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Effects of 5-hydroxytryptamine on human isolated placental chorionic arteries and veins

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1 Effects of 5-hydroxytrypamine (5-HT) on cylindrical segments of human chorionic arteries and veins were investigated. Concentrations of 5-HT (up to 3×10^{-6} M) produced concentration-dependent contractions; higher concentrations induced a reduction of the maximal response. These responses were antagonized by methysergide and ketanserin in a non-competitive manner. The contractions elicited by low 5-HT concentrations were more affected by methysergide (10^{-7} M) than by ketanserin (10^{-7} M). Ketanserin apparently increased the responses to high 5-HT concentrations in veins. Arteries appeared to be more sensitive to both drugs than veins. Single concentrations of 5-HT elicited transient contractions in both kinds of vessel.

2 Marked tachyphylaxis was seen in segments exposed to high concentrations of 5-HT or in which a concentration-response curve was determined.

3 Contractions induced by 5-HT were reduced in a Ca^{2+}-free medium. Veins were more affected by the Ca^{2+} antagonists, nifedipine (10^{-7} M), nicardipine (10^{-5} M) and diltiazem (10^{-5} M) than arteries.

4 5-HT (10^{-6} M) enhanced $^{45}Ca^{2+}$ uptake in those vessels in which a concentration-response curve had not been previously determined. In veins, this increase was reduced by the three Ca^{2+} antagonists.

5 The results indicate that 5-HT responses in these vessels were greatly dependent on extracellular Ca^{2+}. A type of 5-HT_1-receptor may mediate responses to low 5-HT concentrations, while higher concentrations may activate 5-HT_2-receptors. 5-HT may desensitize the latter by interconversion between a high affinity and low affinity state, as suggested by others, a change prevented in part by ketanserin.

Introduction

It has been demonstrated that 5-hydroxytryptamine (5-HT) induces a substantial increase in the tone of the human placental vascular bed (Aström & Samelius, 1957) and of isolated umbilical vessels (Altura *et al.*, 1972; Mak *et al.*, 1984). In addition, the concentration of 5-HT in the placental circulation, maternal blood and placental tissue increases from late pregnancy until spontaneous vaginal delivery (Koren *et al.*, 1965; O'Reilly & Loncin, 1967; Jones & Rowsell, 1973). It is suggested that 5-HT may be involved in the closure of the umbilical blood vessels at birth (Mak *et al.*, 1984), and in the pathogenesis of pre-eclampsia (Montenegro *et al.*, 1985).

The umbilical and placental vessels, which lack autonomic innervation (Walker & McLean, 1971; Reilly & Russel, 1977), are appropriate for studying the direct effect of 5-HT on the vascular smooth muscle. In other blood vessels, an indirect adrenergic component contributes to the total action of this amine (Marín & Sánchez, 1980; Marín *et al.*, 1981). The action of 5-HT has been more widely studied on umbilical cord than on placental chorionic vessels. The latter contribute more to placental vascular

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resistance than the larger umbilical cord vessels (Bhargava & Raja, 1970).

The aim of the present study was to analyse the effects of 5-HT in human chorionic arteries and veins, in terms of: (1) the subtype of 5-HT receptor involved, (2) the degree of dependence of 5-HT-induced contractions on extracellular Ca^{2+}, and (3) the effects of different Ca^{2+} antagonists on 5-HT-evoked contraction and $^{45}Ca^{2+}$ influx.

Methods

Vascular preparations

The human placental vessels used in the present study were arteries and veins (1.8–2.3 mm o.d.) of chorionic plate near to the point of insertion of the umbilical cord. The placentas were obtained from full-term normal, vaginal deliveries from apparently healthy women. Immediately following delivery, the vessels were carefully isolated and immersed in Krebs-Henseleit solution (KHS) at 4°C and transported to the laboratory. Then, they were divided into cylindrical segments 5 mm in length, which were cleaned of traces of blood and adherent tissues.

Analysis of drug effects on vascular tone

For isometric tension recording, each vascular cylinder was set up in an organ bath according to the method described elsewhere (Marín *et al.*, 1981). The organ bath contained 6 ml of KHS at 37°C continuously bubbled with 95% O_2: 5% CO_2, which gave a pH of 7.4. Two stainless steel pins, 250 μm in diameter, were passed through the lumen of the vascular segment. One pin was fixed to the organ bath wall while the other was connected to a strain gauge for isometric tension recording. The latter pin was parallel to the former and was movable, thus permitting the application of resting tension in a plane perpendicular to the long axis of the vascular cylinder. The isometric contraction was recorded through a force-displacement transducer (Grass FTO3C) connected to a Grass Model 7D polygraph. The segments were submitted to a tension of 1.5 g (optimal resting tension), which was readjusted every 15 min during a 120 min equilibration period before addition of drugs.

The vessels were exposed, at the beginning of the experiment, to 75 mM K^+ to check their functional integrity. Subsequently, the bath medium was changed several times until the resting tone recovered and then cumulative concentration-response curves (CRCs) to 5-HT were determined.

Contractile responses induced by 5-HT were expressed in mg or as a percentage of the response induced by previous administration of 75 mM K^+. 5-HT concentrations producing 50% of maximum contractile responses (EC_{50}) were calculated according to Fleming *et al.* (1972).

When Ca^{2+} antagonists (nifedipine, diltiazem or nicardipine) were used, they were added to the bath 15 min before and during the administration of 5-HT (10^{-6} M).

To study the effect of extracellular Ca^{2+} on 5-HT contractions, segments were exposed for 10 min to solutions containing different concentrations of Ca^{2+} (2.5, 1, 0.5, 0.25, 0 mM and 0 plus 10^{-3} M EGTA). Furthermore, the effect of Ca^{2+} removal and subsequent Ca^{2+} addition on the 5-HT contraction was investigated; thus, segments were exposed for 10 min to Ca^{2+}-free solution and then 10^{-6} M 5-HT was administered. Once the amine produced its effects, a CRC to $CaCl_2$ was determined.

$^{45}Ca^{2+}$ uptake

$^{45}Ca^{2+}$ uptake in 5 mm segments of chorionic artery and vein was determined by the La^{3+}-method (Godfraind, 1976). Briefly, segments were tied at one end with silk thread to a glass rod, the lumen remaining open. Thereafter, they were immersed in 4 ml of oxygenated KHS at 37°C for 60 min (stabilization period) and placed for different time intervals (30 s to 60 min) in KHS at 37°C containing $^{45}Ca^{2+}$ (0.6 μCi ml^{-1}).

Segments were rinsed with KHS at 4°C for 10–15 s (to remove surface $^{45}Ca^{2+}$) and then incubated with 200 ml of a Ca^{2+}-free solution containing La^{3+} (50 mM) for different periods of time (2 to 60 min). La^{3+} displaces the extracellular Ca^{2+} and blocks Ca^{2+} fluxes (Van Breemen *et al.*, 1972; Godfraind, 1976), therefore, the method gives an estimation of intracellular $^{45}Ca^{2+}$ uptake. Different exposure times to La^{3+} solution were employed to determine the optimum uptake. Finally, the segments were blotted, weighed and digested in vials containing 1 ml of H_2O_2 (30%) at 100°C for 5 h. Two ml of Ready-Solv HP (Beckman) was added and the radioactivity present in the vials measured in a scintillation counter (Beckman LS 2800).

The $^{45}Ca^{2+}$ uptake was calculated from the formula (Turlapaty *et al.*, 1979):

$$^{45}Ca^{2+}\ (\text{mmol kg}^{-1}\ \text{wet wt}) = \frac{\text{c.p.m. in muscle}}{\text{wet wt (kg)}} \times \frac{\text{mmol Ca}^{2+}\ \text{l}^{-1}\ \text{medium}}{\text{c.p.m. l}^{-1}\ \text{medium}}$$

In order to determine the effect of 5-HT (10^{-6} M) on $^{45}Ca^{2+}$ uptake, this amine was added to the bath during incubation of the vessels with $^{45}Ca^{2+}$. Ca^{2+}

antagonists were administered 15 min before, and during, the incubation period (5 min). Influx predominates over efflux or redistribution during this time interval. Once the CRC to 5-HT had been determined a marked desensitization was observed, i.e. the vessels did not respond to 5-HT. Some experiments were designed to investigate if this effect was parallel to a loss of the ability of 5-HT to induce Ca^{2+} influx. After the curve to 5-HT had been constructed, the segments were rinsed several times with KHS until control basal tone was restored (around 20 min) and were then exposed to a solution containing $^{45}Ca^{2+}$ plus 5-HT (10^{-6} M).

Solutions, drugs and statistical evaluation

The composition of KHS was (mM): NaCl 115, KCl 4.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, glucose 11.1, Na_2EDTA 0.03. The Ca^{2+} concentration was changed in modified KHS with no compensation. Ca^{2+}-free KHS was prepared by omitting $CaCl_2$ and in some cases, 1 mM ethyleneglycol-*bis*(beta-aminoethyl-ether) N,N'-tetra-acetic acid (EGTA) was added. La^{3+} (50 mM) solution contained (mM) (Turlapaty *et al.*, 1979): NaCl 118, KCl 5.9, $MgSO_4 \cdot 7H_2O$ 1.2, glucose 10, tris hydroxymethyl-aminomethane 5, $LaCl_3 \cdot 7H_2O$ 50. The final pH was adjusted to 7 with HCl (0.1 M), due to the basicity of $LaCl_3$ solution.

Stock solutions (10^{-3} M) of 5-HT, methysergide and ketanserin were prepared in physiological saline solution (0.9% NaCl) containing 0.01% (w/v) ascorbic acid. Those of nifedipine and nicardipine were prepared in ethanol 99.8% and protected from the light, and that of diltiazem in distilled water. Both dihydropyridines were used, at the appropriate concentrations in KHS, under sodium vapour light. All the stock solutions were kept at −20°C. The effect of Ca^{2+} antagonists could not be reversed after several washing periods. For this reason, the segments were used once, and the same was done with vessels in which a CRC to 5-HT was determined, due to desensitization.

Drugs used were: 5-hydroxytryptamine creatinine sulphate (Sigma), ketanserin tartrate (a gift from Janssen), methysergide bimaleate (a gift from Janssen); ^{45}calcium chloride (specific activity 36.5 mCi mg^{-1}, New England Nuclear); lanthanum chloride (Sigma), nifedipine hydrochloride (a gift from Bayer), nicardipine hydrochloride (a gift from Zambeletti) and diltiazem hydrochloride (a gift from Esteve).

Results are given as means ± s.e.mean. Statistical significance was evaluated by Student's t test for paired or unpaired values and P values of 0.05 or less were considered significant.

Results

Reactivity experiments

5-HT (up to 3×10^{-6} M) induced concentration-dependent contractions in chorionic arteries and veins; higher concentrations produced a reduction of the maximal contraction (Figures 1 and 2). EC_{50} values are shown in Table 1. 5-HT (10^{-6} M) did not induce contractile responses in vessels in which a CRC had been determined in the preceding 60 min or which had been exposed to high concentrations of 5-HT (10^{-4} M). After 2 h, the responses elicited by 5-HT were usually similar to those obtained in the control situation. In contrast, 75 mM K^+ produced contractions in these desensitized vessels, which were similar to those obtained at the beginning of the experiment (Figure 1). Single concentrations of this amine (up to 10^{-6} M) did not usually produce desensitization. Single concentrations of 5-HT elicited transient contractile responses. The maximal contraction was obtained during the first 4–5 min and then the vascular tone began to diminish (Figure 1).

The CRC to 5-HT was determined in the presence (15 min preincubations) of ketanserin (10^{-7} and 10^{-6} M) or methysergide (10^{-7} M), antagonists of 5-HT_2- or both 5-HT_1- and 5-HT_2-receptors, respectively (Figures 2 and 3). These blockers reduced contractions caused by 5-HT; arteries appeared to be more sensitive than veins. Ketanserin apparently increased the contractions to high 5-HT concentra-

Table 1 Effect of methysergide and ketanserin (15 min preincubations) on the concentrations of 5-hydroxytryptamine (5-HT) producing 50% of maximum contractile responses (EC_{50})

	EC_{50} (M)	
	Arteries	*Veins*
Control	2.96 (1.09–7.94) × 10^{-7}	2.55 (0.95–6.46) × 10^{-7}
Methysergide (10^{-7} M)	1.96 (0.7–10) × 10^{-6}*	1.1 (0.26–4.58) × 10^{-6}
Ketanserin (10^{-7} M)	1.97 (0.66–5.68) × 10^{-7}	6.99 (3.36–14.22) × 10^{-7}
Ketanserin (10^{-6} M)	3.05 (1.22–6.72) × 10^{-5}*	5.04 (2.9–8.6) × 10^{-5}*

95% confidence limits are shown in parentheses. $n \geq 6$, in each case. * $P < 0.05$ with respect to control.

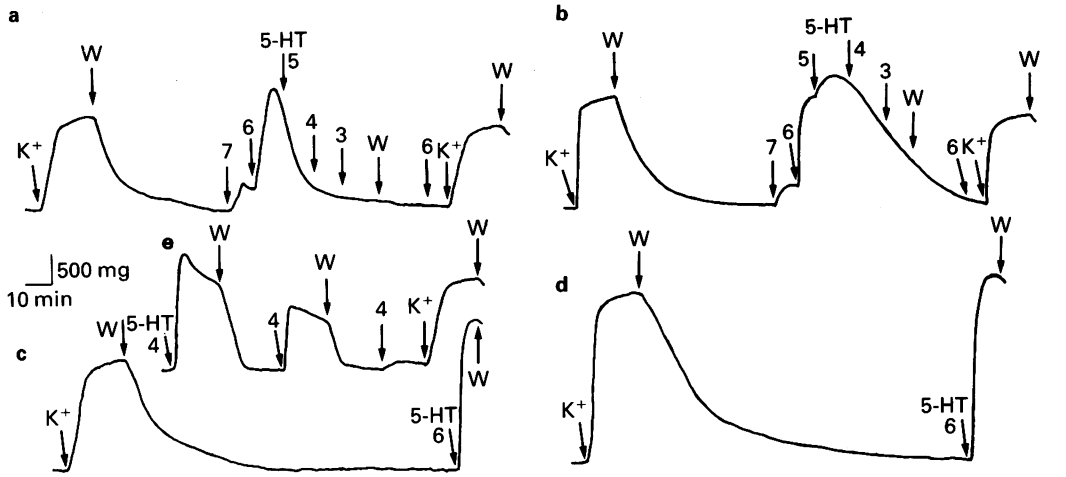

Figure 1 Typical recordings showing the biphasic responses induced by the cumulative administration of 5-hydroxytryptamine (5-HT) in cylindrical segments of human chorionic arteries (a) and veins (b), and the loss of the ability of this amine, but not of K^+ (75 mM), to contract these segments. In segments of artery (c) and vein (d) not exposed to this amine, this desensitization was not observed. The addition of high concentrations of these drugs (10^{-4} M) to arteries also produced a marked desensitization (e). In veins, this process was similar. Single concentrations of 5-HT elicited transient contractions. The concentrations of 5-HT administered are indicated as $-\log_{10}$ M. W = wash.

tions in veins (Figure 3). Neither ketanserin (10^{-8} M) nor methysergide (3×10^{-8} M) modified the contractions caused by 5-HT in either kind of vessel. The presence of 10^{-6} M ketanserin enhanced the EC_{50} values of 5-HT in both types of vessel (Table 1). Increasing the preincubation time with ketanserin from 15 min to 30 or 60 min did not modify the inhibitory effect of this antagonist (results not shown).

The effect of different extracellular Ca^{2+} concentrations on the CRC to 5-HT was analysed. Figure 4 shows that contractions are largely dependent on extracellular Ca^{2+}, but possess a small component independent of this source of Ca^{2+}. In addition, responses induced by 10^{-6} M 5-HT were greatly reduced by Ca^{2+} removal. Subsequent cumulative Ca^{2+} addition produced concentration-dependent contractions (Figure 5).

Contractions induced by 5-HT (10^{-6} M) in arteries and veins were reduced by nifedipine (10^{-7} M),

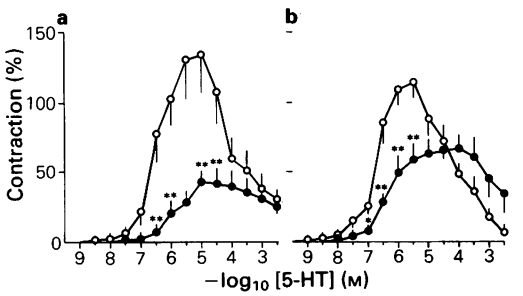

Figure 2 Effect of methysergide (●, 10^{-7} M, $n = 6$) on the concentration-response curve to 5-hydroxytryptamine (5-HT, 0, $n = 8$) in cylindrical segments of human chorionic artery (a) and vein (b). Responses to 5-HT were expressed as percentages of the previous contraction induced by 75 mM K^+ (1340 ± 240 mg in arteries, 1394 ± 218 mg in veins). Values are means and vertical lines show s.e.mean. $*P < 0.05$, $**P < 0.01$.

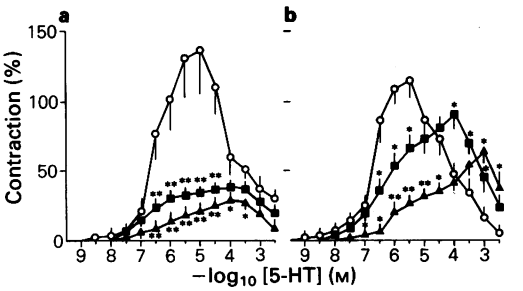

Figure 3 Effect of ketanserin (■, 10^{-7}, and ▲, 10^{-6} M, $n = 6$) on the concentration-response curve to 5-hydroxytryptamine (5-HT, 0, $n = 8$) in cylindrical segments of human chorionic artery (a) and vein (b). Responses to 5-HT were expressed as percentages of the previous contraction induced by 75 mM K^+ (1326 ± 221 in arteries, 1245 ± 166 mg in veins). Values are means and vertical lines show s.e.mean. $*P < 0.05$, $**P < 0.01$.

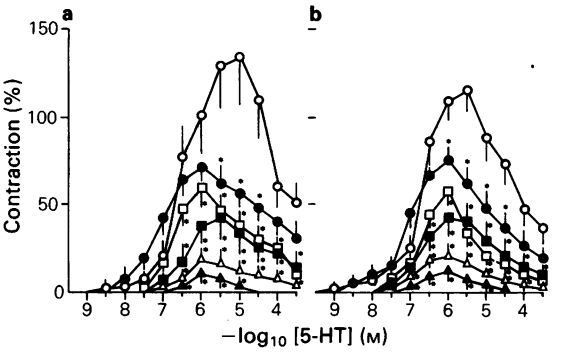

Figure 4 Effect of different Ca^{2+} concentrations (mM) (○, 2.5; ●, 1; □, 0.5; ■, 0.25; △, 0 and ▲, 0 + 1 EGTA; $n = 5$–8) on the concentration-response curve to 5-hydroxytryptamine (5-HT) in segments of human chorionic artery (a) and vein (b). Responses to 5-HT were expressed as percentages of the previous contraction induced by 75 mM K^+ (1250 ± 230 mg in arteries, 1200 ± 210 mg in veins). Values are means and vertical lines show s.e.mean. * $P < 0.05$.

nicardipine (10^{-5} M) and diltiazem (10^{-5} M). Arteries were less sensitive to Ca^{2+} antagonists than veins, the reduction caused by diltiazem in arteries did not reach significance (Figure 6). The concentrations of Ca^{2+} antagonists used were chosen because they produced maximal relaxant responses in segments contracted with 75 mM K^+.

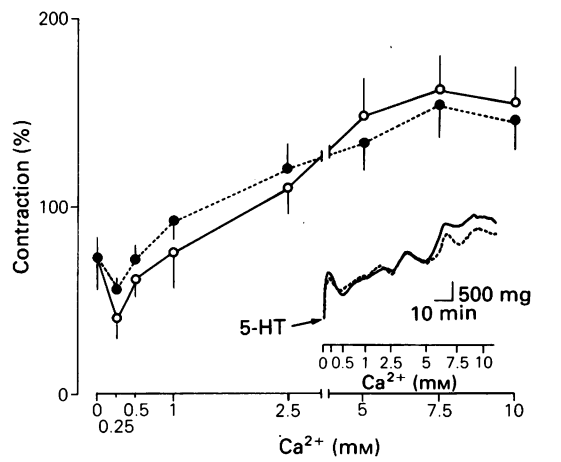

Figure 5 Effect of Ca^{2+} removal and subsequent Ca^{2+} addition on contractions induced by 5-hydroxytryptamine (5-HT, 10^{-6} M) in segments of human chorionic artery (○) and vein (●). Responses to 5-HT were expressed as percentages of the previous contraction induced by 75 mM K^+ (1775 ± 375 mg in arteries, 1550 ± 306 mg in veins). The inset shows typical recording of these effects.

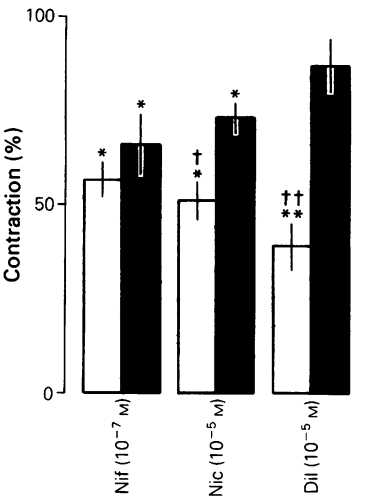

Figure 6 Inhibitory effect of nifedipine (Nif), nicardipine (Nic) and diltiazem (Dil) (15 min preincubations) on the contractions induced by 5-hydroxytryptamine (5-HT, 10 M) in segments of human chorionic artery (solid columns) and vein (open columns). Responses to 5-HT were expressed as percentages of the previous contraction induced by 5-HT (10^{-6} M) (1650 ± 285 mg in arteries, 1870 ± 426 mg in veins). * $P < 0.05$, ** $P < 0.01$, with respect to controls; † $P < 0.05$; †† $P < 0.01$, with respect to the remaining contractions obtained in arteries.

$^{45}Ca^{2+}$ uptake

To measure intracellular $^{45}Ca^{2+}$ uptake it is necessary to remove the large amount stored in the extracellular space and adhering to the plasma membrane. In these experiments, extracellular Ca^{2+} was removed by the addition of La^{3+} (Van Breemen *et al.*, 1972) at a concentration of 50 mM (Godfraind, 1976). For this purpose, the vessels were incubated for 15 min in KHS containing $^{45}Ca^{2+}$ and then rinsed with La^{3+} solution, which produced a rapid $^{45}Ca^{2+}$ loss in the first 10 min. At this time, steady state Ca^{2+} efflux was reached, and after 20 min washing with La^{3+} medium the Ca^{2+} loss was stopped (Figure 7); this incubation time was used in the following experiments determining $^{45}Ca^{2+}$ uptake.

The time course of $^{45}Ca^{2+}$ uptake, with or without 5-HT (10^{-6} M), is illustrated in Figure 7. In the control situation, the $^{45}Ca^{2+}$ content was augmented with time of incubation until 15 min, when the steady-state was reached. 5-HT significantly increased $^{45}Ca^{2+}$ uptake at 5 and 10 min incubations in both kinds of vessel.

The interference by nifedipine (10^{-7} M), nicardipine (10^{-5} M) and diltiazem (10^{-5} M) with the $^{45}Ca^{2+}$

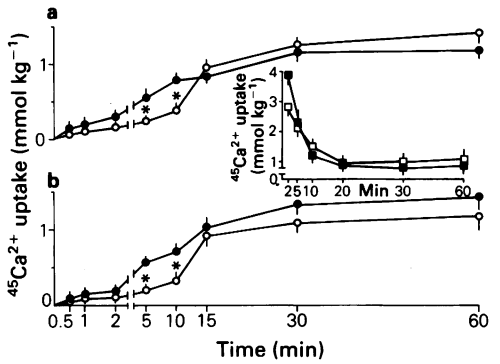

Figure 7 Time course of intracellular $^{45}Ca^{2+}$ uptake in the presence (●) and absence (○) of 5-hydroxytryptamine (5-HT, 10^{-6} M) in segments of human chorionic artery (a) and vein (b), incubated for different times in 4 ml KHS containing $^{45}Ca^{2+}$ and subsequently rinsed with La^{3+} (50 mM) solution for 20 min. The inset represents the time course of Ca^{2+} loss after different periods of washing with this La^{3+} solution in arteries (□) and veins (■). Each point represents the mean and vertical lines show s.e.mean of 6 experiments. * $P < 0.05$.

accumulation induced by 5-HT (10^{-6} M, 5 min incubation) is shown in Table 2. These Ca^{2+} antagonists significantly reduced Ca^{2+} content only in veins; basal $^{45}Ca^{2+}$ uptake was not modified. In addition, we investigated whether there was a reduction in the $^{45}Ca^{2+}$ uptake in desensitized vessels. If 5-HT receptors gate Ca^{2+} entry, then a reduction in Ca^{2+} influx would be predicted in desensitized vessels. Vessels were exposed to cumulative concentrations of 5-HT, and after rinsing with KHS and a period of stabilization in which the tension returned to baseline (around 20 min), $^{45}Ca^{2+}$ and 5-HT (10^{-6} M) were added to the bath. Control vessels were submitted to similar conditions. Uptake was significantly reduced in desensitized arteries (control: 0.67 ± 0.19; desensitized: 0.40 ± 0.06 mmol kg^{-1}; $P < 0.04$, $n = 5$) and veins (control: 0.55 ± 0.08; desensitized: 0.33 ± 0.02 mmol kg^{-1}; $P < 0.03$, $n = 6$).

Table 2 Effect of nifedipine, nicardipine and diltiazem (15 min preincubations) on intracellular $^{45}Ca^{2+}$ uptake (mmol kg^{-1}) induced by 5-hydroxytryptamine (5-HT, 10^{-6} M) in segments of human chorionic artery and vein

	Arteries	*Veins*
Control	0.58 ± 0.08	0.57 ± 0.04
Nifedipine (10^{-7} M)	0.61 ± 0.09	0.45 ± 0.03*
Nicardipine (10^{-5} M)	0.56 ± 0.09	0.47 ± 0.02*
Diltiazem (10^{-5} M)	0.53 ± 0.08	0.43 ± 0.04*

The vessels were incubated for 5 min in 4 ml KHS containing $^{45}Ca^{2+}$, with or without 5-HT, and subsequently rinsed with La^{3+} (50 mM) solution for 20 min.
Values are means ± s.e.mean. $n = 6$ in each situation. * $P < 0.05$.

Discussion

In this study it was shown that 5-HT induces strong, transient contractile responses in chorionic arteries and veins. The cumulative addition of this amine produced concentration-dependent contractions up to 3×10^{-6} M in veins and 10^{-5} M in arteries. The ability of 5-HT to elicit potent and transient increases in tension has been observed in bovine coronary arteries (Ratz & Flaim, 1984) and rabbit thoracic aorta (Purdy *et al.*, 1987). This amine also produces powerful contractions, greater than other agents, in human and animal umbilical or placental vessels (Dyer, 1970; Altura *et al.*, 1972; Nair & Dyer, 1974; Tulenko, 1979; Mak *et al.*, 1984; Maigaard *et al.*, 1986). In addition, it has been demonstrated that the concentration of 5-HT in the maternal blood and blood present in umbilical vessels at term (vaginal delivery) is around 10^{-7} M (in both cases measured in the whole blood) (O'Reilly & Loncin, 1967; Jones & Rowsell, 1973), i.e., similar to our EC_{50} values for 5-HT on chorionic vessels. It has been suggested that 5-HT may contribute to spasm and closure of the umbilical vessels after birth (Tulenko, 1979; Mak *et al.*, 1984). It is interesting to note that the concentration of placental 5-HT increases from the start of pregnancy until delivery. In contrast, monoamine oxidase (MAO) activity is decreased. This might trigger 5-HT release from uterine and placental stores and induce delivery (Koren *et al.*, 1965), and contraction of placental vessels. The increase in placental 5-HT concentration is probably due to an augmentation in the production of 5-HT in the developing foetus, since the placenta appears to lack the ability to synthesize 5-HT (Jones & Rowsell, 1973).

The 5-HT receptors involved in the contractions were investigated, using ketanserin and methysergide, antagonists of 5-HT_2 and both 5-HT_1 and 5-HT_2 receptors, respectively (Van Nueten *et al.*, 1981; Houston & Vanhoutte, 1986). Methysergide diminished responses induced by 5-HT, as did ketanserin. However, methysergide (10^{-7} M) reduced the effects of low 5-HT concentrations while ketanserin (10^{-7} M) did not. This suggests that the high sensitivity component of the effect of 5-HT is mediated through a 5-HT_1-like receptor, while high 5-HT concentrations act through 5-HT_2 receptors. The vessels may, thus, possess two types of 5-HT receptor (5-

HT_1 and 5-HT_2), which mediate the actions of 5-HT. A similar conclusion has been obtained by other authors in human umbilical arteries (Diemer *et al.*, 1985). The antagonism between 5-HT and each of these blockers was non-competitive, since the CRCs to 5-HT were not displaced in a parallel manner to the right, and the maximal response was reduced. Both antagonists have other properties which might account for these results, e.g. ketanserin may block α-adrenoceptors (Brazenor & Angus, 1982; Nishimura *et al.*, 1987). Similar results and conclusions have been obtained in human hand veins (Arneklo-Nobin *et al.*, 1985). Alternatively, 5-HT might stimulate α-adrenoceptors in these vessels (see Purdy *et al.*, 1987). If 5-HT acts by these mechanisms in chorionic vessels, this might explain the non-competitive antagonism previously mentioned. In other vessels, ketanserin and/or methysergide are competitive antagonists (Brazenor & Angus, 1982; Frenken & Kaumann, 1985; Arneklo-Nobin *et al.*, 1985), non-competitive antagonists (Brazenor & Angus, 1982; Arneklo-Nobin *et al.*, 1985) or show no effect (Bradley *et al.*, 1986), supporting the contention that different 5-HT receptor subtypes exist on vascular smooth muscles.

It is interesting to note that the antihypertensive effect of ketanserin (Hedner *et al.*, 1983) occurs at a peak plasma concentration of around 10^{-7} M (Williams *et al.*, 1986). This concentration caused a significant reduction of 5-HT contractions in chorionic vessels. Further, chorionic arteries were more sensitive to both ketanserin and methysergide than veins. Thus, the placental circulation may be markedly affected by the administration of these drugs, especially at the end of delivery when concentrations of 5-HT are high.

The responses induced by 5-HT were decreased with a reduction of Ca^{2+} in the medium. When this ion was removed (with or without EGTA), the responses were greatly diminished. 5-HT (10^{-6} M)-induced contractions were also markedly reduced in a Ca^{2+}-free medium and recovered on subsequent Ca^{2+} addition. These results show that the responses to 5-HT are largely dependent on extracellular Ca^{2+}. The dependence of 5-HT-induced contractions on intracellular and extracellular Ca^{2+} has been observed in rabbit ear and bovine coronary arteries (Maggi *et al.*, 1983; Ratz & Flaim, 1984), whereas others, such as cerebral arteries, are markedly dependent on extracellular Ca^{2+} (Rusch *et al.*, 1985; Marin, 1988).

The ability of 5-HT to induce Ca^{2+} influx was demonstrated by tracer experiments (La^{3+}-method) and by the use of Ca^{2+} antagonists. La^{3+} displaces extracellular Ca^{2+} and blocks Ca^{2+} fluxes (Van Breemen *et al.*, 1972; Godfraind, 1976). Washing chorionic vessels preincubated with $^{45}Ca^{2+}$ with La^{3+} solution displaced most Ca^{2+} in the first 10 min, and Ca^{2+} fluxes were stopped by 20 min. Godfraind (1976) observed in rat aorta, using the same La^{3+} concentration, that there was a rapid loss of $^{45}Ca^{2+}$ in the first 5 min but, subsequently, loss was markedly diminished although not abolished. This discrepancy might be due to vessel differences. Control and stimulated $^{45}Ca^{2+}$ uptake were fast in these vessels, until 15 min of incubation when a steady state was reached. A similar pattern of $^{45}Ca^{2+}$ uptake has been observed in human umbilical vessels (Ozaki *et al.*, 1981).

The effect of Ca^{2+} antagonists on 5-HT-induced $^{45}Ca^{2+}$ influx was studied using a short period of incubation (5 min) when inward flux predominates (Meisheri *et al.*, 1981). 5-HT-stimulated uptake was significantly reduced by nifedipine, nicardipine and diltiazem only in veins. Unstimulated $^{45}Ca^{2+}$ uptake was not affected by these Ca^{2+} antagonists, which agrees with results obtained with verapamil in umbilical vasculature (Ozaki *et al.*, 1981) and with other Ca^{2+} antagonists in different vessels (Cauvin *et al.*, 1983). The ability of these drugs to reduce 5-HT-induced $^{45}Ca^{2+}$ influx in veins was correlated with their greater capacity to inhibit 5-HT contractions in veins compared to arteries (Figure 6). In contrast, ketanserin and methysergide were more effective in blocking 5-HT responses in arteries. These data indicate that although the contraction caused by 5-HT is dependent on extracellular Ca^{2+} in both chorionic arteries and veins, 5-HT might produce a greater depolarization in vein smooth muscle cells and thus be more susceptible to Ca antagonists. The ability of 5-HT to produce depolarization has been observed previously (Harder & Waters, 1983).

In chorionic vessels, 5-HT produced transient contractions, a CRC which was not sigmoid, but biphasic, and pronounced tachyphylaxis. However, the response induced by K^+ in desensitized segments was unaffected. These results suggest that exposure to high 5-HT concentrations produces a rapid and long-lasting desensitization of the receptors. This is supported by the finding that $^{45}Ca^{2+}$ uptake was reduced in this situation. This desensitization appeared to be agonist specific, because responses to histamine or noradrenaline were not diminished (results not shown). It is interesting to note that ketanserin appeared to enhance the contractions to high 5-HT concentrations in veins. These results possibly indicate that desensitization of 5-HT receptors by 5-HT might be due to interconversion of the receptors from a high affinity state to another of low affinity, an interconversion prevented in part by ketanserin. Interconvertible states and this kind of action of ketanserin on 5-HT_2 receptors in their normal active state has been postulated recently (Kaumann & Frenken, 1985; Frenken & Kaumann,

1988). The tachyphylaxis observed in chorionic vessels could have a physiological role in preventing a prolonged reduction of placental blood flow.

In conclusion, 5-HT produced strong transient contractions in human chorionic arteries and veins, largely dependent on extracellular Ca^{2+}. Responses in arteries were more affected by methysergide and ketanserin than those in veins, while the opposite occurred with the Ca^{2+} antagonists, nifedipine, nicardipine and diltiazem. These contractions appear to be mediated by 5-HT_2- and by 5-HT_1-like receptors.

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