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Conversion of angiotensin I to angiotensin II in the human foetoplacental vascular bed

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1 Pressor effects of angiotensin I (AI) and angiotensin II (AII) on the human foetoplacental vasculature were compared in dual-perfused term placental cotyledons in which foetoplacental perfusion pressure was monitored. Arterial injections of 1 nmol doses of AI and AII caused marked increases in perfusion pressure; the mean pressor response to AI was 92.9± 5.8% (mean ± s.e.mean) of the AII response.

2 The angiotensin-converting enzyme inhibitor captopril at 2.2 μM reversibly reduced the AI response to 13.7± 3.2% (mean± s.e.mean) of the AII response, which was unaffected. Saralasin, an AII receptor blocker, at 94 nM reversibly antagonized both AI-and AII-induced increases in foetoplacental perfusion pressure.

3 It is concluded that foetoplacental vasoconstriction elicted by AI is due to its conversion to AII by angiotensin-converting enzyme present in the foetoplacental bed.

Introduction

Angiotensin II (AII), the primary vasoactive component of the renin-angiotensin system, has been measured in human umbilical cord blood (Broughton Pipkin & Symonds, 1977; Lumbers & Reid, 1977). In vaginal deliveries, cord venous blood concentrations of AII were significantly higher than those in cord arterial blood (Broughton Pipkin & Symonds, 1977). Symonds (1979) suggested that the higher cord venous levels of AII may indicate either that angiotensin I (AI) is converted to AII in the placental circulation, or that the complete AII-generating system may occur in the placenta. Although there is as yet no firm evidence to indicate that the human term placenta contains the complete AII-generating system, homogenates of human term placenta were shown to contain kininase activity which inactivated bradykinin and produced AII-like smooth muscle contracting activity from AI (Litorowicz & Malofiejew, 1978). Possible sources of kininase activity in the placental homogenates include syncytiotrophoblast membrane, on which angiotensin-converting enzyme (ACE) has been demonstrated (Defendini *et al.*, 1983), and endothelial cells of the foetoplacental vasculature. In rabbit term placenta angiotensin-converting enzyme has been localized, by immunofluorescence techniques, to the vascular endothelium (Wigger & Stalcup, 1978). Thus, angiotensin-converting enzyme activity in the human term placenta may similarly be present in the foetoplacental vascular bed, and may effect conversion of AI to AII in the foetoplacental circulation. Using dual-perfused human placental cotyledons we showed that AII produced dose-related pressor responses in the foetoplacental vascular bed (Howard & Maguire, 1982; Maguire *et al.*, 1983). Moreover, we found that AI elicited dose-related increases in foetal perfusion pressure in this preparation (unpublished observations). To ascertain the potential of the foetal vascular bed of human term placentas for conversion of AI to AII, we have compared the actions of the two peptides on foetal perfusion pressure in isolated placental cotyledons in the presence and absence of captopril, a specific angiotensin-converting enzyme inhibitor, and saralasin, a specific AII receptor blocker.

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Methods

Human placentas from normal full term pregnancies were obtained immediately after spontaneous delivery or delivery by caesarian section. The foetal circulation and intervillous space of a single cotyledon from each placenta were perfused essentially as de-

scribed by Schneider *et al.* (1972). Preparation and perfusates were maintained at 37°C. Perfusion was performed at constant flow with Earle's salt solution (Earle, 1943) containing 4 g% of dextran, gassed either with 6% carbon dioxide + 94% nitrogen for delivery to the foetal circuit or with 95% oxygen + 5% carbon dioxide for delivery to the maternal circuit. Perfusate gas and pH values were measured with a Radiometer BMS blood gas analyzer and served to indicate adequacy of the maternal perfusion. Perfusion pressure of the foetal circuit was used as an index of foetal vascular resistance, and was monitored via a Statham P23ID transducer and recorded on a Gilson ICT-2H Duograph. At the end of each experiment a 1% solution of Coomassie Blue in 0.9%w/v NaCl solution (saline) was infused via the arterial cannula; the cotyledon demarcated by the dye was dissected and weighed.

Drugs were dissolved in saline and administered via the arterial cannula. One nmol doses of AI and AII were injected in boli of 50 μl. Solutions of captopril and saralasin were infused at 70 μl min^{-1} to give final concentrations of 2.2 μM and 94 nM, respectively. Infusions of captopril and saralasin were commenced 10 min before injections of AI and AII; after infusions were stopped, 30 min elapsed before AI and AII were again administered. Increases in perfusion pressure elicited by 1 nmol doses of AI and AII, and times to maximum pressure increase, were measured in triplicate in each cotyledon. The average increase in perfusion pressure produced by 1 nmol of AII administered before infusion of antagonists was taken as control, equal to 100%, in each cotyledon; responses to AI, and to AI and AII during and after infusion of antagonists, were expressed as percentage of the average control response to AII. Results are shown as means ± s.e.mean of findings in at least three different cotyledons. Statistical analysis was performed using the paired Student's *t* test; where groups showed significantly unequal variances the signed Wilcoxon rank test was used.

AI (Asp^1-Ile^5-Phe^8-angiotensin I) was obtained from Bachem Inc., Torrance, CA; AII (Asp^1-β-amide-Val^5-Phe^8-angiotensin II; Hypertensin) from Ciba-Geigy Corp., Summit, NJ, and saralasin acetate (Sar^1-Ala^8-angiotensin II) from Norwich-Eaton

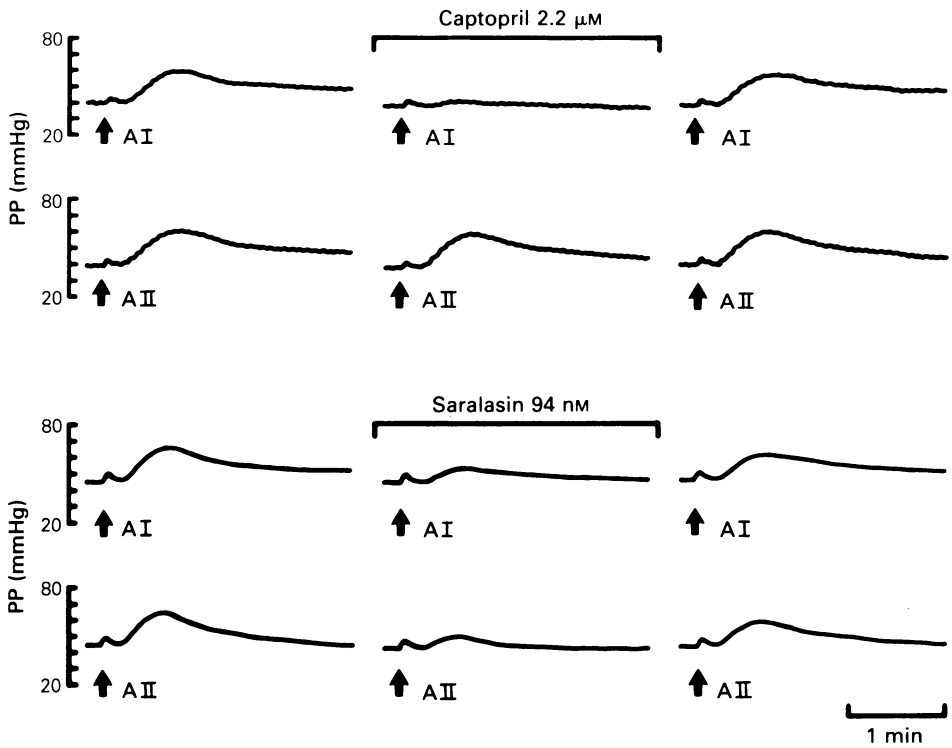

Figure 1 Typical pressor responses of the perfused foetoplacental vascular bed of dual-perfused human term placental cotyledon to 1 nmol doses of angiotensin I (AI) and AII, before, during and after infusion of captopril and saralasin (final concentrations 2.2 μM and 94 nM, respectively). Perfusion pressure (PP) was measured as described under Methods. Injections of AI and AII are indicated by the arrows.

Pharmaceuticals Inc., Norwich, NY. Captopril (D-3-mercapto-2-methylpropanoyl-L-proline, SQ14,225) was supplied by E.R. Squibb & Sons, Inc., Princeton, NJ and dextran (mol.wt 37,000–43,000) was obtained from Chemical Dynamics Corp., South Plainfield, NJ.

Results

Flow rates, pH and gas values of foetal and maternal perfusates

Flow rates of foetal and maternal perfusates were 0.49 ± 0.07 ml $min^{-1}g^{-1}$ and 1.56 ± 0.23 ml $min^{-1}g^{-1}$, respectively ($n = 6$ cotyledons); mean weight of the six cotyledons was 14.3 ± 2.0 g. Perfusates had the following pH and gas values. Foetal arterial: pH 7.34 ± 0.01, PCO_2 43.0 ± 0.5 mmHg. PO_2 27 ± 2 mmHg and foetal venous: pH 7.35 ± 0.02, PCO_2 43.2 ± 1.3 mmHg, PO_2 195 ± 14 mmHg ($n = 6$ cotyledons). Maternal arterial: pH 7.40 ± 0.003, PCO_2 33.2 ± 0.7 mmHg, PO_2 547 ± 6 mmHg and maternal venous: pH 7.35 ± 0.01, PCO_2 38.9 ± 2.6 mmHg, PO_2 331 ± 26 mmHg ($n = 4$ cotyledons). The arteriovenous increase in PO_2 in foetal perfusates (> 150 mmHg) indicated that perfusion of the maternal circuit resulted in substantial maternal to foetal oxygen transfer, consistent with the arteriovenous fall in maternal perfusate PO_2. The foetal arterial pH and gas values approximated the values found for human umbilical arterial blood: pH 7.35, PCO_2 48 mmHg, PO_2 15 mmHg (Longo, 1972).

Vasoconstrictor responses to angiotensin I and angiotensin II

The mean basal foetal perfusion pressure in six dual-perfused cotyledons was 42.2 ± 0.9 mmHg. Injection of bolus doses of 1 nmol of AI and AII in the six cotyledons caused mean increases in perfusion pressure of 20.0 ± 1.8 mmHg and 21.9 ± 2.2 mmHg, respectively, which returned to basal levels within 5–12 min, depending on the cotyledon; within single cotyledons recovery times for both peptides varied by < 2 min. Typical pressor responses to 1 nmol doses of AI and AII are shown in Figure 1. Times to maximum

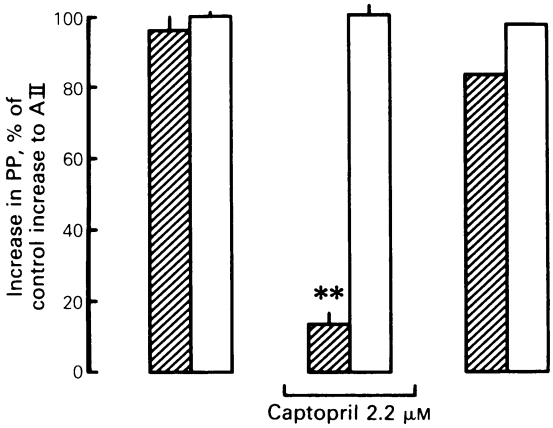

Figure 2 Effect of captopril infusion on pressor responses to angiotensin I (AI) and AII in the perfused foetoplacental vascular bed. Increases in perfusion pressure (PP) elicited in each cotyledon by 1 nmol of AI (hatched columns) and 1 nmol of AII (open columns) before, during and after infusion of captopril (final concentration 2.2 μM) are expressed as a percentage of the average pre-captopril response to AII, which is taken as 100%; in each cotyledon responses were measured in triplicate. Columns represent means and vertical lines s.e.mean for 3 cotyledons before and during captopril infusion, and means for two cotyledons 30 min after cessation of the infusion. $^{**}P < 0.001$, different from the pre-captopril response to AI. Actual increases in PP elicited by 1 nmol doses of AI and AII before captopril infusion were: AI, 17.4 ± 1.9 mmHg; AII, 18.2 ± 2.5 mmHg.

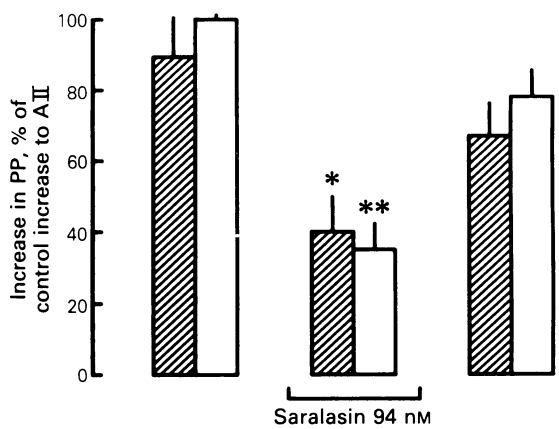

Figure 3 Effect of saralasin infusion on pressor responses to angiotensin I (AI) and AII in the perfused foetoplacental vascular bed. Increases in perfusion pressure (PP) elicited in each cotyledon by 1 nmol of AI (hatched columns) and 1 nmol of AII (open columns) before, during and after infusion of saralasin (final concentration 94 nM) are expressed as a percentage of the pre-saralasin response to AII, which is taken as 100%; in each cotyledon responses were measured in triplicate. Columns represent means and vertical lines s.e.mean for 3 cotyledons before, during and 30 min after cessation of saralasin infusion. $^{*}P < 0.05$ and $^{**}P < 0.001$, different from the pre-saralasin response to AI and AII, respectively. Actual increases in PP elicited by 1 nmol doses of AI and AII before saralasin infusion were: AI, 22.5 ± 2.6 mmHg; AII, 25.5 ± 2.3 mmHg.

pressure increase to AI and AII did not differ significantly (in s, AI 46.9±2.0, AII 45.9±1.8; $P>0.10$; $n=4$ cotyledons). As shown in Figure 1, 2.2 μM captopril reduced the pressor response to AI but did not affect the pressor response to AII. Results from three cotyledons showed that the antagonism by captopril was highly significant for AI ($P<0.001$) and that captopril did not antagonize the AII response ($P>0.05$) (cf. Figure 2). Administration of AI 30 min after the infusion of captopril was stopped elicited pressor responses which were 87.0% of the pre-captopril response (Figure 2).

Pressor responses to AI and AII were reversibly reduced by 94 nM salarasin as shown in Figure 1. In three cotyledons the AII antagonist significantly inhibited the increase in perfusion pressure elicited by both AI ($P<0.05$) and AII ($P<0.001$) (Figure 3). Thirty minutes after cessation of the saralasin infusion, pressor responses to both AI and AII showed substantial recovery of the pre-saralasin responses, as indicated in Figure 3 ($P>0.05$ for AI and AII; AII data were compared by the signed Wilcoxon rank test).

Discussion

Angiotensin I is considered to have little or no direct pressor activity *per se*; its pressor effects are believed to be due substantially or entirely to its conversion by ACE to the potent vasoconstrictor AII (Peach, 1977). In the present study AI was shown to have vasoconstrictor potency in the foetal circuit of the dual-perfused placental cotyledon similar to that of AII, suggesting that AI is rapidly converted to AII during transit through the foetoplacental vascular bed, and *ipso facto*, that ACE is present in the foetoplacental vasculature. Captopril, a potent inhibitor of ACE (Cushman *et al.*, 1977), reduced the pressor response to AI by more than 80%, substantiating the contention that the pressor response to AI was due to its conversion to AII by ACE. Since the pressor response to AII was not affected by captopril, the effect of the drug on the response to AI must not be due to a non-specific diminution of vascular smooth muscle responsiveness. Furthermore, the vascular response to AI substantially recovered after cessation of captopril infusion, in accord with the reversible nature of the inhibition of ACE by captopril (Cushman *et al.*, 1977). We have shown that saralasin, a reversible AII receptor blocker (Pals *et al.*, 1971), inhibits the pressor response of the perfused foetoplacental vascular bed to AII in dual -perfused human placental cotyledons (Howard *et al.*, 1983). That AI is metabolized to AII by ACE present in the foetoplacental vasculature is further supported by our present finding that pressor responses to both peptides were similarly and reversibly antagonized by saralasin. Thus, our findings indicate that the cotyledonary vascular bed of the human term placenta contains considerable ACE activity.

The demonstration that AI is converted to AII in the foetoplacental vascular bed provides a possible explanation for the arteriovenous difference in cord AII levels observed in vaginal deliveries (Broughton Pipkin & Symonds, 1977), but other factors may also contribute to this difference. These factors include degradation of AII on passage through the placenta (Lumbers & Reid, 1978), and the possible foetal release of renin by the placenta. However, although the human term placenta contains renin (Skinner *et al.*, 1968), we find no evidence for the placental release of renin into the foetal circulation, as we have been unable to detect renin in foetal venous perfusates of dual-perfused placental cotyledons, either under the conditions described in this paper or under conditions of maternal hypoxia (T. Hosokawa, R.B. Howard, M.H. Maguire & A.M. Poisner, unpublished findings). That no arteriovenous difference in cord AII concentrations was found in deliveries by elective (pre-labour) caesarian section (Broughton Pipkin & Symonds, 1977), suggests that in vaginal deliveries foetoplacental ACE activity and/or AII degradation may be modulated during labour, resulting in the higher levels of AII found in foetal blood leaving the placenta.

While the functional significance of foetoplacental ACE is not yet clear, its action on AI may contribute to circulating foetal AII; whether circulating AII is involved in regulation of blood pressure in the human foetus, as has been suggested for the sheep foetus (Iwamoto & Rudolph, 1979), is not known. Furthermore, as AII is a potent constrictor in the foetoplacental vascular bed (Howard & Maguire, 1982), *in situ* generation of AII from AI via action of ACE may play a role in the local regulation of foetoplacental blood flow. In conclusion, we have presented evidence for the presence of ACE in the human foetoplacental vascular bed. Foetoplacental ACE may be an important component of the foetal and placental renin-angiotensin system and may contribute to the local regulation of foetoplacental vascular resistance and to circulating foetal AII levels.

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