Studies on the effects of angiotensin II on human chorionic plate arteries and their modification by a calcium antagonist, nitrendipine

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1 Six-point concentration-response curves were initially established for the contractile effect of angiotensin II (AII), at final concentrations of 10^{-11} to 10^{-6} M, on helically-cut strips of human chorionic plate arteries. The strips were then randomly allocated to one of four treatment groups: a control group and three treatment groups ($n = 10$ in each group) which were exposed to carrier or nitrendipine 10^{-11} , 10^{-10} or 10^{-9} M final concentration respectively, before the establishment of second concentration-response curves.

2 The initial responses of the tissues to AII were very similar in the four treatment groups with median EC_{50} s ranging between 2.51×10^{-10} and 4.04×10^{-10} M ($P > 0.8$). The addition of nitrendipine was associated with significant ($P < 0.005$) dose-related inhibition of AII-induced contractions. The median EC_{50} of the control group during the second half of the experiment was 1.77×10^{-7} M, while that of the 10^{-11} M nitrendipine treatment group was 2.16×10^{-7} M. The EC_{50} for the other treatment groups could not be calculated from the curves due to the markedly blunted responses.

3 It is thus suggested that nitrendipine might be effective *in vivo* in the relief of vasospasm of the placental vascular system in pregnancy-induced hypertension.

Keywords calcium antagonists angiotensin II pregnancy, human placenta, human

Introduction

The placental component of the foeto-placental circulation is not innervated and is of comparatively low vascular resistance (see Boddy, 1979). Renin can be synthesized by human chorion (Symonds *et al.*, 1968) and angiotensin I (AI) is generated by human chorio-decidua in tissue culture (Warren *et al.*, 1982); converting enzyme is also present in these tissues (Warren *et al.*, 1984) and conversion of AI to angiotensin II (AII) in the human placental bed has been demonstrated indirectly (Broughton Pipkin & Symonds, 1976). Since AII is the most potent

circulating vasoconstrictor hormone yet described, this capacity for local generation seems likely to be of functional significance. In pregnancy-induced hypertension (PIH) the systemic pressor response to AII is enhanced (Talledo *et al.*, 1968) with a marked arteriolar vasoconstriction and a diminished utero-placental blood flow. The effects of vasodilators in pregnancy are thus of some interest. The dihydropyridines have the pharmacological effect of blocking or reducing calcium ion entry into the cell. We report here a study of the effect of a dihydropyridine, nitren-

dipine (Bay e 5009), on the responses of human chorionic plate arteries to *in vitro* stimulation with AII.

Methods

The placentae used in this study were obtained from the delivery rooms as soon as possible after completion of the third stage of labour. A total of 40 chorionic plate artery strips from 15 women whose parity ranged from one to four and of mean age 26 ± 1.4 years were studied. All the women had had an uneventful antenatal course. The placentae were transported immediately to the laboratory, where three or four sections of the chorionic plate arteries were dissected free and placed in Krebs solution containing 11.1×10^{-3} M glucose (Kitson & Broughton Pipkin, 1981). The resting length of the artery strips ranged between 20–35 mm (mean: 27.5 ± 0.5 mm). Subsequent preparation was as previously detailed (Kitson & Broughton Pipkin, 1981), but since angiotensin II (AII) in low concentrations adheres to untreated glass surfaces, the tissue baths used in this study were modified 5 ml plastic disposable syringes (Becton-Dickinson, England).

The tissues were left to equilibrate for a minimum of 60 min after setting up. A slight modification of the bath system allowed a continuous superfusion of the tissues with Krebs solution at the rate of 0.1 ml min^{-1} during this initial period. The response to 50 mM KCl was determined and the tissues were subsequently allowed to re-equilibrate in the Krebs solution for a minimum of 30 min. Contraction was recorded isotonicly against a constant tension of 500 mg, using modified microtorque potentiometers (Ferranti 8HLI) and was measured to an accuracy of ± 0.04 mm. Cumulative concentration:response curves to AII (Sigma Chemical Company, Poole, Dorset, England) diluted with Krebs solution were then established. Pilot experiments ($n = 6$) had shown that, under these conditions, AII 10^{-11} M induced a response in the majority of tissues, and that the response was maximal in five preparations at 10^{-6} M. Tissues responses were erratic above this concentration, either being unchanged or drifting down. Final concentrations of 10^{-11} , 10^{-10} , 10^{-9} , 10^{-8} , 10^{-7} and 10^{-6} M were thus used, achieved by stepwise addition of concentrated stock solution to the Krebs solution in the tissue bath. The tissues were left in contact with each dilution for a minimum of 20 min. The determination of the concentration-response curves thus required 2 h and this was followed by a recovery period of at least 40 min.

The concentration-response curves to AII were then re-established under one of four different conditions: with only the vehicle for nitrendipine (polyethylene glycol 400) added ($n = 10$) or in the presence of nitrendipine 10^{-11} , 10^{-10} or 10^{-9} M final concentration ($n = 10$ in each group). These concentrations are within the range found in plasma during treatment with nitrendipine (Raemisch & Sommer, 1984). The concentration of nitrendipine or vehicle to be used was determined by a Latin square sequence. The tissues were exposed to nitrendipine for 20 min before the determination of the second dose-response curves. All the experiments in which nitrendipine was added were conducted under sodium light because of the degree of light sensitivity of the substance.

Contractions were measured as the change in length from the stable baseline preceding the first or second concentration-response curve to AII, and have been expressed as percentages of the response to 10^{-6} M AII. The EC_{50} s have been calculated as that concentration of AII required to elicit a contraction 50% of maximum response within the concentration range examined.

Arithmetical means $\pm$ s.e. mean are quoted where the data are normally distributed, while medians are quoted as a measure of central tendency where the data are not normally distributed. Wilcoxon's two sample rank test was used to assess the significance of observed differences between grouped data. Kruskal-Wallis one-way analysis of variance by ranks was used to determine any overall effect of treatment with nitrendipine on the tissue response to AII.

Results

Angiotensin II at 10^{-11} induced contraction in 70% (28) of the tissues during the initial determinations of concentration-response curves. The overall initial EC_{50} was 3.29×10^{-10} M. There was no significant difference in the initial tissue responses to AII in the four treatment groups (Kruskal-Wallis $P > 0.08$; Figure 1, broken lines).

The responses of the nitrendipine-treated tissues to any given concentration of AII were diminished in the second part of the experiment when compared with the control tissues exposed to carrier alone (Figure 1, solid lines). Only 3 tissues, all in the control group, now responded to AII at 10^{-11} M. The EC_{50} for the control group was 1.77×10^{-7} M, compared with 2.16×10^{-7} M for the group treated with nitrendipine 10^{-11} M ($P > 0.1$). The two groups of tissues exposed to

Figure 1 Concentration-response curves for the contraction of helically-cut strips of human chorionic plate artery in response to angiotensin II (AII) obtained (----) during the control period when no tissue was treated with nitrendipine and (—) during the second part of the experiment when three of the four groups of tissues were studied in the presence of nitrendipine [$\circ$, 10^{-11} ; $\blacksquare$, 10^{-10} ; $\square$, 10^{-9} M; $\bullet$, control (untreated) group]. All four second curves showed a significant depression of response, presumably partly to tachyphylaxis, but this was significantly more pronounced overall in the treatment groups. A dose-dependent effect was apparent. Median values of the percentage response to AII are shown, since the data were not normally distributed. ($n = 10$ for each group).

higher concentrations of nitrendipine showed markedly blunted responses (Figure 1) and their EC_{50} s could not be calculated. As Table 1 shows, there was a statistically-significant effect of nitrendipine by comparison with carrier at all doses of AII greater than 10^{-10} M. This effect appeared to be dose-dependent, with the responses in groups B and C being significantly greater than those in group D at 10^{-7} – 10^{-9} M AII, although they did not differ significantly from each other.

Discussion

The vessels of the umbilico-placental unit are known to respond to vasoactive compounds such as serotonin (5-HT), AII and isoprenaline (Tulenکو, 1979); adrenaline and histamine (von Euler, 1938) and the prostaglandins (Hillier & Karim, 1968; Kitson & Broughton Pipkin, 1981; Maigaard *et al.*, 1986). An association between the size of placental vessels and their drug sensitivity has been reported (Tulenکو, 1979) with AII exerting the greatest effect, in molar terms, on the small chorionic plate vessels and villous

Table 1 Comparison between the responses to each concentration of angiotensin II (AII) in control and nitrendipine-treated strips of human chorionic plate artery

Dose of AII (M)	χ^2	P
10^{-9}	18.43	< 0.001
10^{-8}	16.71	< 0.001
10^{-7}	16.54	< 0.001
10^{-6}	8.86	< 0.05

χ^2 was derived from the Kruskal-Wallis H Statistic for the one-way analysis of variance by ranks of the data relating to the evoked response to concentrations of 10^{-9} – 10^{-6} M AII in the four groups of tissues studied. The statistic could not be calculated for doses of 10^{-11} and 10^{-10} M AII because of the general failure to respond of the tissues exposed to nitrendipine.

stem arterioles. As suggested in the Introduction, there is evidence for the local generation of AII itself in the female genital tract and the placenta. Such locally generated AII may act directly on the smooth muscle of the placental vasculature to induce vasoconstriction, and thus influence the placental blood flow. It is also possible that AII complements the known role of the prostaglandins (Hamberg, 1974) in the microregulation of the placental circulation since AII is known to stimulate vasodilator prostanoid synthesis.

Chorionic plate arteries were found to be sensitive to AII stimulation at the very low concentration of 10^{-11} M, during the establishment of the initial curves. This is within the range of the observed value of cord arterial AII concentration in normotensive pregnancy (Broughton Pipkin & Symonds, 1977). Tulenکو (1979) reported a mean EC_{50} of 5.3×10^{-8} M AII for human chorionic plate vasculature. However, the AII in those experiments was only left in contact with the tissues for some 3–4 min, whereas in our experiments 20 min was found to be necessary to reach full contraction for any dose. The marked diminution in response to AII seen in the control tissues during the second part of the experiment was considerably greater than that seen to PGE_2 in the control experiments on identical preparations previously reported (Broughton Pipkin & Chinnery, 1985). This suggests a degree of tachyphylaxis to AII, rather than a response to the carrier *per se*, although the washout period was long. The long time-course of the experiments may also have led to some deterioration of the preparations. Thus the responses of the nitrendipine-treated tissues were compared with those of the control tissues in the second part of the experiment, rather than with their own initial responses.

An inhibitory effect of nitrendipine on AII-induced contractions of chorionic plate arteries has been demonstrated in this study. Although there was no significant difference between the second EC₅₀s of the control group and that of the group treated with nitrendipine 10⁻¹¹ M, a dose-dependent effect of treatment was seen. It appears from Figure 1 that this effect was especially pronounced at low concentrations of AII, within the physiological range. In an earlier study, Broughton Pipkin & Chinnery (1985) have demonstrated the inhibitory effect of nitrendipine on PGE₂-stimulated human chorionic artery strips, and Maigaard *et al.* (1986) have also shown an effect of nitrendipine on the response of vessels from the uteroplacental unit to other agonists.

An enhanced systemic pressor reactivity to AII has been repeatedly shown in patients becoming hypertensive in late gestation (Talledo *et al.*, 1968; Gant *et al.*, 1973). This is presumably secondary to the enhanced contractile response of the resistance vessels (Aalkjaer *et al.*, 1984). The utero-placental blood flow is also diminished in this condition (see MacGillivray, 1983), and

the proteinuric form of the disease is associated with babies small for gestational age. A directly-acting vasodilator, without the tendency to fluid retention of such drugs as minoxidil and hydralazine, could on theoretical grounds be of benefit to both mother and foetus, unlike the sympatholytic agents which can cause a degree of β -adrenoceptor blockade in neonates for several days postnatally (Dumez *et al.*, 1981; Macpherson *et al.*, 1986). Nitrendipine is known to be a potent antihypertensive agent in severe non-pregnant hypertension (Hoffler & Stoepel, 1984). Preliminary data suggest that it readily crosses the guinea pig placenta, which is structurally-similar to that of the human (Broughton Pipkin & Lawrence, unpublished observations). Thus its antihypertensive effects together with its effect in inhibiting the contractile response both to angiotensin II and prostaglandin E₂ (Broughton Pipkin & Chinnery, 1985) of the human chorionic plate vasculature suggests that it could be a useful drug in the treatment of PIH.

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References

- Aalkjaer, C., Johannesen, P., Pedersen, E. B., Rasmussen, A. & Mulvany, M. J. (1984). Morphology and angiotensin II responsiveness of isolated resistance vessels from patients with pre-eclampsia. *Scand. J. clin. lab. Invest.*, **44**, suppl 169, 57-60.
- Boddy, K. (1979). In *Placental transfer*, ed. Chamberlain G. & Wilkinson, A., pp. 49-59. Bath: The Pitman Press.
- Broughton Pipkin, F. & Chinnery, E. (1985). The effect of a new calcium antagonist, nitrendipine, on the responses of isolated strips of human chorionic plate artery to prostaglandins E₂ and E₁. *Br. J. Pharmacol.*, **86**, 705-709.
- Broughton Pipkin, F. & Symonds, E. M. (1976). Angiotensin II and the placenta in normal pregnancy and in pregnancy complicated by hypertension. *J. Physiol.*, **256**, 121-122P.
- Broughton Pipkin, F. & Symonds, E. M. (1977). Factors affecting angiotensin II concentrations in the human infant at birth. *Clin. Sci. mol. Med.*, **52**, 449-456.
- Dumez, Y., Tchobroutsky, C., Hornych, H. & Amiel-Tison, C. (1981). Neonatal effects of maternal administration of acebutolol. *Br. med. J.*, **283**, 1077-1079.
- Gant, N. F., Daley, G. L., Chand, S., Whalley, P. J. & MacDonald, P. C. (1973). A study of angiotensin II pressor response throughout primigravid pregnancy. *J. clin. Invest.*, **52**, 2682-2689.
- Hamberg, M. (1974). Quantative studies on prostaglandin synthesis in man III. Excretion of the major urinary metabolites of prostaglandin F_{1 α} and F_{2 α} during pregnancy. *Life Sci.*, **14**, 247-252.
- Hillier, K. & Karim, S. M. M. (1968). Effects of prostaglandin E₁, E₂, F_{1 α} and F_{2 α} on isolated human umbilical and placental blood vessels. *J. Obstet. Gynaec. Br. Commw.*, **75**, 667-673.
- Hoffler, D. & Stoepel, K. (1984). Nitrendipine in hypertension that is difficult to control. *J. cardiovascular. Pharmacol.*, **6**, S1060-S1062.
- Kitson, G. E. & Broughton Pipkin, F. (1981). Effects and interactions of prostaglandin E₁ and E₂ on human chorionic plate arteries. *Am. J. Obstet. Gynecol.*, **140**, 683-688.
- MacGillivray, I. (1983). *Pre-eclampsia*. The hypertensive disease of pregnancy. London: W. B. Saunders Co. Ltd.
- MacPherson, M., Broughton Pipkin, F. & Rutter, N. (1986). The effect of maternal labetalol on the newborn infant. *Br. J. Obstet. Gynaecol.*, **93**, 539-542.
- Maigaard, S., Forman, A., Brogaard-Hansen, K. P. & Andersson, K. A. (1986). Inhibitory effects of nitrendipine on myometrial and vascular smooth muscle in human pregnant uterus and placenta. *Acta pharmac. tox.*, **59**, 1-10.
- Raemsch, K. D. & Sommer, J. (1984). Pharmacokinetics and metabolism of nitrendipine. In *Nitrendipine*, eds Scriabine, A., Vanov, S. & Deck, K. Baltimore-Munich: Urban & Schwarzenberg.
- Skinner, S. L., Cran, E. J., Gibson, R., Taylor, R.,

- Walters, W. A. W. & Catt, K. J. (1975). Angiotensins I and II, active and inactive renin, renin substrate, renin activity and angiotensinase in human liquor amnii and plasma. *Am. J. Obstet. Gynec.*, **121**, 626–630.
- Symonds, E. M., Stanley, M. A. & Skinner, S. L. (1968). Production of renin by *in vitro* culture of human chorion and uterine muscle. *Nature*, **217**, 1152–1153.
- Talledo, O. E., Chesley, E. C. & Zuspan, F. P. (1968). Renin–angiotensin system in normal and toxemic pregnancies. III. Differential sensitivity to angiotensin II and norepinephrine in toxemia in pregnancy. *Am. J. Obstet. Gynec.*, **100**, 218–221.
- Tulenko, T. N. (1979). Regional sensitivity of vaso-active polypeptides in the human umbilico-placental vasculature. *Am. J. Obstet. Gynec.*, **135**, 629–636.
- von Euler, U. S. (1938). Action of adrenaline and acetylcholine and other substances on nerve free vessels (human placenta). *J. Physiol. (London)*, **93**, 129–143.
- Warren, A. Y., Craven, D. J. & Symonds, E. M. (1982). Production of active and inactive renin by cultured explants from the human genital tract. *Br. J. Obstet. Gynaecol.*, **89**, 628–632.
- Warren, A. Y., Craven, D. J. & Symonds, E. M. (1984). Angiotensin converting enzyme in human fetal membranes, placenta, amniotic fluid and cord venous serum. *Clin. exp. Hypertension*, **B3**, 51–60.

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