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# 5-$HT_1$-like receptors requiring functional cyclo-oxygenase and 5-$HT_2$ receptors independent of cyclo-oxygenase mediate contraction of the human umbilical artery

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**1** The interactions between 5-hydroxytryptamine (5-HT) and the antagonists ketanserin, methysergide and phentolamine were studied in isolated preparations of human umbilical artery (HUA) at physiological oxygen tension ($Po_2 \sim 15$ mmHg) and at high $Po_2$ ($\sim 120$ mmHg).

**2** At physiological $Po_2$ ketanserin, methysergide and phentolamine behaved as silent competitive antagonists of the 5-HT-induced contraction of HUA. $pA_2$ values calculated by Schild analysis were 8.92, 8.52 and 6.37, respectively.

**3** At high $Po_2$, 5-HT-induced contractions were antagonised in a biphasic manner by ketanserin (0.1 $\mu$M); the response to low but not to high concentrations of 5-HT was resistant to blockade by ketanserin. The ketanserin-resistant component was abolished following cyclo-oxygenase inhibition by indomethacin (1 $\mu$M).

**4** At high $Po_2$, methysergide behaved as a partial agonist. Methysergide-induced contractions were inhibited but not abolished by indomethacin, and resistant to 5-$HT_2$ receptor and $\alpha_1$-adrenoceptor blockade.

**5** At high $Po_2$ the component of the response to 5-HT mediated by the ketanserin-resistant receptor was mimicked by the selective 5-$HT_1$-like receptor agonist 5-carboxamidotryptamine (5-CT): 5-CT was 7 fold more potent than 5-HT.

**6** At high $Po_2$ the component of the response to 5-HT mediated by the ketanserin-resistant receptor was antagonised by phentolamine and the selective $\alpha_2$-adrenoceptor antagonist Wy 26703.

**7** These results suggest that (i) at physiological $Po_2$ 5-$HT_2$ receptors almost exclusively mediate contractions induced by 5-HT, and (ii) at high $Po_2$ the agonist potency order of 5-CT > 5-HT > methysergide suggests that ketanserin-resistant responses are mediated by 5-$HT_1$-like receptors which require functional cyclo-oxygenase.

## Introduction

We have previously shown that, *in vitro*, under conditions which mimic the arterial blood-gas status of the human umbilical artery (HUA) *in utero*, 5-hydroxytryptamine (5-HT) causes contraction via a receptor which could be described as 5-$HT_2$ (McGrath *et al.*, 1985). The criteria for this classification relied upon rather non-selective antagonists: methysergide which is non-selective between 5-HT receptor sub-types, and phentolamine which, besides being a non-selective $\alpha$-adrenoceptor antagonist has micromolar affinity for both peripheral 5-$HT_2$ receptors (e.g. rabbit aorta, Apperley *et al.*, 1976) and 5-$HT_1$-like receptors (e.g. dog saphenous vein, Humphrey, 1978). A further examination of the receptor in the HUA was therefore necessary in order to address the guidelines for 5-HT receptor classification, which have more recently been published (Bradley *et al.*, 1986a), central to which are the use of the selective 5-$HT_1$ receptor agonist 5-carboxamidotryptamine, and the selective 5-$HT_2$ receptor antagonist ketanserin.

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A second question we wished to address relates to how varying the oxygen tension ($Po_2$) of the solution bathing the isolated arterial strips might influence the reactivity of the smooth muscle. Under the normally low level of oxygen ($Po_2 \sim 15$ mmHg; Wulf, 1964) the receptor population appears to be homogeneous (McGrath *et al.*, 1985). We have since established that increasing the $Po_2$ induces the isolated HUA to contract by cyclo-oxygenase products (McGrath *et al.*, 1986; MacLennan *et al.*, 1988b); therefore, we wished to examine how cyclo-oxygenase products might influence the expression of the 5-$HT_2$ receptor. In the present study we showed that increasing the $Po_2$ above the physiological level had no apparent effect on 5-$HT_2$ receptor expression in the HUA. However, on elevating the $Po_2$ a second population of 5-HT receptors was 'revealed' which could be classified as 5-$HT_1$-like.

Some of these results have been presented previously at meetings of the British Pharmacological Society (McGrath & MacLennan, 1986; MacLennan & McGrath, 1986).

## Methods

Lengths of umbilical cord (5–30 cm) were cut from the placental portion as soon as practically possible after delivery but normally within 45 min. The cords were placed in de-oxygenated Krebs-bicarbonate saline (composition, mM: NaCl 119, KCl 4.7, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.1) at 4°C for up to 48 h, before being used. The saline was de-oxygenated by pre-gassing with 8% $CO_2$ in nitrogen.

Arteries were dissected free of the surrounding Wharton's jelly in de-oxygenated Krebs solution, to reduce the oxygen tension as far as possible. Longitudinal strips of artery, length 1–1.5 cm, were suspended within 40 ml organ baths containing Krebs saline at 37°C under a force of 1 g. Isometric force was monitored using Grass FT03c transducers and a Grass model 7 polygraph. Unpublished studies suggest the endothelium is not functional in this tissue.

In all experiments the tissues were equilibrated for 2–2.5 h before any experimentation, gassed with 2.5% $O_2$, 8% $CO_2$, balance $N_2$, to mimic the gas tensions and pH of umbilical arterial blood, *in utero*, i.e. $Po_2$, 15 mmHg; $Pco_2$ 50 mmHg; pH 7.28 (Wulf, 1964; Pearson, 1976). $Pco_2$ and pH of the organ baths were monitored by withdrawing samples and analysing them on a blood-gas analyser (Instrumentation Laboratories, model 1302). $Po_2$ was continuously monitored by use of an oxygen electrode (Instrumentation Laboratories, model 1302) and meter (Strathkelvin Instruments, model 781). The oxygen electrode was incorporated into a specially designed organ bath via a side port. It was established that the $Po_2$ measured in one bath was within $\pm 3$ mmHg of the $Po_2$ found for the Krebs solution in any of the three other baths used (when aerated with the same gas mixture, and at a similar rate), throughout the range tested −0 to 600 mmHg.

Agonists and antagonists were studied at two levels of $Po_2$: (i) physiological $Po_2$, ~15 mmHg (2.5% $O_2$), (ii) high $Po_2$, ~120 mmHg (17% $O_2$). The values in parentheses are the % $O_2$ composition of the gas mixtures used to aerate the Krebs solution. The remaining gas was composed of 8% $CO_2$/balance $N_2$. The gas mixtures were made up in Douglas bags using the rotameters from an anaesthetics trolley. The Krebs solution was gassed with these mixtures by a small aquarium pump.

Concentration-response curves (CRCs) to agonists were constructed cumulatively, by increasing the bathing solution concentration by $0.5 \log_{10}$ increments at intervals when the preceding response had reached a plateau – this was at approximately 3 min intervals for all agonists. Response (% of maximum response, g tension or % of a prior 50 mM KCl contraction) was plotted against log(agonist concentration). $pD_2$ values were calculated as $-\log(EC_{50})$, where $EC_{50}$ is the concentration of agonist which gives 50% of the maximum response.

The potency of test agonists was compared to that of 5-HT by first constructing a CRC to 5-HT followed by one for the test agonist in the same preparation. The potency of the test agonist was calculated as the ratio of $EC_x$ (test agonist), over $EC_x$ (5-HT), where x is the level of response, e.g. $EC_{50}$, $EC_{75}$, as given in the text. Where the potency of 5-carboxamidotryptamine (5-CT) relative to 5-HT was examined, the calculated potency was corrected for the time-related change in sensitivity of the tissue by division by the concentration-ratio of a control preparation in which two successive CRCs to 5-HT were constructed. The relative potencies are given as the geometric mean and 95% confidence limits.

### *Antagonist experiments*

*Low* $Po_2$, *~15 mmHg* Four preparations from the one artery were used. In each preparation CRCs to 5-HT or 5-CT were constructed as already described. After washout of the drug (60 min) different concentrations of antagonist were added to three preparations and allowed to equilibrate for 30 min before again constructing a CRC to 5-HT (or 5-CT). One preparation therefore acted as a control to assess the change in sensitivity (with time), which was approximately two fold. Response was calculated as the % of the 1st curve's maximum response and plotted

**Table 1** Parameters of the Schild plot for the interaction of antagonists with 5-hydroxytryptamine (5-HT) and 5-carboxamidotryptamine (5-CT) in the human isolated umbilical artery at physiological $Po_2$ (~15 mmHg)

| *Antagonist* | *Agonist* | $pA_2$ | *Slope* | n |
|---|---|---|---|---|
| Methysergide | 5-HT | 8.52 (8.32–8.72) | 0.94 (0.76–1.12) | 6 |
| Phentolamine | 5-HT | 6.37 (5.88–6.86) | 1.04 (0.82–1.26) | 6 |
| Ketanserin | 5-HT | 8.92 (8.70–9.14) | 0.91 (0.60–1.22) | 6 |
| | 5-CT | 8.77 (8.39–9.15) | 1.19 (0.68–1.70) | 5 |
| Ketanserin (+1 μM indomethacin) | 5-HT | 8.94 (8.22–9.66) | 0.99 (0.66–1.32) | 4 |
| | 5-CT | 9.05 (8.58–9.52) | 1.00 (0.75–1.25) | 4 |

Values are the mean (95% confidence limits) of *n* estimates.

against log(5-HT concentration) (log[5-HT]). For each preparation the concentration-ratio (CR) was calculated as the ratio of the $EC_{50}$ of 5-HT (+ antagonist), over the $EC_{50}$ for 5-HT from the control (1st) curve. The CR was corrected for time-related change in sensitivity by division by the CR of the control preparation. A Schild plot was constructed with the CRs: log(CR − 1) (ordinate scale) was plotted against log(antagonist concentration) (abscissa scale) (Arunlakshana & Schild, 1959). A line of best-fit was found for the points by linear regression (least squares) which gave the slope and an estimate of the $pA_2$ as the intercept of the regression line with the line, log (CR − 1) = 0. In each estimate of a $pA_2$, 4 to 6 preparations were used. The mean values of the slope of the regression line and estimated $pA_2$ are presented together with their respective 95% confidence limits.

*High* $Po_2$, *~120 mmHg* In any one preparation a single concentration of antagonist was studied at higher than physiological $Po_2$ (~120 mmHg) as follows: CRC to 5-HT was constructed at $Po_2$ ~ 15 mmHg. After washout (60 min) the $Po_2$ was increased to ~120 mmHg which sometimes caused a contraction, but which was always transient. The antagonist was added to the bath for 30 min before a 2nd CRC to 5-HT was constructed. A third CRC to 5-HT was constructed in the presence of the same antagonist concentration plus indomethacin (1 μM, 30 min). Response (% of 1st (low $Po_2$) maximum response) was plotted against log[5-HT]. Estimates of antagonist potency were made by calculating the log agonist concentration-ratio as described above. Log agonist CRs were calculated at the $EC_{25}$ and $EC_{75}$ levels in the presence and absence of indomethacin.

Methysergide was an agonist at high $Po_2$ but due to tachyphylaxis two successive CRCs (before and in the presence of the antagonist) could not be constructed. Antagonists were studied as follows: one strip acted as a control and antagonist(s) were added to other strips from the same artery. CRCs to the agonist were constructed simultaneously on all strips.

### *Drugs*

The following drugs were used (source in parentheses): 5-hydroxytryptamine creatinine sulphate (Sigma); indomethacin (Sigma); methysergide bimaleate (Sandoz); (±)-pindolol (Sandoz); phentolamine mesylate (Ciba); prazosin hydrochloride (Pfizer); ketanserin tartrate (Janssen); 5-carboxamidotryptamine (Glaxo); buspirone hydrochloride (Bristol-Myers); Wy 26703 (N-methyl-N-(1,3,4,6,7,11b-hexahydro-2H-benzo-[a]-quinolizin-2-yl)-i-butanesulphonamide HCl, Wyeth). All drugs, with the exception of indomethacin, were dissolved in distilled water. Indomethacin was initially dissolved in absolute ethyl alcohol and diluted further in distilled water. The concentration of vehicle in the organ bath (0.8 mM) did not influence tissue responsiveness.

### *Statistics*

Statistical comparisons of the means of groups of data were made by use of Student's *t* test for paired or unpaired data, where appropriate. A level of probability of $P < 0.05$ was taken to indicate statistical significance.

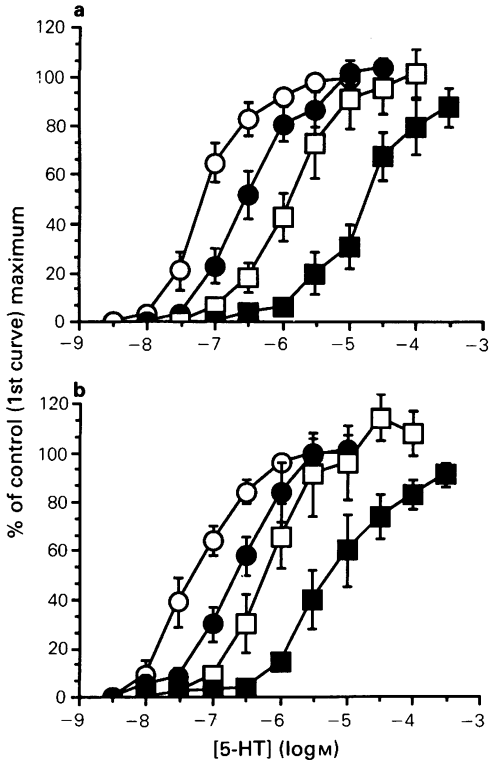


**Figure 1** Log concentration-response curves (CRCs) to 5-hydroxytryptamine (5-HT) at low $Po_2$ in the presence of (a) methysergide ($n = 6$), and (b) phentolamine ($n = 6$). CRCs to 5-HT were constructed twice in each of four preparations from the same artery. In three of the four preparations the second CRC was repeated in the presence of one concentration of antagonist. In the fourth (control) preparation the 1st (○) and second CRCs were constructed without antagonist in order to assess the change in sensitivity to 5-HT with time, which was in (a) 2.0 ± 0.7 fold and in (b) 1.8 ± 0.5 fold. (a) [Methysergide] (●) 10 nM; (□) 100 nM; (■) 1000 nM; $Po_2 = 11 \pm 2$ mmHg. (b) [Phentolamine] (●) 1 μM; (□) 10 μM; (■) 100 μM; $Po_2 = 16 \pm 2$ mmHg. Each point represents the mean and vertical lines show s.e.mean.

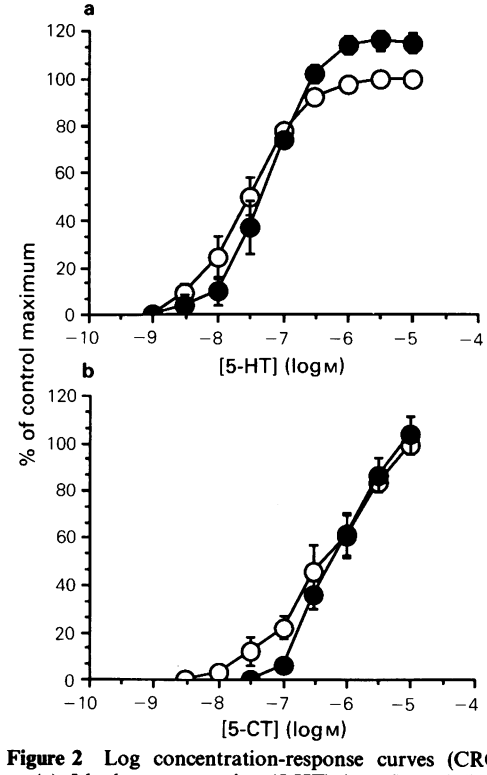


**Figure 2** Log concentration-response curves (CRCs) to (a) 5-hydroxytryptamine (5-HT) ($n = 6$), and (b) 5-carboxamidotryptamine (5-CT) ($n = 5$), at low $Po_2$ in the presence (●) and absence (○) of indomethacin (1 μM). The protocol was to construct a CRC to either 5-HT or 5-CT before and after exposure (30 min) to indomethacin. Indomethacin had no effect on baseline tension. (a) $Po_2 = 16 \pm 2$ mmHg, (b) $Po_2 = 13 \pm 2$ mmHg.

## Results

### *Antagonist studies at physiological* $Po_2$

The parameters of the Schild plots obtained from the interaction between the antagonists employed in this study, and the agonists 5-HT and 5-CT, are summatised in Table 1.

Methysergide and phentolamine were found to be silent competitive antagonists of the 5-HT-induced contraction of the HUA, since antagonism was surmountable and the slopes of the Schild plots were not significantly different from unity (Figure 1). $pA_2$ values for methysergide and phentolamine were 8.52 and 6.37, respectively (mean values). The concentration-response curves appeared, in a qualitative manner, to be displaced in parallel fashion by these antagonists. This was confirmed quantitatively since the log(agonist concentration-ratios) calculated at the level of the $EC_{25}$ and $EC_{75}$ at each antagonist concentration, were not significantly different; for example, in the presence of methysergide (1 μM) the log agonist CR at the $EC_{25}$ and $EC_{75}$ levels were $2.26 \pm 0.2$ and $2.62 \pm 0.28$, respectively (mean ± s.e.mean). In the presence of phentolamine (100 μM) the log agonist CR at the $EC_{25}$ and $EC_{75}$ levels were $2.41 \pm 0.24$ and $2.50 \pm 0.28$, respectively.

The possible role of cyclo-oxygenase products was investigated. Indomethacin (1 μM) alone did not shift the concentration-response curves to 5-HT or 5-CT

(Figure 2). The control concentration-response curve to 5-CT appeared to be slightly biphasic and the responses to lower concentrations of 5-CT were blocked by indomethacin: the 5-CT concentration-response curve thus became monophasic in the presence of indomethacin with a resulting steepening of the concentration-response curve. At higher concentrations, the maximum response to 5-HT was significantly increased by $18 \pm 4\%$ in the presence of indomethacin. However, in paired control tissues not exposed to indomethacin, a smaller but significant increase of the maximum of $7 \pm 3\%$ was obtained (not shown). Indomethacin (1 $\mu$M) caused no change of the resting baseline tension.

The interaction between ketanserin and both 5-HT and 5-CT was investigated in the absence and presence of indomethacin (1 $\mu$M), (Figure 3 and Table 1).

*5-Hydroxytryptamine* Without indomethacin present ketanserin caused a rightward shift of the 5-HT concentration-response curve that was parallel only at concentrations (of 5-HT) which produced greater than (approximately) 30% of the maximum response (Figure 3a), i.e. at physiological $Po_2$ there was a small component of the response to 5-HT that was resistant to ketanserin. Log agonist CRs were calculated from 0.1 $\mu$M ketanserin. At the $EC_{25}$ the mean log agonist CR ($1.33 \pm 0.38$) was smaller than at either the $EC_{50}$ ($1.90 \pm 0.14$) or $EC_{75}$ ($1.89 \pm 0.23$), but the difference was not significant. The Schild plot, which is based on concentration-ratios calculated at the $EC_{50}$, indicated that ketanserin was a competitive antagonist of 5-HT at physiological $Po_2$ since the slope of the regression line (0.91, mean) was not significantly different from unity. The estimated $pA_2$ for ketanserin was 8.92 (mean).

In the presence of indomethacin (1 $\mu$M) ketanserin displaced the 5-HT concentration-response curve in a parallel manner at all concentrations (Figure 3b). The Schild plot had a slope of 0.99 (mean) and the estimated $pA_2$ for ketanserin against 5-HT was 8.94 which was not significantly different from the $pA_2$ obtained in the absence of indomethacin, 8.92. The broken line of Figure 3a (which is the concentration-response curve to 5-HT in the presence of 0.1 $\mu$M ketanserin plus 1 $\mu$M indomethacin – from Figure 3b) highlights the small component of the response to 5-HT that was resistant to 0.1 $\mu$M ketanserin but sensitive to indomethacin.

*5-Carboxamidotryptamine* Ketanserin (3 nM–30 nM) caused biphasic shifts of the 5-CT concentration-response curve similar to those described for 5-HT, i.e. the responses to the lowest concentrations of 5-CT were resistant to blockade by ketanserin but were sensitive to indomethacin. Nevertheless the

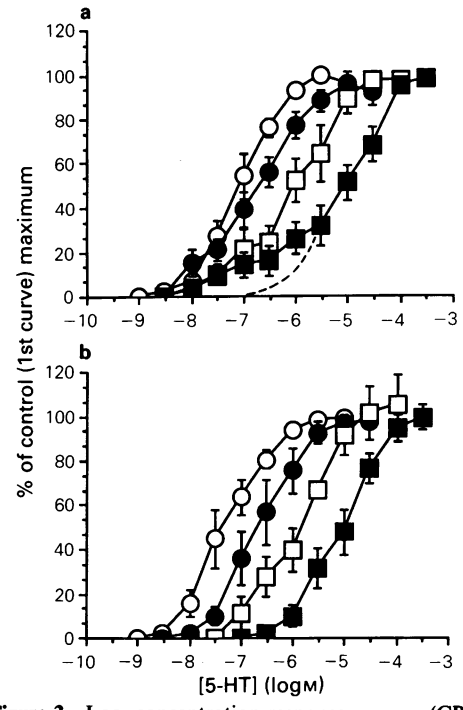


**Figure 3** Log concentration-response curves (CRCs) to 5-hydroxytryptamine (5-HT) at low $Po_2$ ($14 \pm 2$ mmHg), (a) in the presence of ketanserin ($n = 6$), and (b) in the presence of ketanserin plus indomethacin (1 $\mu$M) ($n = 4$). CRCs to 5-HT were constructed twice in each of four preparations from the same artery. In three of the four preparations the second CRC was repeated in the presence of one concentration of ketanserin; (●) 1 nM; (□) 10 nM; (■) 100 nM. In the fourth (control) preparation the 1st (○) and second CRCs were constructed without antagonist in order to assess the change in sensitivity to 5-HT with time, which was in (a) $1.8 \pm 0.3$ fold and in (b) $1.5 \pm 0.5$ fold. The broken line in (a) is the CRC to 5-HT in the presence of 100 nM ketanserin plus 1 $\mu$M indomethacin (from b). Each point represents the mean and vertical lines show s.e.mean.

Schild plot, based on concentration-ratios calculated at the $EC_{50}$, had a slope (1.19) not significantly different from unity. In this set of experiments the slopes of two of five of the individual Schild plot regression lines were considerably greater than unity (1.57 and 1.78), which was due to the rather low concentration-ratios obtained at the lowest antagonist concentration. In the presence of indomethacin (1 $\mu$M), ketanserin (3–30 nM) caused a parallel shift of the 5-CT concentration-response curve. The estimated $pA_2$ values for ketanserin against 5-CT were not significantly different in the presence (9.05) (mean) or absence (8.77) of indomethacin. These $pA_2$ values were not significantly different from the

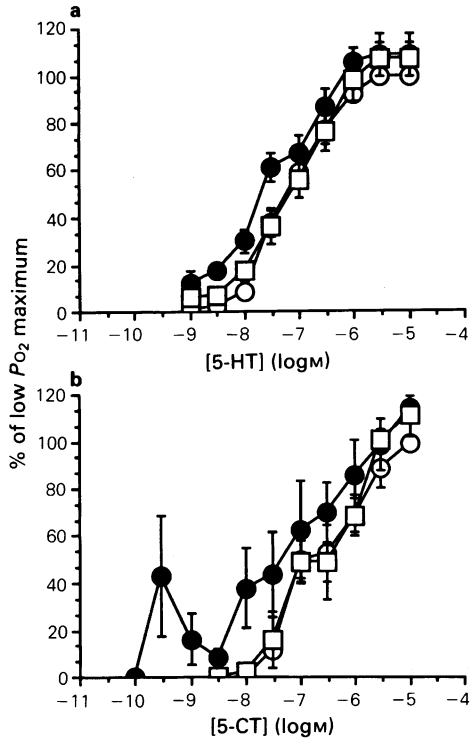


**Figure 4** Log concentration-response curves (CRCs) to (a) 5-hydroxytryptamine (5-HT) ($n = 7$) and (b) 5-carboxamidotryptamine (5-CT) ($n = 5$) at low $Po_2$ (□), and at high $Po_2$ in the presence (○) and absence (●) of indomethacin (1 μM). The protocol was to construct a CRC to either 5-HT or 5-CT at low $Po_2$. Following washout the $Po_2$ was increased and a 2nd CRC constructed in the presence or absence of indomethacin. In those tissues exposed to indomethacin, the drug was added 30 min before raising the $Po_2$. Response (ordinate scale) is expressed as a % of the maximum response to 5-HT or 5-CT at low $Po_2$. Each point represents the mean and vertical lines show s.e.mean. (a) Low $Po_2 = 8 \pm 3$ mmHg, high $Po_2 = 118 \pm 3$ mmHg. (b) Low $Po_2 = 12 \pm 3$ mmHg, high $Po_2 = 119 \pm 4$ mmHg.

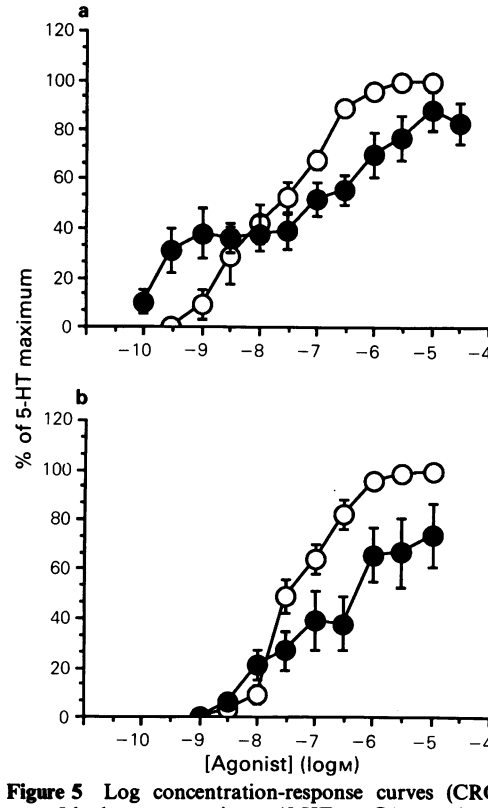


**Figure 5** Log concentration-response curves (CRCs) to 5-hydroxytryptamine (5-HT, ○) and 5-carboxamidotryptamine (5-CT, ●) at (a) low $Po_2$ ($13 \pm 1$ mmHg) ($n = 5$); (b) high $Po_2$ ($123 \pm 1$ mmHg) ($n = 6$). The protocol was to construct a CRC to 5-HT followed by one to 5-CT in each preparation. Response (ordinate scale) is expressed as a % of the maximum response to 5-HT. Each point represents the mean and vertical lines show s.e.mean.

respective values (8.94 and 8.92) in the presence and absence of indomethacin, with 5-HT as the agonist (Table 1).

*Effect of raising the* $Po_2$

The effects, *per se*, of increasing the $Po_2$ from the physiological level (~15 mmHg) to high $Po_2$ (~120 mmHg) on the response to 5-HT and 5-CT were investigated (Figure 4). Concentration-response curves were first constructed at physiological $Po_2$ then re-constructed at high $Po_2$ in the presence or absence of indomethacin (1 μM). In the absence of indomethacin increasing the $Po_2$ contracted the HUA. This contraction was $35 \pm 10\%$ of the maximum contraction to 5-HT but was not maintained and the change in baseline tension before the construction of a 2nd concentration-response curve was $4 \pm 3\%$, which was not significant. Raising the $Po_2$ failed to induce a contraction in the presence of indomethacin.

*5-Hydroxytryptamine* Increasing the $Po_2$ caused a significant leftward shift of the 5-HT concentration-response curve. Assessed at the $EC_{50}$ level the shift was 3.23 fold (geometric mean, 95% confidence limits 1.1–5.3). In paired preparations incubated with indomethacin increasing the $Po_2$ did not significantly increase (or decrease) the potency of 5-HT.

*5-Carboxamidotryptamine* Increasing the $Po_2$ had a significant facilitating effect on the response to 5-CT, which, at high $Po_2$, was found to be distinctly biphasic. The 1st phase of the response lay between 0.1 nM and 10 nM, and the 2nd phase between 10 nM and 10 $\mu$M. The 1st phase of the 5-CT response was very variable between different preparations, both in terms of the magnitude of the response and the degree of fade of the response. At the different levels of $Po_2$ the 5-CT concentration-ratios were calculated at the $EC_{25}$ and $EC_{75}$ levels which were taken as parameters of the 1st and 2nd phases respectively. Increasing the $Po_2$ caused a significant leftward shift: at the $EC_{25}$ level this was by 24.5 (6.8–89.1) fold and at the $EC_{75}$ level the shift was 6.9 fold (1.9–25.1) which was smaller than at the $EC_{25}$ but was still significant. As was found for 5-HT, increasing the $Po_2$ in the presence of indomethacin had no significant effect on the concentration-response curve to 5-CT.

The potency of 5-CT relative to 5-HT was examined at both physiological $Po_2$ and at high $Po_2$ by first constructing a concentration-response curve to 5-HT followed by one to 5-CT. The experiments at the two levels of $Po_2$ were examined in two separate series of experiments (Figure 5 and Table 2). The relative potencies of 5-HT and 5-CT were calculated at the $EC_{25}$ and $EC_{75}$. As described above, the predominant difference between the two levels of $Po_2$ was the distinctly biphasic nature of the 5-CT concentration-response curve at high $Po_2$. These experiments showed that (i) at physiological $Po_2$ 5-CT was approximately equipotent to 5-HT at the $EC_{25}$ but 5 fold less potent at the $EC_{75}$, (ii) at high $Po_2$ 5-CT was 7 fold more potent than 5-HT at the $EC_{25}$ but 4 fold less potent at the $EC_{75}$.

*Antagonist studies at high* $Po_2$ (*~ 120 mmHg*)

The interactions between 5-HT and the different receptor antagonists, in the presence and absence of indomethacin (1 $\mu$M) was investigated by employing only a single concentration of each of the antagonists (Figure 6). Log agonist CRs were calculated at the level of the $EC_{25}$ and $EC_{75}$. These levels were chosen as parameters of the ketanserin-resistant and ketanserin-sensitive components of the concentration-response curve to 5-HT at high $Po_2$. Log agonist CRs in the absence and presence of indomethacin were calculated with reference to the following control concentration-response curves: in the absence of indomethacin the control curve was that for 5-HT at high $Po_2$ (non-paired preparations, from Figure 4a); in the presence of indomethacin the control curve was to 5-HT at physiological $Po_2$ (same preparation). The rationale for the latter calculation was that the concentration-response curves to 5-HT at physiological $Po_2$, and at high $Po_2$ in the presence of indomethacin were not significantly different – there was no significant difference between the two curves at the level of the $EC_{25}$ or $EC_{75}$ (Figure 4a).

Ketanserin (0.1 $\mu$M) caused a distinctly biphasic shift of the 5-HT concentration-response curve at high $Po_2$. The log agonist CR calculated at the $EC_{25}$ ($0.62 \pm 0.12$) was significantly lower than at the $EC_{75}$ ($1.67 \pm 0.18$), indicating that at high $Po_2$ the response to lower concentrations of 5-HT was mediated by a ketanserin-resistant mechanism. The log agonist CR at the $EC_{75}$ (1.67) was not significantly different from that calculated at physiological $Po_2$ ($1.89 \pm 0.23$), indicating that at high $Po_2$ the response to higher concentrations of 5-HT was mediated by a ketanserin-sensitive mechanism. In the presence of indomethacin, ketanserin caused a parallel rightward displacement of the 5-HT concentration-response curve: the log agonist CRs at the $EC_{25}$ ($2.12 \pm 0.14$) and $EC_{75}$ ($1.97 \pm 0.10$) levels were not significantly different. Thus the ketanserin-resistant phase was mediated by an indomethacin-sensitive mechanism (Figure 6a).

At high $Po_2$ methysergide contracted the HUA. At 0.1 $\mu$M this contraction was $16 \pm 8\%$ of the maximum response to 5-HT. This contrasts with the lack of agonist action of methysergide at physiological $Po_2$. Methysergide (0.1 $\mu$M) caused a shift of the

**Table 2** Comparison of the activities of 5-carboxamidotryptamine (5-CT) and 5-hydroxytryptamine (5-HT) at different oxygen tensions ($Po_2$) in the human isolated umbilical artery

| | *5-CT* | | | *5-HT* | | $\frac{EC_x\ 5\text{-}CT}{EC_x\ 5\text{-}HT}$ | | |
|---|---|---|---|---|---|---|---|---|
| $Po_2$ (mmHg) | $EC_{25}$ (nM) | $EC_{75}$ ($\mu$M) | *% of 5-HT maximum* | $EC_{25}$ (nM) | $EC_{75}$ ($\mu$M) | $EC_{25}$ | $EC_{75}$ | n |
| $13 \pm 1$ | 13.2 (1.5–115) | 1.1 (0.26–4.4) | $81 \pm 10$ | 17.8 (12.6–25.1) | 0.22 (0.09–0.51) | 0.64 (0.04–9.1) | 5.4 (1.3–21.4) | 5 |
| $123 \pm 1$ | 0.25 (0.06–1.0) | 0.58 (0.1–2.6) | $88 \pm 8$ | 3.3 (0.9–12.3) | 0.1 (0.05–0.19) | 0.15 (0.02–1.4) | 3.5 (1.6–7.4) | 6 |

Values are geometric mean (95% confidence limits) and mean $\pm$ s.e.mean, from *n* estimates.
Relative potencies were calculated at the $EC_{25}$ and $EC_{75}$.

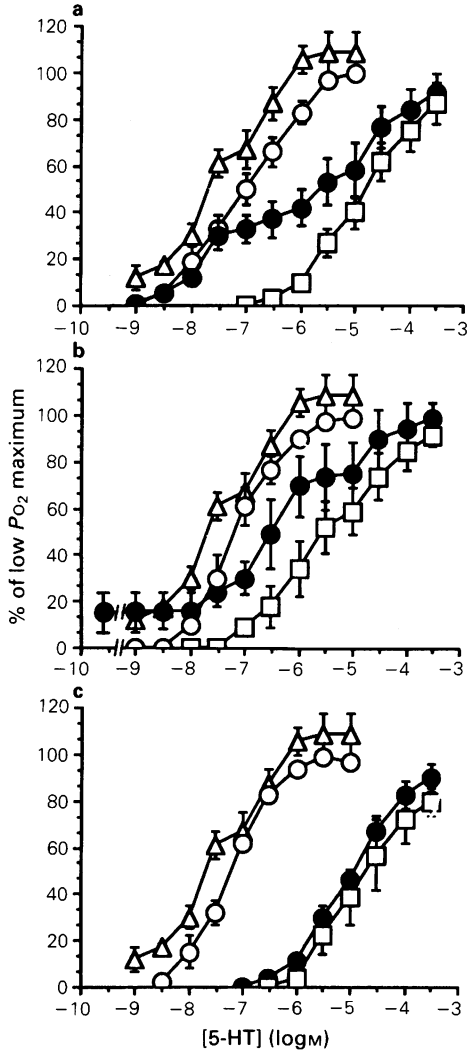


**Figure 6** Interaction between 5-hydroxytryptamine (5-HT) and (a) ketanserin (0.1 $\mu$M), (b) methysergide (0.1 $\mu$M) and (c) phentolamine (100 $\mu$M) at high $Po_2$ in the presence and absence of indomethacin (1 $\mu$M). In each experiment (a, b and c) a CRC to 5-HT at low $Po_2$ ($\sim$15 mmHg), was first constructed (○). Following washout the $Po_2$ was then increased (to $\sim$120 mmHg), antagonist was added and a CRC to 5-HT repeated (●). A third CRC to 5-HT was then constructed in the presence of antagonist plus indomethacin (1 $\mu$M) at the high $Po_2$ (□). A control CRC to 5-HT at high $Po_2$ (△), (from non-paired experiments, Figure 4a) is shown in each figure. The extreme left hand symbol (Figure 4b) indicates the tone induced by methysergide. Responses were calculated as a % of the maximum response to 5-HT at low $Po_2$. (a) [Ketanserin] = 0.1 $\mu$M; low $Po_2$ = 13 $\pm$ 2 mmHg; high $Po_2$ = 117 $\pm$ 1 mmHg ($n$ = 8). (b) [Methysergide] = 0.1 $\mu$M; low $Po_2$ = 14 $\pm$ 2 mmHg; high $Po_2$ = 123 $\pm$ 3 mmHg ($n$ = 6). (c) [Phentolamine] = 100 $\mu$M; low $Po_2$ = 14 $\pm$ 2 mmHg; high $Po_2$ = 127 $\pm$ 2 mmHg ($n$ = 4). Each point represents the mean and vertical lines show s.e.mean. For clarity, error bars are omitted at some points.

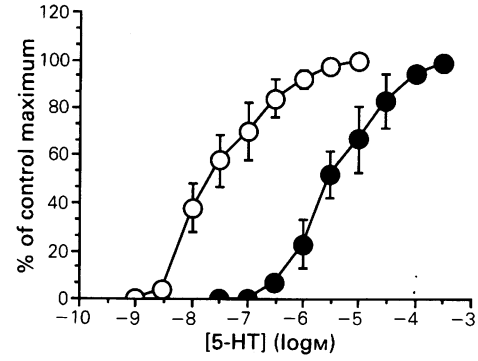


**Figure 7** Log concentration-response curves (CRCs) to 5-hydroxytryptamine (5-HT) ($n$ = 4) at high $Po_2$ (119 $\pm$ 1 mmHg) before (○) and after (●) exposure to Wy 26703 (10 $\mu$M). Each point represents the mean and vertical lines show s.e.mean when these are greater than the height of the symbols.

5-HT concentration-response curve which, in descriptive terms, was biphasic. However, log agonist CRs at the $EC_{25}$ (1.65 $\pm$ 0.51) and $EC_{75}$ (1.58 $\pm$ 0.49) were not significantly different. After incubation with indomethacin, methysergide (0.1 $\mu$M) failed to contract the HUA and the displacement of the 5-HT concentration-response curve was parallel; the log agonist CR at the $EC_{25}$ (1.48 $\pm$ 0.23) and $EC_{75}$ (1.90 $\pm$ 0.18) levels were not significantly different (Figure 6b).

The agonist action of methysergide at high $Po_2$ was investigated further. Cumulative concentration-response curves were constructed to methysergide. The $pD_2$ was 6.82 $\pm$ 0.12. The $pD_2$ for 5-HT at high $Po_2$ was 8.01 $\pm$ 0.26 (non-paired preparations, from Figure 5b). Thus 5-HT was approximately 15 fold more potent than methysergide. The maximum responses to methysergide and 5-HT (relative to a prior contraction induced by 50 mM KCl) were 64 $\pm$ 18% and 222 $\pm$ 26%, respectively. Neither ketanserin (30 nM) nor the selective $\alpha_1$-adrenoceptor antagonist prazosin (30 nM) antagonised the response to methysergide. Prazosin (30 nM) did not antagonise either the 1st or 2nd phase of the 5-HT concentration-response curve at high $Po_2$. Indomethacin (1 $\mu$M) significantly reduced the maximum response to methysergide from 64 $\pm$ 18% to 43 $\pm$ 11% (relative to a prior contraction induced by 50 mM KCl).

The non-selective $\alpha$-adrenoceptor antagonist phentolamine (100 $\mu$M) displaced the 5-HT

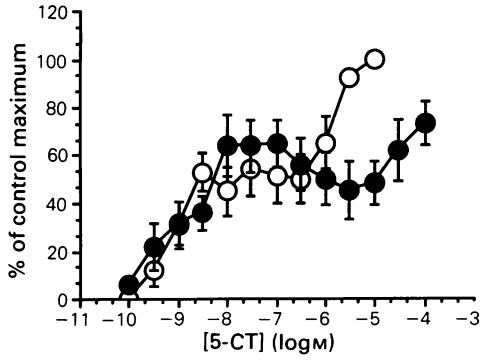


**Figure 8** Log concentration-response curves to 5-carboxamidotryptamine (5-CT) ($n = 5$) at high $Po_2$ ($118 \pm 4$ mmHg) before (○) and after (●) exposure to ketanserin (0.1 $\mu$M). Each point represents the mean and vertical lines show s.e.mean.

concentration-response curve in a parallel manner, either in the absence or presence of indomethacin (Figure 6c). In the absence of indomethacin the log agonist CRs at the $EC_{25}$ and $EC_{75}$ were $2.69 \pm 0.10$ and $2.57 \pm 0.22$ respectively. In the presence of indomethacin the log agonist CRs at the $EC_{25}$ and $EC_{75}$ were $2.57 \pm 0.39$ and $2.57 \pm 0.33$ respectively.

In the absence of indomethacin, the selective $\alpha_2$-adrenoceptor antagonist Wy 26703 (10 $\mu$M) caused a parallel shift of the 5-HT concentration-response curve (Figure 7): log agonist CRs at the $EC_{25}$ ($2.16 \pm 0.24$) and $EC_{75}$ ($1.96 \pm 0.33$) were not significantly different. Thus the ketanserin-resistant phase of the 5-HT concentration-response curve could be antagonised by the $\alpha_1/\alpha_2$-adrenoceptor antagonist phentolamine or by the selective $\alpha_2$-antagonist Wy 26703.

The interaction between 5-CT and ketanserin at high $Po_2$ was investigated. Ketanserin (0.1 $\mu$M) antagonised the 2nd phase (log agonist CR 2.30) but not the 1st phase (log agonist CR $0.2 \pm 0.3$) of the 5-CT concentration-response curve (Figure 8). At the $EC_{75}$ log agonist CR was calculated only from the mean $EC_{75}$ value, since in the presence of ketanserin contractions in some preparations did not reach the level of the $EC_{75}$.

The ligands buspirone (0.1 $\mu$M) and (±)-pindolol (3 $\mu$M) had no affinity (at the given concentrations) for either receptor mediating the response to 5-CT at high $Po_2$; at these concentrations neither ligand contracted the HUA or displaced the 1st or 2nd phase of the 5-CT concentration-response curve.

## Discussion

Under conditions of physiological $Po_2$ the antagonists ketanserin, methysergide and phentolamine interact with 5-HT in a simple, competitive manner suggesting that the 5-HT-induced contraction of the HUA is mediated by a homogeneous population of receptors. At higher $Po_2$, however, the nature of the interaction of these antagonists with 5-HT suggests that a heterogeneous population of 5-HT receptors exists.

It is appropriate at this point to rule out some possible mechanisms of action of 5-HT in the HUA. In other vascular smooth muscle tissues 5-HT can act directly (Apperley *et al.*, 1976) or indirectly (Innes, 1962; Humphrey, 1978; Humphrey *et al.*, 1983) at $\alpha$-adrenoceptors. In the HUA there is only a small functional population of prazosin-sensitive, $\alpha_1$-adrenoceptors that require a higher than physiological $Po_2$ for their expression (MacLennan, 1986). However, the effects of 5-HT at high $Po_2$ were not antagonised by prazosin. The general consensus of opinion is that the HUA is not innervated (Spivack, 1943; Roach, 1972), so that we doubt that 5-HT could possibly displace stored noradrenaline. This evidence rules out a direct or indirect action of 5-HT at $\alpha$-adrenoceptors.

We have not examined a possible contribution of $5\text{-HT}_3$ receptors in the 5-HT-induced contraction of the HUA but, as described above, the HUA is not innervated and as yet peripheral $5\text{-HT}_3$ receptors have been described only on neurones (see Bradley *et al.*, 1986a; Humphrey & Feniuk, 1987). An involvement of $5\text{-HT}_3$ receptors is therefore discounted.

At physiological $Po_2$ ketanserin, methysergide and phentolamine acted as simple competitive antagonists. A comparison of the affinities of these antagonists at the $5\text{-HT}_2$ recognition site in radiolabelled ligand binding studies (Hoyer, 1988) and the $pA_2$ values from the present study in HUA are given in Table 3. It is clear that there is a marked similarity of the affinities of the antagonists for the $5\text{-HT}_2$ recognition site in the CNS and for the receptor in the HUA, suggesting that they are the same. Hence, the receptor for 5-HT in the HUA at physiological $Po_2$ can be classified as a $5\text{-HT}_2$ receptor and confirms our previous study (McGrath *et al.*, 1985).

Further (if somewhat indirect) evidence for describing the receptor as $5\text{-HT}_2$ comes from the similarity of the receptor in the HUA and that in the rabbit aorta, since other studies have shown an identity between the 5-HT receptor in the rabbit aorta and the $5\text{-HT}_2$ recognition site in binding studies (Humphrey *et al.*, 1982; Maayani *et al.*, 1984). The receptors in the HUA and rabbit aorta appear similar both in terms of estimated antagonist affinities (ketanserin, methysergide and phentolamine – Table 3) and of the relative potency of 5-HT and 5-CT in these preparations. In the HUA, at physiological $Po_2$, 5-CT was approximately 5 fold less potent than 5-HT (Table 2) while in the rabbit aorta

**Table 3** Comparison of the affinities of the antagonists ketanserin, methysergide and phentolamine for the 5-$HT_2$ binding site in brain tissue (rat cortex) and the receptor for 5-HT in the human umbilical artery (HUA) at physiological $Po_2$ and rabbit aorta

| *Antagonist* | *Binding site*[1] | *Receptor in HUA*[2] | *Receptor in rabbit aorta*[3] |
|---|---|---|---|
| Ketanserin | 8.86 | 8.92 (8.70–9.14) | 8.67 (8.38–8.95) |
| Methysergide | 8.57 | 8.52 (8.32–8.72) | 8.49 (7.85–9.14) |
| Phentolamine | 6.06 | 6.37 (5.69–7.05) | 6.21 (5.52–6.92) |

[1] Values are $-\log$ D (mean) from Hoyer (1988).
[2] $pA_2$ values estimated from a Schild analysis and are the mean (95% confidence limits).
[3] $pA_2$ values estimated from a Schild analysis and are the mean (95% confidence limits), from Apperley *et al.* (1976) and Feniuk *et al.* (1985).

5-CT was 26 fold less potent than 5-HT (Feniuk *et al.*, 1985).

Increasing the $Po_2$ from physiological levels ($\sim$15 mmHg) to a higher level ($\sim$120 mmHg) resulted in an increased potency of the agonists 5-HT and 5-CT. This effect was particularly evident with 5-CT whose concentration-response curve became distinctly biphasic at high $Po_2$. For either agonist this increased potency was due to an involvement of cyclo-oxygenase products since indomethacin was able to reverse it.

At high $Po_2$, contractions induced by 5-HT and 5-CT were mediated via both 'ketanserin-resistant' and 'ketanserin-sensitive' receptors, i.e. 5-HT receptors in the HUA are heterogeneous. The ketanserin-sensitive receptor is undoubtedly the 5-$HT_2$ receptor: at the level of the $EC_{75}$ (taken as the mid-point location of the 2nd, ketanserin-sensitive, phase) the log agonist CR for either 5-HT or 5-CT in the presence of ketanserin is consistent and similar to that at physiological $Po_2$.

A quantitative comparison of the potency of the two tryptamines was made at the level of the $EC_{25}$, as an estimate of their relative potency at the 'ketanserin-resistant' receptor, and at the $EC_{75}$ as an estimate of their potency at the 5-$HT_2$ receptor. At the 1st (ketanserin-resistant) phase 5-CT was more potent than 5-HT, while at the 5-$HT_2$ receptor the converse was found. These two pieces of evidence, i.e. a resistance to blockade by ketanserin and the higher potency of 5-CT relative to 5-HT, suggest that the receptor through which low concentrations of 5-HT and 5-CT exert their effects can be described as '5-$HT_1$-like' in accordance with the guidelines of Bradley *et al.* (1986a).

This order of potency is similar to that found in other vascular smooth muscle preparations where the receptor has been described as 5-$HT_1$-like. For example, 5-CT is more potent than 5-HT at receptors mediating contraction of saphenous veins from dogs (Feniuk *et al.*, 1985) and rabbits (Martin *et al.*, 1988), and at other receptors that mediate relaxation of porcine vena cava (Trevethick *et al.*, 1986), cat jugular vein (Feniuk *et al.*, 1983) and at the smooth muscle receptor in rabbit jugular vein (Martin *et al.*, 1987). If the $EC_{25}$ for 5-CT at high $Po_2$ (0.25 nM) is taken as an approximation of its affinity at the 5-$HT_1$-like receptor (i.e. the $EC_{50}$ of the 1st phase), then 5-CT has an affinity at this receptor in the HUA greater than that found for each of the receptor types mentioned above.

A further similarity between the 5-$HT_1$-like receptors in the HUA and in other tissues is the partial agonist action of methysergide at high $Po_2$, where it was 15 fold less potent than 5-HT. This similarity extends only to the 5-$HT_1$-like receptors in tissues where the receptor-mediated response is contraction since, in tissues where relaxation is seen (see above), methysergide is a silent, albeit weak, antagonist. Thus, an agonist action of methysergide at 5-$HT_1$-like receptors has been obtained in dog saphenous vein (Apperley *et al.*, 1980), rabbit basilar artery (Bradley *et al.*, 1986b) and rabbit saphenous vein (MacLennan *et al.*, 1988a). In other tissues which may contain a heterogeneous population of 5-$HT_2$ and 5-$HT_1$-like receptors, for example dog coronary (Brazenor & Angus, 1981; 1982) and basilar arteries (Muller-Schweinitzer, 1980), methysergide is a weak agonist, but it has not been clearly shown which receptor mediates this agonism.

Since the response to methysergide was partially sensitive to indomethacin, this suggests an action at the 5-$HT_1$-like receptor. However, in contrast with the additional responses to 5-HT and 5-CT, although the agonist action of methysergide required a higher than physiological $Po_2$, this was only partially antagonised by indomethacin. A possible involvement of 5-$HT_2$ receptors and $\alpha_1$- adrenoceptors was ruled out, as the agonist response was resistant to the selective antagonists ketanserin and

prazosin. However, some further $O_2$-induced factor may be involved: the $\alpha_1$-receptor in this tissue also requires high $Po_2$ for its functional expression and, like methysergide, is resistant to indomethacin (McLennan, 1986). Thus, it is possible that some further action of high $Po_2$ can facilitate the responses to methysergide and adrenaline.

5-HT has been shown to cause liberation of prostaglandin-like substances from the perfused rat lung (Alabaster & Bakhle, 1976) and to increase the activity of cyclo-oxygenase in ram seminal vesicles (Takeguchi *et al.*, 1971), but the nature of the receptors was not identified. In bovine cultured aortic smooth muscle cells (Coughlin *et al.*, 1984) and in the dog saphenous vein smooth muscle (Kokkas & Boeynaems, 1988) the receptor mediating prostacyclin synthesis has been characterized and appears to belong to the 5-$HT_2$ subtype.

In other preparations where a functional response to 5-HT has been shown to be mediated by 5-$HT_1$-like receptors, a direct or indirect action involving cyclo-oxygenase products has been ruled out. Thus, the contraction of dog saphenous vein (Apperley *et al.*, 1980) and rabbit basilar artery (Bradley *et al.*, 1986b) does not involve cyclo-oxygenase products since the response is resistant to indomethacin. Similarly, cyclo-oxygenase products are not involved in the receptor mediating relaxation of cat saphenous vein and guinea-pig ileum (Feniuk *et al.*, 1983). Therefore, unlike the receptor uncovered in the HUA, whose expression relies on cyclo-oxygenase products, other 5-$HT_1$-like receptor-mediated responses do not directly or indirectly involve cyclo-oxygenase products.

Clearly, the 5-$HT_2$ receptor-mediated response is unchanged on moving between oxygen tensions but oxygen somehow unmasks the response to activation of the 5-$HT_1$-like receptor. The only clue to the basis of this modulation by the prevailing oxygen tension is that it requires functional cyclo-oxygenase. Raising the oxygen tension across the same range causes a contraction of the vessel which is blocked not only by indomethacin but also by aspirin and flurbiprofen (McGrath *et al.*, 1986; MacLennan *et al.*, 1988b). It seems reasonable to postulate that oxygen stimulates the formation of cyclo-oxygenase products, which in turn activate the smooth muscle to produce contraction. On prolonged exposure to high $Po_2$ this contraction wanes but presumably activation continues. The simplest explanation for the uncovering of the 5-$HT_1$-like receptor is that it cannot, on its own, activate the contractile process without the additional effect produced by cyclo-oxygenase products. It is likely that this synergism occurs beyond the receptor, possibly at the 2nd messenger. If the effect occurred beyond this stage, then it would not be likely to be specific for the 5-$HT_1$-like compared with the 5-$HT_2$-receptor. We cannot exclude the alternative possibility that the 5-$HT_1$-like receptor itself mediates the formation of a contractile cyclo-oxygenase product, which in turn causes contraction, and that either this synthesis or the resultant action on muscle is oxygen-dependent. Indeed, in the perfused HUA, 5-HT can stimulate the synthesis of thromboxane $A_2$ (Bjoro, 1986) a potent agonist in this tissue (Svensson *et al.*, 1977). However, since we know that oxygen is itself contractile this latter explanation may be over-complicated.

An interesting observation was that phentolamine and the selective $\alpha_2$-adrenoceptor antagonist Wy 26703 (Lattimer *et al.*, 1984) antagonised both the 5-$HT_2$ and 5-$HT_1$-like receptor-mediated responses. The cross-reactivity of $\alpha$-adrenoceptor antagonists and 5-$HT_2$ receptors is well known (Apperley *et al.*, 1976; Kaumann, 1983). Our results support previous studies which have shown that $\alpha_2$-adrenoceptor antagonists possibly interact with modest affinity for 5-$HT_1$-like receptors. Two studies have demonstrated that phentolamine is a competitive antagonist ($pA_2$ 6.11 and 6.05) at the 5-$HT_1$-like receptor in dog saphenous vein (Humphrey, 1978; Curro *et al.*, 1978).

A heterogeneous population of 5-HT receptors mediates contraction of the dog coronary (Frenken & Kaumann, 1985) and basilar arteries (Frenken & Kaumann, 1986) and human saphenous veins (Docherty & Hyland, 1986). In each preparation 5-HT has a high affinity for a ketanserin-resistant receptor (which may be 5-$HT_1$-like but has not been fully characterized) and a somewhat lower affinity for 5-$HT_2$ receptors. In these two arteries from dogs phentolamine has micromolar affinity for the ketanserin-resistant (5-$HT_1$-like) receptor (Muller-Schweinitzer, 1980) while in the human saphenous vein yohimbine weakly antagonised the 5-$HT_1$-like (and 5-$HT_2$) mediated effects of 5-HT (Docherty & Hyland, 1986). Clearly, $\alpha_2$-adrenoceptor antagonists have significant affinity for 5-$HT_1$-like receptors.

Since $\alpha_2$-adrenoceptor antagonists generally have greatest affinity for the 5-$HT_{1a}$ subtype of the 5-$HT_1$ recognition site (see Hoyer, 1988), this may suggest a similarity between the 5-$HT_{1a}$ binding site and the 5-$HT_1$-like receptor in the HUA. However, at concentrations which saturate more than half of these binding sites buspirone and (±)-pindolol had no detectable affinity for the receptor in HUA.

In conclusion, raising the $Po_2$ above the physiological level found *in vivo* in this vessel uncovers a second 5-HT-receptor, besides the 5-$HT_2$ receptor. An analysis of this receptor suggests that it can be described as 5-$HT_1$-like, in accordance with the guidelines of Bradley *et al.* (1986a). It will be interesting to see whether this type of unmasking occurs more generally, possibly highlighting physiological

mechanisms for engaging or disengaging different forms of activation.

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