Br. J. Pharmac. (1985), **86**, 705–709

The effect of a calcium antagonist, nitrendipine, on the responses of isolated strips of human, chorionic plate artery to prostaglandins E_2 and E_1

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1 Six point concentration-response curves were established for the contractile effect of prostaglandin E_2 (PGE_2) on helically-cut strips of human chorionic plate arteries.

2 Tissues were then allocated to one of four treatment groups: a control group and 3 groups exposed to the calcium channel blocker nitrendipine at 10^{-9}, 10^{-7} or 10^{-5} M. The concentration-response curves were then repeated.

3 The addition of nitrendipine was associated with a significant depression of the induced contraction. The contractile response to the lower doses of PGE_2 was replaced by a small relaxation in 63% of the treated tissues.

4 It is suggested that in these tissues PGE_2 exerts its constrictor effect via the receptor-operated channels and that nitrendipine is blocking this effect.

5 Similar experiments performed with PGE_1 showed great variability in the initial response. Nitrendipine 10^{-5} M failed to exert any detectable effect on this response.

6 Pregnancy-induced hypertension is associated with vasoconstriction in both the maternal and placental circulations. There also appears to be a relative excess of vasoconstrictor eicosanoid production. Nitrendipine may be of use in the treatment of this condition.

Introduction

The pathophysiology of pregnancy-induced or associated hypertension (PIH) remains one of the most baffling problems of modern obstetrics. Treatment of the disease is, in consequence, largely empirical. There is today an increasing tendency to question the need for any pharmacological treatment of the mild and moderate forms of the disease. What is not questioned is the need for a rapid-acting, effective, antihypertensive agent, without or with minimal effects on the foetus, which can be used in severe PIH. General, and more recently, cardio-selective, β-adrenoceptor blocking drugs have been widely used, as also have combined α- and β-adrenoceptor blockers, but there is increasing evidence that they can exert pharmacological effects on the foetus (Dumez *et al.*, 1981; Macpherson *et al.*, 1984) which could further jeopardise a foetus already compromised.

The heterogeneous group of drugs known as 'calcium antagonists' act by preventing stimulated increases in the intracellular concentration of calcium ions. Originally used as agents for the treatment of angina pectoris, some of them, such as the dihydropyridines, are now primarily used for their potent vasodilator ability (Opie, 1980). Since intense vasospasm is a feature of severe PIH, it is not unreasonable to suggest that calcium antagonists may be suitable antihypertensive agents for use in this condition. We present here a study of the effects of a dihydropyridine, nitrendipine (BAY e 5009), on the responses of human chorionic plate arteries to prostaglandins E_2 and E_1.

Methods

Placentae from 25 women were studied. The women were in their first to fourth pregnancy and with one exception had enjoyed an uneventful antenatal course. One woman had become hypertensive at 37 weeks gestation and had been treated with labetalol until delivery at 39 weeks. No differences were observed between the data obtained from the 3 tissue strips derived from her placenta and that from the normotensive patients, and the data were therefore included. All women were at full term at the time of delivery.

Placentae were obtained directly from the hospital delivery rooms. They were transported immediately to the laboratory and 3 or 4 sections of chorionic plate artery were carefully dissected free and placed in Krebs solution containing 11.1×10^{-3} M glucose (Kitson & Broughton Pipkin, 1981). Excess connective tissue was trimmed off, and helical strips were cut from each artery as previously described (Kitson & Broughton Pipkin, 1981). The initial length of the strips ranged between 12.5–46 mm (mean 29.8 ± 1.2 mm). The strips were mounted in individual 20 ml tissue baths filled with Krebs solution gassed with 95% O_2: 5% CO_2 and maintained at 37°C throughout. Contraction was recorded isotonically against a tension of 500 mg, using microtorque potentiometers (Ferranti 8HLI). Contraction could be measured to an accuracy of ± 0.04 mm.

The tissues were left to equilibrate for a minimum of 60 min after setting them up; the Krebs solution was changed at 10 min intervals during this period. The response to 50 mM KCl (final dilution) was then determined, and the tissues were subsequently allowed to re-equilibrate in Krebs solution for a minimum of 30 min. Cumulative concentration-response curves to prostaglandin E_2 (PGE_2; supplied in dehydrated alcohol (Upjohn, Crawley, Sussex) and diluted with Krebs buffer) were then established. Final concentrations of PGE_2 7.1×10^{-7}, 2.1×10^{-6}, 6.4×10^{-6}, 2.0×10^{-5}, 6.2×10^{-5} and 2.0×10^{-4} M, were achieved by the stepwise addition of concentrated stock solutions to the Krebs in the tissue bath. The tissues were left in contact with each solution for 20 min. The determination of the concentration-response curves thus required 2 h, and was followed by a recovery period of at least 40 min.

The concentration-response curves to PGE_2 were then re-established under one of four different conditions: with no nitrendipine added (control, $n = 13$) or in the presence of nitrendipine at 10^{-9}, 10^{-7} or 10^{-5} M final dilution ($n = 10$ in each group). Nitrendipine was supplied in liquid form, in a vehicle of citrate, borate and phosphate buffers, polyethyleneglycol 400 and ethanol, by Bayer Germany, Wuppertal. All experiments in which nitrendipine was added were conducted under sodium light because of a degree of light sensitivity of the substance. The concentration of nitrendipine to be used was determined by a Latin Square sequence.

Contractions were measured as the changes in length from the stable baseline preceding the concentration-response curve. PGE_2 at the highest concentration evoked a near-maximal contraction (see Figure 1). To allow between-tissue comparison, responses to the other concentrations have been expressed as percentages of this initial maximal response. EC_{50} values have been calculated as that concentration of PGE_2 required to elicit a contraction 50% of the maximum within the concentration range examined.

Experiments were also carried out in an identical manner to determine the effects of nitrendipine 10^{-5} M on the response of chorionic plate arteries to concentrations of prostaglandin E_1 ranging between 2.8×10^{-6} M and 1.4×10^{-4} M (PGE_1; Upjohn, Crawley, Sussex).

Arithmetic means ± s.e.mean are given where the data are normally distributed. 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. The Kruskal-Wallis one-way analysis of variance by ranks was used to determine any overall effect of treatment with nitrendipine on the response to the prostaglandins.

Results

A total of 43 artery strips from 25 placentae were used. Ten women had been delivered by Caesarean section, 3 of which had been performed as emergencies for foetal indications during labour. Tissues from vaginal and Caesarean deliveries were randomly allocated among the treatment groups. The mean EC_{50} for the tissues derived from emergency Caesarean sections

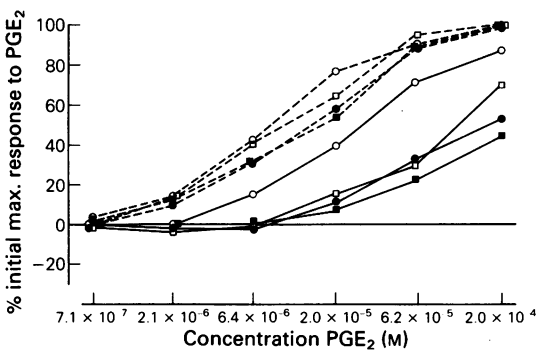

Figure 1 Concentration-response curves for the contraction of helically-cut strips of human chorionic plate artery in response to prostaglandin E_2 (PGE_2) obtained (– – –) during the control period when no tissue was treated with nitrendipine and (———) during the second part of the experiment, when 3 of the 4 groups of tissues were studied in the presence of nitrendipine (□) 10^{-9} (●) 10^{-7} and (■) 10^{-5} M. (○) Control (untreated) group. All 4 second curves showed a significant depression of PGE_2-induced contraction (see Table 1), but this was significantly more pronounced in the treatment groups. There was no statistically-significant difference between the responses of the tissues in the 3 treated groups. Median values of the percentage response to PGE_2 are shown, since the data of the second curves was not normally-distributed. $n = 10$ for each nitrendipine–treated group and $n = 13$ for control group.

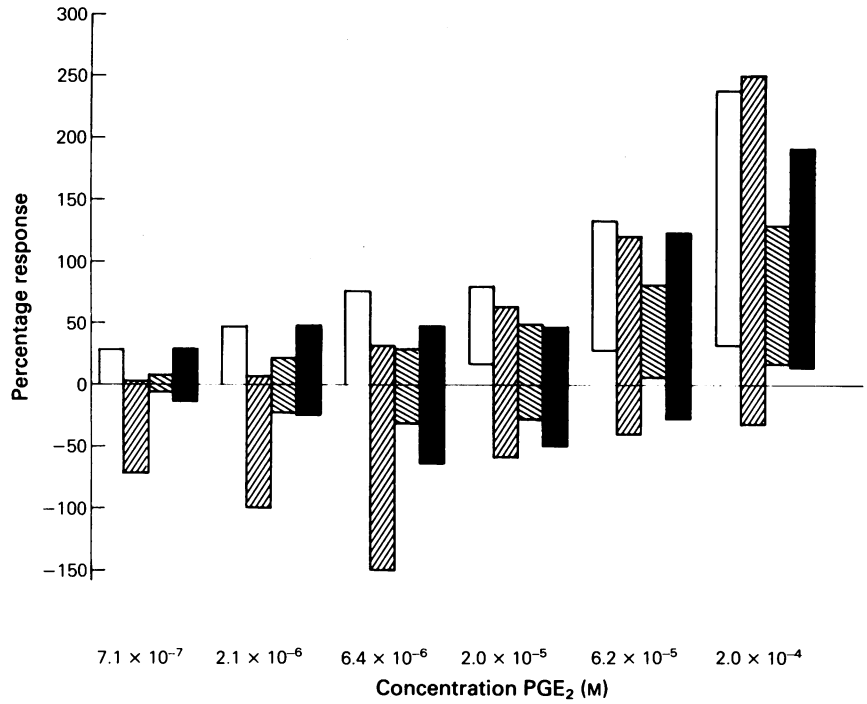

Figure 2 The range of percentage response to prostaglandin E_2 (PGE_2) as a function of the initial maximal response to PGE_2 in the 4 study groups during the second half of the experiment is shown; (□) control (untreated) group; groups treated with nitrendipine (▨) 10^{-9} (▨) 10^{-7} and (■) 10^{-5} M. No relaxation was ever seen during exposure to PGE_2 in the control group, but more than half the tissues in each group treated with nitrendipine showed relaxation during exposure to PGE_2.

Table 1 Comparison between the responses to each concentration of prostaglandin E_2 (PGE_2) in control and nitrendipine-treated strips of human chorionic plate artery

Dose of PGE_2 (M)	χ^2	P
7.1×10^{-7}	5.002	>0.1
2.1×10^{-6}	10.311	<0.025
6.4×10^{-6}	11.910	<0.01
2.0×10^{-5}	15.975	<0.002
6.2×10^{-5}	8.869	<0.05
2.0×10^{-4}	5.345	>0.1

χ^2 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 each of the six concentrations of PGE_2 in the four groups of tissues studied. The data show statistically significant differences between the treatment groups in their responses to 2.1×10^{-6}, 6.4×10^{-6}, 2.1×10^{-5} and 6.2×10^{-5} M PGE_2.

was significantly less (6.8 ± 2.3 compared with $15.1 \pm 1.9 \times 10^{-6}$ M) than that for tissues derived from placentae delivered normally or by elective Caesarean section ($P < 0.02$).

Experiments with prostaglandin E_2

Figure 1 illustrates the first and second concentration-response curves for the four treatment groups used in this experiment. The median EC_{50} of the first curve was somewhat lower in the control group than in the 3 treatment groups, but this difference was not statistically significant (Kruskal-Wallis $\chi^2 = 2.48$, $P > 0.4$). The same analysis also failed to show any significant difference between the responses of the four treatment groups at any concentration during establishment of the first curve.

The addition of nitrendipine to the organ baths caused a small relaxation in 21 of the 30 tissue strips. This ranged between 0.5 and 3 mm and did not appear to be concentration-related. The addition of identical

volumes of the vehicle (see Methods) in 3 separate experiments was without effect on basal tone, and was without evident effect on the concentration-response curve in a single experiment.

Figure 1 shows clearly the effect of nitrendipine at all 3 concentrations. Although there was depression of the induced contraction in the control curve, summarized by a change in EC_{50} from a median of 8.5 to 24.7×10^{-6} M ($P < 0.01$), in no instance did the tissues relax in response to PGE_2. In the three treated groups, however, 19 of the 30 tissue strips relaxed in response to PGE_2 at the 3 lowest concentrations (Figure 2). This relaxation and subsequent contraction made the conventional calculation of EC_{50} values impossible. Comparison between the responses to each concentration of PGE_2 in the control and treated groups was therefore made using one-way analysis of variance by ranks. The derived values are summarized in Table 1. It can be seen that the response at all concentrations except the lowest and highest was significantly different between the four treatment groups, with the control tissues experiencing a much smaller depression of contraction. The change in response in the presence of nitrendipine did not appear to be concentration-dependent over the range of concentrations studied.

Experiments with prostaglandin E_1

These experiments were performed using one concentration (10^{-5} M) of nitrendipine only. The responses to PGE_1, during the establishment of the initial concentration-response curve varied very widely from tissue to tissue in the 10 strips used. Five showed an initial relaxant response to PGE_1, while in four the peak response was not maintained at the highest dose (1.4×10^{-4} M). Classical interpretation of the curves was thus very difficult. In both the control and treatment groups, the second curves showed depression of contraction with respect to the first. The responses in the presence of nitrendipine were similar to those in the control group, and did not show any evidence of the marked differences seen when PGE_2 was used as the agonist.

Discussion

The umbilical and chorionic plate arteries are unique in that they are without innervation (von Euler, 1938; Somlyo *et al.*, 1965). Control of tone in these vessels is thus believed to be humoral in origin, and angiotensin II (AII), 5-hydroxytryptamine, isoprenaline, adrenaline, histamine and various prostaglandins have all been shown to evoke concentration-dependent changes in tone (von Euler, 1938; Hillier & Karim, 1968; Tulenko, 1979; Kitson & Broughton Pipkin, 1981; Abramovich *et al.*, 1983). Previous studies (Hillier & Karim, 1968; Park *et al.*, 1972; Kitson & Broughton Pipkin, 1981) have emphasized the variability of the effects of PGE_1 on the umbilical and placental vasculature, as was noted in these experiments. This is partly dose-dependent, in that Kitson & Broughton Pipkin (1981) formally demonstrated a biphasic concentration-response curve for PGE_1 in the majority of chorionic plate arteries.

Prostaglandin E_2 (PGE_2) consistently constricts chorionic plate arterioles (Hiller & Karim, 1968; Tulenko & Hesson, 1977; Kitson & Broughton Pipkin, 1981). This constrictor response was consistently seen throughout the 43 control curves performed in these experiments; at no time was any relaxant response seen. The overall EC_{50} for the tissues derived from vaginally-delivered placentae was very similar to the value found by Parkin & Broughton Pipkin (1984) ($1.5 \pm 0.2 \times 10^{-6}$ M compared with $1.6 \pm 0.4 \times 10^{-6}$ M). Inspection shows a marginally lower EC_{50} in the earlier data following Caesarean section. The difference was more pronounced in the tissues of this paper and achieved statistical significance. The random treatment allocation had assigned 7 strips from Caesarean section to the control group, while only 4 or 5 were in each of the groups treated with nitrendipine. It is possible that the slightly greater initial sensitivity of the control group (Figure 1) was related to this.

The depression of PGE_2-induced contraction seen in the tissues of the control group during the second part of each experiment presumably reflects a deterioration in condition of the strips with time. Earlier experiments (Kitson & Broughton Pipkin, 1981) had shown that the use of D-glucose 11.1×10^{-3} M, rather than the conventional 5.55×10^{-3} M, was associated with a smaller depression with time; the higher concentration was therefore used in these experiments.

The 1,4-dihydropyridine calcium antagonists are an extremely potent class of antihypertensive drug. They appear to bind to a single, high-affinity site, presumably close to the calcium 'channels' (Bolger *et al.*, 1982). Like all calcium antagonists, they inhibit the flux of extracellular calcium ions across the cell membrane. Calcium appears to gain entry to the cell by two main mechanisms, the 'potential-operated channels' and the 'receptor-operated channels' (Bolton, 1979). Most vasoconstrictor hormones activate the receptor-operated mechanism, and it is probable that PGE_2 in the human utero-placental vasculature is acting in a similar way. Since it is the receptor-operated mechanism which is inhibited by the calcium channel antagonists, an antagonistic effect of nitrendipine would then be expected to occur. The apparent lack of a concentration-dependent effect of nitrendipine may imply that a concentration of 10^{-9} M is already maximally effective on these tissues.

The relaxation observed during exposure to the

lower doses of PGE_2 in the presence of, but never in the absence of, nitrendipine is more difficult to explain. It could be related to continuing relaxation in the absence of effective stimulation by PGE_2. However, this seems improbable since preliminary experiments had indicated that basal conditions would be re-attained in the time allowed. It may be that adenosine 3′:5′-cyclic monophosphate production is stimulated under these circumstances, and is associated with relaxation, as with PGE_1 (Clyman, 1978).

Blood flow through the placenta is diminished in PIH (see Chesley, 1978), suggesting vasoconstriction, such as is also observed on the maternal side. The balance between foetal and maternal vasoconstrictor and vasodilator eicosanoid production is tipped towards production of the constrictors in PIH, by comparison with normal pregancy (for example, Martensson & Wallenburg, 1984). The *in vitro* data presented here suggest that nitrendipine may have a role to play in PIH on the foetal, as well as the maternal, side of the circulation.

We are grateful to Bayer Germany, Wuppertal, for their support in the project.

References

AMBROVICH, D.R., PAGE, K.R. & WRIGHT, F. (1983). Effect of angiotensin II and 5-hydroxytryptamine on the vessels of the human foetal cotyledon. *Br. J. Pharmac.*, **79**, 53–56.

BOLGER, G.T., GENGO, T.J., LUSCHOWSKI, E.N., SIEGEL, H., TRIGGLE, D.J. & JANIS, R.A. (1982). High affinity bindings of a calcium channel antagonist to smooth and cardiac muscle. *Biochem. biophys. Res. Commun.*, **104**, 1604–1606.

BOLTON, T.B. (1979). Mechanism of action of transmitters and other substances in smooth muscle. *Physiol. Rev.*, **59**, 606–718.

CHESLEY, L.C. (1978). *Hypertensive disorders in pregnancy*. New York: Appleton Century Crofts.

CLYMAN, R.I. (1978). Regulation of cyclic nucleotide metabolism in the human umbilical artery. *Adv. Prostaglandin Thromb. Res.*, **4**, 275–283.

DUMEZ, Y., TCHOBROUTSKY, C., HORNYCH, H. & AMIEL-TISON, C. (1981). Neonatal effects of maternal administration of acebutolol. *Br. med. J.*, **283**, 1077–1079.

VON EULER, U.S. (1938). The action of adrenaline, acetycholine and other substances on nerve-free vessels (human placenta). *J. Physiol.*, **93**, 129–143.

HILLIER, K. & KRIM, S.M.M. (1968). Effects of prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ on isolated human umbilical and placental blood vessels. *J. Obstet. Gynaec. Br. Comnw.*, **75**, 667–673.

KITSON, G.E. & BROUGHTON PIPKIN, F. (1981). Effects and interactions of prostaglandins E_1 and E_2 on human chorionic plate arteries. *Am. J. Obstet. Gynec.*, **140**, 683–688.

MACPHERSON, M., BROUGHTON PIPKIN, F. & RUTTER, N. (1984). Effects of maternal labetalol on the newborn infant. *Biol. Neonate*, **45**, 150.

MARTENSSON, L. & WALLENBURG, H.C.S. (1984). Uterine venous concentrations of 6-keto-$PGF_{1\alpha}$ (6-k) in normal pregnant (NP) and pregnancy-induced hypertensive (PIH) women. *Proc. soc. Gynec. Invest. San Francisco*, Abstract 410, p. 423.

OPIE, L.H. (1980). Drugs and the heart: III. Calcium antagonists. *Lancet*, **i**, 806–810.

PARK, M.K., RISHOR, C. & DYER, D.C. (1972). Vasoactive actions of prostaglandins and serotonin on isolated human umbilical arteries and veins. *Can. J. Physiol. Pharmac.*, **50**, 393–399.

PARKIN, A. & BROUGHTON PIPKIN, F. (1984). The effects of mode of delivery and type of anaesthesia on the response of human chorionic plate arteries to prostaglandin E_2. *Am. J. Obstet. Gynec.*, **149**, 573–574.

SOMLYO, A.V., WOO, C. & SOMLYO, A.P. (1965). Responses of nerve-free vessels to vasoactive amines and polypeptides. *Am. J. Physiol.*, **208**, 749–753.

TULENKO, T.N. (1979). Regional sensitivity to vasoactive polypeptides in the human umbilical vasculature. *Am. J. Obstet. Gynec.*, **135**, 629–636.

TULENKO, T.N. & HESSON, L.H. (1977). The regional distribution of drug sensitivity in the human umbilico-placental vasculature. *Proc. Int. Union Physiol. Sci.*, **13**, 766.

(*Received May 16, 1985.*
Revised June 27, 1985.
Accepted July 31, 1985.)