Central angiotensin II-induced pressor responses and neural activity in utero and hypothalamic angiotensin receptors in preterm ovine fetus

Zhice Xu, Lijun Shi, and Jiaming Yao

Harbor-University of California-Los Angeles Medical Center (UCLA) and Research and Education Institute, Harbor-UCLA Medical Center, Torrance, California 90502

Submitted 8 August 2003; accepted in final form 26 November 2003

ANGIOTENSIN II (ANG II) is a potent vasoconstrictive hormone, and a number of studies have demonstrated the importance of the renin-angiotensin system (RAS) in the control of blood pressure (BP) (1, 19, 23). Earlier work has shown that an active RAS is present in the ovine fetus before birth (6, 15). Its major function appears to be the regulation of fetal arterial pressure under conditions of fetal stress, including acute hemorrhage or hypoxia (17). However, study of the development of the central RAS in the regulation of BP in utero is still limited. Notably, most previous work performed in the fetus focused exclusively on actions of angiotensin at the peripheral side in the control of BP in utero (8, 10, 15, 17, 28). Our recent studies have demonstrated an increase of fetal mean arterial pressure (MAP) immediately after an injection of ANG II into the lateral ventricle of the near-term (90% gestational age) ovine fetal brain in utero (36). This observation prompted initial experiments investigating the cardiovascular action of fetal intracerebroventricular injection of octapeptide ANG II.

In the adult, administration of either ANG II or its precursors into the brain induces pressor responses, drinking, and release of hormones (3, 11, 29). A number of studies have confirmed that almost all biological effects of ANG II are expressed via angiotensin subtype 1 (AT1) receptors, not AT2 receptors (21, 31, 32). It has been suggested that more AT2 than AT1 receptors exist in the fetal brain (7). Whether AT1 receptors have developed in the fetal hypothalamus is understudied. Therefore, one purpose of the present study was to determine AT1 receptors in the hypothalamic paraventricular and supraoptic nuclei (PVN and SON, respectively) in the preterm ovine fetus. Intracerebroventricular ANG II has been repeatedly shown to produce a reliable pressor response in the adult animals and stimulate cellular activation marked by c-Fos expression in the central pathways via angiotensin receptors (18, 32). Central angiotensin-stimulated c-Fos expression has been associated with cellular activation in the PVN, the median preoptic nucleus (MePO), the area postrema (AP), and the lateral parabrachial nuclei (LPBN) (33–35). However, no data exist regarding the effect of central action of angiotensin in the fetus at preterm or before near term, and there has been no information about angiotensin receptors in the hypothalamic nuclei like the SON in the fetus. Therefore, the present study was performed to investigate the effect of central ANG II on the fetal pressor response in utero at preterm (70–75% gestational age), to determine whether central application of ANG II can activate the brain pathways in the preterm ovine fetus, to identify where the action sites of ANG II in the preterm fetal brain are during the early developmental stage, and, finally, to examine whether AT1 receptors are expressed in the PVN and SON at preterm. The information gained is important to the understanding of the ontogeny of the brain RAS mechanisms in cardiovascular regulation and provides insight about the development of central angiotensin-related neural pathways before birth.

METHODS

Surgical preparation. All surgical and experimental procedures were approved and governed by the Institutional Animal Care Committee. Time-dated pregnant ewes with singleton fetuses (110 ± 3 days of gestation at test, corresponding to the early third trimester of human gestational age) were used. Animals were injected with ketamine hydrochloride (15 mg/kg im), and general anesthesia was then maintained with 3% isoflurane and 1 l/min oxygen. The uterus was exposed by a midline abdominal incision, and a small hysterotomy...
was performed to provide access to a fetal hindlimb and head. An intracranial cannulae was implanted in the fetal lateral ventricle and held in place with dental cement. Patency of the catheter at insertion was assessed by free flow of cerebrospinal fluid via gravity drainage. The placement of cannulae was verified with histological analysis after the testing was finished. Polyethylene catheters were placed in the maternal and fetal femoral vein and artery and advanced to the inferior vena cava and abdominal aorta, respectively. An intraterine catheter was inserted into the uterus for measuring amniotic fluid pressure as previously reported (33). The fetus was then immersed into the uterus, and the hysterotomy was closed in two layers. All catheters were externalized to the maternal flank and placed in a cloth pouch. Catheters were filled with heparinized saline, and the animals were treated with antibiotics immediately preoperatively and twice daily during the initial 2 days of recovery, gentamicin (8 mg) and oxacillin (33 mg) were administrated intravenously to the fetus, and gentamicin (72 mg), oxacillin (1 g), and chloramphenicol (1 g) were administrated intravenously to the ewe (33).

Cardiovascular testing. For the animals used in the present study, at the time of testing or actual study, both maternal and fetal sheep were unanesthetized. Animals were not used for experiments if their blood pH dropped under 7.3. Testing began with a baseline (~120 to 0 min) followed by a study period (0–100 min). There were two groups of animals. Beginning at time 0, the fetal animals in the control group (n = 5) were injected intracerebroventricularly with isotonic saline (1 ml). The fetuses in the experimental group (n = 5) were intracerebroventricularly injected with ANG II (0.5 μg/kg, Sigma; St. Louis, MO) freshly prepared in isotonic saline (1 ml) on the first day. The control animals were killed after the cardiovascular testing, and the fetal brain was then used for the c-Fos studies as described in c-Fos experiments. For the experimental group, the fetuses were intracerebroventricularly injected with 1.5 μg/kg ANG II on the second day, and the fetal brain was used for c-Fos studies after the cardiovascular testing. Throughout the testing period, maternal and fetal systolic and diastolic pressure, heart rate, and amniotic pressure were monitored continuously. The fetal MAP was corrected against amniotic cavity pressure. BP, heart rate, and amniotic cavity pressure were measured by means of a Beckman R612 (Beckman Instruments; Fullerton, CA) physiological recorder with Statham (Garret; Oxnard, CA) P23 transducers. Analog signals were determined by computer analysis of waveforms using customized pattern recognition. Fetal body weight was estimated as reported previously (26).

c-Fos experiments. At the end of the cardiovascular testing, the animals were anesthetized with pentobarbital intravenously. A midline abdominal incision was made, and the fetal head and neck were exposed. One fetal carotid artery was catheterized with a 16-gauge indwelling catheter. The fetal head was perfused by gravity flow with 0.01 M PBS for 5 min, followed by 4% paraformaldehyde. The brains were removed, postfixed in 4% paraformaldehyde for ~6 h, and then placed in 20% sucrose at room temperature overnight for cryoprotection. The sections (20 μm) were cut through the forebrain and hindbrain on a freezing cryostat (Leica). Every other tissue section was used for Fos-immunoreactive (Fos-ir) staining with the avidin-biotin-peroxidase technique. The Fos primary antibody is against the first 16 amino acids on the NH2-terminal sequence of the Fos protein (Oncogene). Tissue sections were washed twice for 5 min each in 0.1 M PBS and then incubated overnight in the primary antibody (1: 15,000 in antibody buffer with 0.3% Triton X-100). After being washed, the sections were incubated in an anti-rabbit secondary antibody for 1 h at room temperature. The sections were then washed three times again and processed using the Vectastain ABC kit (Vector Labs). The sections were treated in 1 mg/ml diaminobenzidine tetrahydrochloride (Sigma). The reactions were initiated and catalyzed by the addition of 0.02% hydrogen peroxide.

AT1 receptor immunostaining. Additional pregnant sheep (110 ± 3 gestational days) were used. The animals were not treated. To harvest the fetal brain for AT1 receptor study, the animals were prepared and perfused as described above for c-Fos immunostaining. The preterm ovine sheep brains (n = 3) were cut at 20 μm on a cryostat. The tissue sections from the hypothalamus were washed in 0.1 M PBS; they were then incubated in the AT1 primary antibody (1:1.000, Santa Cruz Biotechnology) overnight at 4°C. For the control staining, no AT1 antibody was used in the procedure. The sections for the control and AT1 receptor staining were then processed with the secondary antibody, ABC kit, and dianminobenzidine tetrahydrochloride.

Data analysis. All cardiovascular signals were digitized at 500 Hz and acquired on a computer with WINDAQ acquisition software (Data Q instruments; Akron, OH). Heart rate, systolic and diastolic pressure, and MAP were calculated from the pressure waveforms by means of Advanced CODAS software. The number of Fos-ir-positive cells in the whole PVN, the parvicellular part of the PVN (dorsal and ventral parts of the PVN), and the MePO were counted. The MePO was divided into the dorsal and ventral MePO by the anterior commissure, and the number of positive Fos-ir nuclei was counted separately in both parts. Fos-ir was also counted in the tractus solitarius nuclei (NTS), AP, LPBN, and caudal ventrolateral medulla (CVLM) in the hindbrain. The CVLM was defined as the area of the ventral brain stem between the nucleus reticularis ventralis, the nucleus reticularis, and the nucleus ambiguous (9). At least three sections were used for the counting of Fos-ir nuclei for each experimental group. Positive Fos-ir nuclei were counted in a quantitative manner by observers who were blind to the experimental conditions of the animals.

Statistical analysis was performed with repeated-measures ANOVA (MANOVA) and a t-test. Comparisons before and after treatments were determined with one-way ANOVA, followed by a post hoc test (Tukey’s test). All data are expressed as means ± SE.

RESULTS

Histological analysis confirmed that intracerebroventricular catheters were inserted into the fetal lateral ventricle in all sheep. There was no significant difference of maternal systolic and diastolic pressure, MAP, and heart rate among the control group and experimental groups treated with different doses of ANG II into the fetus (Table 1). There was no significant change in maternal systolic and diastolic pressure, MAP, and heart rate between before and after intracerebroventricular injections (baseline period vs. testing period) of either vehicle or ANG II (Table 1).

Fetal BP. In the control fetal group, there was no significant difference between the baseline and testing periods in systolic pressure [F(24,5) = 0.06], diastolic pressure [F(24,5) = 0.21], MAP [F(24,5) = 0.02], and adjusted MAP [F(24,5) = 0.34] (all P values: not significant). However, intracerebroventricular injection of ANG II (0.5 and 1.5 μg/kg) into the fetus increased fetal BP in the experimental fetuses. Fetal systolic pressure was significantly increased for 30 min after injections of 0.5 and 1.5 μg/kg ANG II [F(24,5) = 4.18 and 2.87, respectively, P < 0.05, Tukey’s test]. The low and high doses of intracerebroventricular ANG II increased fetal systolic pressure from the baseline levels, 39 ± 1 and 35 ± 2 mmHg, to 50 ± 3 and 52 ± 4 mmHg (P < 0.05), respectively, at 15 min after the injections. Fetal diastolic pressure was also enhanced from the baseline levels, 34 ± 1 and 34 ± 2 mmHg (low and high doses of ANG II, respectively), to 44 ± 3 and 48 ± 4 mmHg at 15 min after the intracerebroventricular administration of the peptide. MANOVA demonstrated a significant difference of systolic pressure between the fetuses treated with intracerebroventricular vehicle and ANG II [F(12,2) = 6.61, P < 0.01]. MANOVA (repeated measures) indicated a statistical differ-
ence of fetal MAP \( [F(12,2) = 4.12, P < 0.05] \) and adjusted fetal MAP \( [F(12,2) = 6.33, P < 0.01] \) among the control and experimental groups. Fetal adjusted MAP against amniotic cavity pressure was also enhanced within 10 min after intracerebroventricular injections of ANG II. The fetal MAP and adjusted MAP significantly increased for 10 min after intracerebroventricular administration of 0.5 \( \mu g/kg \) ANG II \( [F(24,5) = 3.40 \ and \ 4.55, \ both \ P < 0.05, \ Tukey's \ test] \). Intracerebroventricular 1.5 \( \mu g/kg \) ANG II increased fetal MAP, and adjusted MAP remained at significantly higher levels for 20 min after the injections \( [F(24,5) = 3.94 \ and \ 4.29, \ both \ P < 0.05, \ Tukey's \ test; \ Fig. 1] \).

**Fetal heart rate.** There was a significant difference indicated by MANOVA for fetal heart rates between the animals treated with intracerebroventricular ANG II and vehicle \( [F(12,2) = 7.78, P < 0.01] \). Intracerebroventricular injection of vehicle had no effect on fetal heart rate \( [F(24,5) = 0.94, P \ value: \ not \ significant] \). However, fetal heart rate was decreased after a change of BP after the injection of ANG II. A significant decrease of heart rate in the fetus was observed at the period between 20 and 30 min after the injection of 0.5 \( \mu g/kg \) ANG II \( [F(24,5) = 3.73, P < 0.05, \ Tukey's \ test] \) and between 20 and 60 min after the application of 1.5 \( \mu g/kg \) ANG II \( [F(24,5) = 3.83, P < 0.05, \ Tukey's \ test; \ Fig. 2] \).

**Fos immunoreactivity.** In the control fetuses after intracerebroventricular injection of vehicle, there were none or a few Fos-ir nuclei in the fetal basal forebrain and hypothalamic structures, including the putative pressor nuclei PVN. However, Fos-ir was significantly increased after the treatment with intracerebroventricular ANG II in the fetal forebrain. Positive Fos-ir nuclei were located in both the dorsal and ventral MePO \( (t = 14.47 \ and \ 7.66, \ respectively, \ both \ P < 0.05) \). Intense Fos-ir was observed in the PVN \( (t = 7.01, P < 0.05) \), including the parvicular part of the PVN \( (t = 7.14, P < 0.05) \) (Figs. 3 and 4). In the fetal hindbrain, there were few Fos-ir nuclei in the AP, NTS, LPBN, and CVLM after the injection of vehicle. However, intracerebroventricular injection of ANG II induced significant c-Fos expression in the AP \( (t = 19.29, P < 0.01) \).

### Table 1. Maternal arterial blood pressure and HR before and after intracerebroventricular injection of vehicle or ANG II into the fetal brain

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>5 min</th>
<th>15 min</th>
<th>20 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>105.0±5.5</td>
<td>105.6±5.1</td>
<td>108.3±4.7</td>
<td>106.9±5.0</td>
<td>106.0±4.8</td>
<td>109.8±5.4</td>
</tr>
<tr>
<td>Group 2</td>
<td>109.4±9.0</td>
<td>110.1±9.4</td>
<td>110.9±10.0</td>
<td>107.2±9.3</td>
<td>109.2±9.4</td>
<td>110.5±9.3</td>
</tr>
<tr>
<td>Group 3</td>
<td>105.0±5.5</td>
<td>105.6±5.1</td>
<td>108.3±4.7</td>
<td>106.9±5.0</td>
<td>106.0±4.8</td>
<td>109.8±5.4</td>
</tr>
<tr>
<td>DP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>66.7±4.2</td>
<td>67.1±4.0</td>
<td>68.2±4.0</td>
<td>68.2±4.1</td>
<td>66.6±4.2</td>
<td>68.8±3.6</td>
</tr>
<tr>
<td>Group 2</td>
<td>65.2±7.2</td>
<td>66.0±6.8</td>
<td>66.5±7.7</td>
<td>63.5±6.8</td>
<td>64.8±6.9</td>
<td>65.8±7.4</td>
</tr>
<tr>
<td>Group 3</td>
<td>66.7±4.2</td>
<td>67.1±4.0</td>
<td>68.2±4.0</td>
<td>68.2±4.1</td>
<td>66.6±4.2</td>
<td>68.8±3.6</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>81.5±6.8</td>
<td>81.0±6.3</td>
<td>79.9±8.0</td>
<td>80.1±7.2</td>
<td>81.9±5.8</td>
<td>78.9±7.6</td>
</tr>
<tr>
<td>Group 2</td>
<td>79.9±8.2</td>
<td>80.7±5.9</td>
<td>80.7±8.6</td>
<td>78.1±6.9</td>
<td>79.6±8.1</td>
<td>82.7±7.8</td>
</tr>
<tr>
<td>Group 3</td>
<td>79.5±5.7</td>
<td>79.9±6.7</td>
<td>81.6±5.2</td>
<td>81.1±6.2</td>
<td>79.7±5.8</td>
<td>82.1±5.9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>85.4±6.1</td>
<td>95.0±6.8</td>
<td>85.0±7.7</td>
<td>92.8±8.0</td>
<td>84.6±6.9</td>
<td>89.2±7.1</td>
</tr>
<tr>
<td>Group 2</td>
<td>79.2±7.7</td>
<td>81.2±7.2</td>
<td>80.6±7.4</td>
<td>81.9±6.4</td>
<td>82.0±7.3</td>
<td>81.8±7.9</td>
</tr>
<tr>
<td>Group 3</td>
<td>90.8±7.7</td>
<td>87.6±6.8</td>
<td>87.4±6.1</td>
<td>89.6±6.6</td>
<td>91.6±6.6</td>
<td>92.2±6.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Group 1, intracerebroventricular injection of vehicle; group 2, intracerebroventricular injection of ANG II (0.5 \( \mu g/kg \)); group 3, intracerebroventricular injection of ANG II (1.5 \( \mu g/kg \)); SP, systolic pressure; DP, diastolic pressure; MAP, mean arterial pressure; HR, heart rate. All \( P > 0.05 \) (no significance) compared with the baseline level.

---

Fig. 1. Effect of intracerebroventricular (icv) injection of vehicle or ANG II on the preterm fetal mean arterial pressure (MAP; \( A \)) and adjusted MAP (\( B \)) 0 min, time of intracerebroventricular injection. *\( P < 0.05 \) compared with the baseline level.
NTS ($t = 12.87, P < 0.01$), LPBN ($t = 8.7, P < 0.05$), and CVLM ($t = 12.24, P < 0.01$) (Figs. 3 and 5).

**AT$_1$ receptor immunoreactivity.** In the control staining, there was no positive immunoreactivity signal in either the PVN or SON in the preterm ovine brain. However, intense AT$_1$ receptor staining was shown in both the PVN and SON (Fig. 6). The positive AT$_1$ receptors were distributed all over these two nuclei in the brain of the preterm ovine fetus. All preterm fetal brains used showed a consistent pattern of positive AT$_1$ receptor signals in the hypothalamus.

**DISCUSSION**

This is the first study to investigate the cardiovascular effect of central ANG II in the preterm fetus. The results demonstrated that intracerebroventricular application of ANG II could produce reliable pressor responses in the preterm ovine fetus. Moreover, intracerebroventricular application of ANG II induced neural activation marked with Fos-ir in the fetal central cardiovascular pathways, indicating that the brain RAS is functional as early as at preterm. In addition, AT$_1$ receptors were demonstrated in the fetal PVN and SON.

Our recent study (36) has shown that intracerebroventricular application of ANG II caused an increase of systolic and diastolic pressure, MAP, and adjusted MAP in the near-term ovine fetus. In the present preterm fetal study, intracerebroventricular ANG II increased fetal systolic, diastolic, and mean arterial pressures, and adjusted MAPs were dose dependent. The RAS exists intact in both central and peripheral systems. Many studies have shown that central angiotensin-produced pressor responses occur within the first 10 min after the administration of ANG II in the adult and near-term fetal animal (12, 25, 32). The present study demonstrated that the fetal systolic pressures, diastolic pressures, MAP, and adjusted MAP increased within 10 min after the intracerebroventricular injection of ANG II. Thus the latency for the intracerebroventricular ANG II-stimulated pressor response in the preterm ovine fetus is comparable with that observed in the adult. Despite the fact that latency of the central angiotensin-induced cardiovascular response at preterm was similar to that in both the near-term fetus and the adult, a difference was noted in the time period of the ANG II-increased fetal blood pressure between the younger and older fetus. In the present preterm study, intracerebroventricular ANG II increased fetal BP, including systolic pressure and MAP, and adjusted MAP was kept at significantly higher levels within 20 min after intracerebroventricular administration of the peptide. However, a similar dose of intracerebroventricular ANG II elevated fetal BP, which remained at a significantly higher level for a longer period in the near-term fetus (36). This difference can be considered as demonstrating that the central angiotensin mechanism-regulated pressor responses in the preterm have not matured completely.

The change of fetal heart rate after intracerebroventricular injection of ANG II is interesting. Notably, fetal MAP was increased to peak levels within 10–20 min after intracerebroventricular application of ANG II. Fetal heart rate was decreased during the same period and was further decreased to significantly slower levels until 60 min after the intracerebroventricular injections. Whereas the pressor effect of intracerebroventricular ANG II has been well established, the response of heart rate to central ANG depends on species, action sites, injection routes, and other conditions (3, 4, 13, 20, 25). For example, an intracerebroventricular injection of ANG II agonist induced a dose-dependent increase in heart rate and arterial...
BP in conscious trout (20). ANG II also interacts with parasympathetic pathways to modulate the baroreflex control of heart rate. This accounts for its ability to increase BP without causing a reflex bradycardia in adults (25). However, injections of angiotensin into the hindbrain caused depressor and bradycardic effects (4). Administration of intracisternal bolus doses of ANG II produced short-duration dose-dependent increases in BP accompanied by bradycardia in conscious rabbits (13). In the present study, the peak of the pressor response was within 10–20 min after the intracerebroventricular injections and followed by the peak of the fetal bradycardia between 20 and 60 min. It was noted that central ANG II increased fetal MAP and adjusted MAP was associated with the bradycardia in the present study. This time-dependent pattern suggests that the fetal bradycardia is likely a subsequent event immediately after ANG II-induced hypertension. Thus the results indicate that fetal baroreflex has been functional in the face of the challenge at preterm and suggest a relative maturity of fetal sympathetic and parasympathetic systems in utero.

An important question of central angiotensin action during developmental stages is where the action sites in the brain are. The Fos experiments in the present study demonstrated cellular activation in the MePO and PVN in the fetal forebrain and the AP, LPBN, NTS, and CVLM in the fetal hindbrain. Autonomic mechanism is the major contribution to the increased fetal BP by intracerebroventricular injection of ANG II (6). Many nuclei and central regions that process signals for the control of BP have been identified in the adult. Presumably, every region and area in the pathway can potentially influence signal integration in the pressor net. However, certain nuclei with angiotensin receptors, especially AT1 receptors (14, 37), have been demonstrated to be particularly critical to cardiovascular responses. Most notable among these are the nuclei in the anterior third ventricle region, the MePO, and hypothalamus-PVN, particularly the parvicellular part of the PVN (34, 35, 37). The present results showed that intracerebroventricular ANG II induced FOS-ir in the dorsal and ventral MePO and in the PVN, including the parvicellular part. Associated with the

Fig. 4. Fos-ir induced by intracerebroventricular ANG II in the fetal MePO and PVN. A and B: MePO; C and D: PVN. A and C: the animal was injected with intracerebroventricular vehicle; B and D: the animal was treated with intracerebroventricular ANG II. AC, anterior commissure; 3V: third ventricle.
increased fetal MAP in the same animals at preterm, the FOS-ir data suggest that the critical areas in the central angiotensin-related sympathetic pathways for the regulation of cardiovascular mechanisms are functional during the preterm period in utero. The present study is the first to demonstrate that central angiotensin can stimulate c-Fos expression in the preterm fetal brain. Intense Fos-ir produced after the intracerebroventricular injection of ANG II was also observed in the AP, NTS, LPBN, and CVLM in the preterm fetal hindbrain. There is a possibility

Fig. 5. Fos-ir induced by intracerebroventricular ANG II in the fetal NTS and LPBN. A and B: LPBN. C and D: NTS. A and C: intracerebroventricular vehicle; B and D: intracerebroventricular ANG II. scp, Superior cerebella peduncle.

Fig. 6. Angiotensin subtype 1 receptor-immunoreactive signals in the supraoptic nuclei (SON) and PVN of the preterm ovine fetus. Left: SON; right: PVN.
that the increased BP and baroreflex may induce c-Fos expression in the brain (5, 16, 30). c-Fos expression induced by increased BP and baroreflex is mainly located in the hindbrain, particularly in the NTS, LPBN, and CVLM (5, 16, 22, 30). As the site of termination of baroreceptor inputs, together with the CVLM neurons, the NTS plays an important role in mediating the baroreflex. The c-Fos expression in those areas has been reported previously during acute elevations in arterial pressure induced by ANG II (22, 24). These studies have also shown the critical role of baroreceptor input in mediating central changes in neuronal activity when arterial pressure is acutely increased. In the present study, the increase of Fos-ir in the NTS and CVLM during intracerebroventricular application of ANG II is consistent with the concept that the NTS and CVLM are critical to angiotensin-mediated baroreflex. The Fos-ir in the fetal hindbrain support the finding that the fetal hindbrain structures in the central pathways start to function in the face of ANG II stimulation and/or ANG II-induced pressor in the hindbrain at preterm.

In situ receptor binding studies have demonstrated a widespread and heterogeneous distribution of angiotensin receptors and provided evidence for the existence of at least two subtypes, AT1 and AT2, in the brain (11, 27). In the adult brain, AT1 receptors predominate in the regulation of cardiovascular homeostasis (31, 32). By activating these receptors, central ANG II activates the “pressor” network. In the pressor net, a high level of AT1 receptor mRNA expression has been demonstrated in the adult hypothalamus (20). Almost all of the known biological effects of ANG II are mediated by AT1 receptors, whereas the exact physiological function of AT2 receptors remains unclear. A previous study (7) has indicated that the rat fetus expresses more AT2 than AT1 receptors in the brain. Despite this, few data exist indicating that the rat fetus expresses more exact physiological function of AT2 receptors remains unclear. A response induced by intracerebroventricular ANG II in the preterm is consistent with the concept that the NTS and CVLM are critical to angiotensin-mediated pressor mechanism is functional pathways located in the hypothalamus.

The results of the present study provide evidence that the central angiotensin-mediated pressor mechanism is functional as early as at preterm and that AT1 receptors are present in the fetal PVN and SON. The data suggest that the putative areas in the central sympathetic and parasympathetic pathways for pressor and baroreflex are relatively intact at preterm. The information gained provides insight that the development of central regulatory systems for angiotensin-mediated pressor responses start during fetal life as early as at preterm and that the in utero development of a RAS mechanism is important for fetal cardiovascular homeostasis.

ACKNOWLEDGMENTS

This study was conducted at the biomedical research facilities of the Research and Education Institute at Harbor-University of California-Los Angeles (UCLA) Medical Center.

GRANTS

This study was supported by a March of Dimes Research Grant, an External Research Grant from Philip Morris, Inc., and a UCLA Faculty Grant Program Award (to Z. Xu).

REFERENCES