Fos expression in brain stem nuclei of pregnant rats after hydralazine-induced hypotension

KATHLEEN S. CURTIS,1 J. THOMAS CUNNINGHAM,1,2 AND CHERYL M. HEESCH2,3

Departments of 1Physiology and 3Veterinary Biomedical Sciences and
2Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO 65211

Curtis, Kathleen S., J. Thomas Cunningham, and Cheryl M. Heesch. Fos expression in brain stem nuclei of pregnant rats after hydralazine-induced hypotension. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R532–R540, 1999.—Fos and dopamine β-hydroxylase immunoreactivity were evaluated in the brain stems of 21-day pregnant and virgin female rats injected with either hydralazine (HDZ; 10 mg/kg iv) or vehicle. HDZ produced significant hypotension in both groups, although baseline blood pressure was lower in pregnant rats (96 ± 2.5 mmHg) than in virgin female rats (121 ± 2.8 mmHg). There were no differences in Fos immunoreactivity in the brain stems of pregnant and virgin female rats after vehicle treatment. HDZ-induced hypotension significantly increased Fos expression in both groups; however, the magnitude of the increases differed in the caudal ventrolateral medulla (CVL), the area postrema (AP), and the rostral ventrolateral medulla (RVL). Fos expression after HDZ in pregnant rats was augmented in noncatecholaminergic neurons of the CVL but was attenuated in the AP and in noncatecholaminergic neurons in the RVL. These results are consistent with differences in the sympathetic response to hypotension between pregnant and virgin female rats and indicate that the central response to hypotension may be different in pregnant rats.

baroreflex; sympathetic activation

PREGNANCY IN HUMANS and other animals is associated with striking alterations in body fluid homeostasis and cardiovascular regulation that may include changes in baroreflex function. A number of studies have examined baroreflex function during pregnancy in species such as rat, rabbit, sheep, and dog (3, 4, 8, 10, 22, 25, 33, 34). However, some controversy exists regarding alterations in baroreflex function during pregnancy. For example, baroreflex control of heart rate has been reported to be blunted (3), augmented (8), or unchanged (22, 33) during pregnancy. These conflicting findings could be attributable to anesthetic effects, stage of pregnancy, or species differences. Alternatively, it is possible that the discrepant findings may be related to whether blood pressure was increased or decreased to evaluate baroreflex function (e.g., Refs. 3, 8). Recently, studies examining baroreflex function over the full range of changes in blood pressure demonstrated that the curve relating blood pressure to heart rate (4) or sympathetic nerve activity (10, 34) was shifted to the left and that the set point was shifted to a lower pressure. These studies also revealed that pregnancy-induced differences in baroreflex function were especially pronounced when blood pressure was decreased. More specifically, the ability to increase sympathetic nerve activity in response to a hypotensive challenge appears to be blunted during pregnancy (34).

Several possible mechanisms could account for pregnancy-induced changes in baroreflex function, including changes in sympathetic efferent activity, in the sensitivity of baroreceptors, or in the central processing of baroreceptor information. For example, the activation of central pathways associated with the control of sympathetic outflow may be altered by hormonal changes associated with pregnancy. The major progesterone metabolite, 3α-hydroxy-dihydroprogesterone (3α-OH-DHP), has been reported to potentiate central GABA mechanisms (37), and GABAergic inhibition plays an important role in the baroreflex pathway (12). Consistent with these observations, when 3α-OH-DHP was given to virgin female rats either by systemic administration (34) or by microinjection into the rostral ventrolateral medulla (RVL) (21), the increase in renal sympathetic nerve activity in response to a hypotensive challenge was blunted similarly to that seen in late-term pregnant rats. Thus the attenuated increase in sympathetic nerve activity in response to a hypotensive challenge during pregnancy may reflect differences in the central processing of information related to control of sympathetic outflow.

A number of studies have used c-fos immunocytochemistry as a method for evaluating neuronal activation in the central nervous system in response to cardiovascular challenges in the conscious animal. Studies examining hypotension (1, 6, 18, 31) have consistently reported increased Fos expression in specific brain stem areas associated with the central control of autonomic function; however, it is not known whether hypotension-induced activity in these areas is affected by pregnancy. Therefore, the present study examined Fos immunoreactivity in late-term pregnant rats after acute decreases of blood pressure to determine whether the activation of brain stem areas known to be involved in the central control of sympathetic nerve activity is altered by pregnancy. To determine whether pregnancy differentially affected the activity in catecholamine-containing neurons in response to acute decreases of blood pressure, immunolabeling for dopamine β-hydroxylase (DBH), the enzyme involved in conversion of dopamine to norepinephrine, also was examined in these areas.

METHODS

Surgery and blood pressure recording. Chronic femoral arterial and jugular vein catheters for recording mean arte-
rrial pressure (MAP) and administering drugs, respectively, were implanted in 18-day pregnant and virgin female rats that had been anesthetized with Nembutal (50 mg/kg ip). Rats were permitted to recover for 3 days. MAP was recorded for a 30-min baseline period and then for 90 min after intravenous administration of 10 mg/kg hydralazine (HDZ; n = 5/group) or the isotonic saline vehicle (Veh; n = 5/group). Rats then were deeply anesthetized with Nembutal (50 mg/kg iv) and perfused intracardially with 0.1 M PBS followed by 4% paraformaldehyde. Experiments were conducted in accordance with the guidelines of the American Physiological Society for research involving animals and all experimental protocols were approved by the Institutional Laboratory Animal Care and Use Committee.

Immunocytochemistry. Brains were removed, placed in a 30% sucrose solution, and subsequently were cut into 30-μm sections. Every third brain stem section was processed for Fos and DBH immunoreactivity; thus sections were obtained every 90 μm. After a 60-min rinse in PBS, brain stem sections were placed in a 0.3% hydrogen peroxide solution for 30 min at room temperature. After an additional 30-min PBS rinse, sections were incubated in PBS diluent [3% heat-inactivated horse serum (Sigma, St. Louis, MO) in PBS with 0.25% Triton-100] for 2 h at room temperature. Sections then were incubated in the rabbit anti-c-fos antibody (Oncogene Ab-5; Oncogene Science, Cambridge, MA) diluted 1:30,000 in PBS diluent for 72 h at 4°C. After a 60-min rinse in PBS, sections were incubated in a biotinylated horse anti-rabbit IgG (Vector Laboratories, Burlingame, CA) diluted 1:200 in PBS diluent for 2 h at room temperature. After an additional 60-min rinse in PBS, sections were reacted with an Avidin-peroxidase conjugate (Vectastain ABC Ki; Vector Laboratories) and PBS containing 0.04% 3,3’-diaminobenzidine hydrochloride and 0.04% nickel ammonium sulfate. Sections then were rinsed for 30 min in PBS and incubated in the DBH antibody (Chemicon International, Temecula, CA) diluted 1:1,000 in PBS diluent for 24 h at 4°C. After a 60-min PBS rinse, sections were incubated in an anti-mouse antibody conjugated with Cy3 (Jackson ImmunoResearch, West Grove, PA) for 3 h at room temperature.

Analysis and statistics. Bright-field and fluorescent photomicrographs were taken of at least two representative sections from each area, and Fos and DBH immunoreactive cells were counted by observers blind to experimental condition. Representative sections for each area were chosen and matched between animals based on rostrocaudal anatomical landmarks as described by Paxinos and Watson (39). Fos and DBH immunoreactivity were evaluated in the RVL from the caudal pole of the medial nucleus of the inferior olive to the caudal pole of the facial nucleus (3 representative sections, −12.80 to −11.80 mm from Bregma); in the intermediate ventrolateral medulla (IVL) from the caudal pole of the beta nucleus of the inferior olive to the caudal pole of the dorsal nucleus of the inferior olive (2 representative sections, −13.80 to −13.30 mm from Bregma); in the caudal ventrolateral medulla (CVL) from the level of the pyramidal decussation to the caudal pole of the cap of Kooy of the medial nucleus of the inferior olive (4 representative sections, −14.08 to −14.08 mm from Bregma); in the area postrema (AP) at caudal, middle, and rostral levels of the AP (3 representative sections, −14.08 to −13.68 mm from Bregma); in the middle portion of the nucleus of the solitary tract (nNTS) at the caudal, middle, and rostral levels of the AP (3 representative sections, −14.08 to −13.68 mm from Bregma); and in the caudal nucleus of the solitary tract (cNTS) from the median accessory nucleus to the caudal nucleus of the solitary tract in the commissural and medial subnucleus of the NTS. Numbers of Fos-positive neurons that also were DBH positive, and numbers of Fos-positive neurons that were not DBH positive were averaged for each animal.

Data are presented as group means. Statistical significance was determined by two-way ANOVA (2 × 2 factorial design), or, where variability was high, by Kruskal-Wallis one-way ANOVA on ranks. Pairwise comparisons were made using the Student-Newman-Keuls method only when a statistically significant (P < 0.05) interaction existed in the two-way ANOVA or when a statistically significant difference existed in the Kruskal-Wallis one-way ANOVA on ranks. Pairwise differences were considered to be statistically significant when P < 0.05.

RESULTS

Physiological measurements. Body weights of pregnant rats (n = 10, 317 ± 7.5 g) were significantly greater than those of virgin female rats (n = 10, 246 ± 3.8 g; F (1,16) = 71.0, P < 0.0001). There was no difference in body weight between Veh- and HDZ-treated rats within either the pregnant or virgin groups. MAP in the baseline condition was less in pregnant rats (n = 10, 96 ± 2.5 mmHg) compared with that in virgin female rats (n = 10, 121 ± 2.8 mmHg; F (1,16) = 41.6, P < 0.001). Veh did not affect MAP in either pregnant rats (n = 5, 96 ± 4.0 mmHg) or in virgin female rats (n = 5, 117 ± 2.6 mmHg). HDZ decreased MAP significantly in both groups [pregnant n = 5, 64 ± 2.7 mmHg; virgin n = 5, 72 ± 1.6 mmHg; F (1,16) = 117.3, P < 0.001]; however, the decrease after HDZ was significantly less in pregnant rats (−31 ± 3.5 mmHg) compared with that in virgin female rats (−51 ± 4.4 mmHg; P < 0.05).

Immunocytochemistry. The mean numbers of Fos-positive nuclei after Veh treatment were low in all areas examined, and there were no statistically significant differences between pregnant and virgin female rats after Veh treatment in any area. HDZ treatment significantly increased the total numbers of Fos-positive nuclei in each area evaluated (Fig. 1); however, significant differences in the response to HDZ-induced hypotension between pregnant and virgin female rats were observed in some of the regions.

NTS. The mean numbers of Fos-positive nuclei in both cNTS and mNTS after Veh treatment were similar in virgin female and pregnant rats. HDZ treatment significantly increased the mean numbers of Fos-positive nuclei in both areas to comparable levels (Fig. 1), and the distribution of Fos-positive nuclei after HDZ was similar in virgin female and pregnant rats (Figs. 2 and 3). Although there was Fos immunolabeling ventral to the solitary tract in the ventral subnucleus of the NTS, Fos-positive nuclei were located primarily medial to the solitary tract in the commissural and medial subnuclei of the NTS. Numbers of Fos-positive neurons that also were DBH positive comprised a small proportion (<10%) of the total numbers of Fos-positive neurons regardless of treatment in both pregnant and virgin female rats (data not shown).

AP. The mean numbers of Fos-positive nuclei in AP after Veh treatment were similar in virgin female and pregnant rats. HDZ treatment significantly increased
the mean numbers of Fos-positive nuclei in both groups (Fig. 1), and Fos immunolabeling was distributed throughout the AP. However, the mean numbers of Fos-positive nuclei were significantly less in the AP of HDZ-treated pregnant rats compared with that in HDZ-treated virgin females (Figs. 1 and 3). Numbers of Fos-positive neurons that also were DBH positive comprised a small proportion (10–15%) of the total numbers of Fos-positive neurons regardless of treatment in both pregnant and virgin female rats (data not shown).

CVL. There were no differences in Fos immunolabeling between pregnant and virgin female rats after Veh treatment, and HDZ treatment significantly increased Fos immunolabeling in both groups (Figs. 1 and 4). Neither the mean total number of Fos-positive nuclei nor the mean number of Fos-positive neurons that also were DBH positive were different in HDZ-treated pregnant rats compared with those in HDZ-treated virgin female rats (Fig. 4). In contrast, the mean number of Fos-positive neurons that were not labeled for DBH were significantly greater in the CVL of HDZ-treated pregnant rats compared with that in HDZ-treated virgin female rats (Fig. 4). Figure 5 shows photomicrographs of immunolabeling in the CVL.

IVL. There were no differences in Fos immunolabeling between pregnant and virgin female rats after Veh treatment, and HDZ treatment significantly increased Fos immunolabeling in both groups (Fig. 1). HDZ treatment significantly increased the mean numbers of Fos-positive neurons that also were labeled for DBH in both groups \(F(1,16) = 97.3, 30.7, \) and 221.6, respectively; all \(P < 0.001\). Group effect \(F(1,16) = 26.9\) and group \(\times\) drug interaction \(F(1,16) = 27.8\) were statistically significant in RVL (both \(P < 0.001\)). *\(P < 0.05\) compared with corresponding Veh-treated group; **\(P < 0.05\) compared with HDZ-treated virgin female rats.
RVL. There were no differences in Fos immunolabeling between pregnant and virgin female rats after Veh treatment, and HDZ treatment significantly increased Fos immunolabeling in both groups (Fig. 1, 6). Both the mean total number of Fos-positive nuclei and the mean number of Fos-positive neurons that were not DBH positive were significantly less in HDZ-treated pregnant rats compared with those in HDZ-treated virgin female rats (Fig. 6). In contrast, the mean number of Fos-positive neurons that also were labeled for DBH were not different in HDZ-treated pregnant rats compared with that in HDZ-treated virgin female rats (Fig. 6). Figure 7 shows photomicrographs of immunolabeling in the RVL.

**DISCUSSION**

Pregnancy in rats and other species is associated with altered cardiovascular function, including blunted sympathetic excitation in response to a hypotensive challenge (10, 34). This attenuation of sympathetic nerve activity may reflect changes in the central processing of baroreceptor information during pregnancy. In the present study, we used Fos immunocytochemistry to investigate the activation of areas in the central nervous system involved in the regulation of sympathetic outflow in pregnant and virgin female rats after a hypotensive challenge.

The use of Fos immunocytochemistry is an effective method for evaluating integrated patterns of neuronal activation. A number of studies have employed Fos immunocytochemistry to examine activation in the brain stem of conscious, behaving animals during cardiovascular challenges (1, 6, 18, 31) and have shown consistent, replicable patterns of Fos expression in specific regions within the brain stem. However, there are limitations to the Fos-immunolabeling method (13, 15) that are an important consideration in the interpretation of the data. Synaptic activation elicits Fos expression, but neither the magnitude nor the duration of the stimulus required to produce detectable levels of the Fos protein is well defined. In addition, not all neurons express Fos on activation and Fos expression is not associated with neuronal inhibition.

The present findings suggest that during pregnancy there is an alteration in the activation of central pathways involved in the control of sympathetic outflow. HDZ increased the numbers of Fos-positive nuclei in the NTS to comparable levels in pregnant and virgin female rats (Figs. 1, 2, and 3). Increased Fos expression in the NTS in response to hypotension also has been reported to occur in male rats (e.g., Refs. 6, 18). In those studies, as in the present, Fos-positive neurons in the NTS were located within subnuclei corresponding to the location of aortic baroreceptor terminals (7). Be-
cause baroreceptor activation is decreased with decreased MAP, the stimulus for increased Fos expression in these areas is unclear; however, neither the numbers nor the distribution of neurons in the NTS that express Fos in response to signals associated with hypotension appear to be affected by pregnancy.

HDZ-induced Fos expression in the AP was less in pregnant rats compared with that in virgin female rats (Figs. 1 and 3). Because the AP receives arterial baroreceptor input (14, 28), it is possible that the attenuated Fos expression in the AP of pregnant rats reflects less neuronal excitation after removal of baroreceptor afferent input to the AP. HDZ-induced hypotension also may increase Fos expression in the AP by the actions of circulating ANG II or vasopressin. Consistent with this idea, hemorrhage-induced Fos expression in the AP was decreased when male rats were pretreated with an ANG II receptor antagonist (6). These observations suggest that if activation of the AP during hypotension is mediated by circulating ANG II, then this response could be attenuated in pregnant rats.

Overall, HDZ-induced Fos expression in the CVL was not different in pregnant and virgin female rats (Figs. 1 and 4), although there was a tendency toward greater numbers of Fos-positive nuclei in pregnant rats ($P = 0.097$). Closer examination revealed that HDZ-induced Fos expression in noncatecholaminergic neurons in the CVL of pregnant rats was greater than that in virgin female rats (Figs. 4 and 5). The phenotype of these noncatecholaminergic neurons was not examined; however, neuronal populations in the CVL are known to include a number of neurotransmitters, including GABA (2). GABAergic neurons within the CVL are known to inhibit sympathetic outflow via projections to the RVL (12), but these neurons are excited by an increase in blood pressure (17, 27). Therefore, it seems unlikely
The reproductive state of the rats did not influence a significant increase in Fos-positive neurons (Fig. 1). In addition, the lower absolute MAP after HDZ in pregnant rats might predict a greater increase of Fos immunoreactivity in the sympathoexcitatory area in the RVL rather than less, as actually was observed.

Another explanation for the attenuated Fos immunoreactivity in the RVL of pregnant rats is the influence of changes in circulating levels of reproductive hormones during pregnancy. It is known that GABA provides an important inhibitory influence on sympathoexcitatory neurons in the RVL (12) and that the major metabolite of progesterone, 3α-OH-DHP, positively modulates central GABAergic responses (37). Previous studies demonstrated that acute administration of 3α-OH-DHP to virgin female rats attenuated sympathetic excitatory responses (21, 34) and potentiated baroreflex-mediated inhibition of spinally projecting RVL neurons (29).

Therefore, the attenuated response to HDZ-induced hypotension in the RVL of pregnant rats could be the result of potentiation of GABAergic influences by 3α-OH-DHP, and the apparent specificity of this potentiation to the RVL of pregnant rats may reflect heterogeneity of GABA receptor subunit composition (30). However, a recent study has shown that reflex increases in heart rate in response to hemorrhage in rabbits are attenuated in late pregnancy but are not altered at midgestation, even though levels of progesterone and its metabolites are elevated early in pregnancy (5). It is possible that elevated levels of estrogen, which plateau later in pregnancy, contribute to the effects of progesterone and/or its metabolites on sympathetic outflow. A role for estrogen is supported by a recent report demonstrating
that chronic administration of estrogen to ovariectomized rats potentiates baroreflex sympathoinhibitory responses to hypertension (19). Thus it is likely that the effects of pregnancy on control of sympathetic outflow are the result of combined influences of ovarian hormones.

In addition to inhibitory input, the RVL receives tonic excitatory input (12). For example, a recent study (26) showed that increased blood pressure produced by blockade of the tonic GABAergic inhibition of the RVL was reversed or prevented by injection of ANG II receptor antagonists into the RVL. It seemed possible that the actions of ANG II as a neurotransmitter in the RVL also may be affected by pregnancy. Preliminary studies to evaluate this idea suggest that the tonic excitation to RVL provided by ANG II is not different between pregnant and virgin female rats (20); however, other excitatory inputs have not been examined. Thus both excitatory and inhibitory input to the RVL may be altered during pregnancy, although the source of excitatory input to the RVL is unclear.

Central control of sympathetic outflow has been reported to be modulated by ANG II acting at the AP (16, 9), possibly via pathways involving the RVL (11, 41, 42). In addition, pregnant rats demonstrate blunted pressor responses to systemic administration of ANG II that appear to require reflex neural mechanisms (23). The present findings of decreased Fos in the AP of HDZ-treated pregnant rats suggest the possibility that the contribution of circulating ANG II to the activation of the central nervous system during hypotension also may be reduced during pregnancy.
Although direct comparisons are difficult because of methodological differences, HDZ-induced Fos activation in the brain stems of virgin female rats appears to be comparable to the results reported in earlier studies of hypotension using male rats (1, 6, 18). Chan and Sawchenko (6) reported that hypotension produced by administration of sodium nitroprusside increased Fos immunoreactivity in the NTS as well as in the A1 (CVL) and C1 (RVL) areas of the ventrolateral medulla. Similar to the present findings, Fos-positive neurons that also were labeled for DBH comprised ~15% of the total numbers of Fos-positive cells in the NTS, ~35% of the total numbers of Fos-positive cells in the A1 area, and ~45% of those in the C1 area. These authors reported that Fos immunoreactivity did not increase in the AP after hypotension produced by sodium nitroprusside, despite the robust increase that occurred after hypotensive hemorrhage. However, the duration of decreased MAP with sodium nitroprusside is fairly short (~10 min), and it was noted that the increased Fos immunoreactivity in the AP after hemorrhage was delayed by ~2 h compared with the immunoreactivity in the NTS. It is possible, therefore, that the prolonged hypotension produced by HDZ may be a stimulus of sufficient duration to produce detectable levels of Fos in the AP. Consistent with this idea, Graham et al. (18) reported that HDZ given in similar doses to produce similar decreases in MAP resulted in increased Fos immunoreactivity in the AP, as well as in the NTS, CVL, IVL, and RVL. Similar results also were reported in male rabbits in response to hypotension (31).

In summary, HDZ-induced hypotension resulted in increased Fos immunoreactivity in the AP, NTS, CVL, IVL, and RVL of pregnant and virgin female rats. There were no differences between the groups in nTS, mNTS, or IVL. However, after HDZ, Fos immunoreactivity in noncatecholaminergic neurons in CVL was augmented in pregnant rats, and Fos immunoreactivity in the AP and in noncatecholaminergic neurons in the RVL was attenuated in pregnant rats compared with that in virgin female rats. The decreased Fos expression in the sympathetic cervical area in the RVL is consistent with previous reports of blunted sympathetic nerve activity in pregnant rats in response to hypotensive challenges (10, 34), although additional experiments will be necessary to determine if these RVL neurons project to sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord. In addition, the attenuated Fos immunoreactivity in the AP suggests that decreased neural activity in the AP could contribute to the attenuated HDZ-induced Fos immunoreactivity in the RVL. Further experiments are necessary to fully evaluate the interactions among these brain stem areas in response to hypotension and other cardiovascular challenges during pregnancy, and to determine whether neurosteroid modulation plays a role in these central changes.

Perspectives

There is much evidence for alterations in the regulation of body fluid homeostasis during pregnancy, including decreased vascular responsiveness to pressor agents (22, 36, 38), decreased sensitivity of cardiopulmonary baroreceptors in response to atrial distension (24), and blunted baroreflex responses to hypotension (4, 10, 34). The present study suggests that alterations in the activity of central nervous system areas associated with the control of autonomic function may contribute to the attenuated ability of pregnant rats to increase sympathetic outflow in response to hypotension. Although the mechanism of this alteration remains under investigation, previous research suggests a role for progesterone metabolites (21, 34). It is possible that blunted sympathetic nerve activity in response to hypotensive challenges may have the important functional consequence of preventing rapid reflex increases in MAP during a state when both cardiac output and blood volume are elevated. In other words, changes in the central processing of information that regulates sympathetic outflow may be adaptive during pregnancy. An important issue that remains, however, is whether such alterations also are present in the basal state during pregnancy and may play a permissive role by contributing to changes in body fluid homeostasis that are essential to support a pregnancy. Results from studies employing the Fos immunocytochemistry method may permit an initial assessment of whether, and where, such changes may occur.

The authors thank Dr. Margaret Sullivan for helpful comments and advice, and gratefully acknowledge the expert assistance of Regina Randolph, J. Jennifer Laiprasert, and Sarbani Ghosh.

This work was supported by National Heart, Lung, and Blood Institute Grants RO1–36246 (to C. M. Heesch), KO2–03882 (to J. T. Cunningham), and T32–07094 (to K. S. Curtis).

Address for reprints and other correspondence: C. M. Heesch, Univ of Missouri, Dalton Cardiovascular Res. Ctr., Research Park, Columbia, MO 65211 (E-mail: heeschcm@missouri.edu).

Received 9 December 1998; accepted in final form 30 April 1999.

REFERENCES


