Numerous earlier studies have shown that the central renin-angiotensin system (RAS) is as important as peripheral RAS in the control of body fluid and cardiovascular homeostasis. In adults, there is significant information regarding the effects of central and peripheral angiotensin (ANG) (8, 21). Administration of either ANG II or its precursors into the brain or the peripheral bloodstream produces a pressor response, induces drinking behavior, and causes release of adrenocorticotropic hormone and arginine vasopressin (AVP) (1, 8, 25). A link between peripheral administration of ANG II and AVP release in adult animals has been well established (9). The effect of intravenous ANG II on the hypothalamic-neurohypophysial system has been suggested via circumventricular organs (CVOs), which lack blood-brain barriers (BBB) (28). Furthermore, blockade of the ANG receptors can suppress AVP release into the circulation (3). Although there are a number of studies that have demonstrated the importance of ANG mechanisms in the regulation of AVP in adults, very little is known about the relationship between peripheral ANG and central AVP systems in the fetus. Addressing this question is important not only to prenatal life but also to postnatal health.

Previous fetal studies have demonstrated that an active RAS is present in the fetus before birth, and ANG II has been ascribed important functions in the fetus (11, 13, 14). Its major function appears to be maintenance of fetal arterial pressure under conditions of fetal stress, including acute hemorrhage or hypoxia (13). Recent findings from our (31) and others’ experiments (20) suggest that the central ANG mechanism is important in the control of fetal ingestive behavior and body fluid homeostasis in the near-term fetus in utero. Intracerebroventricular injection of ANG II significantly increased fetal blood pressure, associated with increased c-fos expression in the supraoptic nuclei (SON) without influence on fetal plasma ANG II levels. The fetal plasma AVP levels were also significantly increased by intracerebroventricular injection of ANG II (20). It was not surprising that central application of ANG peptide can act on the hypothalamic nuclei in the brain. However, whether ANG in the peripheral circulation would stimulate the central AVP system and cause AVP release in the fetus was understudied. Few data exist regarding effects of peripheral ANG on the fetal central nervous system (CNS), particularly on the hypothalamic-neurohypophysial system in utero. In the present study, we sought to determine effects of intravenous infusion of ANG II on fetal plasma AVP levels associated with plasma osmolality/sodium and hematocrit at near-term and to determine the influence of peripheral ANG on identified cells (e.g., AVP neurons), specific regions (e.g., SON), and potential connections (e.g., pathways) in the fetal brain with c-fos expression in testing the hypothesis that the fetal hypothalamic-neurohypophysial sys-

Intravenous angiotensin induces brain c-fos expression and vasopressin release in the near-term ovine fetus

Lijun Shi, Fang Hu, Paul Morrissey, Jiaming Yao, and Zhice Xu

Department of Obstetrics and Gynecology, Harbor-UCLA Medical Center and Research and Education Institute, Torrance, California 90502

Submitted 23 June 2003; accepted in final form 8 August 2003

tem and neural pathways between the CVOs and the hypothalamus have been functional during the late gestation.

METHODS

Time-dated pregnant ewes with fetuses (131 ± 3 days gestation on the study day; term for sheep is ~145 days) were used. Animals were housed in individual study cages and in a light-controlled room (12:12-h light-dark cycle) with food and water provided ad libitum. All surgical and experimental procedures had been approved by the Research and Education Institute’s Animal Care Committee.

Surgical preparation. Anesthesia was injected with ketamine hydrochloride (20 mg/kg im), and general anesthesia was maintained with 3% isoflurane and 1 L/min oxygen. The uterus was exposed by midline abdominal incision, and a small hysterotomy was performed to provide access to a fetal hindlimb and head, as reported (20, 31, 33). Polyethylene catheters (1.0 mm ID, 1.8 mm OD for the fetus) were placed in the maternal and fetal femoral vein and artery and advanced to the inferior vena cava and abdominal aorta, respectively. An intrauterine catheter (Corometrics Medical System, Wallingford, CT) was inserted for measuring amniotic fluid pressure. The fetus was then returned into the uterus, and the hysterotomy was closed in two layers. Immediately after surgery, buprenorphine (0.05 mg/kg) was subcutaneously injected around the incision on the ewe. All catheters were externalized to the maternal flank and placed in a cloth pouch. Animals recovered for 5 days after surgery. Immediately preoperatively and twice daily during the initial 2 days of recovery, gentamicin (8 mg) and oxacillin (33 mg) were administered intravenously to the fetus, and gentamicin (72 mg), oxacillin (1 g), and chloramphenicol (1 g) were injected intravenously into the ewe.

Physiological experiments. On the testing day, sheep were allowed a period of 60–100 min to be aclimatized to the testing rooms. When animal heart rates and arterial pressures appeared to be stable, a 60-min baseline was followed by 20 min of intravenous infusion and an additional 60-min period. Maternal and fetal blood samples were collected at ~30 and ~5 min of the baseline period and at 15, 30, and 60 min after intravenous infusion of ANG II or vehicle. All fetal blood samples (3 ml/sample) were replaced with equivalent volumes of heparinized maternal blood withdrawn before the study, and all maternal blood samples were replaced with equivalent volumes of isotonic saline. Blood samples were withdrawn from the fetal and maternal arterial catheters for measurements of blood PO2, PCO2, hemoglobin, and pH on a Radiometer BM 33 MK2-PHM 72 MKS acid-base analyzer system (Radiometer, Copenhagen, Denmark) adjusted to sheep internal temperature (39°C). Blood hematocrit was measured with a microcapillary reader. Plasma osmolality was measured by using freezing point depression on an Advanced Digimatic osmometer (model 3MO, Advanced Instruments, Needham Heights, MA). Plasma electrolyte concentrations were determined by a Nova 5 electrolyte analyzer (Nova Biomedical, Waltham, MA). All experiments were performed on the animals with fetal PO2 ≥ 18.0 and pH ≥ 7.3; otherwise, animals were not used for the studies. Animals were divided into control (n = 5) and experimental (n = 5) groups with computer-randomized selection. Beginning at time 0, ANG II (3.5 μg/kg, 10 ml; Sigma, St. Louis, MO) was infused intravenously to the experimental fetus over 20 min. The intravenous infusion rate and ANG II dose were selected according to previous reports (5, 19, 23) and our preliminary testing. For the control animals, the same volume of isotonic saline (vehicle) was infused intravenously. Maternal and fetal blood pressures were monitored during the testing period by means of a Beckman R612 physiological recorder (Beckman Instruments, Fullerton, CA). Blood pressure and heart rate were determined by computer analysis of waveforms by utilizing a customized pattern recognition algorithm.

Endocrine experiments. Maternal and fetal blood samples were collected into iced tubes containing lithium heparin during the baseline and study periods. Blood samples for AVP assays were centrifuged immediately. The plasma was then stored at −20°C before AVP radioimmunoassay. Plasma AVP concentrations were measured using Sep-Pak C18 cartridge (Waters Associates, Milford, MA) extraction. Samples for AVP extraction were acidified with 1 N HCl and extracted using a modification of the method (14). Acidified plasma samples were added slowly to the columns, and the columns were washed with 0.1% trifluoroacetic acid (TFA). The absorbed AVP was eluted with 50% methanol and 0.1% TFA, and the eluates were dried in a Speed-Vac concentrator. The sensitivity of our AVP antiserum is 1.2 pg AVP/tube with intra-assay and interassay coefficients of variation of 6 and 8%, respectively. AVP recoveries average 70% in our laboratory. All plasma samples were processed together.

Immunohistochemistry experiments: Fos immunoreactivity staining. At the end of the study, animals were anesthetized as described and ventilated with a mixture of isoflurane and oxygen. A middle abdominal incision was made, and the fetal head and neck were exposed. A 16-gauge needle was inserted into one side of the fetal carotid artery for perfusion. The fetuses were perfused via the carotid artery with 0.01 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer under anesthesia. The perfusion period was ~5–7 min, and the fetus was decapitated during perfusion. The brain was removed immediately following perfusion. Postfixation was performed in the same PFA solution for 12 h, after which the brain was placed in 20% sucrose in 0.01 M phosphate overnight. Twenty-micrometer coronal sections were cut through the fetal forebrain on a cryostat. Every other section of the SON and every third section of other parts of the forebrain were used for Fos immunoreactivity (Fos-IR) staining using the avidin-biotin-peroxidase technique (31, 33). The tissue sections were incubated for 20 min in the blocking serum (1:500; M PBS, followed by two washes in 0.01 M PBS (each for 5 min). AVP recoveries average 70% in our laboratory. All plasma samples were processed together.

Fos-IR and AVP-IR double labeling. To characterize the positive Fos-IR cells in the SON, the hypothalamic sections were first processed using the Fos staining procedure. They were then rinsed for 5 min in a 0.5% solution of H2O2 in 0.01 M PBS, followed by two washes in 0.01 M PBS (each for 5 min). To reduce nonspecific background staining, the sections were incubated for 20 min in the blocking serum (1:500; Vector Labs). The selected sections were then incubated in the AVP antibody (1:5,000; Diasorin) overnight, after which anti-rabbit antibody was applied. Detection of immunostaining was performed using a streptavidin-biotin detection system (Vector Laboratories) and nickel-diaminobenzidine as chromogen. The sections were dried, dehydrated in alcohol, and then coverslipped with histological mounting medium (National Diagnostics, Atlanta, GA).
Table 1. Fetal arterial values before and after intravenous infusion of vehicle or ANG II into the fetus

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct, %</td>
<td>(1) 27.38 ± 1.42</td>
<td>27.10 ± 2.06</td>
<td>27.50 ± 1.00</td>
<td>28.30 ± 0.98</td>
</tr>
<tr>
<td></td>
<td>(2) 27.16 ± 0.83</td>
<td>26.75 ± 1.33</td>
<td>27.50 ± 0.84</td>
<td>27.50 ± 1.17</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>(1) 7.98 ± 0.47</td>
<td>8.18 ± 0.44</td>
<td>7.96 ± 0.33</td>
<td>8.08 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>(2) 7.87 ± 0.39</td>
<td>7.85 ± 0.39</td>
<td>7.55 ± 0.46</td>
<td>7.55 ± 0.54</td>
</tr>
<tr>
<td>pH</td>
<td>(1) 7.38 ± 0.01</td>
<td>7.33 ± 0.02</td>
<td>7.35 ± 0.01</td>
<td>7.36 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>(2) 7.38 ± 0.01</td>
<td>7.37 ± 0.01</td>
<td>7.38 ± 0.01</td>
<td>7.38 ± 0.01</td>
</tr>
<tr>
<td>Po2, mmHg</td>
<td>(1) 21.33 ± 1.41</td>
<td>20.06 ± 2.14</td>
<td>21.78 ± 1.10</td>
<td>21.78 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>(2) 25.00 ± 1.39</td>
<td>23.78 ± 1.50</td>
<td>24.08 ± 1.34</td>
<td>22.43 ± 1.50</td>
</tr>
<tr>
<td>Pco2, mmHg</td>
<td>(1) 48.43 ± 1.35</td>
<td>51.92 ± 3.61</td>
<td>48.54 ± 1.26</td>
<td>47.90 ± 0.80</td>
</tr>
<tr>
<td></td>
<td>(2) 48.50 ± 2.68</td>
<td>46.85 ± 1.76</td>
<td>44.05 ± 1.79</td>
<td>45.95 ± 1.63</td>
</tr>
<tr>
<td>Osmolality, mosmol/kg</td>
<td>(1) 303.25 ± 2.07</td>
<td>303.40 ± 1.70</td>
<td>301.40 ± 0.64</td>
<td>301.40 ± 1.46</td>
</tr>
<tr>
<td></td>
<td>(2) 303.25 ± 3.11</td>
<td>300.75 ± 2.37</td>
<td>301.5 ± 0.91</td>
<td>302.63 ± 1.14</td>
</tr>
<tr>
<td>Na+, meq/l</td>
<td>(1) 142.96 ± 0.47</td>
<td>142.98 ± 0.57</td>
<td>142.32 ± 0.65</td>
<td>142.68 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>(2) 141.23 ± 0.69</td>
<td>141.40 ± 0.63</td>
<td>142.83 ± 1.10</td>
<td>141.35 ± 0.52</td>
</tr>
<tr>
<td>K+, meq/l</td>
<td>(1) 3.67 ± 0.17</td>
<td>3.79 ± 0.15</td>
<td>3.62 ± 0.16</td>
<td>3.58 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>(2) 3.92 ± 0.10</td>
<td>3.95 ± 0.15</td>
<td>4.01 ± 0.19</td>
<td>3.92 ± 0.16</td>
</tr>
<tr>
<td>Cl−, meq/l</td>
<td>(1) 110.78 ± 1.67</td>
<td>110.80 ± 0.91</td>
<td>110.74 ± 1.03</td>
<td>109.34 ± 1.04</td>
</tr>
<tr>
<td></td>
<td>(2) 110.18 ± 0.88</td>
<td>109.98 ± 0.36</td>
<td>109.98 ± 1.02</td>
<td>110.75 ± 0.88</td>
</tr>
</tbody>
</table>

Values are means ± SE. (1), iv infusion of ANG II (3.5 μg/kg); (2), iv infusion of vehicle (saline). Hct, hematocrit; Hb, hemoglobin.

Data analysis. The numbers of Fos-IR-positive cells in the median preoptic nucleus (MnPO), the organum vasculosum of the lamina terminalis (OVLT), the subfornical organ (SFO), and the SON were counted in a blinded manner as reported (31, 33). Repeated-measures ANOVA was used to determine differences over time and effects of the treatments. Comparison before and after the treatments was determined by one-way ANOVA followed by Tukey’s test or a t-test. All data are expressed as means ± SE.

RESULTS

Blood values. There was no significant difference in arterial blood pH, Po2, Pco2, hemoglobin, and hematocrit before or after intravenous infusion of ANG II (Table 1). For both the control and the experimental animals, intravenous infusion of ANG II or vehicle had no effect on plasma osmolality or sodium levels (F8,1 = 0.41 and 1.94, respectively, both P = not significant) in the fetal animals. Fetal Cl− and Na+ concentrations were not changed between the control and the experimental groups. All arterial values were within normal ranges and did not vary significantly between the control and experimental groups (all P = not significant).

In maternal animals, intravenous infusion of ANG II or vehicle into the fetus had no effect on plasma osmolality or sodium levels (F8,1 = 0.72 and 1.59, respectively, both P = not significant). Maternal plasma Cl− and K+ concentrations, blood pH, Po2, and Pco2 were not significantly changed between the control and experimental groups (all P = not significant).

There was no difference in maternal mean arterial pressure between the control and experimental groups (P = not significant). Fetal mean arterial pressure (F8,1 = 27.80, P < 0.01) was increased, and heart rate (F8,1 = 12.51, P < 0.01) was decreased after administration of ANG II.

Plasma AVP assay. There was no difference in plasma AVP levels between the control and the experimental ewes before intravenous ANG II infusion (F8,1 = 0.36, P = not significant). However, the fetal AVP concentrations were significantly higher in the ANG II-infused fetuses than in the vehicle-treated animals (F8,1 = 29.7, P < 0.01). In the control group, intravenous infusion of vehicle did not change the maternal and fetal plasma AVP levels (F20,4 = 0.98 and 0.43, respectively, P = not significant, baseline period vs. period after iv infusion). The intravenous ANG II significantly increased fetal plasma AVP levels (F20,4 = 15.4, P < 0.01, baseline period vs. the period after iv infusion; Fig. 1). Fetal plasma AVP increased significantly within 15 min (from 3.47 ± 0.21 to 8.57 ± 0.75 pg/ml) after intravenous infusion of ANG II, and the peak level of plasma AVP was observed at 30 min (11.42 ± 1.23 pg/ml) after intravenous infusion of ANG II. There was no change in maternal plasma AVP after ANG II infusion into the fetus.

Fos-IR and AVP-IR. In the control fetuses infused with the vehicle, there was little or no Fos-IR in the fetal basal forebrain structures. However, intravenous

Fig. 1. Effect of intravenous (iv) infusion of vehicle or ANG II (3.5 μg/kg) on fetal plasma arginine vasopressin (AVP) levels. *P < 0.01 compared with baseline level.
infusion of ANG II (3.5 μg/kg) induced Fos-IR in several regions in the fetal forebrain, including the SON in the hypothalamus. There was a significant difference of Fos-IR in the SON between the intravenously vehicle- and the intravenously ANG II-infused fetuses (t = 7.04, P < 0.01; Figs. 2 and 3). Fos-IR was observed in all regions of the SON, including the dorsal and ventral parts. Fos-IR induced by the intravenous infusion of ANG II was also restricted in the MnPO, the OVLT, and the SFO in the fetus (dorsal MnPO: t = 11.29; ventral MnPO: t = 9.0; OVLT: t = 9.83; SFO: t = 29.4, all P < 0.01).

Positive AVP-IR was detected in the SON at near-term in the ovine fetus. In the control fetus, little Fos-IR was present in the SON, and there was no colocalization of AVP-IR and Fos-IR in the fetal SON. However, the intravenous ANG II increased Fos-IR in the bilateral SON. Many positive Fos-IR were in coexistence with the supraoptic AVP-IR neurons. There was no difference in the total counting of AVP-IR in the SON between the control and the treated fetuses (t = 3.25, P = not significant). Double labeling of Fos-IR and AVP-IR was significantly higher in the ANG II-infused fetuses than in the control animals (t = 6.14, P < 0.001; Fig. 4). Two-thirds of the AVP-IR-positive neurons in the SON were labeled with Fos-IR in the fetuses treated with intravenous ANG II. However, quite a few positive Fos-IR cells in the SON were not AVP-containing neurons.

DISCUSSION

The present study provides new information that intravenous ANG II can cause fetal AVP release at near-term and that ANG II in the peripheral circulation can act at the fetal CNS and activate the AVP neurons at 90% gestational age in utero. The data indicate that ANG II in the fetal peripheral circulation may play an important role in central neuroendocrine regulation.

A previous fetal study (10) has shown that there is a fetal cardiovascular response to peripherally administered ANG II during the last third of gestation. Although the studies in adults have demonstrated that the intravenous infusion of ANG II effectively stimulated the AVP release (9), it remains unknown whether intravenous or peripheral ANG II can influence the fetal hypothalamic-neurohypophysial system in the brain in utero. Central injections of ANG II have been shown to increase plasma AVP in the near-term ovine fetus (20). Despite this information, little is known about the effect of fetal peripheral ANG on the developing brain and vasopressinergic pathways in utero. The results in the present study show that the fetal plasma AVP concentrations were changed within 15 min after an intravenous infusion of ANG II, and the increased level was more than threefold at 30 min after the infusion.

![Fig. 2. Effect of iv ANG II on Fos immunoreactivity (Fos-IR) induced in the fetal forebrain. SFO, subfornical organ; MnPO, median pre-optic nucleus; OVLT, organum vasculosum of the lamina terminalis; SON, supraoptic nuclei. *P < 0.01.](http://ajpendo.physiology.org/)

![Fig. 3. Fos-IR induced by intravenous ANG II in the SON. A: control fetus infused with vehicle. B: fetus infused with ANG II (3.5 μg/kg). Magnification ×20.](http://ajpendo.physiology.org/)
The peripheral and central actions (4, 26) of AVP complement each other and highlight the importance of this hormone in regulating body fluid balance. It is well known that hypertonicity is a potent stimulus in the control of AVP secretion and release (6, 7). Hyper tonic saline infusions into maternal ewes increase both maternal and fetal plasma osmolality and stimulate fetal AVP secretion (33). Direct application of hypertonic NaCl intravenously into the fetus also increases both plasma osmolality and sodium concentrations, which results in activation of the fetal AVP neurons associated with an increase of plasma AVP levels at near-term (33). In the present study, the fetal physiological status remained stable under the condition of intravenous infusion of ANG II, as arterial values (pH, PCO2, PO2, and plasma electrolytes) were not changed. Particularly, the unchanged fetal plasma osmolality and sodium levels indicate that osmotic mechanisms are unlikely to have been involved in the intravenous ANG II induction of the fetal AVP release in the present study.

AVP is also released in response to fluid volume change (8). Hypotension and hypovolemia provoke an increase of AVP. Typically, hemorrhage-increased plasma AVP has been demonstrated (29). In the present study, fetal blood hematocrit and hemoglobin were not influenced by the exogenous ANG II in the circulation, indicating an unchanged fluid volume. There was no difference between the control and the experimental ovine fetuses, as both animals were infused with the same volume of vehicle or ANG II solution. Therefore, the results do not support the possibility that a potential fluid volume change under the condition of the treatments may contribute to AVP release in utero. In addition, it is well established that hypotension facilitates whereas hypertension inhibits AVP release (12). The fetal blood pressure was increased following intravenous infusion of ANG II. This does not agree with the notion that the alteration of fetal blood pressure may be a cause to influence fetal plasma AVP levels.

In addition to hypertonicity and fluid volume mechanisms, an important mediator of the rise in plasma AVP is ANG mechanisms. An increase of AVP concentrations is facilitated by both central and peripheral ANG II (8, 9, 16). The intravenous ANG II evoked AVP release in adult sheep (9), likely by stimulation of the hypothalamic-neurohypophysial system via CVOs (e.g., SFO, OVLT) (28). These central nuclei lack a BBB and are accessible to the peripheral hormonal signal. In the present study, a significant increase of the fetal plasma AVP concentrations after intravenous ANG II administration suggests that central vasopressinergic pathways may participate in the AVP response.

Previous studies by our laboratory (31) demonstrated that intracerebroventricular injection of ANG II in near-term ovine fetuses caused c-fos expression in the hypothalamus and that fetal plasma AVP levels were also significantly increased. It was not a surprise that central application of the ANG peptide can act at the central AVP system in the brain. However, whether ANG from the peripheral side would stimulate the AVP neurons inside the fetal brain was unknown. Thus those studies were the first to demonstrate that intravenous ANG II infusion not only increases fetal plasma AVP levels but also induces cellular activation marked with c-fos expression in the fetal CNS.

ANG II is a polar molecule unable to penetrate the BBB under normal physiological conditions (8). Although it was argued that the BBB mechanisms in the developing brain might be different in important respects from those in the adult brain, it is noted that the underlying morphological feature of the barrier is the presence of tight junctions that are located between cerebral endothelial cells and between choroid plexus epithelial cells. These junctions are present in blood vessels in the fetal brain. They are effective in restricting the entry of proteins from blood into the fetal brain and cerebrospinal fluid, at least at 90% of gestational
age (22). A previous study demonstrated that the BBB is relatively impermeable to low-molecular-weight amino acids even at 60% of gestation in sheep (24). Therefore, it is likely that some structures that lack the BBB in the brain may play a critical role in the integration of the signals in the control of AVP neurons.

Many ANG II-sensitive neurons have been found to be present in highly vascularized nervous structures, the CVOs, where the BBB is deficient. They are therefore accessible to circulating polar molecules such as ANG II. There are many ANG II-sensitive receptors in the CVOs, particularly in the OVLT and SFO (8). The special character of the BBB and the richness of ANG receptors in the CVOs provide anatomic and pharmacological conditions in response to the ANG from the circulation. The CVOs have many connections with other regions in the brain. The SFO and OVLT project directly or via the MnPO and periventricular tissue, known as the anterior third ventricular region, to the preoptic, anterior hypothalamic, and limbic structures in the control of body fluid and cardiovascular homeostasis. Destruction of these CVO structures in adult animals impairs neuroendocrine functions such as those related to vasopressinergic and oxytocinergic systems (12, 15, 17). In the present study, we have found that Fos-IR were restricted in the OVLT and SFO after intravenous infusion of ANG II in the unanesthetized fetal animals. This suggests that the fetal brain, particularly the fetal CVOs, is functional in the face of the hormonal signal from the periphery, at least at near-term. Interestingly, supraoptic cells in the bilateral SON also expressed c-fos. It is well known that the hypothalamic neurons are the main source contributing to AVP concentration in the bloodstream (2, 27, 30), and a number of cells in the SON are AVP-containing neurons.

On microscopic inspection, all regions of the bilateral SON, including dorsal and ventral parts, expressed positive c-fos after intravenous ANG II. Detailed analysis revealed that two-thirds of AVP cells in the fetal SON were colocalized with Fos-IR. These results are, to our knowledge, the first to demonstrate that peripheral ANG II can induce c-fos expression in AVP neurons in the fetal hypothalamus. Previous studies have demonstrated that c-fos induced by central ANG II in the adult rat hypothalamus can occur in oxytocin neurons (32). For example, in the rat SON, colocalizations of oxytocin and c-fos, or AVP and c-fos, were 80 and 60%, respectively (32). The distribution of Fos-IR throughout the dorsal SON in the present study suggests that the oxytocin neurons may be candidates for the activated non-AVP cells. Further studies are needed to identify these fetal ANG II-activated cells during early development.

Functional and retrograde tracing studies have shown the anatomic connections between the forebrain CVOs and the hypothalamic SON in adult animals (18, 34). The SFO and OVLT have projections to the MnPO. All of these three brain structures have anatomic projections to the hypothalamus (8). Whether the fetal pathways between the SFO/OVLT and the SON have been developed remained a question. Although very little information exists regarding the neural development of these important pathways in the fetal brain, the results gained in the present study support the hypothesis that the pathway between the forebrain CVOs and the hypothalamus has been relatively intact and has functioned by near-term, as evidenced by the peripheral ANG II-induced cellular activation in the hypothalamic AVP neurons associated with an increase of fetal plasma AVP concentrations. Notably, the SFO, OVLT, and MnPO were also activated by peripheral ANG II with c-fos expression. When it is considered that a relatively complete BBB has developed in the fetal brain at near-term, it appears that the CVOs may act as signal-integrated centers for the relationship between ANG II in the circulation and the cellular activation in the hypothalamic-neurohypophysial system in utero. However, further studies are needed to confirm the ontogeny of neural pathways between the CVOs and the hypothalamus.

In conclusion, the results demonstrate that the fetal AVP neurons in the SON are activated by intravenous ANG II in association with increased plasma AVP. It is very likely that peripheral ANG II acts on the fetal supraoptic AVP neurons via the forebrain CVOs that lack the BBB. In light of this, the data support the hypothesis that pathways from the SFO, OVLT, and MnPO to the AVP-containing neurons in the hypothalamus have been established and are functional during late gestation. The present study provides novel information for understanding the development of the central hypothalamic-neurohypophysial system in response to peripheral ANG II in utero.

These studies were conducted at the biomedical research facilities of the Research and Education Institute at Harbor-UCLA Medical Center. We thank Sicheng Xu for technical support.

DISCLOSURES

The research described in this article was supported by a March of Dimes research grant and an External Research Grant from Philip Morris, USA, Inc., to Z. Xu.

REFERENCES