Effect of captopril on bradykinin inactivation by human foetal placental circulation

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- 1 The inactivation of bradykinin on passage across the human foetal placental circulation was investigated in six full-term human placentas.
- 2 The placentas were perfused with a modified Krebs-Henseleit solution and placenta perfusion pressure was recorded.
- 3 Samples collected at the arterial inflow and at the venous effluent were assayed on the isolated guinea-pig ileum as an estimation of bradykinin activity.
- 4 Bradykinin (100 ng ml^{-1}) was infused through the foetal placental vessels before and during captopril $4 \times 10^{-7} \text{ M}$.
- 5 Bradykinin produced a transient increase in placental vascular resistance that was not potentiated by captopril.
- 6 Bradykinin activity was completely abolished after passage through the foetal placental circulation, and the inactivation was blocked by captopril.
- 7 These data suggest that angiotensin I converting enzyme (kininase II) might occur in the foetal placental vessels.

Keywords captopril bradykinin converting enzyme kininase II
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Introduction

The mechanisms that control human foetal placental blood flow are at the moment not completely understood. Humoral components must be important in that control, since placental vessels are devoid of adrenergic innervation (Spivack, 1943; Baljet & Drukker, 1981). Umbilical and placental blood vessels are sensitive to bradykinin and to angiotensin II (Altura *et al.*, 1972; Tulenko, 1979; Abramovich & Wright, 1983) and there is evidence that the renin-angiotensin and kallikrein-kinin systems play a role in the physiological adjustments of

the foetal circulation not only at birth but also during the early postpartum period (Broughton Pipkin & O'Brien, 1978; Melmon *et al.*, 1968). Angiotensin I converting enzyme, a substance involved with inactivation of bradykinin (Erdo, 1976), is present in the placental vascular network of rabbits (Wigger & Stalcup, 1978). However, there is no information concerning the occurrence of that enzyme in human foetal placental vessels. Therefore we decided to study whether bradykinin could be inactivated when perfused through the human foetal placental

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circulation. The results indicate that angiotensin I converting enzyme might occur in the human foetal placental circulation.

Methods

We studied six full-term human placentas (450–690 g) obtained from spontaneous deliveries at the Hospital Geral de Bonsucesso in Rio de Janeiro. Placentas from mothers with eclampsia, hypertension, diabetes, Rh incompatibility, or other overt disease were not included in this study. All placentas were used on the day of delivery. Immediately after delivery each placenta was immersed in warm saline to maintain the temperature and to prevent drying. A cannula was inserted into each of the umbilical arteries and into the umbilical vein close to the placental plate, and blood was washed out of the vascular tree by gentle injection of 100 to 200 ml of warm Krebs solution containing heparin (Liquemin Roche, 0.02 mg ml⁻¹). The placenta was then transported directly to our laboratory, placed inside an opened plastic bag full of warm Krebs solution and immersed in a constant-temperature (37°C) bath. The two arterial umbilical cannulae were joined by a Y-connector and perfused as a single unit, using a pulsatile pump (Harvard 1405) and a modified warm Krebs-Henseleit solution aerated with 5% CO₂ in oxygen and containing (in mM) NaCl 116.2; KCl 4.7; CaCl₂ 1.8; KH₂PO₄ 1.17; MgSO₄ 1.16; NaHCO₃ 22.1; and glucose 7.8. The pH of the perfusion fluid on the inflow side was 7.4 and the temperature was about 37°C. The venous effluent was kept for further pharmacological analyses (see below) and not re-perfused. The interval between delivery and an establishment of perfusion was no longer than 45 min. The placenta perfusion pressure was measured with a Statham pressure transducer (P23 Db) inserted between the pump and the Y-connector and continuously recorded on a Hewlett-Packard polygraph (HP 7702 B). The pump frequency in all experiments was fixed at 110 strokes min⁻¹ and the stroke volume was adjusted at the beginning of the experiment to give a mean perfusion pressure of about 25 mm Hg. Since the volume output from the pump was kept constant during the experimental period, changes in vascular resistance in the placenta could be recorded as pressure changes. One hour after starting infusion with warm Krebs solution (37°C) (equilibration period) bradykinin was added to the reservoir of perfusion Krebs solution.

In order to study the inactivation of bradykinin by the human foetal placental circulation, brady-

kinin (Sigma B3259, 100 ng ml⁻¹) was infused during 45 min through the placental vessels, and during this period samples were collected at the arterial inflow close to the Y-connector and at the venous cannula. At the end of this period, bradykinin was removed from the Krebs perfusion solution and captopril (Squibb, 4 × 10⁻⁷ M) was added to it. Thirty minutes later, arterial and venous samples were collected and then bradykinin was re-introduced, to be perfused together with captopril for another period of 45 min. Arterial and venous samples were also collected at intervals during bradykinin plus captopril perfusion. As a control for deterioration of added bradykinin during perfusion through the warm coils, a sample of the Krebs solution containing bradykinin, was collected before adding it to the warm reservoir, referred to as bradykinin control. All samples were collected in small glass vials and stored frozen at -20°C for further assay on the guinea-pig isolated ileum.

The samples of perfusion fluid were assayed at 37°C on the guinea-pig isolated ileum mounted as described by Perry (1970). Isometric contractions were measured with a Hewlett-Packard force transducer (HP FTA-10) and recorded on a HP polygraph (7702B). A segment of the guinea-pig ileum was suspended in an organ bath containing a modified Krebs solution of the same composition as that used to perfuse the placenta plus captopril 4 × 10⁻⁷ M. In five preliminary experiments, concentration-response curves to bradykinin were constructed in order to obtain the EC₅₀ of bradykinin in our experimental conditions (11.2 ± 1.1 ng ml⁻¹). About ten times the EC₅₀ was used as the concentration of bradykinin to perfuse the placenta. To assay the bradykinin activity in the ileum, 2 ml samples of the perfusion fluid were added to the bath (final volume 20 ml). The responses induced by the samples were expressed in terms of percentage of the effect induced by the bradykinin control. The data were analysed by means of Student's *t*-test (paired) and significance was set at *P* < 0.05. The results are presented as mean ± s.e. mean.

Results

Placental perfusions

The mean placental perfusion pressure was 24.4 ± 1 mm Hg and the mean placental flow was 20.2 ± 1.2 ml min⁻¹. One hour after starting the placental perfusion, bradykinin (100 ng ml⁻¹) was added to the warm Krebs perfusion solution. In all experiments bradykinin produced, within

a few minutes after starting perfusion, a significant but transient increase in placental vascular resistance of $77.6 \pm 14.2\%$. The delay was almost entirely accounted for by the time required for the drug to circulate through the coils and to reach the placental vessels. Subsequently the placental perfusion pressure returned to basal values 19.6 ± 3.6 min after starting the infusion with bradykinin (Figure 1, BK).

Immediately after removal of bradykinin from the Krebs perfusion solution, captopril (4×10^{-7} M) was added to the Krebs perfusion solution. The concentration of captopril used was higher than that found by Gaudreau *et al.* (1980) to inhibit completely the activity of kininase II in isolated vessels. Captopril alone produced no change in the placental perfusion pressure (Figure 1, CAPT). Thirty minutes later, bradykinin was added to the solution containing captopril and, as in the beginning of the experiment, produced a significant but transient increase in placental vascular resistance of $40.6 \pm 8.2\%$ (Figure 1, BK-CAPT). The pressor effect of bradykinin on the foetal placental circulation during captopril infusion was significantly smaller than before captopril. The perfusion pressure returned to its basal values 17.3 ± 3.0 min after beginning of bradykinin infusion in the presence of captopril. The duration of bradykinin pressure effects was not affected by captopril infusion (Figure 1).

Bradykinin assay on the guinea-pig ileum

The control samples of Krebs with bradykinin (10 ng ml^{-1} in the organ bath) produced in all experiments a contraction of the guinea-pig ileum of 2.7 ± 0.3 g. Samples collected at the arterial inflow (A) close to the Y-connector before bradykinin infusion were inactive on the guinea-pig ileum. The samples collected at the venous cannula (V) before bradykinin infusion produced in all experiments a contraction that corresponded to $37.2 \pm 9.6\%$ of the bradykinin control response. Samples collected at the

arterial inflow during bradykinin infusion, produced responses that were not different from bradykinin control, indicating that bradykinin was not degraded by warming from room temperature to 37°C . The samples collected at the venous cannula during bradykinin infusion, on the other hand produced contractions that were significantly smaller than the bradykinin control response ($57.7 \pm 6.5\%$, 44.8 ± 10.8 and $40.7 \pm 9.4\%$ of the control bradykinin after 15, 30 and 45 min, respectively). None of these responses was significantly different from the response produced by a venous sample collected during perfusion with Krebs not containing bradykinin. These results indicate the bradykinin was virtually completely inactivated by the human foetal placental circulation after only 15 min of perfusion. Perfusion with Krebs solution containing captopril and collected from the arterial inflow was inactive on the guinea-pig ileum. The sample collected at the venous cannula 30 min after beginning of captopril infusion produced a response that was not significantly different from that obtained from the venous effluent when no drugs had been added to the Krebs perfusion solution. Samples collected at the arterial inflow during captopril plus bradykinin produced responses that were not different from the bradykinin control response. Samples collected at the venous cannula 15, 30 and 45 min after the beginning of bradykinin plus captopril produced responses that were identical to those obtained from samples taken simultaneously from the arterial inflow. These results indicate that captopril prevented the inactivation of bradykinin that otherwise would have occurred during passage through the human foetal placental vessels (Figure 2).

Discussion

The renin-angiotensin system may play a very important role in the control of circulation during

Figure 1 Effect of bradykinin on the perfusion pressure of the isolated human foetal placenta before (BK) and during captopril treatment (BK + CAPT). The arrows represent the moment of sample collection at the arterial inflow and at the venous cannula.

Figure 2 Effect induced by samples collected at the arterial inflow (■) and at the venous cannula (□), before and during captopril (4×10^{-7} M) infusion, on the isolated guinea-pig ileum, expressed as percentage of the bradykinin control response. A, sample collected at the arterial inflow; V, sample collected at the venous cannula; BK, sample collected 15, 30 and 45 min after beginning of bradykinin infusion. Left side of figure refer to results obtained before captopril, and right side of figure to results obtained during captopril infusion. Each column represents the mean and vertical lines show s.e. means. An asterisk indicates a significant difference ($P < 0.05$) between venous and arterial samples.

pregnancy, since maternal renin substrate, renin and angiotensin II concentrations are elevated (Brown *et al.*, 1966; Broughton Pipkin & Symonds, 1977; Tetlow & Broughton Pipkin, 1983). In the foetal circulation at birth, this system is also hyperactive, since foetal plasma renin substrate and renin and angiotensin II concentrations are higher than in normal adults (Soveri *et al.*, 1975; Broughton Pipkin & Symonds, 1977; Tetlow & Broughton Pipkin, 1983). Angiotensin I, formed by the action of renin on angiotensinogen (renin substrate) is converted enzymatically to angiotensin II by a peptidyl dipeptidase, also called angiotensin I converting enzyme or kininase II, which inactivate bradykinin (Erdos, 1976).

Vane and collaborators have demonstrated that conversion of blood-born angiotensin I to angiotensin II is mainly catalysed by angiotensin I converting enzyme on the luminal surface of small blood vessels in lungs (Vane, 1969). However, since the intrauterine foetal pulmonary blood flow is limited (Rudolph & Heiman, 1970), and angiotensin I converting enzyme activity is lower in the lungs of neonatal and foetal lamb than in ewes (Hebert *et al.*, 1972), extrapulmonary conversion of angiotensin I to angio-

tensin II has to be considered. Extrapulmonary localization of angiotensin I converting enzyme in human foetal tissue has been demonstrated by Johnson & Erdos (1975), who showed the presence of kininase II in endothelial cells from human umbilical cord vein. Broughton Pipkin & Symonds (1977) have shown that in humans, blood angiotensin II in cord venous outflow was substantially higher than in the cord arterial blood. On the basis of these results, the authors suggested that the human placenta may possess considerable converting enzyme activity. The possibility that the foetal placenta may be a major site of angiotensin I conversion *in utero* has also been raised by Stalcup and collaborators (1978), since angiotensin I converting enzyme was present in the placental vascular network of foetal rabbits (Wigger & Stalcup, 1978). Our results confirm that hypothesis, since bradykinin is completely inactivated when perfused through the human foetal placental circulation. Further support for involvement of angiotensin I converting enzyme comes from the present observation that when the placenta is perfused with captopril, a substance that selectively inhibits that enzyme, the placental inactivation of bradykinin is completely abolished, and also by the

demonstration that angiotensin I produces a dose dependent vasoconstrictor in human foetal placental circulation that is shifted to the right by captopril (Soares de Moura & Solano Vale, 1984). Since in our experiments the placenta is perfused with Krebs solution instead of blood, the inactivation of bradykinin is independent of any plasma enzyme and may reflect the presence of kininase II in the endothelium of the placental vessels.

The vasopressor effect of bradykinin is probably induced by an increase in tone of the resistance vessels of the placental vasculature, since Tulenko (1979) has shown that bradykinin has a potent vasoconstrictor action on placental arterioles. However, an action of bradykinin on placental veins cannot be discarded, since this substance contracts rabbit vena cava (Gulati *et al.*, 1967) and human umbilical veins (Altura *et al.*, 1972).

The mechanism of the vasoconstrictor action of bradykinin is at the moment not clear. Prostaglandins might be involved, since this substance releases prostaglandin $F_{2\alpha}$ from mesenteric vessels (Limas, 1977). As shown by Soares de Moura *et al.* (1975), prostaglandin $F_{2\alpha}$ has a potent vasoconstrictor effect on the isolated human placenta. On the other hand, direct effect of bradykinin on vascular smooth muscle has been suggested by Toda (1977), who showed that the vasoconstrictor effect of bradykinin on isolated canine vessels does not depend on prostaglandin release, and is apparently not related to adrenergic, cholinergic or histaminergic mechanisms.

The failure of captopril to potentiate the pressor effect of bradykinin in our experiments

could be due to tachyphylaxis of the bradykinin pressor effect. Tachyphylaxis has been shown to occur when this polypeptide is repeatedly injected into the circulation of human foetal placenta with short intervals between injections (Soares de Moura & Solano Vale, 1984). Figure 1 shows that tachyphylaxis to bradykinin begins very shortly after the bradykinin infusion, since the placental perfusion pressure returned to basal values despite continued infusion of bradykinin through the placental circulation. Probably the lack of captopril action on the pressor effect of bradykinin during the second infusion of bradykinin was due to the incomplete recovery of the placental vessels from the bradykinin tachyphylaxis that occurred during the first bradykinin infusion period. Our experiments also indicate that some active substance is released from the placental circulation, since the venous effluent from the human foetal placenta produces an increase in tone of the isolated guinea-pig ileum even when no drugs have been added to the perfusion Krebs solution (Figure 2). Kininase II seems to be inactive on this substance, since the effects of the venous effluent were insensitive to captopril.

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