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Characterization of the receptor mediating contraction of human umbilical artery by 5-hydroxytryptamine

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1 The 5-hydroxytryptamine (5-HT) receptor in human umbilical artery was found to be similar to that in rabbit aorta. The pD_2 was 7.45, pA_2 for methysergide 8.63 and pA_2 for phentolamine 6.21.

2 Noradrenaline gave only very weak contractions at non-physiological concentrations. Amidephrine and xylazine did not contract human umbilical artery. It is concluded that there is no significant population of functional α-adrenoceptors in this vessel.

3 The implications of these findings are discussed in relation to the control of the umbilical circulation.

Introduction

It is known that 5-hydroxytryptamine (5-HT) is a potent agonist in the human umbilical artery (HUA) (Altura *et al.*, 1972). Since no previous quantitative assessment of the receptor mediating this contraction seems to have been made, we have undertaken this *in vitro*, at gas tensions found in umbilical arterial blood *in utero*. In addition the effects of α-adrenoceptor agonists were tested. A preliminary account of these results has been published (McGrath *et al.*, 1984).

Methods

Cords from full term pregnancies were obtained from the Queen Mother's Hospital immediately following delivery. The cords were transported to the laboratory in cold de-oxygenated Krebs-bicarbonate solution (composition (mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.1) and the vessels were dissected out while the cord was still immersed in this solution. This keeps the gas tensions at foetal levels but allows cooling, which, on its own, may lead to a contraction through the release of prostaglandins (Boura *et al.*, 1979). Nevertheless, this method has provided viable preparations.

Longitudinal strips of artery were set up *in vitro*, in 50 ml organ baths containing Krebs solution at 37°C, and the isometric tension was monitored using Grass FT03c transducers and a Grass Model 7 polygraph.

The time taken from delivery until the tissue was set up in the organ baths was approximately 1 h.

The Krebs solution was bubbled with CO_2 6–8%; the balance of the gas mixture was N_2. This was in order to mimic umbilical arterial blood gas tensions: $P_{O_2} = 20$ mmHg; $P_{CO_2} = 50$ mmHg and pH = 7.28 (Pearson, 1976). (The natural tendency for atmospheric oxygen to equilibrate with the solution raises the P_{O_2} above zero). Gas tensions of the Krebs solution were monitored by analysing samples on an IL 213 blood-gas analyser.

Analysis of responses to 5-hydroxytryptamine

In each preparation, two cumulative concentration-response curves to 5-HT were constructed, separated by 30 min. The first was the control and the second was in the presence of an antagonist. Responses were expressed as a percentage of the maximum of the control and the dose-ratio was calulated for responses at the level of the EC_{50}. (pD_2 values were calculated as $-\log(\text{control } EC_{50})$). It was not practical to construct several concentration-response curves at successive antagonist concentrations so dose-ratios (DR) from separate experiments were correlated by linear regression as $\log(DR-1)$ vs. $-\log(\text{antagonist concentration})$ and extrapolated to $\log(DR-1) = 0$ to arrive at an estimate of pA_2.

The drugs used in this study were: 5-hydroxytryptamine creatinine sulphate (5-HT; Sigma); methysergide bimaleate (Sandoz); phentolamine mesylate (Ciba); (−)-noradrenaline bitartrate (Sigma); (−)-amidephrine hydrochloride (Mead-Johnson) and xylazine hydrochloride

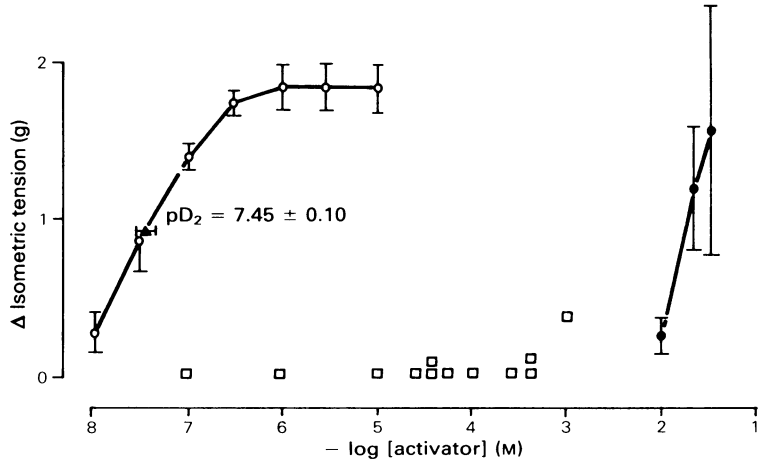

Figure 1 Concentration-response curves for contraction of human umbilical artery by 5-hydroxytryptamine (○) ($n = 18$); noradrenaline (□) (single responses), and KCl (●) ($n = 20$). Vertical lines show s.e.mean.

(Bayer). Stock solutions were made up in distilled water and added to the 50 ml organ baths in volumes not greater than 0.5 ml.

Results

KCl (10–40 mM) produced concentration-related contractions (Figure 1). In the first 20 experiments, the contraction to KCl (20 mM) was used as a test for viability of the preparations. This precaution was considered necessary because noradrenaline (NA) produced such weak and inconsistent responses (see below). When the failure was traced to NA rather than the preparations, the tissues were no longer challenged with KCl. There was no evidence to suggest that prior exposure to KCl affected responses to other agonists.

NA was tested at physiological levels (3–30 nM) but the threshold for contraction was greater than 10 μM and the contraction to 100 μM was much smaller than that to 5-HT (100 nM) or KCl (20 mM) (Figure 1). The synthetic α-adrenoceptor agonists, amidephrine (10 μM; α_1) or xylazine (100 μM; α_2) produced no contraction.

Contractions to 5-HT (1 μM), were larger than to

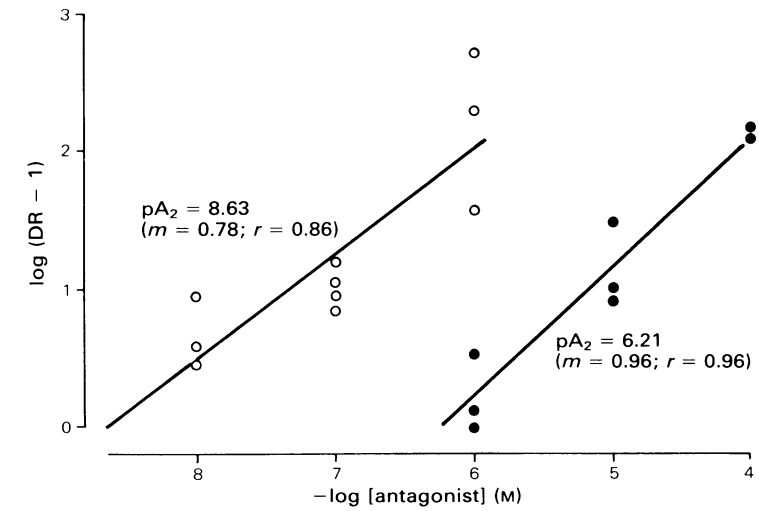

Figure 2 Schild analysis for the actions of methysergide (○) and phentolamine (●) against 5-hydroxytryptamine. Linear regression line calculated by least squares method. m = slope of linear regression line; r = correlation coefficient for slope of regression line.

Table 1 Comparison of the pA_2 values and regression lines for the Schild plots for antagonists versus 5-hydroxytryptamine in human umbilical artery and those in rabbit aorta

	Human umbilical artery (present study)		*Rabbit aorta* (Apperley *et al.*, 1976)	
	Methysergide	*Phentolamine*	*Methysergide*	*Phentolamine*
pA_2	8.63 (7.97–9.29)	6.21 (5.88–6.54)	8.49 (7.85–9.14)	6.21 (5.52–6.90)
Slope	0.78	0.96	0.78	0.93
r	0.86	0.96		
Significance	$0.05 < P < 0.001$	$P > 0.001$		

Figures in parentheses are 95% confidence limits for pA_2 values.

any other stimulus tested. (KCl (30 mM) produced an equally large response in some but not all preparations). The pD_2 for 5-HT was 7.45 ± 0.10 ($n = 18$). Concentration-response curves to 5-HT were expressed as a percentage of the control maximum. Methysergide and phentolamine produced parallel shifts to the right of the concentration-response curves to 5-HT. Phentolamine (10 μM) significantly increased ($P < 0.05$) the maximum responses to 5-HT. The mean increase was $25 \pm 3\%$ ($n = 3$): neither phentolamine (1 μM and 100 μM) nor any concentration of methysergide produced a significant increase. Plots of $\log(DR - 1)$ versus $-\log$[antagonist] yielded significant linear regression lines with slopes not significantly different from unity, thus suggesting competitive antagonism (Figure 2).

Discussion

The effects of agonists indicate no significant population of α-adrenoceptors (α_1 or α_2) in the HUA. Although NA produces small contractions, neither amidephrine nor xylazine had any effect. A 5-HT receptor is, however, present. According to the pA_2 values for methysergide and phentolamine this receptor is similar to the one mediating contraction by 5-HT in the rabbit aorta (Apperley *et al.*, 1976; see Table 1). On current usage it might be regarded as a 5-HT_2 receptor (see Humphrey *et al.*, 1982).

To our knowledge there is no information available on circulating levels of 5-HT in the human foetus or whether this could play a physiological or pathological role. The high sensitivity of the umbilical artery to 5-HT suggests that its release into the foetal circulation, by a drug or, perhaps, by an anaphylactic reaction, would reduce or cut off the blood supply leading to growth retardation or abortion. Similarly, any drug with agonism at these receptors and which reached the foetal circulation would have similar consequences, as has been postulated previously for lysergic acid diethylamide (Gant & Dyer, 1971). On the other hand 5-HT antagonists, if there was a 5-HT involvement in spontaneous abortion, might lead to its suppression and hence to the continued development of a foetus which might otherwise have been rejected.

At concentrations found in the foetal circulation we found no evidence that NA could constrict the HUA at foetal blood-gas tensions. This may explain why the relatively high concentrations of NA in the foetal circulation during labour (when compared with post-natal or adult levels) do not appear to correlate with foetal distress, as they might if NA were able to constrict the umbilical vessels and hence reduce the foetal blood supply from the placenta (Inglis *et al.*, 1981).

These results illustrate that a dominant role for α-adrenoceptors in blood vessels is not ubiquitous. It would be interesting to know to what extent vascular α-adrenoceptors are functional in the human foetus and in the new-born child. Are the umbilical vessels a special case or do they represent one end of a spectrum of sub-sensitivity which protects the foetus against the high circulating level of catecholamines? Do the high circulating levels of catecholamines, or an accompanying factor, lead to the sub-sensitivity or down-regulation?

The lack of α-adrenoceptors and preponderance of 5-HT receptors make this an interesting and useful preparation for the study of human vascular 5-HT_2 receptors.

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