37]. Data of all 15–18 fields were transferred to a Microsoft Excel spreadsheet and then averaged and presented as the relationship of average power (Y axis) to frequency (X axis) using Microsoft Excel. To allow for comparison between animals power was normalized as % maximum.

To evaluate the contribution of AT₁ and EAA inputs to the effects of Bic on the heart rate component of RSNA autospectra, high frequency power density (HF) in the presence of Bic was expressed as a percentage of HF power density prior to Bic (HF after Bic/ HF before Bic X 100) under the three conditions: control (Bic₁), after AT₁ X (Bic₂) and after AT₁X +Kyn (Bic₃). Effects of Bic on low frequency (LF) power density were similarly normalized (LF after Bic/ LF before Bic X 100) and compared among the three conditions.

Student's t-tests were used to compare resting MAP and HR between nonpregnant and pregnant rats. Paired t-tests were used to compare responses to Ang II before and after AT₁ receptor blockade in control experiments. Responses to microinjection of Bic into the PVN before, after AT₁X and after AT₁X+Kyn in nonpregnant and pregnant rats were compared by two-way ANOVA with repeated measures on one factor. Within each group responses to Bic before, after AT₁X and after AT₁X+Kyn were compared by one-way ANOVA for repeated measures. When appropriate a logarithmic transformation was performed prior to statistical analysis in order to obtain a normal distribution of the data. Student-Newman-Keuls post hoc test was performed following ANOVA. P < 0.05 was considered significant. Values are presented as means ± SE.

3. Results

3.1 Control Experiments in Nonpregnant Rats

3.1a. Animals—Preliminary experiments were performed in eight nonpregnant rats (body weight = 236 ± 5.7g) to verify efficacy of AT₁ receptor blockade using L158,809 in the PVN (n= 3) and to determine responses to repeated Bic injections into the PVN (n=5). Following surgical preparation, resting MAP was 96 ± 4.4 mmHg and HR was 297 ± 9.8 beats/min in these rats.

3.1b. AT₁ Receptor Blockade in PVN—In three nonpregnant rats microinjection of A II into the PVN resulted in a significant increase in MAP (+9 ± 1.3 mmHg). Microinjection of L158,809 into the PVN (AT₁X) did not change baseline MAP, HR or RSNA. However, 20 minutes after AT₁X, pressor responses to A II in the PVN were no longer significant (+3 ± 0.5 mmHg). HR and RSNA were not significantly changed by microinjection of A II into the PVN either before or after AT₁X. These experiments verified that the dose of L158,309 used in subsequent protocols blocked responses to AT₁ receptor activation in the PVN.

3.1c. Time Control Experiments: Responses to Repeated Unilateral GABA_A Receptor Blockade in the PVN—In five nonpregnant rats, unilateral microinjection of Bic significantly increased MAP, RSNA and HR. Responses were maximal within approximately 2 minutes and all values returned to control levels within 30 – 45 minutes.

Mean responses are shown in Fig. 1. Responses to Bic were tested three times: Bic₁, Bic₂ (70 – 90 min after Bic₁) and Bic₃ (50 min after Bic₂). Increases in MAP, RSNA and HR were not different among the three Bic injections. Thus, excitatory responses due to disinhibition of the PVN were reproducible for the length of the experimental protocol.

3.2 Experimental Protocol: Effects of Pregnancy on Responses to GABA_A Receptor Blockade in the PVN

3.2a. Animals—Pregnant rats (P, n = 6, 342 ± 8 g) weighed more than nonpregnant rats (NP, n = 6, 249 ± 8.4 g). Pregnant rats had 8 ± 1 fetuses and average fetal weight was 4 ± 0.1 g, consistent with day 21 of pregnancy [38–40]. Similar to previous studies, resting mean arterial pressure (MAP) was lower (P = 83 ± 3.9; NP = 98 ± 2.1 mm Hg) and resting heart rate (HR) was higher (P = 369 ± 9.5; NP = 326 ± 13.4 bpm) in pregnant compared to nonpregnant female rats.

3.2b. Effects of pregnancy, AT₁ receptor blockade and ionotropic EAA receptor blockade on responses to injection of bicuculline in the PVN—Figure 2 contains examples of responses to Bic before and after AT₁ receptor blockade and after subsequent additional blockade of ionotropic glutamate receptors in a representative rat. Note that prior to AT₁ and EAA receptor blockade, Bic produced an increase in RSNA with a prominent bursting pattern. This change in discharge pattern of RSNA due to Bic appeared to be attenuated by L158,809 and Kyn.

Microinjections of AT₁X and subsequent EAA receptor blocker (Kyn) in the PVN did not change resting values in either group. In response to AT₁X: ΔMAP: NP = −4 ± 1.1; P = −3 ± 1.6 mm Hg. ΔHR: NP = −9 ± 3; P = −11 ± 3 beats/min. ΔRSNA: NP = −9 ± 4.2; P = −6 ± 1.9 %. In response to Kyn: ΔMAP: NP = −2 ± 1.9; P = −6 ± 1.7 mm Hg. ΔHR: NP = −4 ± 1.2; P = −3 ± 0.9 beats/min. ΔRSNA: NP = 0 ± 3.3; P = −1 ± 0.5 %.

Responses to Bic in the PVN (Fig. 3) were affected by pregnancy and by the antagonists (main effects of pregnancy and treatment, two-way ANOVA). Pressor, tachycardic and sympathoexcitatory responses to disinhibition of the PVN were attenuated by pregnancy. One-way ANOVA within the NP group revealed that AT₁ receptor blockade attenuated, and the addition of EAA blockade (AT₁X + Kyn) further reduced the pressor response to Bic in the PVN. In pregnant rats, AT₁X reduced the pressor response to Bic in the PVN, but the addition of Kyn had no further effect (Fig. 3A). Changes in HR due to disinhibition of the PVN also were attenuated by pregnancy and the antagonists (Fig. 3B). In NP rats, one-way ANOVA revealed that AT₁X attenuated, and AT₁X + Kyn eliminated, tachycardia to Bic in the PVN. In contrast, the decrease in HR responses to Bic did not reach statistical significance in the P group. Pregnancy suppressed increases in RSNA due to Bic in the PVN, (Fig. 3C). In both NP and P rats, AT₁X reduced sympathoexcitation due to Bic in the PVN, and the addition of Kyn had no further effect.

3.2c. Effects of Bic, AT₁ and EAA receptor blockade on bursting pattern of renal nerve discharge—It is evident in the raw RSNA trace (Fig. 2) that microinjection of Bic into the PVN had profound effects on the pattern of sympathetic discharge. This effect of Bic appeared to be attenuated by AT₁X and eliminated by AT₁X + Kyn. Since total nerve activity does not provide specific information regarding bursting pattern, spectral analysis of RSNA was performed.

Spectral analysis results from a representative rat are provided in Figure 4. Prior to microinjection of Bic (Fig. 4A), maximum power density for RSNA (100%) occurred at the heart rate related frequency (5–7 Hz). During the maximum pressor response to Bic, although a peak was evident at heart rate frequency, the relative power density at low

frequency (1–2 Hz) clearly dominated (Fig. 4B). After recovery of arterial pressure and RSNA to initial levels (30–45 min after Bic), the cardiac-related component in RSNA again predominated (not shown). Figure 4C contains absolute power density before and after Bic in the PVN for the traces shown in Fig. 4A and 4B. It appears that absolute power at heart rate frequency (high frequency peak) was well maintained, and the major effect of Bic was to increase the low frequency power density peak. This pattern of RSNA spectra was evident in both nonpregnant and pregnant rats.

It was possible to perform autospectral analysis on RSNA from 5 NP and 5 P rats. Two-way ANOVA on normalized values before and after Bic (as shown for one rat in Fig. 4A & B) revealed a main effect of Bic to decrease the relative contribution of the high frequency component (NP, 100% to $51 \pm 11.7\%$; P, $89 \pm 8.3\%$ to $34 \pm 8.9\%$) [$F(1,8) = 55.2$, $P < 0.001$] while increasing the relative contribution of the low frequency component (NP, $41 \pm 12.5\%$ to 100%; P, $59 \pm 14.9\%$ to $100 \pm 0.0\%$) [$F(1,8) = 26.2$, $P < 0.001$]. This effect of Bic on the RSNA autospectra was not different between NP and P rats. In order to compare effects of prior AT₁ and EAA receptor blockade on RSNA, autospectral responses to Bic (HF and LF components) were normalized as a percentage of baseline power to indicate the relative effects of Bic in the PVN under the three conditions: control (Bic₁); in the presence of AT₁X (Bic₂) and in the presence of AT₁X + Kyn (Bic₃). A value of 100% would indicate no effect of Bic, while a value less than 100% suggests a relative decrease, and a value greater than 100% suggests a relative increase in power due to Bic. Two-way ANOVA revealed a main effect of the treatments (AT₁ and EAA antagonists) to reverse the effects of Bic on the autospectra (Fig. 5), with no difference between NP and P rats.

3.3 Histological verification of injection site

Estimated locations of microinjection sites in all rats used in these experiments are shown in Figure 6. Bic was injected into the left PVN in all rats. However, for clarity the location of microinjections in the PVN of nonpregnant rats are indicated on the left side of diagrams, and the location of microinjections in pregnant rats are indicated on the right side. All microinjections occurred within 1.6 mm to 2.12 mm caudal to bregma, mainly in the lateral and ventrolateral regions of the PVN (Fig. 6).

4. Discussion

A major finding of the current experiments is that increases in MAP, HR and RSNA due to disinhibition of the PVN with Bic were attenuated in pregnant rats. In addition, prior treatment with the AT₁ receptor antagonist, L158,809, in the PVN attenuated increases in MAP and RSNA due to Bic. In nonpregnant, but not pregnant rats, subsequent blockade of ionotropic glutamate receptors with Kyn in the PVN further decreased MAP and HR responses to Bic. Similar to previous reports [34,37], the bursting pattern of sympathetic nerve activity was altered by microinjection of Bic into the PVN. However, Bic had a similar effect on the pattern of sympathetic discharge in pregnant and nonpregnant rats. Thus, differential effects on the bursting pattern of RSNA cannot account for decreased responses to GABA_A receptor blockade in the PVN in pregnancy. These results are consistent with decreased tonic GABAergic inhibition of the PVN in pregnant rats, which could result in a greater role for the PVN in maintenance of resting efferent sympathetic nerve activity in pregnancy. Lastly, while both AT₁ and EAA receptors contribute to excitatory responses following Bic in the PVN of nonpregnant rats, the relative contribution of AT₁ receptors appears to be greater in pregnant rats.

The PVN is an important site of neuroendocrine and autonomic integration, and can influence sympathetic nerve activity through direct projections to the IML, and through projections to the RVLM which in turn projects to the IML [9,10]. Although a number of

neurotransmitters and peptides and their respective receptors have been identified in the PVN, in regard to regulation of presympathetic neurons within the PVN, GABA has been identified as the predominant inhibitory transmitter, while the major excitatory influences appear to be glutamate and A II [27,28].

Global inhibition of the PVN with the long-acting GABA_A agonist, muscimol, has been reported to result in modest decreases in MAP and sympathetic tone [12,15,16], or to have no effect [17,18]. This could be interpreted to indicate that the contribution of the PVN to resting sympathetic nerve activity is minor. However, it is important to consider that the output of the PVN and the final effect on sympathetic nerve activity is the result of a complex interaction among inhibitory and excitatory inputs, which may be well-balanced in an unchallenged physiological state. Changes in this balance could contribute to both physiological and pathophysiological adaptations. Microinjection of GABA_A [11–13], but not GABA_B [22], receptor antagonists into the PVN results in substantial sympathoexcitation in rats, indicating significant tonic GABA_A receptor mediated inhibition of the PVN. The sympathoexcitation following GABA_A receptor blockade within the PVN is dependent on underlying excitatory influences, including EAA mediated by both NMDA and non-NMDA ionotropic glutamate receptors [16,41] and A II mediated by AT₁, but not AT₂, receptors [35].

Drug concentrations used in the current experiments were based on published literature and preliminary experiments. The concentrations of Bic [22,34,35,37,41], Kyn [17,37,41,42], and L158,809 [35] used are within the range used by others to block GABA_A, ionotropic glutamate, and AT₁ receptors in the PVN of male rats. Sympathoexcitatory responses to Bic in the PVN are eliminated by prior ipsilateral administration of the GABA_A agonist, muscimol, and by midbrain transection [34], which is consistent with disinhibition of presympathetic projection neurons by Bic in the PVN. The concentration of Kyn used has been shown to block responses to microinjection of glutamate in the PVN [41]. In the current experiments, prior treatment with L158,809 blocked the responses to microinjection of A II into the PVN. Thus, drug treatments used in the current study have been shown to specifically block the appropriate receptor populations.

In nonpregnant female rats, excitatory responses to disinhibition of the PVN with the GABA_A antagonist, Bic, were of a similar magnitude as those previously reported in male rats [34,35,37,41]. Also, similar to previous reports in males [35], prior blockade of AT₁ receptors in the PVN attenuated sympathoexcitation due to Bic in the PVN. The remaining response to Bic in the PVN following AT₁X was further reduced by subsequent blockade of ionotropic glutamate receptors with Kyn in nonpregnant female rats, consistent with reports in males suggesting that both A II [35] and glutamate [16,37,41] contribute to excitatory responses due to disinhibition of the PVN. Compared to nonpregnant rats, increases in MAP, HR, and RSNA following Bic in the PVN were attenuated in near term pregnant rats. In addition, AT₁ receptor blockade greatly reduced the responses to Bic in the PVN and the addition of Kyn was without effect in pregnant rats. Thus, although indirect, these data suggest that the relative importance of AT₁ receptors may be greater in pregnant compared to nonpregnant rats.

The decrease in excitatory responses to Bic in the PVN indicates that endogenous GABA in the PVN is less effective in pregnant compared to nonpregnant rats. This difference could be due to a decrease in GABAergic inputs, attenuated release of endogenous GABA, or alterations in GABA_A receptors within the PVN of pregnant rats. Alternately, since responses to disinhibition of the PVN are dependent on underlying baseline excitatory inputs and Kyn had no additional effects on responses to Bic in pregnant rats, it is possible that decreased excitatory transmission within the PVN could account for attenuated responses to

Bic in pregnancy. Although data from the current experiments do not distinguish between these possibilities, evidence from other studies is supportive of decreased inhibition rather than decreased excitation of the PVN in pregnant animals. In the PVN, the neuromodulator nitric oxide contributes to tonic inhibition of presympathetic neurons predominantly through a presynaptic action to facilitate release of GABA [43]. In other conditions in which resting sympathetic nerve activity is increased, decreased nitric oxide and the resultant decrease in GABAergic inhibition of presympathetic neurons in the PVN likely contributes to elevated sympathetic tone [23,25]. Recently our laboratory demonstrated that activity and expression of neuronal nitric oxide synthase (nNOS) was decreased in the PVN of near term pregnant compared to nonpregnant rats [40]. Decreased nNOS would be expected to decrease NO production, resulting in decreased GABA release and thus to decreased tonic GABAergic inhibition in the PVN of pregnant rats. Thus, the current observation of attenuated responses to inhibition of GABA_A receptors in the PVN in pregnant rats is consistent with the previously observed decrease in nNOS in the PVN.

In addition, preliminary experiments from our laboratory indicate that sympathoexcitatory responses mediated through the subfornical organ require the PVN and are augmented in pregnant compared to nonpregnant rats [44], which is also consistent with decreased tonic inhibition of the PVN in pregnancy. Functionally, sympathetic innervation of the kidney is required for the decreased diuresis and natriuresis seen in late pregnant rats, suggesting that resting RSNA is augmented in pregnancy [45]. Taken together, these data favor a decrease in tonic inhibitory influences, rather than a decrease in tonic excitatory influences, to explain attenuated responses to Bic in the PVN of pregnant rats. In addition to increasing MAP, HR, and RSNA, similar to previous reports in male rats [34,37], we observed that the bursting pattern of RSNA was substantially changed by Bic in the PVN of both pregnant and nonpregnant female rats. Since the change in bursting pattern of sympathetic nerve activity due to Bic has been reported to increase vasoconstrictor effectiveness of sympathetic outflow [37], we considered the possibility that differences in effects of Bic on MAP between nonpregnant and pregnant rats could be due to differential effects on RSNA frequency components. However, changes in the autospectra of RSNA due to Bic in the PVN were similar between nonpregnant and pregnant rats and therefore cannot account for the attenuated pressor and sympathoexcitatory responses to Bic seen in pregnant animals. Previous studies indicated that ionotropic glutamate receptor blockade prevented the effects of Bic on the bursting pattern of sympathetic nerve activity [34,37]. Interestingly, our data suggest that AT₁ receptor blockade in the PVN also blunts the change in RSNA autospectra due to Bic.

Similar to results in male rats, microinjection of either Kyn [17,37,41,42] or L158,809 [35] alone into the PVN had no effect on resting MAP, HR, or RSNA in either pregnant or nonpregnant female rats. However, sympathoexcitatory effects following disinhibition of the PVN with Bic were clearly dependent on angiotensin and glutamate. A complex interaction among excitatory and inhibitory influences on presympathetic neurons in the PVN determines the resting membrane potential, and thus the excitability of PVN output neurons. It is likely that with all inputs intact, GABAergic inhibition predominates and the depolarizing influence due to activation of ionotropic glutamate and AT₁ receptors is not sufficient to produce action potentials. However, when GABAergic inhibition is removed, the influence of these excitatory inputs becomes evident. Therefore, although blockade of EAA and AT₁ receptors alone has no effect, these inputs are critical for excitatory responses in the PVN.

The mechanism responsible for decreased GABAergic inhibition of the PVN in pregnant animals is not known. In normal pregnancy plasma levels of A II are elevated [2,46]. Accordingly, we and others have considered that elevated circulating A II might contribute

to increased sympathetic nerve activity in pregnancy. Consistent with this idea, the pressor response to intracerebroventricular (icv) A II is augmented in pregnant rats [47] and blockade of angiotensin AT₁ receptors with losartan administered intravenously (iv) [48] or icv [49] decreased arterial blood pressure in pregnant, but not nonpregnant rabbits. In addition, our laboratory has demonstrated that with or without intact arterial baroreflexes, forebrain directed (intracarotid artery) L158,809, the non-peptide AT₁ antagonist used in the current study, resulted in greater depressor responses in pregnant compared to nonpregnant rats [50]. Although the experiments described above suggest that an enhanced CNS sympathoexcitatory action of A II is likely in pregnancy, the exact site of this action has not been determined. Unlike A II, which is a peptide that does not cross the blood brain barrier, the nonpeptide AT₁ receptor antagonists losartan and L158-809 readily cross the blood brain barrier, and therefore have access not only to circumventricular organs (CVOs), but also to AT₁ receptors within the brain parenchyma [51,52]. Thus, the CNS site of action following iv [48], icv [49], and intracarotid artery [50] administration of AT₁ blockers is not clear.

Blood borne A II exerts CNS effects through CVOs which are devoid of a blood-brain barrier, such as the forebrain subfornical organ (SFO). In the forebrain, circulating A II activates AT₁ receptors located on SFO neurons that project to the PVN [26,27]. Activation of the SFO to PVN pathway has been shown to increase release of A II within the PVN [53] and to excite spinally-projecting presympathetic neurons in the PVN [54,55]. PVN neurons which project to the IML and RVLM, and are therefore likely involved in sympathoexcitation, are activated by both A II and glutamate [17,35,56,57]. Water deprivation, which elevates circulating A II and increases sympathetic nerve activity, increases AT₁ and EAA excitation of the PVN [17]. Importantly chronic iv infusion of A II results in decreased GABAergic inhibition [58] and sustained activation of the PVN [59]. Taken together these data suggest that the SFO to PVN pathway is a likely link between peripheral and central A II systems and is subject to modulation by changes in circulating A II. Thus, it is possible that elevated levels of plasma A II in pregnant animals could result in increased sympathetic outflow by mechanisms involving the PVN, similar to those discussed above. Another consideration is that estrogen levels are elevated in pregnancy [33,60] and presympathetic neurons in the PVN are rich in the beta subtype of estrogen receptor (ER β) [61,62]. Therefore ER β receptor mechanisms in the PVN of pregnant rats could participate in adaptations resulting in potentiated sympathoexcitation in pregnant rats.

In summary, although many factors may contribute to elevated sympathetic nerve activity in pregnancy, there is a substantial body of evidence supportive of a CNS role of circulating angiotensin II. The current experiments demonstrate attenuated endogenous GABAergic inhibition of the PVN in near term pregnant rats. Presympathetic neurons in the PVN can affect sympathetic nerve activity indirectly through projections to the RVLM or directly through projections to the IML. Similar to interactions within the PVN, the final output of preganglionic sympathetic neurons in the IML is dependent on a balance among various excitatory and inhibitory inputs [20,28]. Since we previously demonstrated increased inhibition of the RVLM in pregnancy [4], we propose that the balance between the two pathways may be shifted in pregnancy, such that the direct projections from the PVN to the IML are of increased relative importance and contribute to maintenance of sympathetic outflow in pregnant compared to nonpregnant animals.

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References

1. Lindheimer, MD.; Katz, AI. Renal physiology and disease in pregnancy. In: Seldin, DW.; Giebisch, G., editors. *The Kidney: Physiology and Pathophysiology*. New York: Raven Press; 1992. p. 3371-432.
2. Hanssens M, Keirse M, Spitz B, Van Assche FA. Angiotensin II levels in hypertensive and normotensive pregnancies. *Br J Obstet Gynaecol*. 1991; 98:155–61. [PubMed: 2004051]
3. Masilamani SME, Heesch CM. Effects of pregnancy and progesterone metabolites on baroreflex control of sympathetic outflow and heart rate in conscious rats. *Am J Physiol Regul Integr Comp Physiol*. 1997; 272:R924–R934.
4. Kvochina L, Hasser EM, Heesch CM. Pregnancy increases baroreflex independent GABAergic inhibition of the RVLM in rats. *Am J Physiol Regul Integr Comp Physiol*. 2007; 293:R2295–R2305. [PubMed: 17898121]
5. Curtis KS, Cunningham JT, Heesch CM. Fos expression in brain stem nuclei of pregnant rats after hydralazine-induced hypotension. *Am J Physiol Regul Integr Comp Physiol*. 1999; 277:R532–R540.
6. Cohen WR, Galen LH, Vega-Rich M, Young JB. Cardiac sympathetic activity during rat pregnancy. *Metabolism*. 1988; 37:771–7. [PubMed: 3405094]
7. Lucini D, Strappazon P, Vecchia LD, Maggioni C, Pagani M. Cardiac autonomic adjustments to normal human pregnancy: insight from spectral analysis of R-R interval and systolic arterial pressure variability. *J Hypertens*. 1999; 17:1899–904. [PubMed: 10703887]
8. Greenwood JP, Scott EM, Stoker JB, Walker JJ, Mary DASG. Sympathetic neural mechanisms in normal and hypertensive pregnancy in humans. *Circulation*. 2001; 104:2200–4. [PubMed: 11684631]
9. Badoer E. Hypothalamic paraventricular nucleus and cardiovascular regulation. *Clin Exp Pharmacol Physiol*. 2001; 28:95–9. [PubMed: 11153547]
10. Swanson LW, Sawchenko PE. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Annu Rev Neurosci*. 1983; 6:269–325. [PubMed: 6132586]
11. Tagawa T, Dampney RA. AT₁ Receptors Mediate Excitatory Inputs to Rostral Ventrolateral Medulla Pressor Neurons From Hypothalamus. *Hypertension*. 1999; 34:1301–7. [PubMed: 10601134]
12. Zhang K, Patel KP. Effect of nitric oxide within the paraventricular nucleus on renal sympathetic nerve discharge: role of GABA. *Am J Physiol Regul Integr Comp Physiol*. 1998; 275:R728–R734.
13. Martin DS, Segura T, Haywood JR. Cardiovascular responses to bicuculline in the paraventricular nucleus of the rat. *Hypertension*. 1991; 18:48–55. [PubMed: 1860711]
14. Kiely JM, Gordon FJ. Role of rostral ventrolateral medulla in centrally mediated pressor responses. *Am J Physiol Heart Circ Physiol*. 1994; 267:H1549–H1556.
15. Dampney RAL, Tagawa T, Fontes MAP. Evidence for a tonic glutamergic input from the hypothalamic paraventricular nucleus to rostral VLM pressor neurons (Abstract). *FASEB J*. 2000; 14:A623.
16. Li YF, Jackson KL, Stern JE, Rabeler B, Patel KP. Interaction between glutamate and GABA systems in the integration of sympathetic outflow by the paraventricular nucleus of the hypothalamus. *Am J Physiol Heart Circ Physiol*. 2006; 291:2847–56.
17. Freeman KL, Brooks VL. AT₁ and glutamatergic receptors in paraventricular nucleus support blood pressure during water deprivation. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 2007; 292:1675–82.
18. Stocker SD, Keith KJ, Toney GM. Acute inhibition of the hypothalamic paraventricular nucleus decreases renal sympathetic nerve activity and arterial blood pressure in water-deprived rats. *Am J Physiol Regul Integr Comp Physiol*. 2004; 286:R719–R725. [PubMed: 14670808]
19. Yang Z, Coote JH. Paraventricular nucleus influence on renal sympathetic activity in vasopressin gene-deleted rats. *Exp Physiol*. 2007; 92(1):109–17. [PubMed: 17012145]
20. Stocker SD, Osborn JL, Carmichael SP. Forebrain osmotic regulation of the sympathetic nervous system. *Clin Exp Pharmacol Physiol*. 2008; 35:695–700. [PubMed: 18067592]

21. Martin DS, Haywood JR. Reduced GABA inhibition of sympathetic function in renal-wrapped hypertensive rats. *Am J Physiol Regul Integr Comp Physiol.* 1998; 44:R1523–R1529.
22. Li DP, Pan HL. Role of γ -Aminobutyric Acid GABA_A and GABA_B Receptors in Paraventricular Nucleus in Control of Sympathetic Vasomotor Tone in Hypertension. *J. Pharmacol. Exp. Ther.* 2007; 320:615–26.
23. Zhang K, Li YF, Patel KP. Blunted nitric oxide-mediated inhibition of renal nerve discharge within PVN of rats with heart failure. *Am J Physiol Heart Circ Physiol.* 2001; 281:H995–H1004. [PubMed: 11514264]
24. Zhang K, Li YF, Patel KP. Reduced endogenous GABA-mediated inhibition in the PVN on renal nerve discharge in rats with heart failure. *Am J Physiol Regul Integr Comp Physiol.* 2002; 282:R1006–R1015. [PubMed: 11893604]
25. Zheng H, Mayhan WG, Bidasee KR, Patel KP. Blunted nitric oxide-mediated inhibition of sympathetic nerve activity within the paraventricular nucleus in diabetic rats. *Am J Physiol Regul Integr Comp Physiol.* 2006; 290:R992–R1002. [PubMed: 16322352]
26. McKinley, MJ.; McAllen, RM.; Davern, P.; Giles, ME.; Penschow, J.; Sunn, N.; Ushigome, A.; Oldfield, BJ. The sensory circumventricular organs of the mammalian brain. New York: Springer; 2003.
27. Ferguson AV, Washburn DLS, Latchford KJ. Hormonal and neurotransmitter roles for angiotensin in the regulation of central autonomic function. *Exp Biol Med.* 2001; 226:85–96.
28. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci.* 2006; 7:335–46. [PubMed: 16760914]
29. Haim S, Shakhar B, Rossene E, Taylor AN, Ben-Eliyahu S. Serum levels of sex hormones and corticosterone throughout 4- and 5-day estrous cycles in Fischer 344 rats and their simulation in ovariectomized females. *J Endocrinol Invest.* 2003; 26:1013–22. [PubMed: 14759076]
30. Butcher RL, Collins WE, Fugo NW. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17 β throughout the 4-day estrous cycle of the rat. *Endocrin.* 1974; 94:1704–8.
31. Kobayashi M, Akahane M, Minami K, Moro M, Ajisawa Y, Inoue Y, Kawarabayashi T. Role of oxytocin in the initiation of term and preterm labor in rats: Changes in oxytocin receptor density and plasma oxytocin concentration and the effect of an oxytocin antagonist, L-366,509. *Am. J. Obstet. Gynecol.* 1999; 180:621–7.
32. Francis K, Meddle SL, Bishop VR, Russell JA. Progesterone receptor expression in the pregnant and parturient rat hypothalamus and brainstem. *Brain Res.* 2002; 927:18–26. [PubMed: 11814428]
33. Bridges RS. A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat. *Endocrin.* 1984; 114:930–40.
34. Kenney MJ, Weiss ML, Patel KP, Wang Y, Fels RJ. Paraventricular nucleus bicuculline alters frequency components of sympathetic nerve discharge bursts. *Am J Physiol Heart Circ Physiol.* 2001; 281:H1233–H1241. [PubMed: 11514292]
35. Chen QH, Toney GM. Responses to GABA-A receptor blockade in the hypothalamic PVN are attenuated by local AT₁ receptor antagonism. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2003; 285:R1231–R1239.
36. Paxinos, G.; Watson, C. The Rat Brain in Stereotaxic Coordinates: Compact. 3. New York: Academic Press; 1997.
37. Kenney MJ, Weiss ML, Mendes T, Wang Y, Fels RJ. Role of paraventricular nucleus in regulation of sympathetic nerve frequency components. *Am J Physiol Heart Circ Physiol.* 2003; 284:H1710–H1720. [PubMed: 12521941]
38. Ericsson A, Saljo K, Sjostrand E, Jansson N, Prasad PD, Powell TL, Jansson T. Brief hyperglycemia in the early pregnant rat increases fetal weight at term by stimulating placental growth and affecting placental nutrient transport. *J Physiol.* 2007; 581:1323–32. [PubMed: 17430988]
39. Foley CM, Stanton JJ, Price EM, Cunningham JT, Hasser EM, Heesch CM. GABA_A α_1 and α_2 receptor subunit expression in rostral ventrolateral medulla in nonpregnant and pregnant rats. *Brain Res.* 2003; 975:196–206. [PubMed: 12763608]

40. Heesch CM, Zheng H, Foley CM, Mueller PJ, Hasser EM, Patel KP. Nitric oxide synthase activity and expression is decreased in the paraventricular nucleus of pregnant rats. *Brain Res.* 2009; 1251:140–50. [PubMed: 19041855]
41. Chen QH, Haywood JR, Toney GM. Sympathoexcitation by PVN-injected bicuculline requires activation of excitatory amino acid receptors. *Hypertension.* 2003; 42:725–31. [PubMed: 12900439]
42. Li DP, Pan HL. Glutamatergic inputs in the hypothalamic paraventricular nucleus maintain sympathetic vasomotor tone in hypertension. *Hypertension.* 2007; 49:916–25. [PubMed: 17309953]
43. Stern JE. Nitric oxide and homeostatic control: an intercellular signalling molecule contributing to autonomic and neuroendocrine integration? *Prog. Biophys Mol Bio.* 2004; 84(2–3):197–215.
44. Kvochina L, Phaup G, Hasser EM, Heesch CM. AT-1 receptors in the paraventricular nucleus (PVN) are required for sympathoexcitation due to angiotensin II in the subfornical organ of nonpregnant and pregnant rats (Abstract). *FASEB J.* 2009; 23 In Press.
45. Patel KP, Zhang PL. Role of renal nerves in renal responses to acute volume expansion during pregnancy in rats. *Proc Soc Exp Biol Med.* 1993:203.
46. Brooks VL, Quesnell RR, Kane CM, Keil LC. Hemodynamic and hormonal responses to hemorrhage in conscious rabbits at mid- and late gestation. *Am J Physiol Regul Integr Comp Physiol.* 1998; 275:R1082–R1090.
47. Hines T, Porter J. Role for central angiotensin II in control of blood pressure during pregnancy. *Am J Physiol Regul Integr Comp Physiol.* 1989; 257:R1457–R1461.
48. Brooks VL, Welch LS, Kane CM. Role of angiotensin II in altered baroreflex function of conscious rabbits during late pregnancy. *Am J Obstet Gynecol.* 2001; 184:476–82. [PubMed: 11228506]
49. O'Hagan K, Skogg KA, Stevenson J. AT₁ receptor block does not affect arterial baroreflex during pregnancy in rabbits. *Am J Physiol Heart Circ Physiol.* 2001; 280:H1996–H2005. [PubMed: 11299199]
50. Heesch CM, Phaup G, Kvochina L, Hasser EM, Foley CM. Arterial baroreceptor inputs are important for enhanced role of circulating angiotensin II in maintenance of arterial pressure in pregnancy (Abstract). *FASEB J.* 2006; 20:A774.
51. Li Z, Bains JS, Ferguson AV. Functional evidence that the angiotensin antagonist losartan crosses the blood-brain barrier in the rat. *Brain Res Bull.* 1993; 30:33–9. [PubMed: 8420632]
52. Polidori C, Ciccocioppo R, Pompei P, Cirillo R, Massi M. Functional evidence for the ability of angiotensin AT₁ receptor antagonists to cross the blood-brain barrier in rats. *Eur J Pharmacol.* 1996; 307:259–67. [PubMed: 8836613]
53. Wright JW, Roberts KA, Stubley LA, Hanesworth JM, Harding JW. Hypothalamic angiotensin release in response to AII or glutamic acid stimulation of the SFO in rats. *Brain Res Bull.* 1993; 31:649–54. [PubMed: 8100178]
54. Bains JS, Ferguson AV. Paraventricular nucleus neurons projecting to the spinal cord receive excitatory input from the subfornical organ. *Am J Physiol Integr Comp Physiol.* 1995; 268:R625–R633.
55. Li DP, Chen SR, Pan HL. Angiotensin II stimulates spinally projecting paraventricular neurons through presynaptic disinhibition. *J Neurosci.* 2003; 23:5041–9. [PubMed: 12832527]
56. Ferguson AV, Washburn DLS. Angiotensin II: A Peptidergic Neurotransmitter in Central Autonomic Pathways. *Prog Neurobiol.* 1998; 54:169–92. [PubMed: 9481797]
57. Cato MJ, Toney GM. Angiotensin II excites paraventricular nucleus neurons that innervate the rostral ventrolateral medulla: an in vitro patch-clamp study in brain slices. *J Neurophysiol.* 2005; 93:403–413. [PubMed: 15356186]
58. LaGrange LP, Toney GM, Bishop VS. Chronic angiotensin II infusion attenuates the renal sympathoinhibitory response to acute volume expansion. *Am J Physiol Regul Integr Comp Physiol.* 2003; 284:R1098–R1107. [PubMed: 12626370]
59. Davern PJ, Head GA. Fos-Related Antigen Immunoreactivity After Acute and Chronic Angiotensin II-Induced Hypertension in the Rabbit Brain. *Hypertension.* 2007; 49:1170–7. [PubMed: 17339536]

60. Garland HO, Atherton JC, Baylis C, Morgan MRA, Milne CM. Hormone profiles for progesterone, oestradiol, prolactin, plasma renin activity, aldosterone and corticosterone during pregnancy and pseudopregnancy in two strains of rat: correlation with renal studies. *J Endocrinol.* 1987; 113:435–44. [PubMed: 3305759]
61. Bingham B. Androgen and estrogen receptor-beta distribution within spinal-projecting and neurosecretory neurons in the paraventricular nucleus of the male rat. *J Comp Neurol.* 2006; 499:911. [PubMed: 17072840]
62. Stern JE, Zhang W. Preautonomic neurons in the paraventricular nucleus of the hypothalamus contain estrogen receptor β . *Brain Res.* 2003; 975:99–109. [PubMed: 12763597]

Figure 1.

Responses to repeated GABA_A receptor blockade in the PVN. Unilateral microinjection of bicuculline (Bic₁) into the PVN of 5 nonpregnant rats significantly increased mean arterial pressure (**1A**, MAP), heart rate (**1B**, HR) and renal sympathetic nerve activity (**1C**, RSNA). All values returned to resting levels within approximately 45 minutes (not shown). Seventy – 90 minutes after Bic₁, Bic was microinjected again into the PVN (Bic₂), and 45 – 50 minutes later microinjection of Bic was repeated (Bic₃). Responses were not different among the three Bic injections.

Figure 2.

Analog recording from a representative rat. It appears that the increase in arterial pressure (AP) and HR due to Bic in the PVN was attenuated by AT₁ receptor blockade (AT₁X) and eliminated by the combination of AT₁ and EAA receptor blockade (AT₁X + Kyn). The raw RSNA trace demonstrates effects of Bic in the PVN on the pattern of sympathetic discharge, which appears to be attenuated by AT₁X and Kyn.

Figure 3.

GABAergic inhibition of the PVN is decreased in pregnant (P) rats. Two-way ANOVA revealed main effects of pregnancy on MAP [$F(1,20)=5.3$, $P<0.04$], HR [$F(1,20)=5.1$, $P<0.05$] and RSNA [$F(1,20)=6.0$, $P<0.03$] responses to Bic in the PVN. There were also main effects of the treatments on MAP [$F(2,20)=28$, $P<0.001$], HR [$F(2,20)=9.8$, $P<0.001$], and RSNA [$F(2,20)=12.7$, $P<0.001$] responses to Bic. **3A.** The pressor response to Bic was attenuated in pregnant rats (†). In nonpregnant rats AT_1 receptor blockade attenuated, and the addition of EAA blockade eliminated, the effects of Bic in the PVN. In pregnant rats, AT_1X reduced the pressor effects of Bic in the PVN, while Kyn had no additional effect on the response to Bic. **3B.** HR responses were attenuated by pregnancy (†). The decrease in tachycardia due to the antagonists reached statistical significance in the nonpregnant group only. AT_1X attenuated and $AT_1X + Kyn$ eliminated effects of Bic. **3C.**

Increases in RSNA due to Bic were less in pregnant rats ($\dagger$), and AT₁X attenuated Bic-induced sympathoexcitation in both nonpregnant and pregnant rats. The addition of Kyn had no further effect.

[Bic₁= control; Bic₂= in presence of AT₁X; Bic₃= in presence of AT₁X + Kyn; * different from Bic₁ within group; **different from Bic₁ & Bic₂ within group]

Figure 4.

Individual autospectra from one representative experiment. Power density of the signal (y-axis) is expressed as a function of frequency (x-axis) before (panel A) and after (panel B) microinjection of Bic in the PVN. Autospectra in 4A & B were normalized to maximum power during the recording period (100%). The high frequency peak corresponded to the rat's HR. Absolute power density values before (solid line) and after (dotted line) Bic in this same animal are indicated in 4C.

Figure 5.

Contribution of AT₁ and EAA receptors to autospectra changes due to Bic in the PVN. In the absence of AT₁ and EAA receptor blockade, Bic in the PVN decreased the relative contribution of high frequency power (HF) and increased the relative contribution of low frequency power (LF) to the autospectra of RSNA (see text). This is represented by a HF component less than 100% of baseline and a LF component greater than 100% of baseline in both nonpregnant and pregnant rats (Bic₁, Fig. 5A & B). Two-way ANOVA revealed no effect of pregnancy on relative contribution of HF and LF components, but a main effect of treatment (AT₁X and AT₁X + Kyn) on both the HF (5A) [$F(2,16) = 13.8$, $P < 0.001$] and LF components (5B) [$F(2,16) = 5.0$, $P < 0.02$] of responses to Bic. [\$ = Main effect of treatment]

Figure 6.

PVN microinjection sites. Approximate injection sites in PVN are indicated on adapted diagrams from a rat brain atlas [39]. All microinjections were made into the left PVN, but for clarity injection sites for nonpregnant rats (NP) are shown on the left and injection sites for pregnant rats (P) are on the right. Open squares = NP Ang II in control experiments; open triangles = NP, Bic time controls; open circles = NP, Bic + antagonists; closed circles = P, Bic + antagonists.