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Further characterization, by use of tryptamine and benzamide derivatives, of the putative 5-HT_4 receptor mediating tachycardia in the pig

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1 It has recently been shown that the tachycardic response to 5-hydroxytryptamine (5-HT) in the anaesthetized pig, being mimicked by 5-methoxytryptamine and renzapride and blocked by high doses of ICS 205-930, is mediated by the putative 5-HT_4 receptor. In the present investigation we have further characterized this receptor.

2 Intravenous bolus injections of the tryptamine derivatives, 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$), 5-methoxytryptamine (3, 10 and 30 $\mu g\,kg^{-1}$) and α-methyl-5-hydroxytryptamine (α-methyl-5-HT; 3, 10, 30 and 100 $\mu g\,kg^{-1}$), resulted in dose-dependent increases in heart rate of, respectively, 25 ± 2, 48 ± 3 and 68 ± 3 beats min^{-1} (5-HT; $n = 35$); 15 ± 1, 32 ± 2 and 57 ± 3 beats min^{-1} (5-methoxytryptamine; $n = 30$); 6 ± 4, 18 ± 6, 34 ± 6 and 64 ± 11 beats min^{-1} (α-methyl-5-HT; $n = 3$).

3 The increases in heart rate following i.v. administration of certain substituted benzamide derivatives were genereally less marked and not dose-dependent: 1 ± 5, 11 ± 3 and 10 ± 5 beats min^{-1} after 300, 1000 and 3000 $\mu g\,kg^{-1}$ of metoclopramide, respectively, ($n = 8$); 21 ± 4, 19 ± 2 and 2 ± 2 beats min^{-1} after 100, 300 and 1000 $\mu g\,kg^{-1}$ of cisapride, respectively, ($n = 5$); 6 ± 2, 14 ± 2, 37 ± 6, 43 ± 8 and 34 ± 10 beats min^{-1} after 10, 30, 100, 300 and 1000 $\mu g\,kg^{-1}$ of zacopride, respectively, ($n = 6$); and 1 ± 1, 2 ± 1 and 5 ± 2 beats min^{-1} after 300, 1000 and 3000 $\mu g\,kg^{-1}$ of dazopride, respectively, ($n = 4$). These drugs behaved as partial agonists, antagonizing the responses to 5-HT and 5-methoxytryptamine dose-dependently.

4 The 5-HT_3 receptor agonist 1-phenyl-biguanide (100, 300 and 1000 $\mu g\,kg^{-1}$) induced only slight increases in heart rate of 1 ± 1, 6 ± 2 and 11 ± 1 beats min^{-1}, respectively, ($n = 3$). These effects were not antagonized by the selective 5-HT_3 receptor antagonist granisetron (3 $mg\,kg^{-1}$). In addition, 1-phenyl-biguanide (1000 $\mu g\,kg^{-1}$) did not modify the tachycardia induced by either 5-HT- or 5-methoxytryptamine.

5 High doses (3 $mg\,kg^{-1}$) of ICS 205-930, a 5-HT_3 receptor antagonist with an indole group and devoid of effects on porcine heart rate *per se*, antagonized the stimulatory effects of 5-HT, 5-methoxytryptamine, α-Me-5-HT, metoclopramide, cisapride, zacopride, dazopride and 1-phenyl-biguanide. However, the 5-HT_2 receptor antagonist ketanserin (0.5 $mg\,kg^{-1}$), the 5-HT_3 receptor antagonists granisetron (3 $mg\,kg^{-1}$) and MDL 72222 (3 $mg\,kg^{-1}$) and the dopamine D_2 receptor antagonist domperidone (3 $mg\,kg^{-1}$) had no antagonist activity.

6 The above results support our contention that 5-HT, 5-methoxytryptamine, α-Me-5-HT and the substituted benzamide derivatives increase porcine heart rate by a direct action on the cardiac pacemaker, via the activation of a putative 5-HT_4 receptor. The pharmacological profile of this novel 5-HT receptor is similar (neurones from mouse brain colliculi and human heart) or, perhaps, even identical (guinea-pig cholinergic neurones) to other putative 5-HT_4 receptors.

Introduction

5-Hydroxytryptamine (5-HT) can exert multiple cardiac effects including both increases and decreases in heart rate. In most species, bradycardia induced by 5-HT is mediated by 5-HT_3 receptors, via the activation of the von Bezold Jarisch reflex. In marked contrast, the mechanism of 5-HT-induced tachycardia is notoriously species-dependent and is mediated, directly or indirectly, either by 5-HT_1-like (cat), 5-HT_2 (rat, dog) or 5-HT_3 (rabbit, dog) receptors, or by tyramine-like (guinea-pig) or unidentified mechanisms (see Saxena, 1986; Saxena & Villalón, 1990). In the pig, we have reported that the 5-HT-induced tachycardia is mediated by a novel receptor type which differs from 5-HT_1-like, 5-HT_2 and 5-HT_3 receptors (Duncker *et al.*, 1985; Bom *et al.*, 1988), but resembles the putative 5-HT_4 receptor (Villalón *et al.*, 1990) mediating stimulation of adenylate cyclase in both mouse embryo colliculi neurones and guinea-pig hippocampal membranes (Dumuis *et al.*, 1988; 1989; Clarke *et al.*, 1989). In the present study, we have further characterized the porcine heart 5-HT receptor using several agonist and antagonist drugs, including some substituted benzamide derivatives.

Methods

General

After an overnight fast, 40 young Yorkshire pigs (15–20 kg) were sedated with azaperone (120 mg, i.m.) and metomidate (120–150 mg, i.v.). After intubation, the animals were connected to a respirator for intermittent positive pressure ventilation with a mixture of room air (70%) and O_2 (30%). The anaesthesia was maintained with a continuous infusion of pentobarbitone sodium (15–20 $mg\,kg^{-1}\,h^{-1}$, i.v.). Aortic blood pressure and heart rate were recorded with, respectively, a

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Statham pressure transducer and a tachograph. All drugs were injected into the right jugular vein. The body temperature of the animals was maintained around 37°C by using an electric blanket and arterial blood gases and pH were kept within the normal limits (Po_2 > 90 mmHg; Pco_2 30–40 mmHg; pH 7.35–7.45) by adjusting respiratory rate and tidal volume or by infusing 4.2% sodium bicarbonate solution.

Experimental protocol

After the animals had been in a stable haemodynamic condition for at least 45 min, they received intravenous bolus injections of 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$) and 5-methoxytryptamine (3, 10 and 30 $\mu g\,kg^{-1}$). Subsequently, several doses of α-methyl-5-HT (3, 10, 30 and 100 $\mu g\,kg^{-1}$), 1-phenyl-biguanide (30, 100, 300 and 1000 $\mu g\,kg^{-1}$), metoclopramide (300, 1000 and 3000 $\mu g\,kg^{-1}$), cisapride (30, 100, 300 and 1000 $\mu g\,kg^{-1}$), zacopride (10, 30, 100, 300 and 1000 $\mu g\,kg^{-1}$) or dazopride (300, 1000 and 3000 $\mu g\,kg^{-1}$) were given, and after each dose or the highest dose (1-phenyl-biguanide), the responses to 5-HT and 5-methoxytryptamine were elicited again (for number of experiments and other specifications, see Results). In addition, tachycardic responses to 5-HT- and 5-methoxytryptamine were induced before and after pretreatment with 3 $mg\,kg^{-1}$ of either MDL 72222, granisetron or domperidone. In another set of experiments, the tachycardic responses to 5-HT, 5-methoxytryptamine and α-methyl-5-HT were analyzed before and after pretreatment with either ketanserin (0.5 $mg\,kg^{-1}$) or ICS 205-930 (3 $mg\,kg^{-1}$). Lastly, the dose of each benzamide derivative eliciting the maximum increase in heart rate was chosen and given to a new group of animals (without previous administration of any of the benzamide derivatives) after pretreatment with 3 $mg\,kg^{-1}$ of ICS 205-930.

The interval between the different doses of the compounds used as agonists and/or antagonists depended on the duration of tachycardia produced by the preceding dose, as in each case we waited until heart rate had returned completely or nearly to baseline values. The dose-intervals for the different drugs were as follows; tryptamine derivatives, between 5 and 15 min; benzamide derivatives and 1-phenyl-biguanide, usually between 15 and 30 min, but sometimes even longer than 60 min (cisapride and zacopride); and other antagonists (ICS 205-930, MDL 72222, granisetron, ketanserin and domperidone), between 10 and 15 min. The dosing with ICS 205-930 was cumulative (given as 0.3, 0.7 and 2.0 $mg\,kg^{-1}$), whereas that with all other drugs was sequential.

Drugs

The drugs used in this study were: cisapride (gift: Dr J.A.J. Schuurkes, Janssen Pharmaceutica, Beerse, Belgium), (±)-dazopride (A.H. Robbins Co., Richmond, VA, U.S.A.), domperidone (gift: Dr J.A.J. Schuurkes, Janssen Pharmaceutica, Beerse, Belgium), granisetron (gift: Dr G.J. Sanger, Smith Kline Beecham, Harlow, U.K.), 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Company, St. Louis, MO, U.S.A.), ketanserin tartrate (gift: Dr J.M. Van Nueten, Janssen Pharmaceutica, Beerse, Belgium), 5-methoxytryptamine hydrochloride (Janssen Chimica, Beerse, Belgium), (±)-S-α-methyl-5-HT (gift: Dr P.P.A. Humphrey, Glaxo Group Research, Ware, U.K.), metoclopramide hydrochloride (Pharmacy Department, Erasmus University, Rotterdam, The Netherlands), 1-phenyl-biguanide (Research Biochemicals Inc., Nathick, MA, U.S.A.), 1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate (MDL 72222; gift: Merrel-Dow Research Institute, Strasbourg, France); (3α-tropanyl)-1H-indole-3-carboxylic acid ester (ICS 205-930), and (±)-zacopride (A.H. Robbins Co., Richmond, VA, U.S.A.). The doses of cisapride, dazopride, 5-HT, 5-methoxytryptamine, α-methy-5-HT, 1-phenyl-biguanide and zacopride are given as free base.

Data presentation and analysis

All data in the text, figures and tables are presented as mean ± s.e.mean. The peak changes in heart rate induced by the different doses of both tryptamine- and benzamide derivatives were determined. The increases in heart rate just before and after a particular antagonist drug were compared by Duncan's new multiple range test, once an analysis of variance (randomized block design) revealed that the samples represented different populations (Saxena, 1985). The effects of agonist drugs in the different groups of animals were compared by use of the unpaired Student's t test. A P value of 0.05 or less (two-tailed) was considered statistically significant.

Results

Initial blood pressure and heart rate changes by 5-HT agonist drugs

Baseline values of mean arterial blood pressure and heart rate in the 40 pigs were 84 ± 4 mmHg and 101 ± 4 beats min^{-1}, respectively. The changes induced in mean arterial blood pressure by each 5-HT agonist drug were: 5-HT (−18 ± 1, −18 ± 1 and −16 ± 1 followed by +2 ± 1 mmHg after 3, 10 and 30 $\mu g\,kg^{-1}$, respectively; n = 35), 5-methoxytryptamine (−20 ± 1, −18 ± 2 and −15 ± 2 followed by +2 ± 1 mmHg after 3, 10 and 30 $\mu g\,kg^{-1}$, respectively; n = 30), α-methyl-5-HT (−20 ± 1, −14 ± 2, +10 ± 3 and +41 ± 8 mmHg after 3, 10, 30 and 100 $\mu g\,kg^{-1}$, respectively; n = 3), metoclopramide (+5 ± 2, +8 ± 1 and +3 ± 3 mmHg after 300, 1000 and 3000 $\mu g\,kg^{-1}$, respectively; n = 8), cisapride (+4 ± 4, +11 ± 2, +5 ± 3 and −8 ± 5 mmHg after 30, 100, 300 and 1000 $\mu g\,kg^{-1}$, respectively; n = 5), zacopride (+5 ± 2, +10 ± 2, +14 ± 3 and +12 ± 2 mmHg after 10, 30, 100 and 300 $\mu g\,kg^{-1}$, respectively; n = 6), dazopride (+1 ± 1, +2 ± 1 and +4 ± 2 mmHg after 300, 1000 and 3000 $\mu g\,kg^{-1}$, respectively; n = 4), and 1-phenyl-biguanide (0 ± 0, 0 ± 0, +4 ± 1 and +9 ± 2 after 30, 100, 300 and 1000 $\mu g\,kg^{-1}$, respectively; n = 3). These effects were not evaluated further.

As shown in Figure 1, intravenous bolus injections of the above-mentioned 5-HT agonist drugs caused increases in heart rate of diverse magnitude; the order of potency was 5-HT ≥ 5-methoxytryptamine > α-methyl-5-HT > zacopride > cisapride > metoclopramide = 1-phenyl-biguanide >

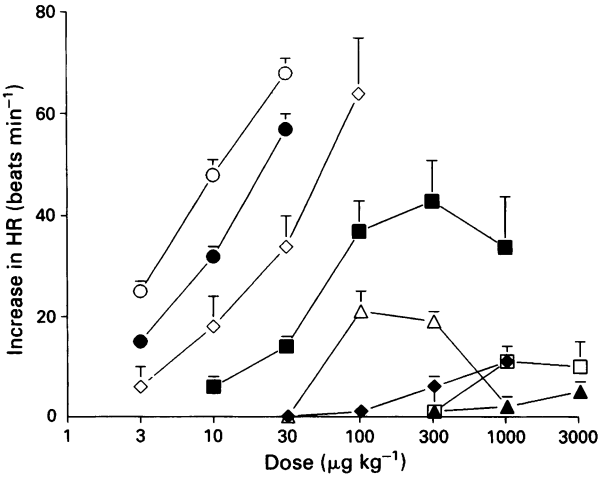

Figure 1 The tachycardic responses to 5-HT (○, n = 35), 5-methoxytryptamine (●, n = 30), α-methyl-5-HT (◇, n = 3), zacopride (■, n = 6), cisapride (△, n = 5), metoclopramide, (□, n = 8), 1-phenyl-biguanide (◆, n = 3) and dazopride (▲, n = 4) in the anaesthetized pig.

dazopride. At the doses used, the duration of action of cisapride (>60 min at 100 μg kg^{-1}) was longer than that of zacopride (17 ± 1, 23 ± 1, 43 ± 17 and 50 ± 8 min), metoclopramide (11 ± 1, 15 ± 2 and 21 ± 5 min), dazopride (1 ± 1, 5 ± 2 and >30 min), 1-phenyl-biguanide (0 ± 0, 0 ± 0, 5 ± 3 and 30 ± 3 min), 5-methoxytryptamine (5.9 ± 0.3, 9.6 ± 0.5 and 13.7 ± 0.6 min), 5-HT (2.2 ± 0.2, 4.5 ± 0.3 and 7.6 ± 0.7 min) or α-methyl-5-HT (0.4 ± 0.1, 0.9 ± 0.1, 4.3 ± 1.3 and 7.3 ± 1.3 min).

Modification of tachycardia in response to 5-HT and 5-methoxytryptamine induced by benzamide derivatives

In a previous publication, we have reported that the tachycardia induced by repeated administrations of 5-HT and 5-methoxytryptamine remained essentially unchanged in control animals receiving physiological saline (Villalón *et al.*, 1990). In marked contrast, the administration of several doses of either zacopride, cisapride, metoclopramide or dazopride antagonized the tachycardia induced by 5-HT (Figure 2) or 5-methoxytryptamine (Figure 3) in a dose-dependent manner; the order of potency for blockade of both 5-HT- and 5-methoxytryptamine-induced tachycardia was similar to that of their tachycardic response (see above): zacopride = cisapride ≫ metoclopramide > dazopride.

Tachycardia induced by benzamide derivatives after ICS 205-930

Because of the fact that the responses to the higher doses of the benzamide derivatives were usually less than the maximum response achieved (see Figure 1), the dose of each benzamide derivative eliciting the maximum increase in heart rate was administered to animals after treatment with 3 mg kg^{-1} of ICS 205-930. This dose of ICS 205-930 antagonizes the tachycardic responses to 5-HT, 5-methoxytryptamine and renzapride, but not that to isoprenaline (Villalón *et al.*, 1990). As shown in Figure 4, the increase in heart rate induced by either zacopride, cisapride, dazopride or metoclopramide was markedly antagonized by ICS 205-930.

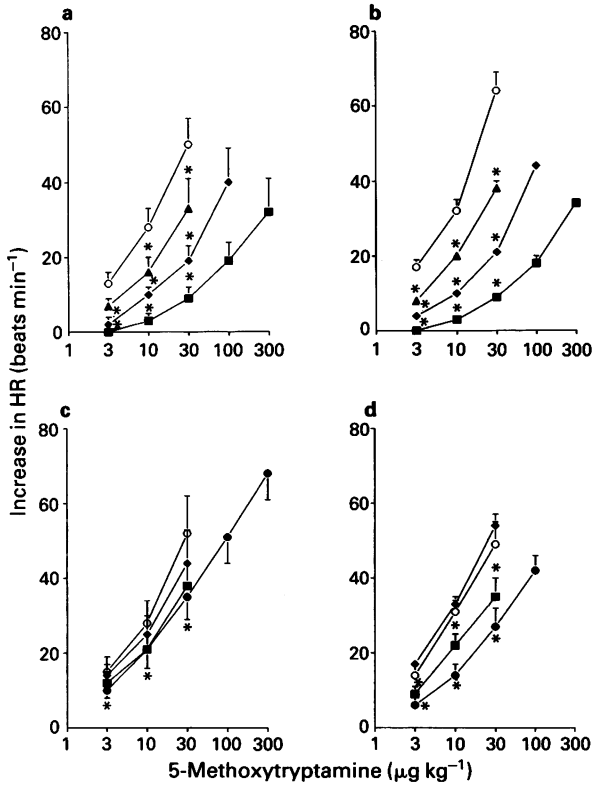

Figure 3 The effects of (a) zacopride (n = 4), (b) cisapride (n = 4), (c) dazopride (n = 4) and (d) metoclopramide (n = 5) on the tachycardic responses to 5-methoxytryptamine. The doses of the antagonists were: (○), 0 mg kg^{-1} (control); (▲) 0.1 mg kg^{-1}; (◆) 0.3 mg kg^{-1}; (■) 1.0 mg kg^{-1} and (●) 3.0 mg kg^{-1}. * Significantly different from the corresponding control response to 5-methoxytryptamine (P < 0.05).

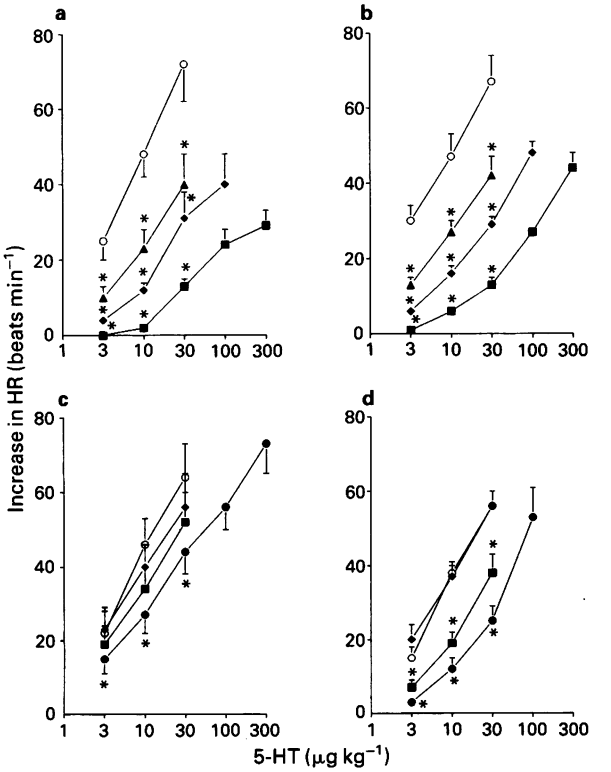

Figure 2 The effects of (a) zacopride (n = 5), (b) cisapride (n = 5), (c) dazopride (n = 4) and (d) metoclopramide (n = 8) on the tachycardic responses to 5-HT. The doses of the antagonists were: (○) 0 mg kg^{-1} (control); (▲) 0.1 mg kg^{-1}; (◆) 0.3 mg kg^{-1}, (■) 1.0 mg kg^{-1} and (●) 3.0 mg kg^{-1}. * Significantly different from the corresponding control response to 5-HT (P < 0.05).

Tachycardia induced by 5-HT or 5-methoxytryptamine after administration of some agonist and antagonist drugs

Inasmuch as all putative antagonists at this novel cardiac receptor also display high affinity for the 5-HT$_3$ receptors, we decided to investigate the effect of high doses of other selective 5-HT$_3$ receptor agonists and antagonists on the tachycardic responses induced by 5-HT or 5-methoxytryptamine; the

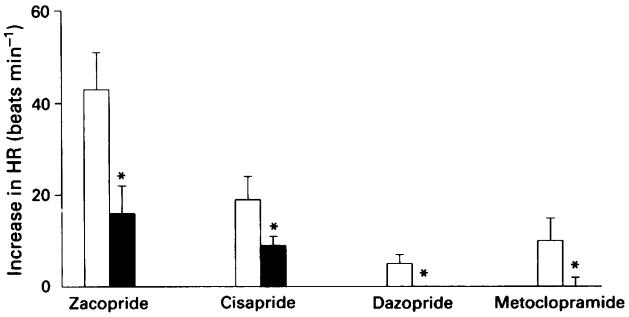

Figure 4 The tachycardic responses to zacopride (0.3 mg kg^{-1}), cisapride (0.3 mg kg^{-1}), metoclopramide (3 mg kg^{-1}) and dazopride (3 mg kg^{-1}) in untreated control pigs (open columns, n = 6, 5, 8 and 4, respectively) and pigs treated with ICS 205-930 (solid columns, n = 6, 6, 5 and 6, respectively). * Significantly different from the corresponding response in the untreated animals (P < 0.05).

Table 1 Effect of MDL 72222, granisetron, 1-phenyl-biguanide or domperidone on 5-HT- and 5-methoxytryptamine-induced increases in heart rate in the pig

			Increase in heart rate (beats min^{-1})					
	Dose		$3\,\mu g\,kg^{-1}$		$10\,\mu g\,kg^{-1}$		$30\,\mu g\,kg^{-1}$	
Antagonist	($mg\,kg^{-1}$)	n	Before	After	Before	After	Before	After
5-HT								
MDL 72222	3.0	6	26 ± 5	23 ± 6	49 ± 9	40 ± 8	69 ± 12	63 ± 11
Granisetron	3.0	3	32 ± 10	31 ± 10	58 ± 14	51 ± 13	77 ± 17	69 ± 16
1-Phenyl-biguanide	1.0	3	32 ± 10	33 ± 10	58 ± 14	56 ± 15	77 ± 17	74 ± 18
Domperidone	3.0	6	26 ± 5	29 ± 6	49 ± 9	50 ± 7	69 ± 12	69 ± 6
5-Methoxytryptamine								
MDL 72222	3.0	6	18 ± 4	18 ± 4	35 ± 6	33 ± 5	61 ± 10	57 ± 10
Granisetron	3.0	3	15 ± 2	14 ± 2	32 ± 7	28 ± 5	62 ± 8	54 ± 8
1-Phenyl-biguanide	1.0	3	15 ± 2	14 ± 3	32 ± 7	30 ± 7	62 ± 8	60 ± 8
Domperidone	3.0	6	18 ± 4	18 ± 5	35 ± 6	38 ± 5	61 ± 10	58 ± 7

All data are mean ± s.e.mean. None of the responses after antagonist drugs differed significantly from those before antagonist ($P > 0.05$).

selective dopamine$_2$ (D_2) receptor antagonist domperidone, as some benzamide derivatives (metoclopramide) also show affinity for D_2 receptors. As shown in Table 1, the responses to both 5-HT and 5-methoxytryptamine remained unchanged after administration of MDL 72222 ($3\,mg\,kg^{-1}$), granisetron ($3\,mg\,kg^{-1}$), 1-phenyl-biguanide ($1\,mg\,kg^{-1}$) or domperidone ($3\,mg\,kg^{-1}$).

Since α-methyl-5-HT (a 5-HT$_2$ and, to some extent, 5-HT$_1$-like receptor agonist) did induce quite consistent increases in heart rate (see Figure 1), we explored by pharmacological means the possible mechanisms involved in such an effect. In control animals where 5-HT and 5-methoxytryptamine were administered before and after the last set of injections of α-methyl-5-HT (3, 10, 30 and $100\,\mu g\,kg^{-1}$), the tachycardic responses induced by both 5-HT (3, 10 and $30\,\mu g\,kg^{-1}$) and 5-methoxytryptamine (3, 10 and $30\,\mu g\,kg^{-1}$) remained unchanged after α-methyl-5-HT [for 5-HT: 40 ± 9, 66 ± 11, and 88 ± 13 beats min^{-1} before and 36 ± 5, 62 ± 10 and 82 ± 12 beats min^{-1} after α-methyl-5-HT ($n = 3$), respectively; for 5-methoxytryptamine: 21 ± 7, 37 ± 8 and 67 ± 11 beats min^{-1} before and 17 ± 5, 32 ± 7 and 59 ± 11 beats min^{-1} after α-methyl-5-HT ($n = 3$), respectively]. Likewise, the increases in heart rate induced by 5-HT, 5-methoxytryptamine and α-methyl-5-HT were unaffected by ketanserin ($0.5\,mg\,kg^{-1}$), but were markedly antagonized by ICS 205-930 ($3\,mg\,kg^{-1}$) (Figure 5).

Lastly, it may be noted that the 5-HT$_3$ receptor agonist 1-phenyl-biguanide (30, 100, 300 and $1000\,\mu g\,kg^{-1}$) induced a small increase in porcine heart rate ($n = 3$); this effect was not blocked after administration of $3\,mg\,kg^{-1}$ of the selective 5-HT$_3$ receptor antagonist granisetron (0 ± 0, 1 ± 1, 6 ± 2 and 11 ± 1 beats min^{-1} before and 0 ± 0, 5 ± 3, 11 ± 3 and 16 ± 1 beats min^{-1} after granisetron, respectively). Notwithstanding, this effect appeared to be antagonized by $3\,mg\,kg^{-1}$ of ICS 205-930 (0 ± 0, 5 ± 3, 11 ± 3 and 16 ± 1 beats min^{-1} before and 0 ± 0, 3 ± 1, 4 ± 1 and 7 ± 1 beats min^{-1} after ICS 205-930, respectively).

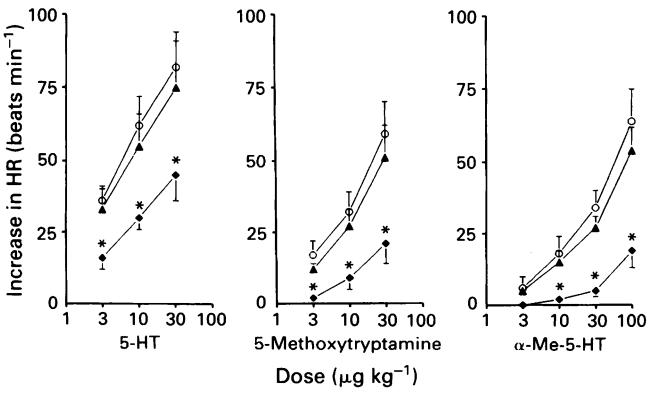

Figure 5 The tachycardic responses to 5-HT ($n = 3$), 5-methoxytryptamine ($n = 3$) and α-methyl-5-HT (α-Me-5-HT) ($n = 3$) before (control, ○) and after injections of ketanserin ($0.5\,mg\,kg^{-1}$, ▲), and ICS 205-930 ($3\,mg\,kg^{-1}$, ◆). * Significantly different from response in untreated animals ($P < 0.05$).

Discussion

We have shown that the 5-HT-induced tachycardia in the pig is neither mimicked by agonists at 5-HT$_1$-like (5-carboxamidotryptamine, 8-hydroxy-2-(di-n-propylamino)tetralin, RU 24969) and 5-HT$_3$ (2-methyl-5-HT) receptors, nor antagonized by drugs that act at various receptors: 5-HT$_1$ and/or 5-HT$_2$ (methiothepin, methysergide, ketanserin); 5-HT$_3$ (MDL 72222, ICS 205-930); adrenoceptors (phenoxybenzamine, propranolol); dopamine (haloperidol); histamine (mepyramine, cimetidine) (see Duncker *et al.*, 1985; Bom *et al.*, 1988). More recently, we found that the tachycardic effects of 5-HT in the pig, being mimicked by 5-methoxytryptamine and renzapride (Villalón *et al.*, 1990), but not by indorenate or sumatriptan (Villalón *et al.*, 1991), and blocked by high doses ($> 1\,mg\,kg^{-1}$) of ICS 205-930 (Villalón *et al.*, 1990), are mediated by a putative 5-HT$_4$ receptor which resembles the one mediating increases in adenosine 3':5'-cyclic monophosphate (cyclic AMP) in mouse embryo colliculi neurones and guinea-pig hippocampal membranes (Dumuis *et al.*, 1988; 1989; Clarke *et al.*, 1989). The present investigation extends these findings and clearly demonstrates that the porcine heart 5-HT receptor (i) can be stimulated by α-methyl-5-HT and some benzamide derivatives; (ii) does not resemble either 5-HT$_2$, 5-HT$_3$ or dopamine receptors; and (iii) resembles that present on the guinea-pig enteric neurones (Craig & Clarke, 1990) and human heart (Kaumann *et al.*, 1990).

Agonist action of α-methyl-5-HT and some benzamide derivatives on the porcine heart 5-HT receptor

Like 5-HT and 5-methoxytryptamine, it was observed that α-methyl-5-HT behaved as a potent agonist and elicited a dose-dependent tachycardia in the pig. The drug was also short-lasting in action and was devoid of any antagonist action against 5-HT or 5-methoxytryptamine. In contrast, the tachycardic action of the benzamide derivatives zacopride, cisapride, metoclopramide and dazopride, was less marked, but longer-lasting, and not strictly dose-dependent. In addition, each of these drugs antagonized the effects of 5-HT and 5-methoxytryptamine in a dose-dependent manner. It has to be emphasized that the tachycardic effects of 5-HT and 5-methoxytryptamine were not 'masked' by the increase in heart rate induced by the benzamide derivatives as the responses to 5-HT and 5-methoxytryptamine were elicited at the time when

the tachycardic effect of the benzamides had worn off (data not shown). Therefore, as previously found with renzapride (Villalón *et al.*, 1990), the benzamide derivatives employed here also behaved as partial agonists at the 5-HT$_4$ receptors in the porcine heart.

In our previous investigation, tachyphylaxis was observed with the tachycardic effect of renzapride (Villalón *et al.*, 1990). Though the present study was not designed for this purpose, we did observe tachyphylaxis in some preliminary experiments with the benzamide derivatives used here. Indeed, for this reason the antagonist effect of ICS 205-930 against the tachycardia induced by zacopride, cisapride, dazopride and metoclopramide was analyzed separately in control animals and in animals pretreated with ICS 205-930 (see Figure 4).

Lack of resemblance of the porcine heart 5-HT receptor with either 5-HT$_2$, 5-HT$_3$ or dopamine D$_2$ receptors

Both 5-methoxytryptamine and α-methyl-5-HT can interact with 5-HT$_2$ (and 5-HT$_1$-like) receptors (Richardson & Engel, 1986; Martin *et al.*, 1987; Hoyer, 1988). However, the tachycardic action of 5-methoxytryptamine and α-methyl-5-HT, as well as that of 5-HT (see also Bom *et al.*, 1988), was not modified after a dose of ketanserin (0.5 mg kg^{-1}) that is sufficient to block 5-HT$_2$ receptors (Van Nueten *et al.*, 1981; Saxena & Lawang, 1985). Therefore, the possibility that 5-HT$_2$ receptors might be involved in these effects is practically ruled out. Unlike ketanserin, 3 mg kg^{-1} of ICS 205-930 markedly antagonized the tachycardic responses to 5-HT, 5-methoxytryptamine and α-methyl-5-HT (see Figure 5), which again suggests the involvement of the 5-HT$_4$ receptor.

Admittedly, ICS 205-930 and the benzamide derivatives used here have the ability to block potently 5-HT$_3$ receptors (for references see Fozard, 1990). However, as previously discussed (Bom *et al.*, 1988; Villalón *et al.*, 1990), several results of this study clearly indicate that this novel 5-HT receptor in the pig heart does not belong to the 5-HT$_3$ receptor family. Firstly, besides the potency of 5-HT as a distinguishing factor, both α-methyl-5-HT and 5-methoxytryptamine are totally inactive at 5-HT$_3$ receptors (Richardson *et al.*, 1985; Richardson & Engel, 1986; Fozard, 1990). Secondly, the selective 5-HT$_3$ receptor agonists 2-methyl-5-HT and 1-phenyl-biguanide (Fozard, 1990) were practically inactive in the stimulation of 5-HT$_3$ receptors (Bom *et al.*, 1988; present results). Thirdly, high doses of other selective 5-HT$_3$ receptor antagonists (except ICS 205-930) such as granisetron (an indazole derivative; Sanger & Nelson, 1989; Fozard, 1990) or MDL 72222 (a dichlorobenzoate derivative; Fozard, 1984; 1990) were completely inactive (see Table 1). Lastly, it must be taken into consideration that the affinity of ICS 205-930 for the 5-HT$_4$ receptor involved in the stimulation of cyclic AMP production in mouse embryo colliculi neurones is much lower (pK$_i$ = 6–6.3; Dumuis *et al.*, 1989) than its affinity for 5-HT$_3$ receptors (pA$_2$ = 8–10; Richardson *et al.*, 1985; Richardson & Engel, 1986).

Most of the benzamide derivatives analyzed in the present study are currently used as prokinetic drugs (Schuurkes *et al.*, 1985; Alphin *et al.*, 1986; Cooper *et al.*, 1986; van Daele *et al.*, 1986; Sanger, 1987). Apart from metoclopramide, which also displays high affinity for central dopamine receptors (Cooper *et al.*, 1986), the other benzamide derivatives are devoid of important dopamine blocking activity. However, ICS 205-930, zacopride and other 5-HT$_3$ receptor antagonists are able to inhibit the release of dopamine by 5-HT and 2-methyl-5-HT in the central nervous system (Blandina *et al.*, 1988; Tricklebank, 1989). It is for these reasons, although haloperidol had been found ineffective (Bom *et al.*, 1988), that we decided to determine whether domperidone, a potent D$_2$ receptor antagonist (Kohli *et al.*, 1983) with gastrokinetic action, antagonizes the 5-HT-induced tachycardia or itself causes tachycardia in the pig. As shown in Table 1, domperidone (3 mg kg^{-1}) did not modify the tachycardic responses to either 5-HT or 5-methoxytryptamine. Therefore, the positive chronotropic effect induced by the tryptamine- and benzamide derivatives in the pig heart is unrelated to a possible action via dopaminergic pathways and/or receptors. Moreover, since domperidone failed to affect basal heart rate in the pig, the drug does not interact with the pig heart 5-HT$_4$ receptor.

Resemblance of the porcine heart 5-HT receptor to other putative 5-HT$_4$ receptors

At the 5-HT$_4$ receptor identified in the neurones from mouse embryo colliculi on the basis of increase in cyclic AMP, 5-methoxytryptamine, 5-carboxamidotryptamine (low affinity) and certain benzamide derivatives (renzapride, metoclopramide, cisapride), but not α-methyl-5-HT or 2-methyl-5-HT, are agonists; and ICS 205-930 (in high concentrations), but not MDL 72222, granisetron or ondansetron, acts as an antagonist (Dumuis *et al.*, 1988; 1989; Clarke *et al.*, 1989). The pharmacological characteristics of this receptor, though exhibiting several similarities, differ in some important respects. For example, 5-carboxamidotryptamine, apparently because of its low affinity, does not show activity in the pig heart (Duncker *et al.*, 1985; Bom *et al.*, 1988) in doses which are highly active in the cat heart (Saxena *et al.*, 1985; Connor *et al.*, 1986). Secondly, α-methyl-5-HT, which has little activity on the neurones from mouse embryo colliculi (Dumuis *et al.*, 1988; 1989), is highly active in our experiments. Thirdly, the agonist potency order reported by Dumuis *et al.* (1989) using mouse embryo colliculi (cisapride > renzapride > zacopride > 5-HT > metoclopramide), differs from that found in the pig heart (5-HT > 5-methoxytryptamine > α-methyl-5-HT > zacopride ≥ renzapride > cisapride > metoclopramide > dazopride; indorenate and sumatriptan, inactive at 1 and 3 mg kg^{-1}) (Villalón *et al.*, 1990; 1991; present results). Lastly, the benzamide derivatives cisapride and renzapride, which are full agonists at the mouse brain receptor, behaved as partial agonists at the pig heart receptor. Several possible explanations for these differences in agonist potencies may include: use of 'second messenger' (cyclic AMP) and functional (tachycardia) responses; tissue-dependent factors such as the number of receptors and coupling efficiency; and/or drug-dependent factors such as the affinity of 5-HT and related agonists for each of these novel receptors.

The 5-HT$_4$ receptor may also mediate the 5-HT-induced enhancement of cholinergic activity in the guinea-pig isolated ileum (Sanger, 1987; Craig & Clarke, 1990) and ascending colon (Elswood *et al.*, 1990), as well as relaxation of the rat oesophagus (Baxter & Clarke, 1990). As in the present experiments, the tryptamine derivatives 5-methoxytryptamine, α-methyl-5-HT and some benzamides mimic, and ICS 205-930 antagonizes 5-HT at the 5-HT$_4$ receptor in the guinea-pig gastrointestinal tract (Craig & Clarke, 1990; Elswood *et al.*, 1990) and the rat oesophagus (Baxter & Clarke, 1990). Moreover, the order of potency at the cholinergic neurones in the guinea-pig ileum (5-HT > 5-methoxytryptamine > renzapride > α-methyl-5-HT > zacopride = cisapride; Craig & Clarke, 1990) is practically identical to that found by us in the porcine heart.

The 5-HT$_4$ receptor is also apparently involved in the inotropic action of 5-HT, mediated via cyclic AMP increase in the human atria. The positive inotropic response to 5-HT is not modified by ketanserin, methysergide, lysergide, methiothepin, yohimbine (±)-propranol, (−)-pindolol or MDL 72222, but is blocked by a high concentration (2 μM) of ICS 205-930 (Kaumann *et al.*, 1990). The precise role of these receptors in cardiac function and cardiovascular pathologies remains to be determined.

In summary, the present investigation demonstrates that the tachycardic response to i.v. administered 5-HT in the anaesthetized pig can be mimicked by the tryptamine derivatives 5-methoxytryptamine and α-methyl-5-HT, and to a lesser extent by the partial agonist benzamide derivatives (in order of potency) zacopride, cisapride, metoclopramide and dazop-

ride. High doses of ICS 205-930, but not ketanserin, granisetron or MDL 72222, acted as an antagonist. These results further confirm the involvement of a putative 5-HT_4 receptor in the positive chronotropic action of 5-HT in the anaesthetized pig.

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