11β-Hydroxysteroid Dehydrogenase Type 2 Activity in Hypothalamic Paraventricular Nucleus Modulates Sympathetic Excitation

Zhi-Hua Zhang, Yu-Ming Kang, Yang Yu, Shun-Guang Wei, Thomas J. Schmidt, Alan Kim Johnson, Robert B. Felder

Abstract—Aldosterone stimulates the sympathetic nervous system by binding to a select population of brain mineralocorticoid receptors (MR). These MR have an equal affinity for corticosterone that is present in substantially higher concentrations, but are held in reserve for aldosterone by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2), which converts corticosterone to an inactive metabolite. Thus, colocalization of MR and 11β-HSD-2 activity may help identify brain regions that mediate the effects of aldosterone. The present studies tested the hypothesis that 11β-HSD-2 activity regulates MR-mediated responses in the paraventricular nucleus (PVN) of the hypothalamus, a forebrain region implicated in sympathetic regulation. Real-time–polymerase chain reaction revealed the presence of 11β-HSD-2 mRNA in PVN. In anesthetized adult male Sprague-Dawley rats, microinjection of the 11β-HSD-2 inhibitor carbenoxolone (CBX) into PVN increased mean arterial pressure, heart rate, and renal sympathetic nerve activity. Intracerebroventricular injections of CBX excited PVN neurons and increased mean arterial pressure, heart rate, and renal sympathetic nerve activity. The ability of CBX to increase sympathetic activity by inhibiting 11β-HSD-2, thereby permitting corticosterone to activate MR, was confirmed by the following: Intracerebroventricular glycyrrhizic acid, another 11β-HSD-2 inhibitor, mimicked the sympathoexcitatory effects of CBX; the sympathoexcitatory effects of CBX were blocked by spironolactone, a MR antagonist. Neither CBX nor glycyrrhizic acid elicited a response in adrenalectomized rats. These findings suggest that MR in PVN contribute to sympathetic regulation and may be activated by aldosterone or corticosterone (or cortisol in humans) depending on the state of 11β-HSD-2 activity. (Hypertension. 2006;48:127-133.)

Key Words: aldosterone • corticosterone • brain

Selective antagonism of mineralocorticoid receptors (MR) significantly reduces morbidity and mortality in patients with established heart failure1 or with reduced left ventricular function following acute myocardial infarction.2 The mechanisms for these beneficial effects of MR antagonists have not been fully elucidated. Recent studies in rats with ischemia-induced heart failure suggest that blocking MR in the brain may play an important role. Thus, intracerebroventricular (ICV) administration of the MR antagonist spironolactone (SL) reduces renal sympathetic nerve activity (RSNA),3,4 intracerebroventricular injections of CBX excited PVN neurons and increased mean arterial pressure, heart rate, and renal sympathetic nerve activity. The central nervous system sites of action of aldosterone and MR antagonists are still poorly defined. MR are widely distributed in the brain6 and are largely occupied by corticosterone,7 which is present in higher concentrations than aldosterone8 and binds to MR with equal affinity.7 The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2), which has a more restricted distribution in the brain,9–11 rapidly converts corticosterone to an inactive metabolite, increasing the likelihood of aldosterone binding to MR. The coexpression of 11β-HSD-2 with MR may therefore identify brain regions that are particularly sensitive to aldosterone. Consistent with that hypothesis, a recent study found that the abundance of 11β-HSD-2 protein in the nucleus tractus solitarius,11 a cardiovascular afferent integrative center in the hindbrain, correlates closely with the nuclear translocation of MR in response to systemically administered aldosterone.12

The paraventricular nucleus (PVN) of the hypothalamus is a well recognized cardiovascular integrative center of the forebrain that contributes to the augmented sympathetic drive in heart failure. PVN neurons express MR,13 but 11β-HSD-2 activity has not previously been described in this region of the brain. Using sensitive molecular and physiological tech-
niques, we tested the hypothesis that the PVN might also be a target for the central actions of aldosterone.

Materials and Methods

General

Experiments were performed on 59 adult male Sprague-Dawley rats (300 to 350 g). Ten rats had been adrenalectomized (ADX) by Harlan (Indianapolis, IN) and were shipped to us 7 days after surgery. The ADX rats were allowed 3 to 5 days to adapt to their new environment before study. Animals were housed in The University of Iowa Animal Care Facility where they were exposed to a normal 12:12-hour light-dark cycle and were provided with tap water and rat chow ad libitum. ADX rats were also provided with 0.9% NaCl. Studies were performed in accordance with the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society. The experimental procedures were approved by the University of Iowa Institutional Animal Care and Use Committee.

Drugs Administered

Glycyrrhizic acid (GA) and carbenoxolone (CBX) are both potent 11β-HSD-2 inhibitors, but with important differences. CBX has the potential to block gap junctions; GA does not, and has been used to control for the gap junction blocking effect of CBX. From a practical standpoint, GA is stickier than CBX and so less amenable to microinjection via micropipettes.

CBX, GA, hexamethonium bromide, and SL were purchased from Sigma (St. Louis, Mo). CBX, GA, and SL were initially dissolved in absolute ethanol and then diluted with artificial cerebrospinal fluid to a desired concentration, with the final ethanol concentration <1%. The same volume of the vehicle (VEH) <1% ethanol in artificial cerebrospinal fluid) was administered as a control for the ICV injections and tissue microinjections. Hexamethonium was dissolved in saline for intravenous injection. The doses of CBX and GA used in this study were derived from previous related studies and optimized in preliminary experiments.

Real-Time Polymerase Chain Reaction

Real-time polymerase chain reaction (PCR) was performed to determine whether messenger RNA for 11β-HSD-2 is expressed in PVN. Eight rats were euthanized with an overdose of urethane. The brains were removed and cut into 450-μm coronal sections. A punch biopsy was obtained from right and left PVN and from right and left cortex (in the same section) using a 15-gauge needle stub (ID 1.5 mm), yielding 600 to 800 mg of each tissue. Total RNA was isolated from PVN and cortex using TRI reagent (Molecular Research Center Inc, Cincinnati, Ohio). cDNAs were synthesized using TaqMan reverse transcription reagents (Applied Biosystems). PCR primers and TaqMan probes for 11β-HSD-2 were designed using the computer program Primer Express (Applied Biosystems). The sequences of primers and probe for 11β-HSD-2 were the following: forward primer, 5'-TGGAACCTCCAAGGCAGCTA-3'; reverse primer, 5'-TGATACCCCAGGGAAGCAGTT-3'; probe, 5'-TGCACTGCTCATGGACACATTCAGCTG-3'. Real-time PCR was performed using a previously described method. To obtain a calibration curve, we amplified the amount of plasmid (PCR2.1-TOPO vector; Invitrogen) encompassing rat 11β-HSD-2 cDNA. The standard curve for 11β-HSD-2 was generated using serially diluted solutions (10^5, 10^6, 10^7, 10^8, 10^9, and 10^10 copies) of plasmid. The target message in unknown samples was

Figure 1. Comparison of 11β-HSD-2 mRNA expression (real-time PCR) in the PVN of hypothalamus vs brain cortex. Values are expressed as mean±SEM; n=number of rats; *P<0.05 vs cortex.

Figure 2. A, Representative tracing illustrating the effects on HR, RSNA, and AP of microinjecting the 11β-HSD-2 inhibitor CBX bilaterally into PVN. RSNA insets: fast time base tracings of baseline and peak RSNA, illustrating the bursting quality of discharge (time bar 1 s). B, Grouped data showing the changes from baseline (Δ) in HR, RSNA, and MAP elicited by bilateral PVN microinjections of CBX vs VEH. Arrows indicate time of PVN microinjection; n=number of injections; *P<0.05.
quantified by measuring the threshold cycle (Ct) and by using a calibration curve to determine the starting target message quantity. Quantification of 11βHSD-2 mRNA was expressed as copy number per nanogram of total RNA.

**Electrophysiology/Microinjection**

The general methods have been described previously. Forty-two rats were anesthetized with urethane (1.5 g/kg IP). Supplemental urethane (0.1 to 0.3 g/kg IP or IV) was administered if spontaneous increases in arterial pressure (AP), heart rate (HR), or respiratory rate, or responses to a noxious stimulus, were observed during surgery or experimental recording. Recording sessions began at least an hour after completion of the surgical preparation.

- To test the hypothesis that 11β-HSD-2 activity in PVN helps regulate sympathoexcitation, AP, HR, and RSNA were monitored during bilateral microinjections (100 nL over 10 s) of CBX or VEH directly into the PVN (7 rats), or of CBX into surrounding tissue (4 rats). Stereotaxic coordinates for PVN injections (CBX and VEH in 5 rats; CBX only in 2 rats) were 1.8 mm caudal to bregma; 0.4 mm lateral to midline; 7.6 mm ventral to the brain surface. CBX injections into surrounding tissue were made at positions 0.5 to 1.0 mm rostral, lateral, dorsal, and caudal to this site (4 bilateral injections/rat, 45 to 60 minutes between injections).
- To confirm that the sympathoexcitatory effects of CBX are mediated by MR, the AP, HR, RSNA, and PVN neuronal activity were monitored during ICV injections (2 µL over 10 s) of CBX or VEH. Some rats receiving ICV CBX were pretreated with the MR antagonist SL, administered ICV 10 minutes before CBX. Stereotaxic coordinates for PVN recording were 1.6 to 2.1 mm posterior to bregma, 0.3 to 0.5 mm from midline, and 7.0 to 8.0 mm ventral to dura.

**Baseline Values for Microinjection Studies**

<table>
<thead>
<tr>
<th>Study Protocol</th>
<th>MAP (mm Hg)</th>
<th>HR (bpm)</th>
<th>RSNA (mv)</th>
<th>PVN Firing (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBX (ICV, n=8)</td>
<td>89.3±3.9</td>
<td>325.4±9.5</td>
<td>9.1±0.7</td>
<td>2.1±0.3</td>
</tr>
<tr>
<td>GA (ICV, n=6)</td>
<td>87.3±5.0</td>
<td>333.7±13.3</td>
<td>9.8±1.3</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>SL + CBX (n=6)</td>
<td>93.7±2.6</td>
<td>347.4±11.7</td>
<td>10.0±1.3</td>
<td>2.4±0.7</td>
</tr>
<tr>
<td>CBX (PVN, n=7)</td>
<td>90.3±3.2</td>
<td>310.0±8.5</td>
<td>9.0±1.0</td>
<td></td>
</tr>
<tr>
<td>CBX (Non-PVN, n=16)</td>
<td>92.8±3.5</td>
<td>325.1±10.2</td>
<td>9.4±1.4</td>
<td></td>
</tr>
<tr>
<td>VEH (PVN, n=5)</td>
<td>88.3±2.8</td>
<td>307.1±9.1</td>
<td>8.9±1.2</td>
<td></td>
</tr>
<tr>
<td>ADX + CBX (n=5)</td>
<td>69.1±5.1*</td>
<td>298.8±7.5</td>
<td>8.8±0.7</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>ADX + GA (n=5)</td>
<td>67.6±5.2*</td>
<td>303.6±9.9</td>
<td>8.2±0.7</td>
<td>2.5±1.0</td>
</tr>
<tr>
<td>VEH (ICV, n=6)</td>
<td>94.0±3.3</td>
<td>323.0±9.1</td>
<td>10.2±1.6</td>
<td>2.2±0.3</td>
</tr>
</tbody>
</table>

*P<0.05, vs VEH (ICV).

![Figure 3](https://hypertension.ahajournals.org/issue/10/119/Zhang-11beta-HSD2.pdf)

**Figure 3.** A, Representative tracings showing the response of a PVN neuron and simultaneously recorded HR, RSNA, and AP to ICV administration of the 11β-HSD-2 inhibitor CBX (15 µg). B, CBX-induced sympathoexcitatory responses were abolished by pretreatment with ICV SL (20 µg). RSNA insets in A and B: fast time base tracings of baseline and peak RSNA illustrating the bursting quality of discharge (time bar 1 sec). Boxed insets in A and B: post-stimulus time histograms illustrating pulse-related activity of the PVN neuron. C, Grouped data showing that the increases (Δ) from baseline in MAP, HR, RSNA, and PVN neuronal activity induced by ICV CBX are prevented by pretreatment with the MR antagonist SL (P<0.01). Arrows indicate time of CBX injection; n=number of injections; *P<0.01.
• To confirm that the sympathoexcitatory effects of CBX are independent of its gap junction blocking properties, AP, HR, and RSNA were monitored during ICV injections (2 μL over 10 s) of GA or VEH.

• To confirm that the responses to CBX and GA are corticosterone-dependent, AP, HR, and RSNA were monitored during ICV injections (2 μL over 10 s) of GA, CBX, or VEH in ADX rats.

At the end of each experiment, the net value of renal nerve activity was determined by subtracting the background noise recorded after injection of the ganglion blocker hexamethonium (30 mg/kg IV). The recordings of rectified and integrated RSNA, single unit PVN neuronal activity, mean arterial pressure (MAP), and HR were analyzed using methods described previously. Data were averaged over 1-minute intervals, and a 3-minute average of baseline activity was used as a control for responses to injection of CBX or GA. Absolute values were used to calculate the changes in MAP and HR, and a percent change from baseline was used to calculate changes in integrated RSNA. The relationship of PVN neuronal discharge to the cardiac cycle was determined by post-stimulus time histograms, triggered by the peak of the arterial pressure pulse across a 3-minute interval.

The last PVN recording or tissue microinjection site in each experiment was marked with pontamine sky blue, and the anatomic locations of other recording and microinjection sites were extrapolated with respect to this reference point. All sites were plotted on schematic tracings of the PVN, based on the rat atlas of Paxinos and Watson.

Immunohistochemistry

CBX (15 μg in 2 μL, 5 rats) or VEH (2 μL, 4 rats) was administered ICV. Two hours later, rats were anesthetized and transcardially perfused with PBS and 4% paraformaldehyde. Brain tissues were removed and processed for c-fos expression using the avidin–biotin–peroxidase complex technique, as previously described. A rabbit polyclonal anti-Fos antiserum (Santa Cruz Biotechnology, Santa Cruz, Calif) was used. The c-fos positive cells in posterior magnocellular, medial parvocellular, and dorsal parvocellular regions of PVN were counted manually in 2 adjacent coronal sections, one of which included the maximal expanse of PVN. An average value for the number of c-fos positive neurons counted in the 2 sections, reported as c-fos positive neurons/100 μm², was used for data analysis.

Data Analysis

Statistical significance among multiple comparisons was determined by 1-way or 2-way repeated-measures ANOVA followed by post-hoc Tukey test. Paired t test was used for comparison between peak responses and baseline. Other group data were compared by t test. Values are presented as means±SEM. P<0.05 was considered to be statistically significant.

Results

Expression of 11β-HSD-2 mRNA in PVN

Real-time PCR analysis revealed the presence of 11β-HSD-2 mRNA in the PVN and in cortex sampled at the same stereotaxic level. The expression of 11β-HSD-2 mRNA was 2.6-fold higher (P<0.01, 8 rats) in PVN compared with cortex (Figure 1).

Sympathoexcitatory Effects of 11β-HSD-2 Inhibition

Microinjection of the 11β-HSD-2 inhibitor CBX (2 μg, n=7) into PVN produced a sympathoexcitatory response (Figure 2A, 2B) characterized by significant (P<0.05) increases from baseline (Table) in RSNA (27.4±5.8% MAP (8.4±2.5 mm Hg), and HR (26.2±6.2 bpm). The same volume of VEH (100 nL, n=5) elicited no significant response (Figure 2B). Microinjection of CBX into sites dorsal, lateral, and anterior to PVN did not significantly alter MAP (1.3±0.2 mm Hg), HR (4.3±3.8 bpm), or RSNA (3.2±1.8%). Microinjection of CBX into the dorsomedial hypothalamic nucleus, 1.0 mm caudal to PVN, did induce a mild increase in MAP (5.7±0.2 mm Hg), HR (16.4±2.9 bpm), and RSNA (16.8±2.6%) in 2 of the 4 rats tested.
ICV CBX (Figure 3) elicited significant ($P<0.05$) increases from baseline (Table) in PVN neuronal firing (from $2.1\pm 0.3$ to $7.2\pm 1.3$ spikes/s), RSNA (by $84\pm 21\%$), MAP ($9.8\pm 2.5$ mm Hg), and HR ($89.8\pm 14.7$ bpm). The onset latency of these responses was $2–3$ minutes, with peak responses at $\sim 40$ to $50$ minutes and durations $\sim 90$ minutes. The same volume of VEH (ICV, n=6) had no effects. Pretreatment with SL ($20\ \mu g$ ICV, n=6), prevented the CBX-induced increases in PVN activity (from $2.4\pm 0.7$ to $2.1\pm 1.1$ spikes/s), RSNA (4.5$\pm 7.0\%$), MAP ($-0.9\pm 2.7$ mm Hg), or HR ($22.0\pm 9.4$ bpm). SL alone had no effect on baseline values (Table) of MAP, HR, or RSNA.

ICV injection of GA (10 $\mu g$, n=6; Figure 4) also significantly ($P<0.01$) increased PVN neuronal activity ($1.8\pm 0.3$ to $7.7\pm 1.6$ spikes/s), RSNA (106.3$\pm 28.9\%$), MAP (29.3$\pm 7.8$ mm Hg), and HR (73.0$\pm 11.2$ bpm) from baseline (Table).

In ADX rats, neither CBX (15 $\mu g$ ICV, n=5 rats) nor GA (10 $\mu g$ ICV, n=5 rats) elicited a significant change in MAP, HR, RSNA, or PVN neuronal activity (data not shown). Consistent with earlier reports,27–29 the ADX rats had lower baseline blood pressures, but HR, RSNA, and PVN neuronal activity were not significantly different from VEH-treated intact rats (Table).

![Figure 5](image-url)

**Figure 5.** Schematic reconstruction of recording and microinjection sites within PVN and surrounding regions. A, Recording sites in intact rats. PVN neurons responsive to CBX alone (●) and unresponsive to SL+CBX (○); PVN neurons responsive to GA (●); PVN neurons unresponsive to VEH (△). B, Recording sites in ADX rats. PVN neurons unresponsive to CBX (○) and GA(△). C, Microinjection sites for CBX (■) and vehicle (●) within PVN; microinjection sites of CBX in areas surrounding PVN (○). All microinjections were made bilaterally. The distance (mm) from bregma is indicated. Sections are modified from Paxinos and Watson.24 AH indicates anterior hypothalamic nucleus; f, fornix; 3V, third ventricle.

The PVN microinjection and PVN recording sites are shown in Figure 5. The PVN neurons recorded were distributed throughout the nucleus. The majority of recorded PVN neurons (21/30 tested) had a pulse-related discharge pattern (see insets in Figures 3A, 3B, 4A).

**Locations of PVN Neurons Excited By ICV Injection of CBX**

Immunohistochemistry of brain tissue harvested 2 hours after ICV administration of CBX (5 rats) revealed increased c-fos expression in neurons throughout the PVN, compared with VEH (4 rats). Similar increases were observed in all 3 regions in which the neurons were counted (Fig 6). Areas outside PVN but at the same stereotaxic level had scattered c-fos expression. However, there was no difference between CBX- and VEH-treated rats.

**Discussion**

Novel findings of this study are: (1) mRNA for 11β-HSD-2 is expressed in the PVN of the hypothalamus; and (2) inhibition of 11β-HSD-2 activity in PVN elicits a sympathetically-mediated cardiovascular response in normal rats. A logical implication of these findings is that PVN neurons are aldosterone-sensitive.

Other forebrain regions express 11β-HSD-2 activity,9,10 but, to the best of our knowledge, this report is the first to suggest that aldosterone might act directly on PVN neurons endowed with 11β-HSD-2–protected MR. The lack of evidence for 11β-HSD-2 expression in PVN in previous studies9–11 likely reflects the sensitivity of the methods used. We used real-time PCR to demonstrate the presence of mRNA for 11β-HSD-2 in PVN, and chemical inhibition of 11β-HSD-2 activity with CBX to demonstrate the presence of functionally significant levels of 11β-HSD-2 in PVN. These 2 lines of evidence leave little room to doubt that 11β-HSD-2 is expressed and enzymatically active in PVN.

The cardiovascular and autonomic responses to ICV injections of CBX or GA are more dramatic than the responses elicited by microinjecting CBX directly into PVN. This is the expected result, since other brain regions expressing 11β-HSD-2–9–11 are likely recruited by the ICV injection. This interpretation is consistent with the observation that ICV injection of CBX elicits a widespread increase in c-fos expression throughout PVN. The recently identified sodium- and aldosterone-sensitive solitary tract nucleus neurons,12 which were noted to have ascending projections, may be one source of extrinsic input to PVN.

The differences in the magnitude and timing of the sympathetic responses to ICV CBX and GA, as compared with the responses to PVN microinjection of CBX, probably reflect differences in experimental conditions (eg, drug solubility, tissue penetration, or numbers of neurons activated). They have no bearing on the relative influence of 11β-HSD-2 activity in any specific brain region on sympathetic regulation under normal or pathophysiological conditions.

Most of the spontaneously discharging neurons we recorded in PVN had cardiac-related activity. Pulse-related activity was recently described in a small group of pre-autonomic neurons in dorsal, lateral, and ventrolateral PVN that were identified by their response to antidromic stimula-
tion of the spinal cord. In that study, neurons in other regions of PVN were not tested. Thus, it remains unknown whether pulse-related activity is an exclusive property of presympathetic neurons. If so, our data would argue that presympathetic neurons are scattered diffusely throughout the PVN. An intermingling of preautonomic and neuroendocrine neurons is consistent with the integrative role of the PVN and with retrograde tracing studies that have demonstrated a broad distribution of presympathetic PVN neurons.

Finally, our results are consistent with the emerging concept that nongenomic as well as genomic effects may be elicited by activation of classical MR. The cardiovascular and sympathetic responses to the ICV or PVN injection of 11β-HSD-2 inhibitors appear to be too rapid in onset to be mediated by genomic actions of MR, and yet are blocked by SL. Early effects of MR stimulation that are responsive to classical MR antagonists have also been described in kidney, colon, and vasculature. Corticosterone activation of aldosterone-sensitive MR is an appealing explanation for the early onset of cardiovascular and sympathetic responses after ICV administration of CBX or GA. In human arteries, application of CBX permits cortisol to induce early nongenomic MR effects.

That said, the precise mechanism by which corticosterone (or cortisol) binding to MR can induce an aldosterone-like response is the subject of continued investigation and speculation. The complexity of this issue is illustrated by the well known ability of glucocorticoids to inhibit aldosterone effects, presumably by competitively occupying the MR. For example, the ICV infusion of corticosterone blocks the hypertension induced by ICV infusion of aldosterone. It has recently been suggested that another effect of 11β-HSD-2—the conversion of NAD to NADH—may be important in determining the outcome of glucocorticoids binding to MR. By mechanisms not fully understood, an altered redox state induced by inhibition of 11β-HSD-2 activity may enable corticosterone to function as a MR agonist.

Perspectives

It is clear from the present experiments that 11β-HSD-2 activity in the PVN and other brain regions effectively prevents corticosterone from inappropriately activating cardiovascular presympathetic neurons. It remains to be determined whether altered 11β-HSD-2 activity in PVN contributes to the increased sympathetic drive that occurs in cardiovascular disease states. It is noteworthy, however, that the same physiological stressors that downregulate 11β-HSD-2 activity in peripheral tissues (eg, angiotensin II and tumor necrosis factor) are upregulated in the PVN in heart failure. In a preliminary study, we found that 11β-HSD-2 mRNA is reduced in PVN of rats with experimentally induced heart failure. Thus, it is conceivable that the beneficial effects of a MR antagonist in experimental heart failure may be ascribed, at least in part, to blocking the effects of corticosterone on brain MR that are normally reserved for aldosterone by 11β-HSD-2 activity.

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Disclosures

None.

References


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