Further characterization of the 5-HT receptor mediating vascular relaxation and elevation of cyclic AMP in porcine isolated vena cava

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1 5-Hydroxytryptamine (5-HT) and 5-carboxamidotryptamine (5-CT) produce both smooth muscle relaxation and elevation of tissue adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels in isolated rings of neonatal porcine vena cava. We now present studies attempting to characterize in more detail the 5-HT receptor mediating these responses.

2 Both 5-HT and 5-CT relaxed porcine isolated vena cava rings (EC_{50} values 200 nM and 4 nM respectively) and elevated tissue cyclic AMP levels (EC_{50} values 1500 nM and 16 nM respectively). For both responses 5-CT was approximately 50–100 fold more potent than 5-HT.

3 Both 5-CT-induced smooth muscle relaxation and cyclic AMP elevation were potently and specifically antagonized to a similar extent by methiothepin, methysergide and spiperone.

4 At concentrations up to 1 μ M, 8-hydroxy-2-(di-n-propylamino) tetralin, buspirone, ipsapirone, n,n-dipropyl-5-CT, cyanopindolol, RU24969, ketanserin, GR38032 and GR43175 were devoid of both agonist and antagonist activity for both responses.

5 These findings suggest that the same 5-HT₁-like receptor mediates both smooth muscle relaxation and elevation of cyclic AMP. This receptor is unlike any known 5-HT₁ ligand binding site or adenylate cyclase-coupled 5-HT receptor in brain tissues.

Introduction

Functional 5-hydroxytryptamine (5-HT) receptors comprise at least 3 classes, designated '5-HT₁-like', 5-HT₂ and 5-HT₃ (Bradley *et al.*, 1986). 5-HT₁-like receptors are characterized by a high sensitivity to the agonists 5-HT and 5-carboxamidotryptamine (5-CT), and to the antagonist methiothepin, and are insensitive to 5-HT₂ and 5-HT₃ receptor antagonists, such as ketanserin and GR38032 (1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-1-yl)methyl]4H-carbazol-4-one), respectively. However, it has been claimed that 5-HT₁-like receptors (as opposed to 5-HT₁ binding sites) can be subdivided (Humphrey *et al.*, 1988). This study is part of our continued work to characterize further the putative subtypes within the 5-HT₁-like receptor class.

Relaxant responses to 5-HT and 5-CT, associated with an activation of adenylate cyclase, have previously been described in neonatal porcine isolated abdominal vena cava ring preparations (Trevethick *et al.*, 1984; 1986). Both the relaxant and adenosine

3':5'-cyclic monophosphate (cyclic AMP) responses to 5-CT were antagonized by methysergide (pA_2 values 7.88 and 7.95, respectively) and on the basis of the available data, appeared to be mediated by 5-HT₁-like receptors. Since then, a new generation of drug tools have emerged which show a differential selectivity for various 5-HT receptors and ligand binding sites (Fozard, 1987). In particular, compounds such as the 5-HT_{1A} ligands, 8-hydroxy-2-(di-n-propyl-amino) tetralin (8-hydroxy-DPAT), buspirone, ipsapirone and n,n-dipropyl-5-CT, the non-selective 5-HT_{1A} and 5-HT_{1B} ligands, cyanopindolol and RU24969, the 5-HT₃ receptor antagonist GR38032 (Butler *et al.*, 1988) and the novel 5-HT₁-like receptor agonist, GR43175 (Humphrey *et al.*, 1988), have fueled the interest in 5-HT receptor subtypes and their characterization. Indeed, the advent of such compounds has led to a proliferation in the number of 5-HT₁ binding sites identified, and four subtypes are currently recognised, denoted 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} (Heuring & Peroutka, 1987). In addition, renewed interest in the

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transducing mechanisms for these binding sites has led to studies describing 5-HT_{1A} responses that are both positively and negatively coupled to adenylate cyclase (Roth & Chuang, 1987), and more recently, to accounts of 5-HT_{1D} receptor-mediated inhibition of adenylate cyclase activity (Hoyer & Schoeffer, 1988).

We have therefore re-examined and attempted to characterize comprehensively the 5-HT receptors mediating the relaxant and cyclic AMP responses in porcine vena cava ring preparations. Some of these results have previously been communicated to the British Pharmacological Society (Sumner *et al.*, 1987).

Methods

Measurement of vascular relaxation

Lengths of abdominal vena cava (~25 mm) were removed from neonatal pigs (large white variety, 1–20 days of age, supplied by Glaxo animal services) that were killed by captive-bolt pistol and exsanguination. No attempts were made to remove the endothelial cell layer from these vessels.

Each vena cava was dissected to obtain 4 rings of 1–2 mm in length which were individually mounted in 10 ml organ baths for isometric recording of tension changes using a Dynamometer UF1 isometric transducer (± 57 g range) connected to a Lectromed 8-channel pen recorder. The preparations were maintained at 37°C in a gassed (95% O₂, 5% CO₂), modified Krebs solution (Apperley *et al.*, 1976), under a resting tension of 0.2–0.5 g. Throughout the experiment a rest period of 60 min (with changes of Krebs solution at 15 min intervals) was allowed between successive treatments of the tissue.

After an initial 60 min equilibration period, a cumulative concentration-effect curve for the contractile agonist (spasmogen) was established, successive concentrations being applied when the response to the previous concentration had plateaued. From this curve, the concentration of spasmogen required to attain 50–70% of its own maximal response was established, and this concentration was used to induce tone throughout the experiment.

After a rest period, the preparations were contracted to a constant tone (0.5–1 g) with the predetermined concentration of spasmogen. In all preparations, a cumulative concentration-effect curve to 5-HT was established, successive concentrations being applied when the response to the previous dose had reached a plateau. When two consecutive concentrations of 5-HT produced no greater relaxation (usually maximal at 10 μ M), the tissues were washed

with fresh Krebs solution to remove both 5-HT and the spasmogen.

Agonist potency measurements

After another rest period, the preparations were again contracted to a constant tone. On one ring (control) a cumulative concentration-effect curve to 5-HT was repeated to assess changes in sensitivity of the tissue to 5-HT (mean agonist concentration-ratio 0.97 ± 0.46 , $n = 22$). On the remaining three rings, the relaxant effects of test agonists were examined. In each case a cumulative concentration-effect curve to the agonist was constructed up to a maximum of 10 μ M. A 5 min interval was employed if a particular concentration of agonist failed to relax the preparation. At the end of the curve for a test agonist, a single concentration of 10 μ M 5-HT was applied to ascertain the maximal relaxant response to 5-HT in the presence of the first agonist.

Relaxant responses were expressed as a percentage relaxation of spasmogen-induced tone, this being the difference between the resting and sustained tensions (0.5–0.75 g); 5-HT and 5-CT usually produced 100% relaxation of this tone. The concentration of agonist required to produce 50% relaxation of this tone (the EC₅₀) was calculated for 5-HT and the test compound in each preparation, together with the maximum relaxation evoked by the test compound as a percentage of that evoked by 10 μ M 5-HT. The equipotent concentration-ratio (ECR) for the test compound relative to 5-HT (5-HT = 1) was calculated by dividing the EC₅₀ value for the compound by the EC₅₀ value for 5-HT. Where quoted, the pEC₅₀ value is the negative logarithm₁₀ of the EC₅₀ value.

In most cases, EC₅₀ and equipotent concentration-ratio values were obtained by data analysis using the logistic curve-fitting programme ALLFIT (De Lean *et al.*, 1978).

Antagonist potency measurements

Potential antagonists of the relaxant response were tested by examining their ability to antagonize the relaxation induced by 5-CT in preparations contracted with either histamine or the stable thromboxane A₂ mimetic U-46619 (Coleman *et al.*, 1981).

For these experiments, four ring preparations were set-up as above, and a suitable concentration of spasmogen was determined as described. Cumulative concentration-effect curves to 5-CT were then obtained in each preparation. After washing, three rings were exposed to vehicle or antagonist (up to a maximal concentration of 10 μ M), whilst the fourth remained untreated (control). After 30 min the prep-

arations were contracted to constant tone, and a cumulative concentration-effect curve to 5-CT was repeated in all four preparations. After washing, the three test preparations were exposed to higher concentrations of antagonists before generating another concentration-effect curve to 5-CT. Usually four 5-CT concentration-effect curves (one control and three curves in the presence of increasing concentrations of antagonist) were generated per ring preparation.

After correcting for any sensitivity changes for successive 5-CT concentration-effect curves, concentration-ratios were calculated by dividing the EC_{50} for 5-CT in the presence of the antagonist by the EC_{50} for 5-CT in the absence of antagonist. In control preparations, the mean concentration-ratio over four consecutive 5-CT concentration-effect curves was 1.23 ± 0.51 ($n = 16$). Concentration-ratio values were then used to calculate pA_2 and slope values by linear regression analyses of Schild plots (Arunlakshana & Schild, 1959). Compounds were considered to be inactive as antagonists of 5-CT if they produced concentration-ratio values of less than 2.0 at $1 \mu M$.

Measurement of cyclic AMP formation

Rings of 2–3 mm in length of freshly isolated vena cava were incubated for 30 min at $37^\circ C$ in gassed (95% O_2 , 5% CO_2), modified Krebs solution (Apperley *et al.*, 1976) containing the cyclic AMP phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine ($100 \mu M$), and test antagonists, if appropriate. The rings were then exposed to vehicle control or test agonist for a further 2 min, after which they were rapidly frozen in liquid nitrogen to terminate cyclic AMP formation.

The cyclic AMP was extracted (mean efficiency 95%) by homogenisation in absolute ethanol using a glass-glass tissue grinder (Jencons Scientific). The extracts were centrifuged at $15,000 g$ for 2 min and the supernatants contained in the ethanol removed and dried under a gentle stream of nitrogen gas. The remaining extracted pellets were dissolved in sodium hydroxide (1 M) for the subsequent determination of protein contents by the method of Lowry *et al.* (1951). When dry, the ethanol extracts were redissolved in a buffer (pH 7.5) comprising Tris-HCl (50 mM) and EDTA (4 mM), and were assayed for cyclic AMP content using a commercially available competitive binding-assay kit (Amersham International plc).

Results are expressed as pmol cyclic AMP formed mg^{-1} tissue protein. The small number of rings available from each vessel (maximum of 10) dictated a protocol in which parts of agonist concentration-effect curves were generated in different experiments,

and these data were then subsequently pooled to generate full concentration-effect curves. For this reason, points on these curves were obtained from between 20 and 60 experiments. In order to calculate equipotent concentration-ratios (ECR) relative to 5-HT, the mean EC_{50} (concentration of agonist producing 50% of its own maximum response) of the test agonist was divided by the mean EC_{50} value for 5-HT. These EC_{50} values were obtained by 'ALLFIT' analysis of the data.

In order to obtain approximate pA_2 values for antagonists, two point concentration-effect curves for 5-CT were generated in the absence and presence of two antagonist concentrations, and concentration-ratios were estimated at an arbitrary point drawn approximately midway through the concentration-effect curves. The concentration-ratios were calculated by dividing the concentration of 5-CT required to produce a response at this point in the presence of antagonist by that required for 5-CT in the absence of antagonist. These estimates were used to calculate pA_2 and slope values, which were subsequently meaned. In the case of methiothepin, pA_2 values were estimated from a single ratio at a methiothepin concentration of 10 nM, since the higher concentration produced a non-parallel shift in the 5-CT concentration-effect curve.

Drugs

5-Hydroxytryptamine creatine sulphate, 3-isobutyl-1-methylxanthine (IBMX) and histamine hydrochloride (Sigma), spiperone and ketanserin (Janssen), forskolin (Calbiochem), buspirone hydrochloride (Bristol-Myers), ipsapirone hydrochloride (Troponewerke), prostaglandin E_2 (PGE_2 , Upjohn), methiothepin maleate (Hoffman La Roche), metergoline (Farmitalia), methysergide hydrogen maleate (Sandoz) and mesulergine (a kind gift from Dr G. Engel, Sandoz). The following compounds were synthesized by Chemistry Research, Glaxo Group Research, Ware, U.K.: 5-carboxamidotryptamine maleate, α -methyl-5-HT maleate, 2-methyl-5-HT sulphate, n,n -dipropyl-5-CT, 8-hydroxy-DPAT (8-hydroxy-2-(di- n -propylamino) tetralin hydrobromide), RU24969 (5-methoxy-3 (1,2,3,6-tetrahydro-4-pyridinyl)1H indole), ($\pm$)-cyanopindolol, U-46619 (11, 9, epoxymethano - PGH_2), GR43175 (3 - (2 - di-methylamino)ethyl-N-methyl-1H-indole-5-methane sulphonamide) and GR38032 (1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-1-yl)methyl] 4H - carbazol-4-one).

Drug stocks were prepared in distilled water or Krebs buffer, except for RU24969, cyanopindolol, ketanserin, spiperone and GR38032 (dissolved in 2 M acetic acid), and forskolin (dissolved in dimethylacetamide). Stock solutions were diluted in

Krebs buffer, and equivalent vehicle controls were included where appropriate.

Statistics

Values are expressed as mean $\pm$ s.e.mean of n observations, which is also the number of animals used. Student's unpaired t test was used to assess the significance of differences between mean values.

Results

Relaxant response

5-HT and 5-CT produced a concentration-dependent relaxation (pEC_{50} values of 6.7 and 8.4, respectively) of preparations contracted with α -methyl-5-HT (Figure 1a). This relaxation was endothelial-independent since endothelial removal by balloon catheterisation abolished relaxant responses to acetylcholine, but did not significantly alter the pEC_{50} value for 5-HT (7.1 ± 0.3 , $n = 4$) or 5-CT (8.5 ± 0.2 , $n = 4$). When either histamine or U-46619 were used as the spasmogen, 5-HT (but not 5-CT) elicited a more complex relaxant response (Figure 1b), and hence relaxant agonist potencies relative to 5-HT were determined in α -methyl-5-HT-contracted preparations (Figure 2 and Table 1). Under these conditions, 2-methyl-5-HT (pEC_{50} 5.2) and n,n -dipropyl-5-CT (pEC_{50} 5.0) were weak agon-

Figure 1 Relaxant responses to 5-hydroxytryptamine (5-HT) and 5-carboxamidotryptamine (5-CT) in endothelium-intact porcine vena cava ring preparations contracted with α -methyl-5-HT (10^{-7} M, a) or U-46619 (10^{-8} M, b). The symbol ($\blacklozenge$) indicates application of contractile agent and washout at the end of the cumulative concentration-response curve to 5-HT or 5-CT.

Figure 2 Tryptamine agonist-induced relaxation of porcine isolated vena cava ring preparations contracted with α -methyl-5-hydroxytryptamine (α -methyl-5-HT). Results are means $\pm$ s.e.mean of at least four determinations. Data shown for 5-carboxamidotryptamine (5-CT, $\bullet$), 5-HT ($\blacktriangle$), 2-methyl-5-HT ($\blacksquare$) and n,n -dipropyl-5-CT ($\blacktriangledown$).

ists. The weak relaxant activity of 2-methyl-5-HT was not antagonized by the 5-HT₃ antagonist GR38032 (agonist concentration-ratio at $10 \mu\text{M} = 0.87 \pm 0.21$, $n = 3$). RU24969, 8-hydroxy-DPAT, buspirone and ipsapirone showed little or no agonist activity at concentrations up to $10 \mu\text{M}$ and, at a concentration of $1 \mu\text{M}$, failed to antagonize the re-

Table 1 Relaxant activity in isolated ring preparations of neonatal porcine vena cava contracted with α -methyl-5-HT

Compound	pEC_{50}	Equipotent concentration-ratio
5-HT	6.7 ± 0.3	1.0
5-CT	8.4 ± 0.3	0.03 ± 0.01
2-methyl-5-HT	5.2 ± 0.1	52 ± 2
n,n -dipropyl-5-CT	5.0 ± 0.3	51 ± 11
8-hydroxy-DPAT	< 5	> 100
RU24969	< 5	> 100
Buspirone	< 5	> 100
Ipsapirone	< 5	> 100
GR43175	< 4	> 500
IBMX	5.8 ± 0.4	8.0 ± 5
Forskolin	7.4 ± 0.3	0.36 ± 0.12

Results are means $\pm$ s.e.mean of the negative logarithm₁₀ of the EC_{50} value (pEC_{50}) obtained from at least three experiments. Equipotent concentration-ratios are expressed relative to 5-HT (see Methods).

Table 2 Antagonism of 5-carboxamidotryptamine (5-CT)-induced relaxation and elevation of tissue cyclic AMP levels in piglet vena cava ring preparations

Compound	Response			
	Relaxation pA_2	Slope	Cyclic AMP pA_2	Slope
Methysergide	7.9 ± 0.2	0.95 ± 0.11	7.9 ± 0.3	1.03 ± 0.23
Spiperone	7.5 ± 0.3	0.87 ± 0.11	7.7 ± 0.3	1.07 ± 0.13
Methiothepin	9.0 ± 0.2	$1.50 \pm 0.85^*$	$8.8 \pm 0.3^{**}$	
Metergoline	8.3 ± 0.3	0.89 ± 0.32		
Mesulergine	7.1 ± 0.1	0.96 ± 0.17		

Concentration-effect curves for 5-CT were obtained in the absence and presence of three (relaxant responses) or two (cyclic AMP responses) concentrations of antagonist. Concentration-ratios, estimated at the EC_{50} level where possible, were used to calculate pA_2 and slope values. Results are means $\pm$ s.e.mean of at least 3 determinations.

* Significantly different from unity ($P < 0.05$ see Figure 3.).

** Calculated only at the lower concentration of antagonist due to non-parallelism of the agonist concentration-effect curve at the higher antagonist concentration (See Figure 7).

laxant activity of 5-CT (agonist concentration-ratios of 1.73 ± 0.37 , 1.92 ± 0.62 , 2.10 ± 0.38 and 1.43 ± 0.65 , respectively, $n \geq 3$). The novel 5-HT₁-like receptor agonist GR43175 displayed no significant agonist or antagonist (versus 5-CT) activity at concentrations up to $10 \mu M$.

In order to avoid interference with the spasmogen, potential antagonists were assessed against 5-CT in preparations contracted with either U-46619 or histamine (the relaxant profile of 5-CT was identical for all spasmogens employed – see Figure 1a and b). Under these conditions, relaxant responses to 5-CT were antagonized competitively by methysergide, spiperone, metergoline and mesulergine (Table 2) and in an unsurmountable fashion by methiothepin (Figure 3). None of these compounds significantly antagonized (at $10 \mu M$) relaxant responses to PGE₂ (concentration-ratios < 2). Relaxant responses to 5-CT were not affected by the 5-HT₂ antagonist ketanserin, the 5-HT₃ antagonist GR38032 or by the non-selective 5-HT_{1A} and 5-HT_{1B} ligand, cyanopindolol (agonist concentration-ratio estimates at $1 \mu M$ were 0.87 ± 0.14 , 1.68 ± 0.22 and 1.64 ± 0.36 respectively, $n = 3$).

Cyclic AMP responses

The basal control level of cyclic AMP in isolated ring preparations of neonatal porcine vena cava was 6.3 ± 1.7 pmol mg⁻¹ protein ($n = 73$).

The time-course for the elevation of tissue cyclic AMP levels evoked by 5-HT ($10 \mu M$) or 5-CT ($1 \mu M$) was similar to that required for relaxation and was comparable to that previously obtained (Trevethick *et al.*, 1984). Maximal elevation of tissue cyclic AMP levels was attained after a 2 min exposure to the

agonists, and hence this exposure time was adopted throughout this study.

Both 5-HT (0.1 – $10 \mu M$) and 5-CT (1 – 1000 nM) increased basal levels of cyclic AMP in a concentration-dependent manner with a maximum increase of about 4 fold (Figure 4). Forskolin ($10 \mu M$),

Figure 3 Antagonism of 5-carboxamidotryptamine (5-CT)-induced relaxation by methiothepin. Porcine vena cava rings contracted with histamine or U-46619 were exposed to 5-CT after pretreatment in the absence (●) and presence of methiothepin at concentrations of 3×10^{-9} M (○), 1×10^{-8} M (△) and 3×10^{-8} M (□). The inset shows the Schild plot obtained using concentration-ratios, necessarily in this case, estimated at the 40% relaxation level. These data are from a single representative experiment for which the calculated pA_2 and slope values were 8.7 and 1.70, respectively.

Figure 4 Concentration-effect curves for 5-carboxamidotryptamine (5-CT) and 5-hydroxytryptamine (5-HT)-induced elevation of porcine vena cava ring cyclic AMP levels. Porcine isolated vena cava rings were exposed to 5-HT (●) or 5-CT (▲) at various concentrations, and the cyclic AMP contents extracted and assayed. Data are expressed relative to control cyclic AMP levels of $7.13 (\pm 2.8) \text{ pmol mg}^{-1}$ tissue protein. Results are means, and vertical lines indicate s.e.mean, of 20–60 experiments, from which EC_{50} values for 5-HT and 5-CT were $1.5 \pm 1.2 \mu\text{M}$ and $16 \pm 8 \text{ nM}$, respectively.

Figure 5 Effects of various agonists ($10 \mu\text{M}$) on porcine vena cava ring cyclic AMP levels. Rings were incubated with: (1) U-46619, (2) α -methyl-5-hydroxytryptamine (α -methyl-5-HT) (3) 2-methyl-5-HT, (4) 8-hydroxy-2-(di-n-propylamino) tetralin, (5) GR43175, (6) buspirone, (7) ipsapirone, (8) RU24969, (9) n,n-dipropyl-5-carboxamido-tryptamine (n,n-dipropyl-5-CT), (10) 5-HT or (11) 5-CT ($1 \mu\text{M}$). Results are means, and vertical lines indicate s.e.mean, from 6–20 determinations, and are compared with a control of $7.3 (\pm 2.9) \text{ pmol mg}^{-1}$ protein. *Significantly different from control ($P < 0.05$).

Table 3 Effects of 5-HT, 5-CT and GR43175 on forskolin and PGE_2 -induced elevation of porcine vena cava ring cyclic AMP content

Treatment	Cyclic AMP content (pmol mg^{-1} protein) in the presence of:	
	Forskolin ($10 \mu\text{M}$)	PGE_2 ($10 \mu\text{M}$)
Control	178 ± 33	22 ± 8
5-HT	$380 \pm 57^*$	35 ± 12
5-CT	$415 \pm 72^*$	$41 \pm 9^*$
GR43175	169 ± 43	32 ± 14

The effects of a single concentration ($10 \mu\text{M}$) of 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT) and GR43175 on the elevation of tissue cyclic AMP levels evoked by forskolin ($10 \mu\text{M}$) or prostaglandin E_2 (PGE_2 , $10 \mu\text{M}$) were determined. Results are means $\pm$ s.e.mean of three experiments. The mean control level of cyclic AMP in the absence of stimulation was $6.3 \pm 1.7 \text{ pmol mg}^{-1}$ protein.

* Significantly different from the control ($P < 0.05$).

a direct activator of adenylate cyclase, elicited a 20–50 fold increase in cyclic AMP levels (data not shown).

Of the agonists examined, only 5-HT and 5-CT produced a marked elevation of cyclic AMP levels at concentrations up to $10 \mu\text{M}$, although a significant elevation was produced by $10 \mu\text{M}$ n,n-dipropyl-5-CT (Figure 5). The equipotent molar concentration-ratio (ECR) for 5-CT (0.01) relative to 5-HT (1.0) was similar to that seen for the relaxant response (ECR 0.02). 5-Hydroxytryptamine, 5-CT or GR43175 did not produce any significant inhibition of forskolin- or PGE_2 -stimulated adenylate cyclase activity at concentrations up to $10 \mu\text{M}$ (Table 3).

The elevation of cyclic AMP levels induced by 5-CT was prevented by $10 \mu\text{M}$ methiothepin, methysergide and spiperone, but not by $10 \mu\text{M}$ ketanserin, GR38032, cyanopindolol or 8-hydroxy-DPAT (Figure 6). Metergoline and mesulergine were not examined. We have attempted to provide pA_2 estimates for spiperone, methysergide and methiothepin (Table 2). These were based on concentration-ratios estimated from 2-point concentration-effect curves to

Figure 6 Antagonism of 5-carboxamidotryptamine (5-CT, 1 μ M)-stimulated cyclic AMP accumulation in porcine vena cava rings treated with 10 μ M antagonist (1) ketanserin, (2) GR38032, (3) cyanopindolol, (4) 8-hydroxy-2-(di-n-propylamino) tetralin, (5) methysergide, (6) methiothepin, (7) spiperone. Results are means, and vertical line indicate s.e.mean, from 6–8 determinations, for which the control and 5-CT-stimulated cyclic AMP levels were 6.3 ($\pm$ 1.7) and 26.5 ($\pm$ 6.3) pmol mg⁻¹ protein, respectively. *Significantly different from control (P < 0.01).

5-CT in the absence and presence of two concentrations of antagonist (Figure 7), a protocol dictated by limited tissue supplies. As was seen in the 'relaxant' studies, spiperone and methysergide appeared to act in a competitive manner, whereas antagonism by methiothepin was clearly unsurmountable at a high concentration.

Discussion

The results of this study confirm and extend those of Trevethick *et al.* (1984, 1986), and provide evidence that a 5-HT₁-like receptor in porcine vena cava ring preparations mediates both the smooth muscle relaxation response and the elevation of cyclic AMP response seen with 5-HT and 5-CT. That these responses are mediated by a 5-HT₁-like receptor has been illustrated by the high sensitivity to the agonist 5-CT, the selective antagonism by methiothepin (albeit unsurmountable) and methysergide, and by a lack of sensitivity to the 5-HT₂ and 5-HT₃ antagonists ketanserin and GR38032 respectively (see Bradley *et al.*, 1986).

Surprisingly, 2-methyl-5-HT (an agonist normally considered to be selective for 5-HT₃ receptors) produced some relaxation of preparations contracted with α -methyl-5-HT, albeit at concentrations one

Figure 7 Antagonism of 5-carboxamidotryptamine (5-CT)-induced elevation of cyclic AMP levels in porcine vena cava ring preparations by methysergide (a), spiperone (b) and methiothepin (c). Two-point 5-CT concentration–effect curves were generated in the absence (■) and the presence of two concentrations of antagonist: methysergide 3×10^{-8} M (▲) and 3×10^{-7} M (●), spiperone 1×10^{-7} M (▲) and 1×10^{-6} M (●), and methiothepin 1×10^{-8} M (▲) and 1×10^{-7} M (●). Each point is the mean, and vertical lines indicate s.e.mean, of 6–8 determinations. pA₂ and slope values were estimated in each individual experiment, and the mean values are shown in Table 2.

thousand-times greater than those required for 5-CT. This action of 2-methyl-5-HT was not, however, antagonized by the 5-HT₃ antagonist GR38032, and may reflect weak agonist activity at this 5-HT₁-like receptor (the specificity of this response has not been investigated). No significant elevation in tissue cyclic AMP levels was seen with 2-methyl-5-HT. However, the fact that the concentration-effect curves for 5-HT and 5-CT for elevation of tissue cyclic AMP levels lay to the right of the corresponding curves for relaxation, suggests that cyclic AMP measurements may be a less sensitive index of agonist activity. Nevertheless, there was generally an excellent agreement between the pharmacology of the relaxant and cyclic AMP responses in terms of agonist and antagonist potencies, implying that a common receptor is involved.

A number of groups have described effects of 5-HT and 5-CT on adenylate cyclase activity in brain tissues. Both stimulant (Barbaccia *et al.*, 1983; Shenker *et al.*, 1983; 1987; Markstein *et al.*, 1986) and inhibitory (DeVivo & Maayani, 1986; Weiss *et al.*, 1986; Hoyer & Schoeffter, 1988) effects have been described and assigned to various types of 5-HT₁ binding site (for review see Roth & Chuang, 1987). Based upon the agonist potencies of 5-CT and 8-hydroxy-DPAT, together with antagonism by spiperone, many of these adenylate cyclase effects have been ascribed to activation of 5-HT_{1A} receptors. The cyclic AMP response that we have characterized in porcine vena cava appears to be distinct from these brain 5-HT receptors. Thus we exclude a role for 5-HT_{1A} receptors because, although spiperone antagonized the actions of 5-CT, other potent 5-HT_{1A} ligands, such as 8-hydroxy-DPAT, buspirone, ipsapirone and *n,n*-dipropyl-5-CT (Fozard, 1987) were either very weak or inactive as agonists or antagonists at concentrations up to 1 μ M. In addition, we have obtained no evidence for a 5-HT receptor-mediated inhibition of forskolin- or PGE₂-activated adenylate cyclase activity in porcine vena cava preparations, thereby precluding a negatively-coupled 5-HT receptor. The non-selective 5-HT_{1B} ligands, cyanopindolol and RU24969 (Fozard, 1987), were similarly devoid of appreciable activity in our study. The possibility that 5-HT_{1C} or

5-HT_{1D} sites could mediate the effects of 5-HT, as might be suggested by the antagonist potencies of mesulergine and metergoline (Table 2), is excluded on the basis of the high affinity of spiperone for the porcine vena cava receptor as compared to its low affinity at 5-HT_{1C} and 5-HT_{1D} binding sites (Heuring & Peroutka, 1987).

The 5-HT₁-like receptor present in porcine vena cava preparations is very similar if not identical to that found to mediate smooth muscle relaxation in cat isolated saphenous vein and guinea-pig ileum preparations (Feniuk *et al.*, 1983; 1984; Kalkman *et al.*, 1986). 5-CT appears to be a relatively selective agonist for this receptor and is competitively antagonized by spiperone ($pA_2 \sim 7.5$) and methysergide ($pA_2 \sim 7.8$). However, vascular relaxation can also be mediated by an endothelial 5-HT receptor (Leff *et al.*, 1987; Martin *et al.*, 1987). At this receptor subtype, 5-HT and α -methyl-5-HT are more potent agonists than 5-CT, and are antagonized by methiothepin and methysergide, but not by spiperone. We have preliminary data that also suggest the presence of endothelial 5-HT receptors in porcine vena cava preparations (Sumner & Humphrey, 1988). Under the experimental conditions purposefully employed in this study, however, we have no evidence for a contribution of the endothelium to the relaxant activities of any of the compounds examined.

In addition to these receptors mediating smooth muscle relaxation, a 5-HT₁-like receptor which mediates smooth muscle contraction and inhibition of neuronal transmitter release has been described, and this receptor is selectively activated by the novel agonist GR43175 (Feniuk *et al.*, 1979; 1985; Humphrey *et al.*, 1988). Hence, the lack of effect of GR43175 in the porcine vena cava (as an agonist or antagonist) provides further evidence for the proposed sub-division of 5-HT₁-like receptors (Humphrey *et al.*, 1988).

In conclusion, this study has demonstrated that porcine vena cava contains a smooth muscle 5-HT₁-like receptor, activation of which leads to adenylate cyclase activation and smooth muscle relaxation. This receptor appears to be unlike any known 5-HT₁ binding site and is dissimilar to those 5-HT₁-like receptors activated by GR43175.

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