Br. J. Pharmacol. (1993), **110**, 1023–1030

Characterization of the 5-HT_4 receptor mediating tachycardia in piglet isolated right atrium

Andrew D. Medhurst & [1]*Alberto J. Kaumann

SmithKline Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD & *Clinical Pharmacology Unit, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ

1 In order to explore whether 5-HT_4 receptor subtypes exist, we have characterized further the 5-HT_4 receptor that mediates tachycardia in the piglet isolated right atrium. All experiments were carried out in the presence of propranolol (400 nM) and cocaine (6 μM). We used tryptamine derivatives, substituted benzamides and benzimidazolone derivatives as pharmacological tools.

2 Tachycardia responses to 5-hydroxytryptamine (5-HT) were mimicked by other tryptamine derivatives with the following order of potency: 5-HT > 5-methoxytryptamine > α-methyl-5-HT = bufotenine > 5-carboxamidotryptamine = tryptamine (after treatment with pargyline) > 5-methoxy-*N*,*N*-dimethyltryptamine > 2-methyl-5-HT.

3 The substituted benzamides were all partial agonists relative to 5-HT except (−)-zacopride which was a full agonist. The stimulant potency order was renzapride > cisapride = (−)-zacopride > metoclopramide > (+)-zacopride.

4 The benzimidazolone derivatives had contrasting effects. BIMU 8 (endo-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-(1-methyl(ethyl-2-oxo-1H-benzimidazole-1-carboxamide hydrochloride) was a full agonist relative to 5-HT whilst BIMU 1 (endo-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazole-1-carboxamide hydrochloride) was a partial agonist with low intrinsic activity compared to 5-HT but had similar potency. We estimated a pK_B of 7.9 for BIMU 1 antagonism of 5-HT-induced tachycardia. DAU 6215 (N-endo-8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)-2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxamide, hydrochloride) had no chronotropic activity and was found to be a simple competitive antagonist with a pK_B of 7.1

5 SB 203186 (1-piperidinyl)ethyl 1H-indole 3-carboxylate) was a potent antagonist with a pK_B of 8.3. The affinity of SB 203186 was approximately 20 times higher than that of tropisetron (ICS 205-930; pK_B = 6.9) and DAU 6215 (pK_B = 7.0). GR113808 (([1-[2-[methylsulphonyl amino]ethyl]-4-piperidinyl] methyl 1-methyl-1H-indole-3-carboxylate) and SDZ 205-557 ((2-diethylaminoethyl)2-methoxy-4-amino-5-chloro-benzoate) also antagonized 5-HT-induced tachycardia but not by simple competitive blockade.

6 The sinoatrial 5-HT_4 receptor in the piglet has a pharmacological profile that correlates well with 5-HT_4 receptors characterized in rat oesophagus, guinea-pig ileum and colon, mouse embryonic colliculi neurones and human atrium.

Keywords: 5-HT_4 receptors; piglet right atrium; tachycardia; tryptamines; benzamides; benzimidazolones; SB 203186; SDZ 205-557; DAU 6215; GR113808

Introduction

5-HT_4 receptors have been identified in a number of tissues including the brain, gastrointestinal tract and the heart (atrium and sinoatrial node). They were first described in primary cultures of mouse embryonic colliculi neurones (Dumuis *et al.*, 1988) and in guinea-pig hippocampal membranes (Shenker *et al.*, 1987; Dumuis *et al.*, 1988) where they mediate 5-hydroxytryptamine (5-HT)-induced stimulation of adenylyl cyclase activity.

Gastrointestinal 5-HT_4 receptors are present in guinea-pig stomach (Buchheit & Bertholet, 1992), ileum (Craig & Clarke, 1990; Eglen *et al.*, 1990) and colon (Elswood *et al.*, 1991; Wardle & Sanger, 1992) where they mediate contractile responses to 5-HT and/or increases in electrically-evoked twitch contractions induced by 5-HT. Stimulation of 5-HT_4 receptors in rat oesophagus causes relaxation (Baxter *et al.*, 1991; Reeves *et al.*, 1991) which occurs via adenylyl cyclase activation (Ford *et al.*, 1992).

5-HT_4 receptors in the heart mediate positive chronotropic effects of 5-HT in adult and newborn pigs (Villalon *et al.*, 1990; 1991; Kaumann, 1990) as well as mediating 5-HT-induced increases in contractile force, adenosine 3′:5′-cyclic monophosphate (cyclic AMP) content and cyclic AMP-dependent protein kinase stimulation in paced human right and left atrial preparations (Kaumann *et al.*, 1990; 1991a; Sanders & Kaumann, 1992). 5-HT_4 receptors also mediate positive inotropic effects and increases in cyclic AMP by 5-HT in piglet left atria (Kaumann *et al.*, 1991b).

Three families of compounds have agonist activity at some or all of the 5-HT_4 receptors described. 5-HT and other tryptamines including 5-methoxytryptamine (5-MeOT) often act as full agonists (Dumuis *et al.*, 1988; Craig & Clarke, 1990) whilst benzamide derivatives like cisapride and renzapride are sometimes full agonists (Dumuis *et al.*, 1989) but usually partial agonists at 5-HT_4 receptors (Kaumann, 1990; Kaumann *et al.*, 1991a).

Recently azabicycloalkyl benzimidazolone derivatives (Turconi *et al.*, 1991) have also been shown to be potent full or partial agonists at 5-HT_4 receptors (Dumuis *et al.*, 1991; Baxter & Clarke, 1992).

Tropisetron (ICS 205-930) has been extensively used as an antagonist of 5-HT_4 receptors despite its relatively low affinity and poor selectivity (reviewed by Bockaert *et al.*, 1992a). Several compounds have recently been reported to be more potent and selective 5-HT_4 antagonists than tropisetron, including SDZ 205-557 ((2-diethylaminoethyl)2-methoxy-4-amino-5-chloro-benzoate) (Buchheit *et al.*, 1991; 1992). DAU 6215 (N-(endo-8-methyl-8-azabicyclo[3.2.1]-oct-

[1] Author for correspondence.

3-yl)-2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxamide, hydrochloride) (Turconi *et al.*, 1991; Dumuis *et al.*, 1991; Baxter & Clarke, 1992), SB 203186 ((1-piperidinyl)ethyl 1H-indole 3-carboxylate) (Kaumann *et al.*, 1992; Parker *et al.*, 1993) and GR113808 ([1-[2-[methylsulphonyl amino]ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate (Grossman *et al.*, 1993).

The present study was designed to characterize more fully the 5-HT_4 receptor described by Kaumann (1990) which mediates 5-HT-induced tachycardia in spontaneously beating right atria of newborn piglets. Using the various agonist and antagonists described above, we have compared the pharmacological properties of 5-HT_4 receptors in porcine sinoatrial node with those described for rat oesophagus (Baxter *et al.*, 1991; Baxter & Clarke, 1992), guinea-pig ileum and colon (Craig & Clarke, 1990; Eglen *et al.*, 1990; Wardle & Sanger, 1992), mouse embryonic colliculi neurones (Dumuis *et al.*, 1988; 1989; 1991) and human atrium (Kaumann *et al.*, 1990; 1991a; Sanders & Kaumann, 1992).

Methods

Male and female piglets (2–5 days old) were obtained from local farms and anaesthetized with halothane. The heart was excised and immediately washed free of blood with warm solution containing (mM): Na^+ 125, K^+ 5, Ca^{2+} 2.25, Mg^{2+} 0.5, Cl^- 98.5, SO_4^{2-} 0.5, HCO_3^- 34, HPO_4^{2-} 1, EDTA 0.04, and aerated with 95% O_2 and 5% CO_2. The right atrium was dissected in warm solution and mounted in a 50 ml organ bath using the apparatus described by Blinks (1965). The organ bath contained the solution described above at 37°C, supplemented with (mM): Na^+ 15, fumarate 5, pyruvate 5, L-glutamate 5, glucose 10 and continuously gassed with 95% O_2 and 5% CO_2 for the duration of the experiment. The water used throughout was deionised and twice distilled in glass.

Each spontaneously beating right atrium was suspended at a resting length just sufficient for measurable development of force. Care was taken not to overstretch the atrium since tachycardia could result (Blinks, 1956). The atrium was attached to a Swema SG4-45 strain gauge transducer by means of a stainless steel wire and force recorded on a Watanabe polygraph.

All experiments were carried out in the presence of 400 nM (±)-propranolol (to avoid possible indirect β-adrenoceptor-mediated effects due to noradrenaline; Kaumann, 1990), 6 μM cocaine (to block neuronal uptake; Kaumann *et al.*, 1990) and 200 μM ascorbate (to decrease oxidation of 5-HT). In some experiments atria were pretreated with pargyline (50 μM) for half an hour followed by washout to inhibit irreversibly monoamine oxidase (MAO).

A single cumulative concentration-effect curve was determined on each atrium by sequential addition of tryptamines, benzamides or benzimidazolones to the bath in amounts that increased the total concentration in steps of 0.5 log unit. Enough time was allowed for each effect to reach equilibrium. Control 5-HT curves were constructed in parallel tissues. In some experiments a concentration-effect curve for a partial agonist was followed by one for 5-HT in the presence of the highest concentration of the partial agonist. To assess the intrinsic activity of other agonists with respect to 5-HT, 600 μM 5-HT was administered after completion of a concentration-effect curve to an agonist (and in the presence of the latter).

To study the effects of antagonists separate atria were incubated with tropisetron, DAU 6215, SDZ 205-557, GR113808 or SB 203186 for at least 60 min before determination of the agonist concentration-effect curve. Control 5-HT curves were determined in other tissues in parallel.

All experiments were terminated by the addition of a saturating concentration of (−)-isoprenaline (200 μM) still in the presence of test agonist or antagonist, and 5-HT (600 μM). This (−)-isoprenaline concentration was calculated to surmount completely the antagonism by (±)-propranolol (Kaumann *et al.*, 1980).

The positive chronotropic effects of drugs were expressed as a percentage of the total increase in beating rate caused by agonist or agonist combination plus 200 μM (−)-isoprenaline (Δ HR % ΔIso). This procedure provided an independent way of estimating maximum increases in beating rates induced by 5-HT, other agonists, and partial agonists (Kaumann, 1990).

Agonist potency

The ability of agonists to induce tachycardia was expressed in absolute terms as pEC_{50} values relative to their individual maxima ($pEC_{50} = -\log EC_{50}$ concentration of agonist required to produce half-maximal effect).

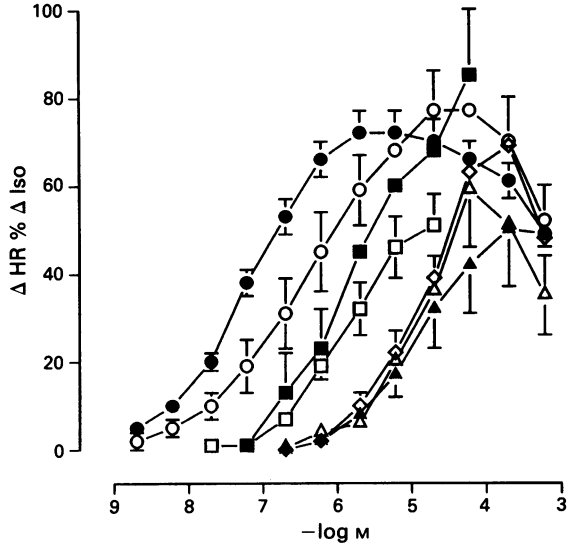

Figure 1 Concentration-effect curves to 5-HT (●, $n = 33$), 5-MeOT (○, $n = 7$), α-methyl-5-HT (■, $n = 7$), bufotenine (□, $n = 4$), tryptamine (◇, $n = 7$), 5-MeO-*N*,*N*-DMT (△, $n = 4$) and 5-CT (▲, $n = 4$) in piglet right atrium. Data are means ± s.e.mean. The chronotropic effects of agonists are expressed as a percentage of the increase in beating rate caused by agonist plus 200 μM isoprenaline (Δ HR % Δ Iso). 5-CT data from Kaumann (1990). For abbreviations, see text.

Table 1 Agonist potency and intrinsic activity in piglet right atrium

Agonist	*pEC_{50}*[a]	*Intrinsic activity*	n
5-HT	7.13 ± 0.1	1.0	33
5-MeOT	6.36 ± 0.3	1.07	7
α-Methyl-5-HT	5.90 ± 0.1	1.18	7
Bufotenine	5.95 ± 0.04	0.71	4
Tryptamine	4.87 ± 0.14	0.96	7
5-MeO-*N*,*N*-DMT	4.69 ± 0.21	0.82	4
5-CT	4.89 ± 0.13	0.69	4
Renzapride	6.70 ± 0.11*	0.69	4
Cisapride	6.44 ± 0.1*	0.39	4
(−)-Zacopride	6.41 ± 0.12	0.94	6
(+)-Zacopride	5.27 ± 0.24	0.33	4
Metoclopramide	5.73 ± 0.1	0.29	4
BIMU 8	6.70 ± 0.12	0.89	8
BIMU 1	7.13 ± 0.05	0.39	6

[a]$pE_{50} = -\log$ concentration of agonist required to produce 50% of the maximum increase in heart rate; mean ± s.e.mean.
*Data from Kaumann (1990). For abbreviations, see text.

Drug-receptor constants

The equilibrium dissociation constant K_B ($-\log K_B = pK_B$) for the antagonist-5-HT$_4$ receptor complex was calculated from

$$pK_B = \log (CR-1) - \log [B] \quad (1)$$

where CR is the concentration-ratio of agonist used in the presence and absence of antagonist (B). The assumption of simple competition (i.e. slope of unity) between antagonist and agonist for 5-HT$_4$ receptors was checked with a Schild plot (Arunlakshana & Schild, 1959).

The equilibrium dissociation constant Kp ($-\log K$p = pKp) was estimated by the method of Marano & Kaumann (1976), and Lemoine & Kaumann (1982). Kp was estimated from the slope of a weighted plot which relates equieffective concentrations of 5-HT in the absence (A_2) and presence (A_3) of a partial agonist P, $A_2 = i + mA_3$, where i is the ordinate intercept. The slope m of the regression equals $m = 1 - yp$, where the fractional 5-HT$_4$ receptor occupancy yp by the partial agonist P is given by [P]/([P] + Kp). pKp was calculated from

$$\log (1/m - 1) = \log [P] - \log K\text{p} \quad (2)$$

For simple competition between a 5-HT molecule and a partial agonist molecule for one 5-HT$_4$ receptor molecule, equation (2) has a slope of one, as in the Schild equation.

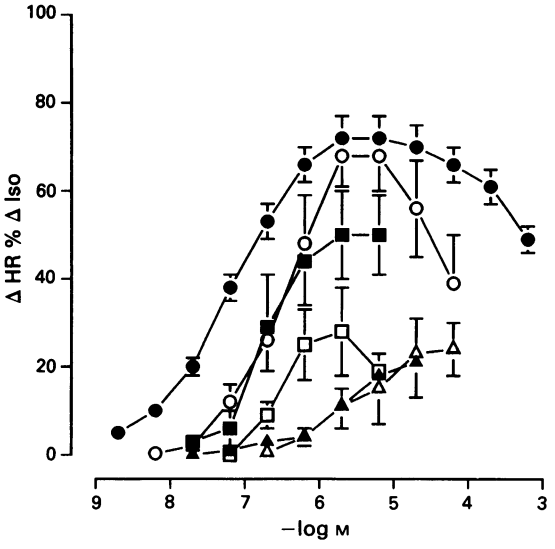

Figure 2 Concentration-effect curves to 5-HT (●, $n = 33$), (−)-zacopride (○, $n = 6$), renzapride (■, $n = 4$), cisapride (□, $n = 4$), (+)-zacopride (△, $n = 4$) and metoclopramide (▲, $n = 4$) in piglet right atrium. Data are means ± s.e.mean. The chronotropic effects are expressed as a percentage of the increase in beating rate caused by agonist plus 200 μM isoprenaline (Δ HR % Δ Iso). Renzapride and cisapride data from Kaumann (1990).

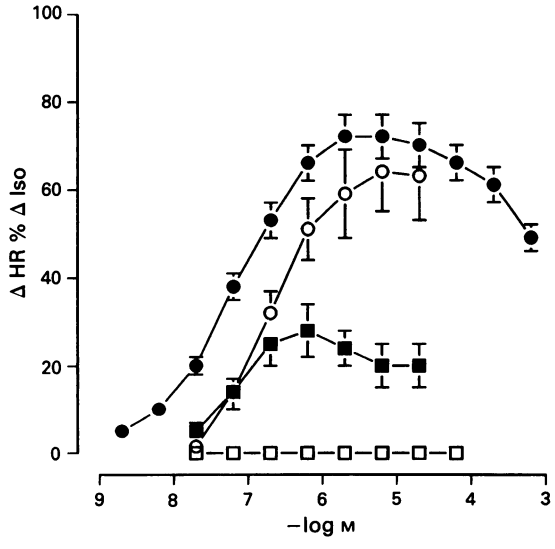

Figure 3 Concentration-effect curves to 5-HT (●, $n = 33$), BIMU 8 (○, $n = 8$), BIMU 1 (■, $n = 4$) and DAU 6215 (□, $n = 4$) in piglet right atrium. Data are means ± s.e.mean. The chronotropic effects are expressed as a percentage of the increase in beating rate caused by agonist plus 200 μM isoprenaline (Δ HR % Δ Iso). For abbreviations, see text.

Drugs

5-Hydroxytryptamine hydrochloride (5-HT), 5-methoxytryptamine hydrochloride (5-MeOT), 5-methoxy-N,N-dimethyltryptamine (5-MeO-N,N-DMT), (−)-isoprenaline hydrochloride, tryptamine hydrochloride, cocaine hydrochloride, bufotenine monoxalate and parglyine hydrochloride were all purchased from Sigma (UK).

α-methyl-5-hydroxytryptamine maleate (α-methyl-5-HT), 2-methyl-5-hydroxytryptamine maleate (2-methyl-5-HT) and tropisetron were obtained from RBI (Natick, MA, U.S.A.) and ascorbic acid from BDH (UK). The following drugs were gifts: (±)-propranolol hydrochloride from ICI Pharmaceuticals (Macclesfield, Cheshire), BIMU 1 (endo-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazole-1-carboxamide hydrochloride), BIMU 8 (endo-N-(8-methyl-8-azabicyclo[3.2.1)oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1H-benzimidazole-1-carboxamide hydrochloride) and DAU 6215 (N-(endo-8-methyl-8-azabicyclo [3.2.1]-oct-3-yl)-2,3-dihydro-2-oxo-1H-benzimidazole-1- carboxamide, hydrochloride) from Istituto de Angeli (Milan, Italy), cisapride from Janssen (Beerse, Belgium), and GR 113808 (([1-[2-[methylsulphonyl amino]ethyl]-4]piperidinyl] methyl 1-methyl-1H-indole-3-carboxylate) from Glaxo Group Research (U.K.). (−)-and (+)-zacopride, renzapride hydrochloride, metoclopramide, SB 203186 ((1-piperidinyl)ethyl 1H-indole 3-carboxylate), SDZ 205-557 ((2-diethylamino-ethyl)2-methoxy-4-amino-5-chloro-benzoate) and 5-carboxamidotryptamine (5-CT) were synthesized in-house.

All stock solutions were made up in distilled water except tropisetron and cisapride (dimethylsulphoxide), 5-HT and (−)-isoprenaline (0.2 mM ascorbate).

Results

Agonist studies

Tryptamines The tryptamines 5-HT, 5-MeOT, α-methyl-5-HT, 5-CT, bufotenine, tryptamine (after pargyline pretreatment), and 5-MeO-N,N-DMT, were all chronotropic agonists in piglet right atrium. Concentration-effect curves to these agonists are shown in Figure 1 and pEC_{50} ($= -\log EC_{50}$) and intrinsic activity values are displayed in Table 1. The maximal effect of 5-HT was 72% of the effect of 200 μM isoprenaline. 5-MeOT, α-methyl-5-HT, 5-MeO-N,N-DMT and tryptamine were all full agonists relative to 5-HT whilst bufotenine and 5-CT were partial agonists. The 5-HT$_3$ receptor agonist, 2-methyl-5-HT, was a weak partial agonist with a pEC_{50} of ~3.9 (experiment not shown); its intrinsic activity could not be assessed since it caused depression of heart rate which could not be overcome by 5-HT or isoprenaline.

Concentration-effect curves to 5-HT, 5-CT, 5-MeOT and 5-MeO-*N*,*N*-DMT ($n = 3$) were unaffected by treatment of the right atria with the irreversible monoamine oxidase inhibitor, pargyline (not shown) and so pargyline pretreatment was not used in subsequent experiments. Pargyline was used routinely, however, in experiments with tryptamine since no response was obtained when pargyline was absent.

Substituted benzamides The 2-methoxy-4-amino-5-chloro-substituted benzamides renzapride, cisapride, (−)-zacopride,

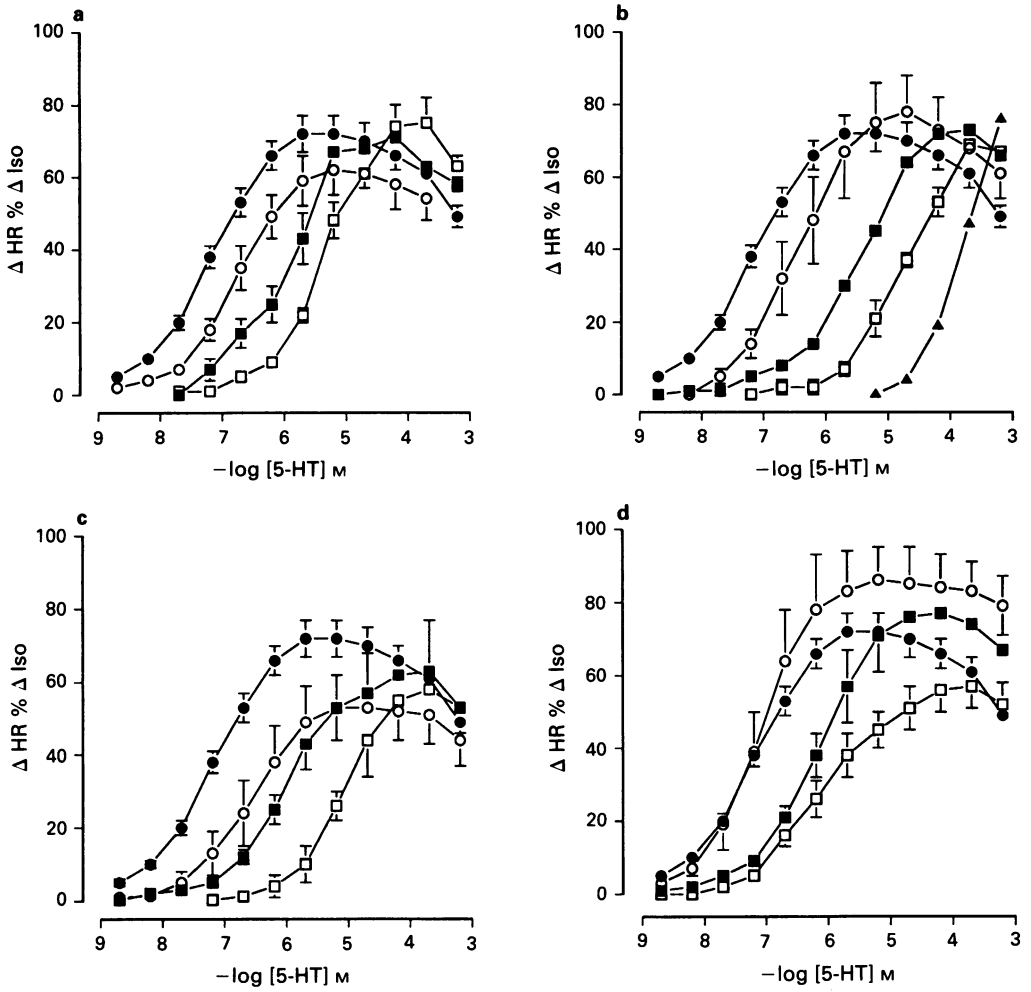

Figure 4 Concentration-effect curves to 5-HT in the absence (●, $n = 33$) and presence of (a) 0.1 (○, $n = 4$), 1.0 (■, $n = 6$) and 10 μM (□, $n = 5$) DAU 6215; (b) 0.02 (○, $n = 3$), 0.2 (■, $n = 2$), 2.0 (□, $n = 3$) and 10 μM (▲, $n = 2$) SB 203186; (c) 0.1 (○, $n = 5$), 1.0 (■, $n = 4$) and 10 μM (□, $n = 3$) SDZ 205-557; and (d) 0.002 (○, $n = 4$), 0.01 (■, $n = 4$) and 0.1 μM GR113808 (□, $n = 8$) in piglet right atrium. Data are means ± s.e.mean. The chronotropic effects of 5-HT are expressed as percentage of the increase in beating caused by agonist or agonist in the presence of antagonist, plus 200 μM (−)-isoprenaline (Δ HR % Δ Iso). For abbreviations, see text.

Table 2 Binding characteristics and affinity values of antagonists and partial agonists for 5-HT_4 receptors in piglet right atrium

Antagonists	pK_B *or* pKp[a]	*Slope*[d]	n
SB203186	8.26 ± 0.09	0.96 ± 0.09	10
DAU 6215	7.00 ± 0.10	0.85 ± 0.12	15
Tropisetron	6.89 ± 0.12[b]	1.09 ± 0.30	7
SDZ 205-557		0.58 ± 0.07	12
GR 113808		non-linear	16
Partial agonists			
BIMU 1	7.93 ± 0.11	1.08 ± 0.10	25
Renzapride	7.95 ± 0.13[c]	–	6
Metoclopramide	6.00 ± 0.5[c]	–	4

[a]pK_B or $pKp = -\log$ equilibrium dissociation constants; K_B, estimated using equation (1); Kp, estimated using equation (2).
[b]Data from Kaumann (1990).
[c]Estimated from single concentrations, 0.5 μM renzapride and 20 μM metoclopramide.
[d]Mean slope ± s.e.mean.

(+)-zacopride and metoclopramide were less potent agonists than 5-HT in causing tachycardia in piglet right atria (Figure 2). Only (−)-zacopride was a full agonist with respect to 5-HT. Renzapride was a potent partial agonist ($-\log EC_{50} = 6.7$) with an intrinsic activity of 0.7 (with respect to 5-HT), whilst (+)-zacopride, metoclopramide and cisapride were less potent partial agonists and had intrinsic activities of less than 0.4 (Table 1).

Renzapride and metoclopramide antagonized the effects of 5-HT so equilibrium dissociation constants for partial agonists (pKp) were calculated (see below). Above 60 μM, metoclopramide caused a dramatic decrease in heart rate.

Azabicycloalkyl benzimidazolone derivatives The azabicycloalkyl benzimidazolone derivative BIMU 8 was a potent full agonist relative to 5-HT in piglet right atrium (Figure 3). BIMU 1 was a chronotropic partial agonist of low intrinsic activity (Table 1, Figure 3). BIMU 1 also antagonized the effects of 5-HT, so that a pKp for partial agonist activity was calculated (see below).

DAU 6215 showed no chronotropic effects with concentrations of up to 60 μM (Figure 3) and caused some decrease in heart rate above this concentration. DAU 6215 was also tested for antagonist properties (see below).

Antagonist studies

Tropisetron (3 μM) antagonized the positive chronotropic effects of 5-MeOT ($n = 5$), α-methyl-5-HT ($n = 4$) and 5-MeO-N,N-DMT ($n = 3$), the log concentration-ratios being 1.28 ± 0.30; 1.33 ± 0.17 and 0.50 ± 0.30 respectively.

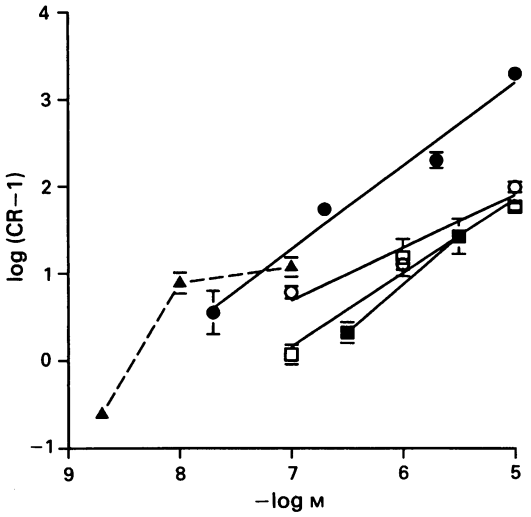

Figure 5 Schild plots from antagonism of 5-HT-induced tachycardia by DAU 6215 (□), SDZ 205-557 (○), SB 203186 (●), GR113808 (▲) and tropisetron (■) in piglet right atrium. Mean slopes ± s.e.mean: 0.96 ± 0.09 (SB 203186), 0.58 ± 0.07 (SDZ 205-557), 1.09 ± 0.3 (tropisetron) and 0.85 ± 0.12 (DAU 6215). A mean slope was not calculated for GR113808 because the plot was non-linear.

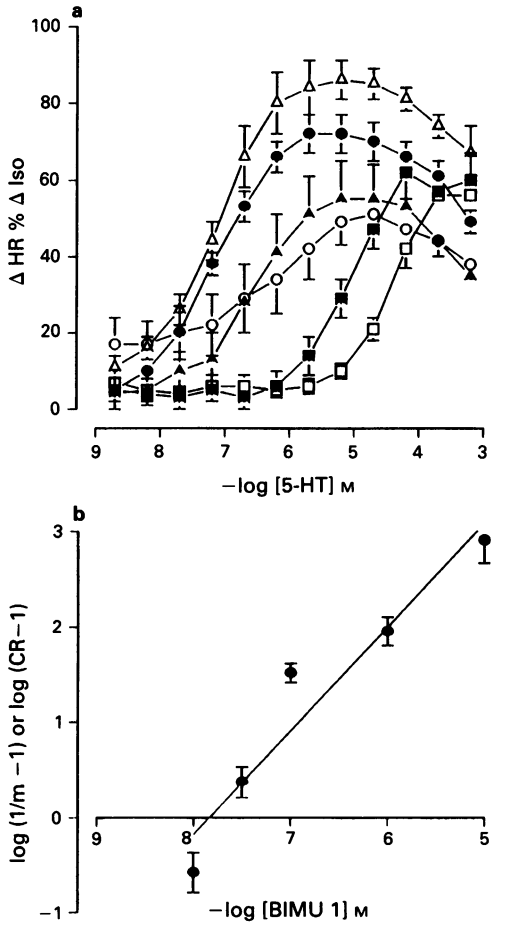

Figure 6 (a) Concentration-effect curves to 5-HT in the absence (●, $n = 33$) and presence of 0.01 (△, $n = 5$), 0.03 (▲, $n = 4$), 0.1 (○, $n = 5$), 1.0 (■, $n = 6$) and 10 μM (□, $n = 5$) BIMU 1 in piglet right atrium; data are means ± s.e.mean, chronotropic effects are expressed as a percentage of the increase in beating caused by agonist alone or in the presence of antagonist, plus 200 μM (−)-isoprenaline (Δ HR % Δ Iso). (b) Double log plot for simple competitive inhibition by BIMU 1; data from (a), calculated using equations (1) and (2); mean slope ± s.e.mean = 1.08 ± 0.10.

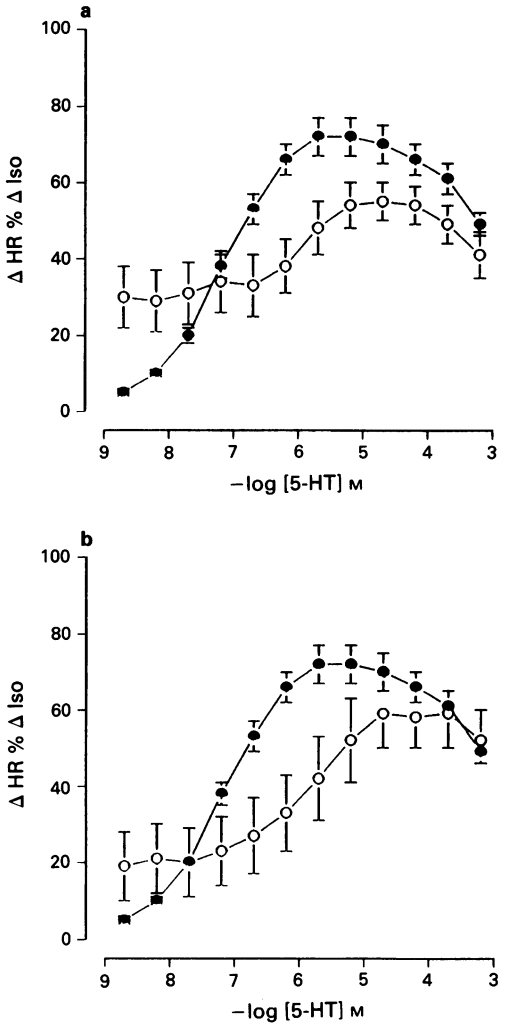

Figure 7 Concentration-effect curves to 5-HT in the absence (●, $n = 33$) and presence of (a) 0.5 μM renzapride (○, n = 6) and (b) 20 μM metoclopramide (○, $n = 4$) in piglet right atrium. Data are means ± s.e.mean. The chronotropic effects of 5-HT, renzapride and metoclopramide are expressed as a percentage of the increase in beating caused by agonist alone or in the presence of antagonist, plus 200 μM (−)-isoprenaline (Δ HR % Δ Iso).

Tropisetron (3 μM) also antagonized BIMU 8-induced increases in heart rate ($n = 4$) but the blockade was partially unsurmountable with up to 60 μM BIMU 8 (data not illustrated). Responses to tryptamine ($n = 4$) were completely abolished with 3 μM tropisetron (not shown).

DAU 6215 (0.1–10 μM) and SB 203186 (0.02–10 μM) caused parallel rightward shifts of concentration-effect curves to 5-HT (Figure 4a and b). The slopes of the Schild regression were not significantly different from unity and yielded estimated pK_B values of 7.0 and 8.3 respectively (Figure 5, Table 2). SDZ 205-557 (0.10–10 μM) also caused rightward displacement of the 5-HT curve (Figure 4c) but the slope of the Schild regression was only 0.58 (Figure 5) so a meaningful estimate of pK_B for SDZ 205-557 could not be made. Similarly, a meaningful pK_B estimate for GR113808 could not be made because although 0.01 and 0.1 μM shifted the 5-HT curve to the right, 0.1 μM also tended to reduce the maximum response to 5-HT and did not produce a significantly greater shift than 0.01 μM (Figure 4d). Since 0.002 μM GR113808 only caused marginal blockade and because of the lack of concentration-dependent antagonism with 0.01 and 0.1 μM, the resultant Schild plot was non-linear (Figure 5).

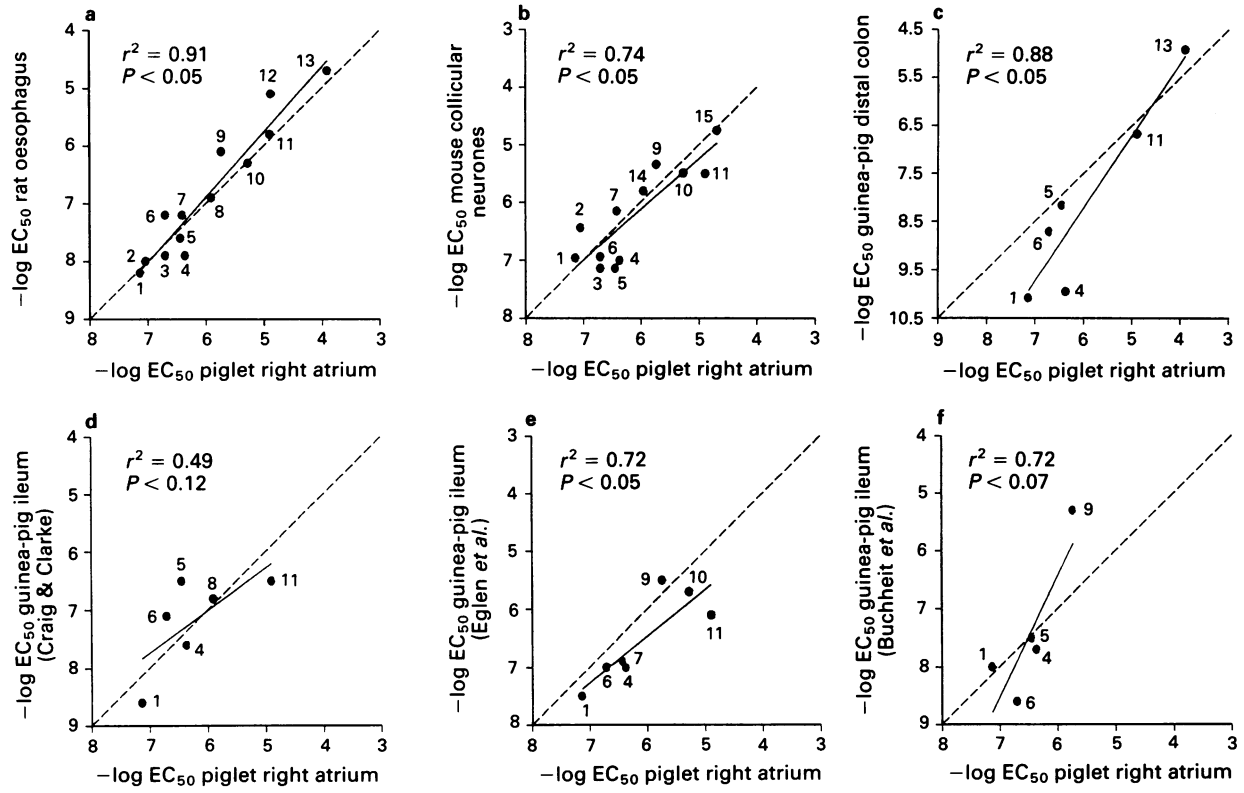

Figure 8 Squared correlation coefficients (r^2) and regression lines for 5-HT_4 receptor agonists in piglet right atrium and (a) rat oesophagus (Baxter *et al.*, 1991, Baxter & Clarke, 1992); (b) mouse embryonic colliculi neurones (Dumuis *et al.*, 1988; 1989; 1991); (c) guinea-pig distal colon (Wardle & Sanger, 1992); (d), (e) and (f) guinea-pig ileum (Craig & Clarke, 1990; Eglen *et al.*, 1990; Buchheit & Buhl, 1991, respectively); (1) 5-HT; (2) BIMU 1; (3) BIMU 8; (4) 5-MeOT; (5) cisapride; (6) renzapride; (7) (−)-zacopride, (8) α-methyl-5-HT; (9) metoclopramide; (10) (+)-zacopride; (11) 5-CT; (12) tryptamine; (13) 2-methyl-5-HT; (14) bufotenine, and (15), 5-MeO-*N*,*N*-DMT. For abbreviations, see text.

Partial agonist studies

BIMU 1 (Figure 6), renzapride (0.5 μM) and metoclopramide (20 μM) (Figure 7) also shifted 5-HT concentration-effect curves to the right. Since they had some agonist activity p*K*p values were estimated with equation (2) (Lemoine & Kaumann, 1982) although in some experiments, where they caused little or marginal stimulation, affinity values were estimated with equation (1) (Arunlakshana & Schild, 1959). The slope of the double log plot for BIMU 1 according to equations (1) and (2) was not significantly different from unity (Figure 6). The estimated p*K*p value for BIMU 1 was 7.9 (Table 2). p*K*p values were 7.9 for renzapride and 6.0 for metoclopramide as estimated from single concentrations (Table 2).

Discussion

The aim of our study was to compare piglet sinoatrial 5-HT_4 receptors (Kaumann, 1990) with 5-HT_4 receptors in other peripheral tissues and CNS. To exclude 5-HT_4 receptor-unrelated factors that determine agonist potency we used cocaine (which blocks tissue uptake of 5-HT thereby making more 5-HT available to the receptors, Kaumann *et al.*, 1990) and propranolol (which blocks β-adrenoceptors thereby preventing possible action of released noradrenaline, Kaumann, 1990). In some cases we also blocked monoamine oxidase (MAO).

The irreversible MAO inhibitor, pargyline, did not alter 5-HT-evoked responses which is consistent with the observations of Baxter *et al.* (1991). The responses to 5-MeOT, 5-CT and 5-MeO-N,N-DMT were also unaffected by pargyline. Conversely, no chronotropic responses were obtained with tryptamine unless pargyline was present, indicating a particular susceptibility of tryptamine to deamination by MAO. A similar effect of MAO inhibition was observed with tryptamine interacting with 5-HT_2 receptors of bovine large coronary artery (Frenken & Kaumann, 1988) indicating that the enzyme avidly oxidizes tryptamine in the neighbourhood of more than one class of 5-HT receptors.

pEC_{50} values were determined for tryptamine, benzamide and benzimidazolone derivatives for comparison with those obtained in rat oesophagus (Baxter *et al.*, 1991; Baxter & Clarke, 1992), guinea-pig ileum and colon (Craig & Clarke, 1990; Eglen *et al.*, 1990; Buchheit & Buhl, 1991; Wardle & Sanger, 1992) and mouse embryonic colliculi neurones (Dumuis *et al.*, 1988; 1989; 1991) (Figure 8). We assessed agonist potency in absolute terms as pEC_{50} values. This seemed the most relevant method of potency determination for this study, because the data we were using for comparison from other studies also used pEC_{50} values as a measure of agonist potency. Figure 8 shows there is excellent correlation between potency estimates for agonists in piglet atria and those in rat oesophagus and guinea-pig distal colon although the number of compounds compared is small for the latter. A weaker but still reasonable correlation is seen with piglet sinoatrial 5-HT_4 receptors and 5-HT_4 receptors in mouse embryonic colliculi neurones. There is borderline correlation between our data and the guinea-pig ileum data of Eglen *et al.* (1990) and Buchheit & Buhl (1991) whilst a poor correlation is seen with the data of Craig & Clarke (1990). This probably reflects a small sample size or that one drug is an outlier.

The relatively low stimulant potency of tryptamines and benzamides in piglet atria (Figure 8) suggests a relatively low 5-HT_4 receptor reserve or alternatively deficient coupling to biochemical effectors compared to guinea-pig ileum (Craig &

Clarke, 1990; Buchleit & Buhl, 1991) and distal colon (Wardle & Sanger, 1992) or rat oesophagus (Baxter *et al.*, 1991). The advent of a suitable 5-HT$_4$ receptor radioligand may reveal variations in 5-HT$_4$ receptor reserve in the different tissues. Indeed, preliminary autoradiographic studies with the novel 5-HT$_4$ receptor radioligand, [^{3}H]-GR113808, have revealed variations in specific binding to different brain regions of the rat and guinea-pig (Grossman *et al.*, 1993).

A number of tryptamine derivatives are full or partial agonists at 5-HT$_4$ receptors in the CNS (Dumuis *et al.*, 1988), gut (Craig & Clarke, 1990; Baxter *et al.*, 1991; Wardle & Sanger, 1992) and heart (Kaumann, 1990; Kaumann *et al.*, 1990; 1991a,b; Villalon *et al.*, 1990; 1991). We obtained the following order of potency for chronotropic activity: 5-HT > 5-MeOT > α-methyl-5-HT = bufotenine > 5-CT = tryptamine (after pargyline pretreatment) > 5-MeO-*N*,*N*-DMT > 2-methyl-5-HT. All tryptamines tested were full agonists compared to 5-HT except 5-CT, bufotenine and 2-methyl-5-HT.

Benzamides are key ligands for 5-HT$_4$ receptor characterization since they are more selective agonists than the tryptamines (although they can antagonize 5-HT$_3$ receptors). These compounds are usually partial agonists at 5-HT$_4$ receptors (Kaumann, 1990; Kaumann *et al.*, 1991a,b; Baxter *et al.*, 1991; Villalon *et al.*, 1990; 1991) although in mouse embryonic colliculi neurones their intrinsic activity often exceeds that of 5-HT (Dumuis *et al.*, 1989). Results from this study and of Kaumann (1990) in piglet right atria show that benzamides are partial agonists with low intrinsic activity except for (−)-zacopride which was a full agonist relative to 5-HT. The order of potency for these agents was renzapride > cisapride = (−)-zacopride > metoclopramide > (+)-zacopride. The similarity of the eudismic ratio for zacopride is consistent with similar steric configurations of the 5-HT$_4$ receptors in piglet, guinea-pig and rat. The stereoselectivity of the 5-HT$_4$ receptor to isomers of zacopride with a 10 fold potency separation between (−)-zacopride ($pEC_{50} = 6.41$) and (+)-zacopride ($pEC_{50} = 5.27$), is also a feature previously described in rat oesophagus (Baxter *et al.*, 1991) and guinea-pig ileum (Eglen *et al.*, 1990).

Because benzamides were partial agonists in piglet right atria, affinity estimates for renzapride and metoclopramide were obtained from experiments investigating antagonism of the chronotropic effects of 5-HT. The affinity estimate for renzapride ($pK_p = 7.9$) was greater than its stimulant potency estimate ($pEC_{50} = 6.7$) suggesting desensitization of 5-HT$_4$ receptors in addition to competition for the receptors. High renzapride concentrations have previously been shown to desensitize 5-HT$_4$ receptors (Dumuis *et al.*, 1989; Kaumann, 1990). In contrast, the affinity estimate for metoclopramide was similar to its potency estimate suggesting lack of desensitization and supporting the observations of Bockaert *et al.* (1992b) who showed desensitization with renzapride but not with metoclopramide. They proposed that 5-HT$_4$ receptor desensitization was proportional to agonist affinity.

Azabicycloalkyl benzimidazolones have also proved to be useful ligands for characterizing 5-HT$_4$ receptors (reviewed by Turconi *et al.*, 1991). BIMU 8 and BIMU 1 are potent full or partial agonists at the 5-HT$_4$ receptor in mouse colliculi (Dumuis *et al.*, 1991), rat oesophagus (Baxter & Clarke, 1992), and guinea-pig ileum (Rizzi *et al.*, 1992). Similarly, in the present study with piglet atria, BIMU 8 was a full agonist whilst BIMU 1 was a partial agonist relative to 5-HT. BIMU 1 was similar in potency to 5-HT but more potent than BIMU 8 as determined from the corresponding pEC_{50} values. We obtained an affinity estimate from experiments where the 5-HT concentration-effect curve was shifted to the right by BIMU 1. As with renzapride, desensitization seemed to be present since the affinity estimate ($pK_p \simeq 7.9$) for BIMU 1 was greater than the potency estimate ($pEC_{50} \simeq 7.1$), and at high concentrations fade of the BIMU 1-induced responses occurred.

Another benzimidazolone derivative, DAU 6215 was reported to be a weak partial agonist in mouse embryonic colliculi neurones (Dumuis *et al.*, 1991) but a competitive antagonist in rat oesophagus (Baxter & Clarke, 1992). In piglet right atria we found no chronotropic activity with DAU 6215 which at high concentrations even decreased the heart rate. DAU 6215 behaved as a simple competitive antagonist at sinoatrial 5-HT$_4$ receptors with a pK_B of 7.1 yielded from the Schild plot. This figure compared favourably with a pA_2 value of 6.7 in rat oesophagus (Baxter & Clarke, 1992), but was rather higher than the value of 5.5 estimated in mouse embryonic colliculi neurones by Dumuis *et al.* (1991).

Buchheit *et al.* (1992) recently introduced SDZ 205-557 as a 30 fold 5-HT$_4$ receptor selective (compared to 5-HT$_3$) antagonist of 5-HT-evoked contractions in guinea-pig ileum with an affinity of 7.4 whilst Eglen *et al.* (1991) showed that SDZ 205-557 had a pK_B of 7.1 in rat oesophagus. In piglet right atria SDZ 205-557 also antagonized 5-HT-induced tachycardia but this antagonism did not appear to be simple competitive. This may partially reflect a non-specific effect of this drug. We were unable to calculate a meaningful pK_B estimate since the slope of the Schild plot was 0.58.

GR113808 was recently presented as a novel 5-HT$_4$ receptor antagonist with a pA_2 of 9.2 and 9.5 in guinea-pig colon and rat oesophagus respectively (Grossman *et al.*, 1993). On piglet atrium, 2 nM GR113808 caused marginal blockade (Figures 4d and 5) and appeared to be less potent than on gut (Grossman *et al.*, 1993). In the present study we were unable to obtain a pK_B estimate since the Schild plot slope was non-linear, suggesting that the blockade was not simple competitive. There was a reduction in the maximum response to 5-HT with 0.1 μM GR113808 in our experiments, a phenomenon reported in the guinea-pig colon at the same concentration (Grossman *et al.*, 1993). This suggests a non-specific effect of GR113808 in these systems. The novel selective 5-HT$_4$ receptor blocker SB 203186 (Kaumann *et al.*, 1992) was the most potent competitive antagonist investigated in the present study. We obtained a pK_B of 8.3 for SB 203186, 20 fold or more potent than tropisetron and DAU 6215. Similar affinity estimates for SB 203186 have been obtained in piglet left atrium and human atrial strips (Parker *et al.*, 1993) where 5-HT$_4$ receptors have also been demonstrated (Kaumann *et al.*, 1990; 1991a,b).

In conclusion, we have shown that 5-HT$_4$ receptors in piglet right atria are sensitive to the three groups of agonists used to characterize this receptor, as well as being blocked by a number of antagonists reported to be selective for 5-HT$_4$ receptors in the literature. We suggest that piglet sinoatrial 5-HT$_4$ receptors are similar to 5-HT$_4$ receptors of rat oesophagus, guinea-pig ileum and colon, mouse embryonic colliculi neurones and human atrium.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonism. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

BAXTER, G.S. & CLARKE, D.E. (1992). Benzimidazolone derivatives act as 5-HT$_4$ receptor ligands in rat oesophagus. *Eur. J. Pharmacol.*, **212**, 225–229.

BAXTER, G.S., CRAIG, D.A. & CLARKE, D.E. (1991). 5-Hydroxytryptamine$_4$ receptors mediate relaxation of rat oesophageal tunica muscularis mucosae. *Naunyn-Schmied. Arch. Pharmacol.*, **343**, 439–446.

BLINKS, J.R. (1956). Positive chronotropic effect of increasing right atrial pressure in the isolated mammalian heart. *Am. J. Physiol.*, **186**, 299–303.

BLINKS, J.R. (1965). Convenient apparatus for recording contractions of isolated muscle. *J. Appl. Physiol.*, **20**, 755–757.

BOCKAERT, J., ANSANAY, M., SEBBEN, M., FAGIN, L. & DUMUIS, A. (1992b). Characterisation of homologous 5-HT_4 receptor desensitisation in colliculi neurons. *Proceedings 2nd International Symposium on Serotonin from Cell Biology to Pharmacology and Therapeutics*, Houston (USA), Sept 15–18, p. 30. Kluwers Academic Publishers.

BOCKAERT, J., FOZARD, J.R., DUMUIS, A. & CLARKE, D.E. (1992a). The 5-HT_4 receptor: a place in the sun. *Trends Pharmacol Sci.*, **13**, 141–145.

BUCHHEIT, K.-H. & BERTHOLET, A. (1992). 5-HT effects on isolated strips from guinea-pig stomach. *Naunyn-Schmied. Arch. Pharmacol.*, **345**, R111, 442.

BUCHHEIT, K.-H. & BUHL, T. (1991). Prokinetic benzamides stimulate peristaltic activity in the isolated guinea-pig ileum by activation of 5-HT_4 receptors. *Eur. J. Pharmacol.*, **205**, 203–208.

BUCHHEIT, K.-H., GMASE, R. & PFANNKUCHE, H.-J. (1991). SDZ 205-557, a selective antagonist at 5-HT_4 receptors in the isolated guinea-pig ileum. *Eur. J. Pharmacol.*, **200**, 373–374.

BUCHHEIT, K.-H., GMASE, R. & PFANNKUCHE, H.-J. (1992). SDZ 205-557, a selective, surmountable antagonist for 5-HT_4 receptors in the isolated guinea-pig ileum. *Naunyn-Schmied. Arch. Pharmacol.*, **345**, 387–393.

CRAIG, D.A. & CLARKE, D.E. (1990). Pharmacological characterisation of a neuronal receptor for 5-hydroxytryptamine in guinea-pig ileum with properties similar to the 5-hydroxytryptamine$_4$ receptor. *J. Pharmacol. Exp. Ther.*, **252**, 1378–1386.

DUMUIS, A., BOUHELAL, R., SEBBEN, M., CORY, R. & BOCKAERT, J. (1988). A non-classical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. *Mol. Pharmacol.*, **34**, 880–887.

DUMUIS, A., SEBBEN, M. & BOCKAERT, J. (1989). The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT_4) positively coupled to adenylate cyclase in neurones. *Naunyn-Schmied. Arch. Pharmacol.*, **340**, 403–410.

DUMUIS, A., SEBBEN, M., MONFERINI, E., NICOLA, M., TURCONI, M., LADINSKY, H. & BOCKAERT, J. (1991). Azabicycloalkyl benzimidazolone derivatives as a novel class of potent agonists at the 5-HT_4 receptor positively coupled to adenylate cyclase in brain. *Naunyn-Schmied. Arch. Pharmacol.*, **343**, 245–251.

EGLEN, R.M., ALVAREZ, R., WONG, E.H.F., JOHNSON, L., PERKINS, L.A., RAMSEY, S., LOURY, D.N., TANG, H.-M., WALLACE, S. & WHITING, R.L. (1992). Characterisation of the interaction of SDZ 205-557 at putative 5-HT_4 receptors. *Br. J. Pharmacol.*, **105**, 275P.

EGLEN, R.M., SWANK, S.R., WALSH, L.K.M. & WHITING, R.L. (1990). Characterisation of 5-HT_3 and 'atypical' 5-HT receptors mediating guinea-pig ileal contractions *in vitro*. *Br. J. Pharmacol.*, **101**, 513–520.

ELSWOOD, C.J., BUNCE, K.T. & HUMPHREY, P.P.A. (1991). Identification of putative 5-HT_4 receptors in guinea-pig ascending colon. *Eur. J. Pharmacol.*, **196**, 149–155.

FORD, A.P.D.W., BAXTER, G.S., EGLEN, R.M. & CLARKE, D.E. (1992). 5-hydroxytryptamine stimulates cyclic AMP formation in the tunica muscularis mucosae of the rat oesophagus via 5-HT_4 receptors. *Eur. J. Pharmacol.*, **211**, 117–120.

FRENKEN, M. & KAUMANN, A.J. (1988). Effects of tryptamine mediated through 2 states of the 5-HT_2 receptor in calf coronary artery. *Naunyn-Schmied. Arch. Pharmacol.*, **337**, 484–492.

GROSSMAN, C.J., GALE, J.D., BUNCE, K.T., KILPATRICK, G.J., WHITEHEAD, J.W.F., OXFORD, A.W. & HUMPHREY, P.P.A. (1993). Development of a radioligand binding assay for the 5-HT_4 receptor: use of a novel antagonist. *Br. J. Pharmacol.*, **108**, 106P.

KAUMANN, A.J. (1990). Piglet sinoatrial 5-HT receptors resemble human atrial 5-HT_4-like receptors. *Naunyn-Schmied. Arch. Pharmacol.*, **342**, 619–622.

KAUMANN, A.J., BROWN, A.M. & RAVAL, P. (1991b). Putative 5-HT_4-like receptors in piglet left atrium. *Br. J. Pharmacol.*, **102**, 98P.

KAUMANN, A.J., MEDHURST, A.D., BOYLAND, P., VIMAL, M. & YOUNG, R.C. (1992). SB 203186, a potent selective 5-HT_4 receptor antagonist. Proceedings 2nd International Symposium on Serotonin from Cell Biology to Pharmacology and Therapeutics, Houston (U.S.A.), Sept 15–18, p. 44. Kluwers Academic Publishers.

KAUMANN, A.J., MCINERNY, T.K., GILMOUR, D.P. & BLINKS, J.R. (1980). Comparative assessment of β-adrenoceptor blocking agents as simple competitive antagonists in isolated heart muscle: similarity of inotropic and chronotropic blocking potency against isoproterenol. *Naunyn-Schmied. Arch. Pharmacol.*, **311**, 219–236.

KAUMANN, A.J., SANDERS, L., BROWN, A.M., MURRAY, K.J. & BROWN, M.J. (1990). A 5-hydroxytryptamine receptor in human atrium. *Br. J. Pharmacol.*, **100**, 879–885.

KAUMANN, A.J., SANDERS, L., BROWN, A.M., MURRAY, K.J. & BROWN, M.J. (1991a). A 5-HT_4-like receptor in human right atrium. *Naunyn-Schmied. Arch. Pharmacol.*, **344**, 150–159.

LEMOINE, H. & KAUMANN, A.J. (1982). A novel analysis of concentration-dependence of partial agonism. *Naunyn-Schmied. Arch. Pharmacol.*, **320**, 130–144.

MARANO, M. & KAUMANN, A.J. (1976). On the statistics of drug-receptor constants for partial agonists. *J. Pharmacol. Exp. Ther.*, **198**, 518–525.

PARKER, S.G., HAMBURGER, S., TAYLOR, E.M. & KAUMANN, A.J. (1993). SB 203186, a potent 5-HT_4 receptor antagonist, in porcine sinoatrial node and human and porcine atrium. *Br. J. Pharmacol.*, **108**, 68P.

REEVES, J.J., BUNCE, K.T. & HUMPHREY, P.P.A. (1991). Investigation into the 5-hydroxytryptamine receptor mediating smooth muscle relaxation in rat oesophagus. *Br. J. Pharmacol.*, **103**, 1067–1072.

RIZZI, C.A., COCCINI, T., ONORI, L., MANZO, L. & TONINI, M. (1992). Benzimidazolone derivatives: a new class of 5-hydroxytryptamine$_4$ receptor agonists with prokinetic and acetylcholine releasing properties in the guinea-pig ileum. *J. Pharmacol. Exp. Ther.*, **261**, 412–419.

SANDERS, L. & KAUMANN, A.J. (1992). A 5-HT_4-like receptor in human left atrium. *Naunyn-Schmied. Arch. Pharmacol.*, **345**, 382–386.

SHENKER, A., MAAYANI, S., WEINSTEIN, H. & GREEN, J.P. (1987). Pharmacological characterisation of two 5-hydroxytryptamine receptors coupled to adenylate cyclase in guinea-pig hippocampal membranes. *Mol. Pharmacol.*, **31**, 357–367.

TURCONI, M., SCHIANTARELLI, P., BORSINI, F., RIZZI, C.A., LADINSKY, H. & DONETTI, A. (1991). Azabicycloalkyl benzimidazolones: Interaction with serotonergic 5-HT_3 and 5-HT_4 receptors and potential therapeutic implications. *Drugs of the Future*, **16**, 1011–1026.

VILLALON, C.M. DEN BOER, M.O., HEILIGERS, J.A.C. & SAXENA, P.R. (1990). Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT_4 receptor. *Br. J. Pharmacol.*, **100**, 665–667.

VILLALON, C.M., DEN BOER, M.O., HEILIGERS, J.A.C. & SAXENA, P.R. (1991). Further characterisation, by use of tryptamine and benzamide derivatives, of the putative 5-HT_4 receptor mediating tachycardia in the pig. *Br. J. Pharmacol.*, **102**, 107–112.

WARDLE, K. & SANGER, G. (1992). Guinea-pig distal colon: a sensitive model of the putative 5-HT_4 receptor. *Br. J. Pharmacol.*, **105**, 279P.

(Received February 24, 1993
Revised June 23, 1993
Accepted July 9, 1993)