Angiotensin II decreases system A amino acid transporter activity in human placental villous fragments through AT1 receptor activation

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THE CAUSES of fetal intrauterine growth restriction (IUGR) are poorly understood; however, there are likely to be multiple causes, including disturbances of nutrient supply (3, 4, 49). In the second half of pregnancy, an increase in the overall transport capacity of the placenta is important to supply the demands of the growing fetus. Several lines of evidence (38, 45) suggest that amino acid transport from mother to fetus is fundamental for normal fetal growth. Amino acids transported to the fetal circulation are used not only for protein synthesis but also as a metabolic energy source. Indeed, 20–40% of the total energy for fetal growth supplied from the maternal circulation derives from amino acids (3).

The system A amino acid transporter, also known as the Na+/H+ antiporter, mediates transport of neutral amino acids, such as alanine, serine, glutamine, and glycine, in an Na+-coupled fashion (11, 24). System A transporter activity is reduced in the placenta of IUGR pregnancies (12, 15, 16, 19, 21, 34), but this does not appear to be due to reduction in placental expression of mRNA for system A (35). A recent study in rats (8) demonstrated that competitive inhibition of system A amino acid transport by α-(methylamino)isobutyric acid (MeAIB) results in decreased fetal growth, consistent with reduced amino acid transport as a cause, rather than just an effect, of this pregnancy condition. Consistent with impaired placental amino acid transport as a potential cause of IUGR, cord blood (but not maternal blood) concentrations of most essential amino acids are significantly decreased in pregnancies with IUGR fetuses compared with uncomplicated pregnancies with appropriately grown fetuses (2, 5).

The renin-angiotensin system (RAS) is thought to play a crucial regulatory role in the fetoplacental circulation, facilitating adequate placental blood flow for fetal oxygenation and nutrition (40). Apart from the traditional hormonal (circulating) RAS involved in the regulation of blood pressure and salt and fluid homeostasis, the essential components of RAS (including renin, angiotensinogen, angiotensin converting enzyme, and angiotensin receptors) have been identified in many tissues, including the placenta, suggesting that local RAS may modulate placental function (40, 43). The angiotensin II type 1 receptor (AT1-R) predominates over the angiotensin II type 2 receptor (AT2-R) in the human placenta (25, 29, 32), and AT1-R is abundantly present in syncytiotrophoblasts (30, 31, 32), the placental cells responsible for amino acid transport. AT1-R immunoreactivity in normal pregnancy syncytiotrophoblast has a negative correlation with infant birth weight, suggesting that AT1-R signaling is involved in the regulation of fetal growth (30). There is evidence for involvement of altered placental RAS in the pathophysiology of IUGR (28, 29, 37, 47). AT1-R gene expression is reduced in syncytiotrophoblasts of pregnancies with IUGR fetuses, potentially reflecting chronic activation of RAS (28, 32). Concentrations of angiotensin II in umbilical cord blood are increased in IUGR cases compared with appropriate-for-gestational-age infants, but fe-
top placental vascular AT1-R concentrations are not significantly altered, as would be expected from the elevated angiotensin II levels, consistent with inappropriate activation of fetal RAS in IUGR (28). In contrast to idiopathic IUGR, syncytiotrophoblast AT1-R expression is enhanced in preeclampsia, a hypertensive pregnancy disorder frequently accompanied by IUGR (31).

Na\textsuperscript{+}-K\textsuperscript{+}-ATPase provides an essential driving force for Na\textsuperscript{+}-dependent amino acid transport systems, including system A, by pumping Na\textsuperscript{+} out of cells (22). Indeed, Na\textsuperscript{+}-K\textsuperscript{+}-ATPase inhibitors reduce Na\textsuperscript{+}-dependent transport system activity by accumulating Na\textsuperscript{+} within the cells (22, 42). Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is abundant in the microvillous plasma membrane of syncytiotrophoblast (22). Recent studies (4, 23) have demonstrated that Na\textsuperscript{+}-K\textsuperscript{+}+ATPase activity is decreased in IUGR. Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity is reportedly reduced by angiotensin II/AT1-R activation in several cell types, including glomerulosa cells and enterocytes (18, 33, 36, 48).

Despite these data, the effects of angiotensin II on system A amino acid transport activity and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in villous placenta have not been determined. Therefore, we investigated whether angiotensin II alters system A amino acid transport activity and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in individual placental villous fragments via AT1-R binding. We also compared the concentration-response effects of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase inhibitor, ouabain, on system A amino acid transport and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activities in villous tissue. Given the known stimulatory effects of angiotensin II on cellular production of superoxide and other reactive oxygen species (17, 44), we secondarily sought to determine whether antioxidants would reverse the effects of angiotensin II on system A activity.

**MATERIALS AND METHODS**

**Buffers and reagents.** All experiments with villous tissue were carried out using Tyrode’s buffer, as previously described by Jansson et al. (20). Tyrode’s buffer consisted of 135 mM NaCl (or 135 mM choline chloride for sodium-free Tyrode’s buffer), 5 mM KCl, 1.8 mM CaCl\textsubscript{2}, 1.0 mM MgCl\textsubscript{2}, 10 mM HEPES, and 5.6 mM glucose, pH 7.4 (adjusted with NaOH for sodium-containing buffer or KOH for sodium-free buffer). All tissue incubations and dissections were carried out in a buffer consisting of 1 volume of Dulbecco’s modified Eagle’s medium (DMEM) mixed with 3 volumes of Tyrode’s/DMEM (1:3) buffer. Villous fragments were washed twice with 1 ml volume of sodium-containing (-free for nonspecific uptake) Tyrode’s buffer containing [14C]MeAIB (1.7 μCi/ml, 0.05 μM) for 20 min at 37°C. The uptake of [14C]MeAIB was stopped by rinsing (washing) villous fragments in ice-cooled sodium-free Tyrode’s buffer two times for 20 s each. Villous fragments were incubated in 1 ml of distilled H\textsubscript{2}O for 18 h to release the accumulated [14C]MeAIB. After villous fragments were removed, scintillation fluid was added to the aqueous supernatant and mixed and the radioactivity counted. Each villous fragment was incubated in 0.2 ml of 0.3 N NaOH overnight, and the total protein amount was determined by the Bradford method. Total pmol of [14C]MeAIB accumulated was calculated from the radioactive value and normalized to the protein content of its respective villous fragment. System A activity was calculated by subtracting the uptake in sodium-free medium from the uptake in sodium-containing medium and expressed as pmol [14C]MeAIB·mg protein\textsuperscript{-1}·20 min\textsuperscript{-1}.

System A activity assays were completed within 4 h after delivery to avoid significant loss of normal morphology and functional integrity of the villous trophoblast (20, 27, 50). The accumulation of lactate dehydrogenase (LDH) diagnostic from Syncytiotrophoblast (50 μM) was used to estimate cell damage. LDH data were expressed as percentage of maximal releasable LDH, with the latter determined for each experiment as follows. Briefly, after 60 min of preincubation with or without angiotensin II or other effector reagents, the villous fragments were sonicated, 1% (final concentration) of Triton X-100 was added, and the mixture was then vortexed and incubated for 10 min to ensure complete cell lysis. After centrifugation of particulate matter (300 g, 10 min), LDH and protein concentration in the supernatant were measured to obtain the value for maximal LDH per milligram of protein.

**Treatment by effector reagents.** Insulin was used as a positive control for stimulation of system A activity (final concentration 300 ng/ml (20, 26). Angiotensin II stock solution was diluted in 1 ml volume of Tyrode’s/DMEM (1:3) buffer (100 nM). To observe the dose-dependent response of system A to angiotensin II, angiotensin II was diluted to final concentrations as follows: 3, 10, 30, 100, and 500 nM. These concentrations are thought to be appropriate given the relatively high local angiotensin II concentration found in placenta (40). AT1-R blockers [losartan (2 μM) and AT1-R anti-peptide (50 μM)] and an AT2-R blocker [PD-123319 (50 μM)] were used to assess whether angiotensin II receptor activation is involved in the decrease of system A activity. Phenylephrine [α\textsubscript{1}-adrenergic receptor agonist (10 μM)] and U-46619 [thromboxane receptor A\textsubscript{2} antagonist (10 μM)] were used to test AT1-R-independent vasconstrictor effects on system A activity. To investigate the role of oxidative stress on angiotensin II’s effect on system A activity, villous fragments were preincubated with antioxidants, either vitamin C (water soluble vitamin, 100 μM), vitamin E (lipid-soluble vitamin, 100 μM), Tiron...
was linear system A activity in villous fragments from normal placentas. Ouabain-sensitive $Na^+\cdot-K^+$-ATPase activity was measured using the established method of Clarson et al. (7), with some modifications. Villous fragments were preincubated with or without ouabain (1 mM) or other effector reagents. After preincubation, the villous fragments were washed twice with 1 ml volume of sodium-containing Tyrode’s buffer at 37°C for 1 min. Subsequently, the uptake of $^{86}$Rb (1 μCi/ml) as an indicator of $Na^+\cdot-K^+$-ATPase activity was performed in sodium-containing Tyrode’s buffer containing unlabeled MeAIB (1.7 nmol/ml) for 20 min at 37°C. Unlabeled MeAIB was added to keep experimental conditions similar between experiments. The uptake of $^{86}$Rb was stopped by rinsing (washing) villous fragments in ice-cooled sodium-containing Tyrode’s buffer for 10 s. Villous fragments were incubated in 1 ml of distilled $H_2O$ for 18 h to measure for 20 min (i.e., that in sodium-free, choline-containing medium).

RESULTS

System A activity is linear $\approx 180$ min. As shown in Fig. 1, system A activity in villous fragments from normal placentas was linear $\approx 180$ min, after which $Na^+$-dependent uptake (pmol $^{14}$CMeAIB/mg protein) began to plateau. All subsequent experiments were performed with an incubation time of 20 min for the uptake of $^{14}$CMeAIB. None of the agents used in the study significantly altered the nonspecific uptake measured for 20 min (i.e., that in sodium-free, choline-containing medium).

Insulin increases, whereas angiotensin II decreases, placental system A activity. Insulin is known to have a positive effect on placental system A activity and, therefore, was used as a positive control (3). As shown in Fig. 2, a 1-h preincubation with insulin (final concentration 300 ng/ml) increased system A activity (pmol $^{14}$CMeAIB/mg protein)$^{-1}\cdot20$ min$^{-1}$ (means ± SD); control $30.7 \pm 6.8$ vs. insulin $40.5 \pm 3.3$, $P < 0.01$ ($n = 6$ placentas)). In contrast, a 1-h preincubation with angiotensin II (100 nM, $n = 12$) decreased system A activity (pmol $^{14}$CMeAIB/mg protein)$^{-1}\cdot20$ min$^{-1}$; control $30.7 \pm 6.8$ vs. angiotensin II $20.7 \pm 3.3$, $P < 0.001$ ($n = 12$ placentas)). As depicted in Fig. 3A, the decrease in system A activity in response to angiotensin II was dose dependent; system A activity decreased by $-2.9$ pmol MeAIB$\cdot$mg protein$^{-1}\cdot20$ min$^{-1}$ for each increase in dose of angiotensin II ($P < 0.0001$).

Angiotensin II did not increase LDH release into the medium, either expressed as a percentage of maximal releasable LDH (Fig. 3B) or as absolute LDH values (data not shown), indicating that the effect of exogenous angiotensin II is not related to loss of cell viability or extensive cell membrane damage. Angiotensin II treatment minimally increased LDH release in a time-dependent fashion, studied $\approx 6$ h (data not shown).

Suppression of system A activity by angiotensin II is mediated by the AT1-R. AT1-R blockers [losartan (2 μM) and AT1-R anti-peptide (50 μM)] and an AT2-R blocker [PD-123319 (50 μM)] were used to investigate the involvement of angiotensin II receptor activation. As shown in Fig. 4, losartan and AT1-R anti-peptide inhibited the negative effect of angiotensin II on system A activity, but PD-123319 had no effect (pmol $^{14}$CMeAIB/mg protein)$^{-1}\cdot20$ min$^{-1}$ ($n = 6$ placentas); control $28.3 \pm 3.2$; angiotensin II $19.8 \pm 3.3$ ($P < 0.01$ vs. control); losartan + angiotensin II $30.0 \pm 4.5$ ($P = 0.77$ vs. control); AT1-R anti-peptide + angiotensin II $31.2 \pm 6.9$ ($P = 0.71$ vs. control); PD-123319 + angiotensin II $19.7 \pm 3.2$ ($P < 0.01$ vs. control)]. To test for effects of endogenous angiotensin II on system A transport activity, villous fragments from six placentas were also incubated with losartan alone (2 μM). Losartan itself did not alter system A activity (control $28.3 \pm 3.2$ vs. losartan $30.3 \pm 5.2$, $P = 0.90$).

AT-R-independent vasoconstrictors do not affect system A activity. The $\alpha_1$-adrenergic receptor agonist phenylephrine (10 μM) and the thromboxane $A_2$ mimetic U-46619 (10 μM) were
used to rule out vasoconstriction per se as a mechanism for decreases in system A activity of villous fragments. As shown in Fig. 5, neither agonist altered system A activity.

Antioxidants do not prevent angiotensin II-mediated reduction of system A activity. Villous fragments were preincubated with antioxidants [either vitamin C (water-soluble vitamin; 100 μM), vitamin E (lipid-soluble vitamin; 100 μM), Tiron (disulfonic acid, a superoxide scavenger; 100 μM), or DPI (an NADPH oxidase inhibitor; 100 μM)] prior to incubation with angiotensin II (100 nM). Antioxidants had no effect on baseline system A activity (data not shown). As shown in Fig. 6, preincubation with these antioxidants did not prevent the angiotensin II-mediated decrease in placental system A activity.

Ouabain-sensitive Na\(^{+}\)-K\(^{+}\)-ATPase activity in villous explants. Ouabain-sensitive Na\(^{+}\)-K\(^{+}\)-ATPase activity of villous explants was measured at 10, 20, and 40 min using villous fragments from three normal placentas. As shown in Fig. 7A, ouabain-sensitive Na\(^{+}\)-K\(^{+}\)-ATPase activity, calculated as the difference between \(^{86}\)Rb uptake (nmol \(^{86}\)Rb/mg protein) in

Fig. 2. Insulin increases and angiotensin II (ANG II) decreases system A activity of villous fragments. Data are means ± SD (n = 12 control, n = 5 insulin, 300 ng/ml; n = 12 ANG II, 100 nmol/l). *P < 0.01; **P < 0.001 vs. control.

Fig. 3. ANG II decreases system A activity in a dose-response fashion without affecting cell viability. A: dose-response effect of ANG II on system A activity (n = 6). B: % release of lactate dehydrogenase (LDH) in response to ANG II (n = 3). Data are means ± SD. *P < 0.05; **P < 0.01 vs. untreated control.

Fig. 4. The effect of ANG II on system A activity is dependent on activation of the ANG II type 1 receptor (AT1-R). ANG II = 100 nmol/l; losartan = 2 μM; AT1-R blocking peptide (AT1-R BP) = 50 μM; and PD-123319 = ANG II receptor inhibitor. Data are means ± SD (n = 6 placentas/treatment). *P < 0.01 vs. control.

Fig. 5. Vasoconstrictors phenylephrine and U-46619 do not affect system A activity. ANG II = 100 nmol/l; phenylephrine = 10 μmol/l; and U-46619 = 10 μmol/l. Data are means ± SD. *P < 0.01 vs. control.
ouabain-free medium and in ouabain-containing (1 mM) medium (nonspecific uptake), was linear for at least 40 min. All subsequent experiments were performed with an incubation time of 20 min for the uptake of $^{86}$Rb.

**Angiotensin II decreases placental Na\(^+\)-K\(^+\)-ATPase activity by an AT1-R-dependent mechanism.** As shown in Fig. 7B, angiotensin II (100 nM) decreased ouabain-sensitive Na\(^+\)-K\(^+\)-ATPase activity. In contrast, Na\(^+\)-K\(^+\)-ATPase activity was maintained when explants were incubated with angiotensin II and losartan (2 μM). Unlike losartan, however, coincubation with PD-123319 (50 μM) did not prevent the inhibitory effect of angiotensin II [nmol $^{86}$Rb·mg protein \(^{-1}\)·20 min \(^{-1}\); control 73.8 ± 13.4 vs. angiotensin II 48.3 ± 12.3 (P < 0.05); control vs. losartan + angiotensin II 69.1 ± 10.7 (P = 0.54); control vs. PD-123319 + angiotensin II 55.3 ± 14.7 (P < 0.05) (n = 7 placenta each)].

**Ouabain decreases both system A and Na\(^+\)-K\(^+\)-ATPase activities.** The relationship between Na\(^+\)-K\(^+\)-ATPase and system A activities was further explored by examining the dose response effect of ouabain on these two transport systems (n = 4 placenta). As shown in Fig. 8, A and B, system A and Na\(^+\)-K\(^+\)-ATPase activities, respectively, displayed similar ouabain dose-inhibitory response profiles. Furthermore, the degree of reduction of system A and Na\(^+\)-K\(^+\)-ATPase by 100 nM angiotensin II (37.2 vs. 44.2% of maximal response to ouabain, respectively) was not different (Fig. 8, A and B).

**DISCUSSION**

The present study demonstrates that angiotensin II decreases system A amino acid transport activity of term villous placenta through AT1-R activation. AT1-R activation by angiotensin II also decreased ouabain-sensitive Na\(^+\)-K\(^+\)-ATPase activity, and it appears that the effect of angiotensin II on placental system A activity is primarily mediated through angiotensin II’s negative effect on Na\(^+\)-K\(^+\)-ATPase activity. These findings lead us to propose that angiotensin II may be involved in the regulation of amino acid transport across the syncytiotrophoblast plasma membrane and that overactivation of the uteroplacental RAS affects fetal growth in part by limiting amino acid transport.

The technique of using single, isolated, villous fragments to study amino acid uptake has several potential advantages, as previously discussed by Jansson et al. (20). It enables villous transport to be studied in primary (explant) tissue, maintaining microvillous membrane/basal membrane polarization and cell-cell contacts and avoiding potential changes in transporter characteristics that may occur with passaged cells in culture. In addition, as discussed previously (20), defining system A activity as Na\(^+\)-dependent uptake of MeAIB makes it unlikely that nonspecific uptake (i.e., diffusion into interstitial spaces or nonspecific binding to extracellular surfaces) contributes significantly to measured values. Rubidium is commonly used as a substitute for potassium in studies of the ouabain-sensitive Na\(^+\)-K\(^+\)-ATPase (9). Uptake of $^{86}$Rb was rapid and linear over the time course of our experiments, and ouabain inhibited this uptake. These data are consistent with previous reports (22, 23) indicating the presence of Na\(^+\)-K\(^+\)-ATPase maintaining a low intracellular Na\(^+\) concentration and thus the electrochemical gradient for Na\(^+\) across the syncytiotrophoblast plasma membrane.

Activation of the uteroplacental RAS with increased production of angiotensin II may occur in the IUGR infant as a
regulatory response to protect from hypoxemia by inducing redistribution of blood flow to critical organs such as the fetal heart and brain (13, 28, 29, 32, 37). However, this transiently protective effect may pose a serious dilemma for the nutrition of the IUGR fetus in the form of reduced system A amino acid activity. In this regard, the reduced gene and protein expression of AT1-R in syncytiotrophoblast of IUGR placentas may occur as a secondary adaptive response to the adverse effects of enhanced placental RAS (32).

One possible source of excess AT1-R activation in IUGR pregnancies may relate to agonistic IgG autoantibodies in the maternal circulation that are directed at the second extracellular loop of the AT1-R (53). Originally thought to be exclusive to women with preeclampsia, these autoantibodies have recently been shown to track with abnormal second trimester uterine artery Doppler waveform, thus occurring principally in cases of reduced uteroplacental perfusion, many of whom subsequently develop IUGR (with or without preeclampsia) (54). There is evidence that the circulating AT1-R autoantibody, like angiotensin II, stimulates production of reactive oxygen species through AT1-R-dependent activation of NADPH oxidase (10).

Excess production of peroxynitrite anion by nitric oxide in combination with superoxide anion impairs system A amino acid transport activity in villous explants (27). However, we found that angiotensin II-mediated decreases in system A activity are not prevented by antioxidants, and therefore, oxidative stress does not appear to be the primary mechanism by which angiotensin II decreases system A activity. Furthermore, antioxidants alone had no effect on system A activity (data not shown). These data, however, do not rule out possible adverse effects of chronic exposure to reactive oxygen species induced by angiotensin II.

Unlike idiopathic IUGR, preeclampsia is not accompanied by indirect evidence of reduced amino acid transport from maternal to fetal circulation, such as lower amino acid concentration in cord blood or decreased system A amino acid transport activity, compared with normotensive controls with
appropriately grown fetuses despite the high incidence (30–35%) of IUGR in preeclampsia. Indeed, cord blood concentrations of many kinds of amino acids are reportedly increased in preeclampsia compared with normal pregnancy (14), and the expression of system A transporter gene subtypes, ATA1 and ATA2, in preeclamptic placenta is not different from normal pregnancy (35). However, amino acid transport activity is intricately modified by other regulatory hormones such as insulin and leptin (20). In this context it is noteworthy that maternal plasma concentrations and placental production of leptin are increased in women with preeclampsia (1, 46), and leptin has recently been reported (20) to significantly increase system A amino acid transport activity of villous explants.

A previous study (51) showed that human angiotensinogen transgenic female mice mated with human renin transgenic male mice develop hypertension and IUGR in late pregnancy due to angiotensin I generated by human renin secreted from the fetal side to maternal circulation. Moreover, the hypertension and IUGR occurring in this model were prevented by the administration of AT1-R blockers (47). As with our data, this result is consistent with AT1-R activation in the uteroplacental unit as a mediator of IUGR.

Na\(^+\)-K\(^+\)-ATPase provides the key driving force for Na\(^+\)-dependent amino acid transport systems by pumping Na\(^+\) out of syncytiotrophoblast cells (22). It is of interest that others have reported inhibition of taurine uptake (Na\(^+\)- and Cl\(^-\)-dependent amino acid transporter system $\beta$) into human placental villous fragments following short-term incubation with ouabain (6). The activity of Na\(^+\)-K\(^+\)-ATPase in microvillous membranes from IUGR placentas was found to be reduced by 35% compared with controls, whereas protein expression of the Na\(^+\)-K\(^+\)-ATPase $\alpha_1$-subunit was only slightly reduced (10%) (23). IUGR is also associated with decreased activity of several Na\(^+\)-coupled transporters in microvillous membranes, including the system A amino acid transporter (34), the tauire transporter (41), and Na\(^+\)/H\(^+\) exchanger (23). Hence, decreased Na\(^+\)-K\(^+\)-ATPase activity may cause a reduction of Na\(^+\)-coupled transporters in the placenta of IUGR pregnancy. However, the underlying reason for the decreased Na\(^+\)-K\(^+\)-ATPase activity in IUGR placenta is still unknown. Angiotensin II has been shown to decrease Na\(^+\)-K\(^+\)-ATPase activity in several tissues (18, 33, 36, 48), and our data indicate that angiotensin II is also a potent inhibitor of ouabain-sensitive Na\(^+\)-K\(^+\)-ATPase activity in villous explants.

Taken together, our data indicate that activation of the AT1-R by angiotensin II decreases placental system A amino acid transporter activity and suggests that enhanced placental RAS is a potential contributor to reduced system A amino acid transport in IUGR. Hypoxia has recently been shown to decrease system A activity and the expression of ATA1 and ATA2 in cultured term human trophoblast cells, suggesting that hypoxia also contributes to decreased amino acid transport in IUGR (39). The regulation of placental amino acid transport activity is not yet fully understood. Additional work on the regulation of placental amino acid transport may increase our understanding of the pathophysiology of pregnancy disorders accompanied by fetal IUGR.

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REFERENCES

18. Hainouczky G, Cordas G, Hunyady L, Kalapos MP, Balla T, Eneydi P, and Spat A. Angiotensin-II inhibits Na\(^+\)/K\(^+\) pump in rat adrenal...