Br. J. Pharmacol. (1988), **93**, 663–671

5-Hydroxytryptamine-induced tachycardia in the pig: possible involvement of a new type of 5-hydroxytryptamine receptor

A.H. Bom, * D.J. Duncker, [1] P.R. Saxena & * P.D. Verdouw

Department of Pharmacology and * Laboratory for Experimental Cardiology (Thorax Centre), Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

1 The mechanism of 5-hydroxytryptamine (5-HT)-induced tachycardia is 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) receptors, or by an action similar to tyramine (guinea-pig). The present investigation is devoted to the analysis of the positive chronotropic effect of 5-HT in the pentobarbitone-anaesthetized pig.

2 Intravenous bolus injections of 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$) in pigs resulted in dose-dependent increases in heart rate of 24 ± 2, 38 ± 3 and 51 ± 3 beats min^{-1}, respectively ($n = 39$). Topical application of a high concentration of 5-HT (150 $\mu g\,kg^{-1}$ in 5 ml) on the right atrium was also followed by tachycardia (38 ± 6 beats min^{-1}, $n = 4$).

3 A number of drugs which antagonize responses mediated by different 5-HT receptors – phenoxybenzamine, methiothepin, metergoline, methysergide and mesulergine ('5-HT_1-like' and 5-HT_2 receptors), ketanserin, cyproheptadine, pizotifen and mianserin (5-HT_2 receptors), and MDL 72222 and ICS 205-930 (5-HT_3 receptors) – did not attenuate the chronotropic responses to 5-HT.

4 The 5-HT-induced tachycardia was also not affected by antagonists at α- and β-adrenoceptors, muscarinic, nicotinic, histamine and dopamine receptors, and calcium channels.

5 Selective inhibitors of 5-HT-uptake, indalpine and fluvoxamine, themselves increased porcine heart rate and facilitated 5-HT-induced tachycardia both in magnitude and in duration.

6 A number of putative selective agonists at '5-HT_1-like' receptors or their possible subtypes (5-carboxamidotryptamine (5-CT), 8-hydroxy-2-(di-N,N-n-propylamino) tetralin (8-OH-DPAT), BEA 1654 and RU 24969), or at 5-HT_3 receptors (2-methyl-5-HT), elicited no or only a weak tachycardiac response in the pig. RU 24969, but not 8-OH-DPAT, seemed to potentiate the responses to 5-HT, whereas 5-CT slightly inhibited these responses.

7 It was concluded that the tachycardia induced by 5-HT in the pig does not involve the receptors for some common neurotransmitter substances but may be mediated by a new 5-HT receptor type that is clearly different from '5-HT_1-like', 5-HT_2 or 5-HT_3 receptors.

Introduction

Intravenous bolus injections of 5-hydroxytryptamine (5-HT) result in a transient decrease in heart rate (Page, 1958) due to a chemoreceptor reflex (von Bezold-Jarisch reflex) initiated by the stimulation of receptors on sensory vagal afferent fibres (Paintal, 1973). In the rat (Fozard, 1984; Richardson *et al.*, 1985) and the cat (Saxena *et al.*, 1985a) the bradycardiac effect has been shown to be mediated by 5-HT_3 receptors (for nomenclature of 5-HT receptors, see Bradley *et al.*, 1986; Saxena *et al.*, 1986b).

5-HT can also increase heart rate in a variety of non-mammalian and mammalian species, including man (Page, 1958; Hollander *et al.*, 1957; LeMessurier *et al.*, 1959). The tachycardiac action of 5-HT has been analysed in some species and it involves several different mechanisms (Trendelenburg, 1960; Saxena, 1986). In the cat 5-HT-induced tachycardia is mediated by myocardial '5-HT_1-like' receptors (Saxena *et al.*, 1985a; Connor *et al.*, 1986), and in the rat (Saxena & Lawang, 1985; Göthert *et al.*, 1986) and the dog (Feniuk *et al.*, 1981) 5-HT_2 receptors, present on the myocardium and adrenal medulla,

[1] Author for correspondence.

respectively, seem to be involved. The third 5-HT receptor type characterized so far (5-HT_3 receptor), located on the postganglionic cardiac sympathetic fibres, mediates the effects of 5-HT in the rabbit (Fozard, 1984) while, in the guinea-pig, tachycardia has been attributed to β-adrenoceptors (Eglen *et al.*, 1985), probably activated by catecholamines released via a tyramine-like action (De Boer *et al.*, 1986; Dhasmana *et al.*, 1988). High concentrations of 5-HT can also cause tachycardia by such a mechanism in the rat (Göthert *et al.*, 1986) and in the hamster (González Alvarez & García Rodríguez, 1977).

In the present experiments the increase in heart rate caused by 5-HT in the pig has been investigated and the results reveal the involvement of a mechanism which is different from those proposed so far (see above). Preliminary results of this investigation were communicated to the British Pharmacology Society (Duncker *et al.*, 1985).

Methods

General

After an overnight fast, 42 young Yorkshire pigs (25–30 kg, 12–16 weeks old) were initially sedated with 120 mg (i.m.) azaperone and 120–150 mg (i.v.) metomidate. After the animals had been intubated, they were connected to a respirator for intermittent positive pressure ventilation with a mixture (1 : 2) of oxygen and nitrous oxide. The anaesthesia was maintained with a continuous infusion of pentobarbitone sodium (15–20 mg kg^{-1} h^{-1}, i.v.). Aortic blood pressure was recorded with a Statham pressure transducer via a cannula inserted into the left femoral artery. All drugs were injected into the left femoral or jugular vein cannulated for this purpose. The animal's temperature was maintained around 37°C by using an electric blanket and arterial blood gasses 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 30 min, bolus injections of 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$, i.v.) were given at intervals of 10–15 min. Subsequently, one of the several antagonist drugs (for names, doses and number of experiments, see the Drug and Results sections), used to analyse the positive chronotropic effects of 5-HT, was slowly administered over two minutes. About 10 min later, the responses to the three doses of 5-HT were elicited again. In initial experiments it was noticed that several drugs, in doses sufficient for the purpose for which they were employed, did not modify the effects of 5-HT. Therefore, in order to restrict the number of animals to be used for this investigation, it was decided to use more than one such drug, rather than several doses of a particular drug, in any single experiment. The order of their use was varied and a total of 30 animals was used for the above purpose. Furthermore, in 3 animals the reproducibility of the 5-HT-induced tachycardia was checked after a period of 2 h.

In 13 animals (12 new and 1 from the above group) the effect of some drugs thought to be selective agonists at different 5-HT receptors was studied. Lastly, after the above experiments had been completed, on 4 occasions 5-HT was administered locally on the surface of the right atrium.

Data presentation and analysis

All data in the text and tables, unless otherwise stated, are presented as mean ± s.e. mean. The peak change in heart rate by the different doses of 5-HT (and other putative 5-HT receptor agonists) were recorded. The mean ± s.e. mean increases in heart rate by the three doses of 5-HT just before and after a particular antagonist drug were compared by use of Duncan's new multiple range test, once an analysis of variance (randomized block design) revealed that the samples represented different populations (Saxena, 1985). A P value of 0.05 or less (two-tailed) was considered statistically significant.

Drugs

The following drugs used in this study were kindly supplied by the sources mentioned: atropine sulphate (Pharmacy Department, Erasmus University Rotterdam, Rotterdam, The Netherlands), 5-carboxamidotryptamine maleate (5-CT; Dr W. Feniuk, Glaxo, Ware, U.K.), cimetidine hydrochloride (Smith Kline & French, Welwyn Garden City, U.K.), cyproheptadine hydrochloride (Merck, Sharpe and Dohme, Haarlem, The Netherlands), fluvoxamine maleate (Dr W. Wouters, Duphar, Weesp, The Netherlands), haloperidol base (Janssen Pharmaceutica, Beerse, Belgium), histamine phosphate (Pharmacy Department, Erasmus University Rotterdam, Rotterdam, The Netherlands), 8-hydroxy-2-(di-N,N-n-propylamino)tetralin (8-OH-DPAT; Dr J.R. Fozard, Merrell Dow Research Institute, Strasbourg, France), indalpine base (Dr A. Uzan, Pharmuka Laboratories, Gennevilliers, France), isoprenaline sulphate (Pharmacy Department, Erasmus University Rotterdam, Rotterdam, The Netherlands), ketanserin tartrate (Dr J.M. Van

Neuten, Janssen Pharmaceutica, Beerse, Belgium), mepyramine maleate (Rhone-Poulenc, Paris, France), noradrenaline bitartrate (Pharmacy Department, Erasmus University Rotterdam, Rotterdam, The Netherlands), mesulergine hydrochloride (Dr G. Engel, Sandoz, Basle, Switzerland), metergoline base (Farmatalia Carlo Erba, Torino, Italy), methiothepin methane-sulphonate (Hoffman La Roche, Mijdrecht, The Netherlands), 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl-1H-indole succinate (RU 24969; Roussel Laboratories, Hoevelaken, The Netherlands), 2-methyl-5-hydroxytryptamine maleate (2-methyl-5-HT; Dr W. Feniuk, Glaxo, Ware, U.K.), methysergide hydrogen maleate (Sandoz, Basle, Switzerland), mianserin hydrochloride (Organon, Oss, The Netherlands), pizotifen maleate (Sandoz, Basle, Switzerland), phentolamine hydrochloride (Ciba-Geigy, Basle, Switzerland), phenoxybenzamine hydrochloride (dibenzyline; Smith Kline & French, Philadelphia, U.S.A.), propranolol hydrochloride (Imperial Chemical Industries, Rotterdam, The Netherlands), 1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate (MDL 72222; Dr J.R. Fozard, Merrell Dow Research Institute, Strasbourg, France), (3α-tropanyl)-1H-indole-3-carboxylic acid ester (ICS 205-930; Dr G. Engel, Ludwigshafen, F.R.G.). Other drugs were purchased: 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP; Sigma, St. Louis, U.S.A.), 5-hydroxytryptamine creatinine sulphate (5-HT; Merck, Darmstadt, F.R.G.) and hexamethonium bromide (Fluka, Buchs, Switzerland). The doses mentioned in the text refer to the salts of substances except in the case of 5-HT and 5-CT, where they refer to the base. All drugs were dissolved in saline before injection; fresh solutions were prepared for each experiment.

Results

Effects of 5-HT

Intravenous bolus injections of 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$) were given in a total of 39 pigs where the baseline values of heart rate and mean arterial blood pressure were, respectively, 90 ± 3 beats min^{-1} and 78 ± 2 mmHg. None of the three doses of 5-HT produced consistent changes in arterial pressure and, therefore, they were not quantified further. Bradycardia due to the Bezold-Jarisch reflex, as is usual in several species, was also absent, probably due to the level of anaesthesia. These doses of 5-HT, however, induced dose-dependent increases in heart rate of 24 ± 2, 38 ± 2 and 51 ± 3 beats min^{-1}, respectively ($n = 39$). In 3 animals the reproducibility of the tachycardiac effects of 5-HT was tested; the increase in heart rate by the three doses of 5-HT were, respectively, 29 ± 2, 45 ± 2 and 65 ± 4 beats min^{-1} and after an interval of 2 h: 27 ± 3, 38 ± 2 and 59 ± 2 beats min^{-1}.

Topical application of a high concentration of 5-HT (150 $\mu g\,kg^{-1}$ in 5 ml) on the right atrium was followed by an increase in heart rate of 38 ± 6 beats min^{-1} from a basal value of 91 ± 5 beats min^{-1} in 4 pigs.

Tachycardiac effects of 5-HT after some antagonist drugs

To study the involvement of the three types of 5-HT receptors the effects of non-selective ('5-HT_1-like' and 5-HT_2: phenoxybenzamine, methiothepin, metergoline, methysergide and mesulergine) and selective (5-HT_2: ketanserin, cyproheptadine, pizotifen and mianserin; 5-HT_3: MDL 72222 and ICS 205-930) 5-HT receptor antagonists were studied. None of these drugs (except MDL 72222 against the lowest dose of 5-HT) reduced the magnitude of tachycardia induced by 5-HT. One such experiment involving MDL 72222, cyproheptadine and methysergide is shown in Figure 1, while the summary of all the data is presented in Table 1. It may be noted that, instead of an antagonism, phenoxybenzamine (highest dose of 5-HT) and cyproheptadine seemed to facilitate the chronotropic effects of 5-HT.

Since none of the above 5-HT antagonists was capable of reducing the 5-HT-induced tachycardia, a number of other commonly used antagonist drugs were investigated. Table 2 shows that the antagonists at adrenoceptors, cholinoceptors, histamine and dopamine receptors and at voltage-dependent calcium channels did not modify the responses to the three doses (3, 10 and 30 $\mu g\,kg^{-1}$) of 5-HT. The effectiveness of phentolamine, propanolol, hexamethonium and mepyramine was confirmed by blockade of, respectively, the noradrenaline (1 $\mu g\,kg^{-1}$)-induced pressor effect, and isoprenaline (0.1 $\mu g\,kg^{-1}$)-, DMPP (20 $\mu g\,kg^{-1}$)- and histamine (3 $\mu g\,kg^{-1}$)-induced tachycardia; the responses before and after the antagonists involved were respectively: noradrenaline, 36 ± 7 and 18 ± 4 mmHg ($n = 4$); isoprenaline: 48 ± 2 and 1 ± 1 beats min^{-1} ($n = 3$); DMPP, 54 ± 17 and 3 ± 2 beats min^{-1} ($n = 4$) and histamine, 15 ± 7 and 1 ± 1 beats min^{-1} ($n = 4$). The hypotensive response to histamine was also antagonized by mepyramine (-18 ± 2 mmHg before and -2 ± 0 mmHg after mepyramine). Cimetidine did not antagonize the tachycardia caused by histamine indicating that, as in the cat (Owen, 1977), the histamine-induced tachycardia in the pig is mediated by histamine H_1 receptors, probably on the adrenal medulla. The hypertensive response to noradrenaline remaining after phentolamine was apparently due to an

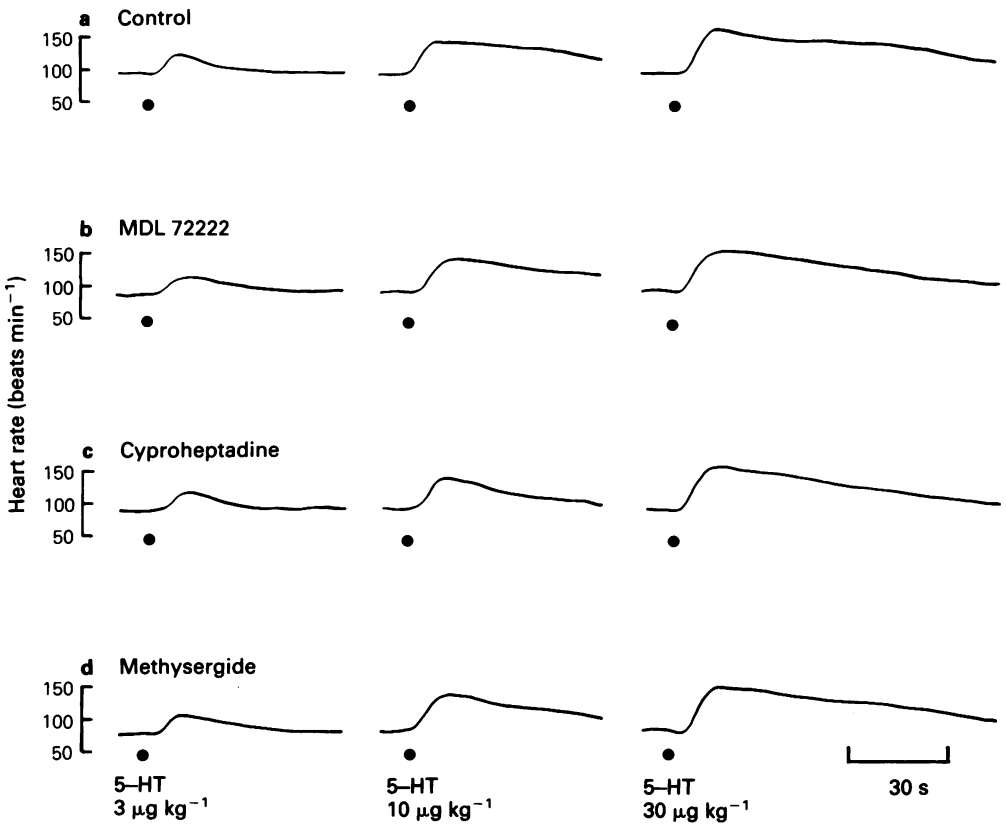

Figure 1 The effects of 5-hydroxytryptamine (5-HT; 3, 10 and 30 $\mu g\,kg^{-1}$) on heart rate in an anaesthetized pig before (a) and after successive administration of MDL 72222 (0.3 $mg\,kg^{-1}$) (b), cyproheptadine (0.5 $mg\,kg^{-1}$) (c) and methysergide (0.5 $mg\,kg^{-1}$) (d). Note that 5-HT caused a dose-dependent tachycardia which was not antagonized by these drugs.

Table 1 Effects of antagonists at different 5-hydroxytryptamine (5-HT) receptors on the 5-HT-induced increases in heart rate (beats min^{-1}) in the pig

			5-HT 3 $\mu g\,kg^{-1}$		*5-HT* 10 $\mu g\,kg^{-1}$		*5-HT* 30 $\mu g\,kg^{-1}$	
Antagonist	*Dose* ($mg\,kg^{-1}$)	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
Phenoxybenzamine	1.0	3	38 ± 13	44 ± 10	46 ± 10	55 ± 12	44 ± 11	60 ± 10*
Methiothepin	0.5	6	22 ± 3	21 ± 4	40 ± 5	39 ± 4	53 ± 5	53 ± 3
Metergoline	0.5	5	29 ± 2	22 ± 3	49 ± 3	46 ± 6	63 ± 4	63 ± 6
Methysergide	0.5	6	26 ± 3	28 ± 3	50 ± 4	49 ± 3	63 ± 5	63 ± 3
Mesulergine	0.3	3	25 ± 1	26 ± 1	40 ± 2	45 ± 2	52 ± 4	56 ± 4
Ketanserin	0.5	4	20 ± 4	18 ± 4	42 ± 8	41 ± 7	58 ± 7	57 ± 7
Cyproheptadine	0.5	5	17 ± 3	23 ± 3*	40 ± 3	45 ± 4*	54 ± 3	60 ± 4*
Pizotifen	0.5	5	29 ± 2	26 ± 2	49 ± 3	45 ± 2	61 ± 5	56 ± 4
Mianserin	0.5	5	24 ± 2	23 ± 2	45 ± 1	44 ± 3	59 ± 3	54 ± 4
MDL 72222	0.3	4	28 ± 4	22 ± 5*	45 ± 4	45 ± 5	60 ± 5	58 ± 6
ICS 205-930	0.3	3	29 ± 2	21 ± 3	43 ± 4	41 ± 2	54 ± 6	56 ± 1

* $P < 0.05$, after vs before.

Table 2 Effects of some common antagonists on the 5-hydroxytryptamine (5-HT)-induced increases in heart rate (beats min^{-1}) in the pig

			5-HT $3\,\mu g\,kg^{-1}$		*5-HT* $10\,\mu g\,kg^{-1}$		*5-HT* $30\,\mu g\,kg^{-1}$	
Antagonist	*Dose* ($mg\,kg^{-1}$)	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
Phentolamine	1.0	5	26 ± 4	24 ± 6	42 ± 4	38 ± 6	58 ± 5	51 ± 12
Propranolol	0.5	5	24 ± 4	27 ± 4	43 ± 3	43 ± 4	60 ± 4	60 ± 3
Atropine	0.5							
+ propranolol	0.5	3	37 ± 4	35 ± 4	50 ± 5	51 ± 1	55 ± 6	58 ± 2
Atropine	1.0							
+ hexamethonium	10.0	4	9 ± 2	10 ± 2	24 ± 11	37 ± 7	32 ± 7	53 ± 8
Cimetidine	1.0	4	38 ± 5	33 ± 3	48 ± 5	47 ± 4	58 ± 5	56 ± 6
Mepyramine	1.0	4	31 ± 5	35 ± 3	45 ± 7	47 ± 5	55 ± 7	53 ± 5
Haloperidol	1.0	3	31 ± 4	35 ± 2	45 ± 5	46 ± 3	51 ± 6	55 ± 4
Verapamil	0.1[a]	3	23 ± 4	23 ± 2	44 ± 2	34 ± 7	58 ± 2	45 ± 5

[a] This dose was followed by an infusion of $0.01\,mg\,kg^{-1}\,min^{-1}$.

increase in cardiac output, since it was eliminated after additional administration of propranolol.

Lastly, indalpine and fluvoxamine, which are known to interfere with the uptake of 5-HT by nerve terminals and blood platelets (Claasen *et al.*, 1977; Le Fur & Uzan, 1977; Ashkenazi *et al.*, 1983), were employed in an attempt to antagonize the tachycardiac responses to 5-HT. However, after administration of these drugs the positive chronotropic responses to bolus injections of 5-HT were not reduced, but, on the contrary, these responses were facilitated both in magnitude and in duration (see Figure 2 for indalpine and Table 3 for both indalpine and fluvoxamine). Furthermore, the two 5-HT-

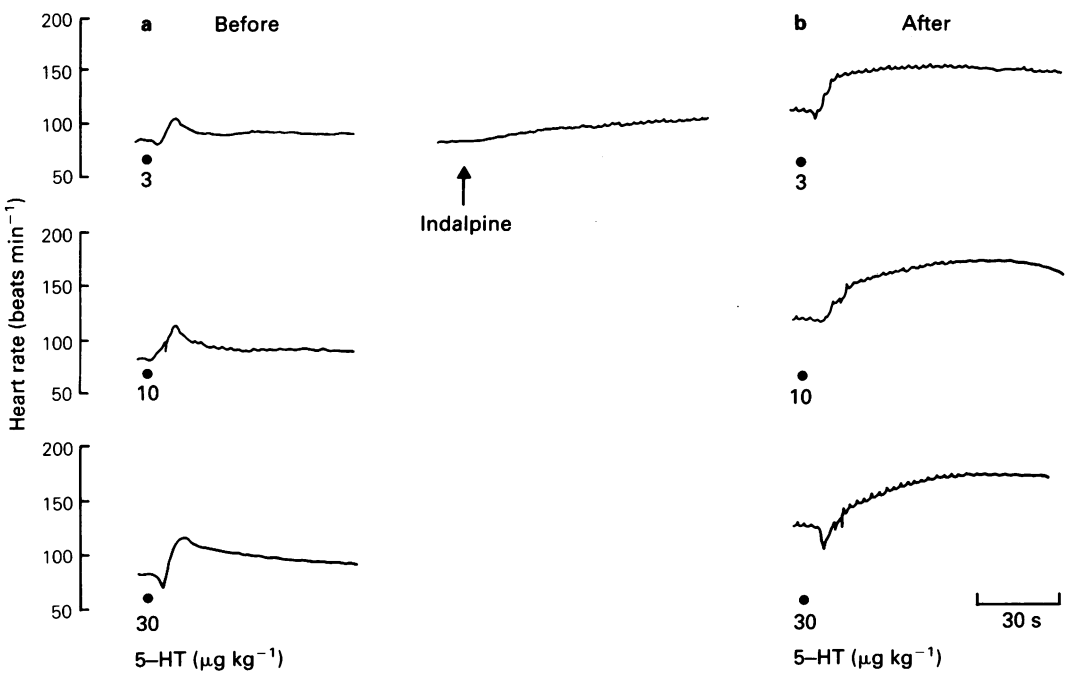

Figure 2 The effects of 5-hydroxytryptamine (5-HT; 3, 10 and $30\,\mu g\,kg^{-1}$) on heart rate in an anaesthetized pig before (a) and after (b) administration of indalpine ($1\,mg\,kg^{-1}$). It can be seen that heart rate increased after indalpine and that this drug potentiated the tachycardiac responses to 5-HT.

Table 3 Effects of 5-hydroxytryptamine (5-HT)-uptake blockers on the 5-HT-induced increases in heart rate (beats min^{-1}) in the pig

			5-HT 3 $\mu g kg^{-1}$		5-HT 10 $\mu g kg^{-1}$		5-HT 30 $\mu g kg^{-1}$	
Antagonist	*Dose* ($mg kg^{-1}$)	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
Indalpine	1.0	3	20 ± 3	48 ± 3*	30 ± 1	58 ± 3*	45 ± 3	54 ± 7*
Fluvoxamine	1.0	4	11 ± 2	26 ± 2*	21 ± 2	40 ± 8*	30 ± 3	48 ± 9*

* $P < 0.05$, after vs before.

Table 4 Effects of putative agonists of 5-hydroxytryptamine (5-HT) receptors on the 5-HT-induced increases in heart rate (beats min^{-1}) in the pig

			5-HT 3 $\mu g kg^{-1}$		5-HT 10 $\mu g kg^{-1}$		5-HT 30 $\mu g kg^{-1}$	
Agonist	*Dose* ($mg kg^{-1}$)	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
RU-24969	1.0	3	14 ± 3	39 ± 5*	13 ± 3	41 ± 5*	24 ± 4	47 ± 8*
8-OH-DPAT	1.0	3	33 ± 13	33 ± 10	44 ± 4	47 ± 4	57 ± 7	61 ± 12
5-CT	0.1	3	27 ± 3	18 ± 2*	38 ± 2	28 ± 2*	59 ± 2	43 ± 1*

* $P < 0.05$, after vs before. 8-OH-DPAT = 8-hydroxy-2-(di-N,N-n-propylamino)tetralin and 5-CT = 5-carboxamidotryptamine.

uptake blockers caused a slowly developing increase in heart rate; the peak tachycardiac effects following indalpine and fluvoxamine were 49 ± 10 ($n = 3$) and 10 ± 5 ($n = 4$) beats min^{-1}, respectively.

Effects of some putative agonists of 5-HT

The effects of intravenous injections of 5-CT, 8-OH-DPAT, BEA 1654, RU 24969 and 2-methyl-5-HT on the heart rate were also studied. Compared to 5-HT, none of these drugs had any profound effect on porcine heart rate (Figure 3). In experiments with RU 24969, 8-OH-DPAT and 5-CT the effect of the highest dose of each compound was also studied on the heart rate responses to 5-HT. The tachycardia caused by 3, 10 and 30 $\mu g kg^{-1}$ of 5-HT was potentiated after administration of RU 24696 (1 $mg kg^{-1}$), remained unchanged after 8-OH-DPAT (1 $mg kg^{-1}$) and was slightly inhibited by 5-CT (0.1 $mg kg^{-1}$) (see Table 4).

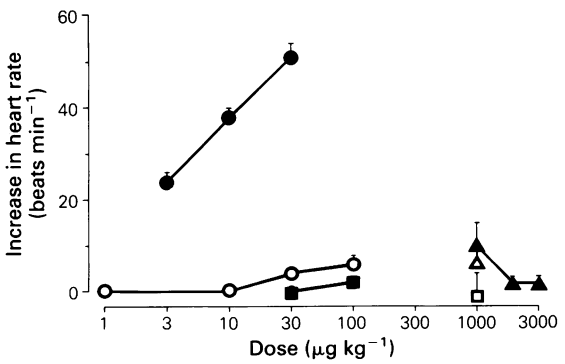

Figure 3. Heart rate responses to a number of agonists at 5-hydroxytryptamine (5-HT) receptors: 5-carboxamidotryptamine (○, $n = 4$), 2-methyl-5-HT (■, $n = 3$), 8-hydroxy-2-(di-N,N-n-propylamino)tetralin (△, $n = 3$), RU-24969 (□, $n = 4$) and BEA 1654 (▲, $n = 3$). Compared to 5-HT (●, $n = 39$), these agents elicited either no or only a slight increase in heart rate. The values of heart rates before administration of different doses of agonists varied between 74 ± 5 and 96 ± 13 beats min^{-1} and did not differ significantly.

Discussion

Consideration of known 5-HT receptors

Until recently 5-HT receptors had been classified on the basis of antagonism by dibenzyline (phenoxybenzamine) and morphine as 'D' and 'M' receptors, respectively (Gaddum & Picarelli, 1957), or on the basis of high binding affinity for [^{3}H]-5-HT and [^{3}H]-spiroperidol as 5-HT_1 and 5-HT_2, respectively (Peroutka & Snyder, 1979). However, neither of these classifications, though correct within themselves, adequately covered the pharmacological actions of 5-HT. Being aware of this drawback and prompted by the recent availability of more selective drug tools, Bradley *et al.* (1986) subdivided 5-HT receptors into three distinct groups named as '5-HT_1-like', 5-HT_2 and 5-HT_3. They advocated 5-CT ('5-HT_1-like') and 2-methyl-5-HT (5-HT_3) as selective agonists, and methiothepin and methysergide ('5-HT_1-like'), ketanserin and cyproheptadine (5-HT_2), and MDL 72222 and ICS 205-930 (5-HT_3)

as antagonists. It is to be noted that while the antagonists for 5-HT_2 and 5-HT_3 are selective, the drugs antagonizing '5-HT_1-like' receptors, being even more effective against the responses mediated via 5-HT_2 receptors, are not. The above agonist-antagonist criteria are clearly fulfilled with respect to 5-HT-induced tachycardia in the cat (5-CT in doses of 0.1 to 1 $\mu g\,kg^{-1}$ mimics, and methiothepin and methysergide, but not ketanserin and cyproheptadine, antagonize) (Saxena *et al.*, 1985a; Connor *et al.*, 1986), the rat (5-CT does not mimic and, ketanserin and cyproheptadine antagonize) (Saxena & Lawang, 1985) and the rabbit (2-methyl-5-HT mimics, and MDL 72222 and ICS 205-930 antagonize) (Fozard, 1984; Richardson *et al.*, 1985). However, in the present investigation in the anaesthetized pig, it was noticed that the above drugs neither mimicked nor antagonized the tachycardiac responses to 5-HT. Therefore, it can be concluded that the positive chronotropic effects of this amine in the pig are not mediated by either the '5-HT_1-like', 5-HT_2 or 5-HT_3 receptors.

Bradley *et al.* (1986) have pointed out that the receptor category denoted as '5-HT_1-like', being devoid of a selective antagonist, is not yet fully characterized but that it appears to be heterogeneous. However, neither the relationship of '5-HT_1-like' receptors with the 5-HT_1 binding sub-sites is clear nor the selectivity of drugs for the subclasses is as yet entirely satisfactory. Richardson & Engel (1986) have nevertheless attempted to subclassify '5-HT_1-like' receptors into three subclasses, namely 5-HT_{1A} (agonists; 8-OH-DPAT and 5-CT; antagonists: spiroperidol and methiothepin), 5-HT_{1B} (agonist: 5-CT; antagonists: 21-009 and methiothepin) and 5-HT_{1C} (agonist: (+)-S-α-methyl-5-HT; antagonists; mesulergine and methiothepin). Although not all of these drugs could be used in this investigation, the ineffectiveness of 8-OH-DPAT, 5-CT, mesulergine and methiothepin does not allow the effect of 5-HT on the porcine heart to be classified into any of the above putative, though not yet convincingly characterized, subclasses. The above conclusion is further supported by the finding that some more drugs which have affinities for 5-HT binding sites – phenoxybenzamine and metergoline (5-HT_1 and 5-HT_2; Peroutka & Snyder, 1979; Leysen *et al.*, 1981), pizotifen (5-HT_2; Leysen *et al.*, 1981; Hoyer *et al.*, 1985), BEA 1654 (5-HT_{1A}; Verdouw *et al.*, 1985; Middlemiss, unpublished), RU 24969 (5-HT_{1A} and 5-HT_{1B}; Hoyer *et al.*, 1985) and mianserin (5-HT_2 and 5-HT_{1C}; Hoyer *et al.*, 1985) – were all ineffective in our experiments.

Lastly, it has to be remarked that the doses of the compounds used were equal to or greater than those found effective in our earlier experiments concerning analyses of functional 5-HT receptors (see Saxena & Verdouw, 1982; 1984; 1985; Saxena *et al.*, 1985a,b; 1986a; Saxena & Lawang, 1985; Verdouw *et al.*, 1984; 1985). These doses also appear to be sufficient on the basis of their estimated affinity constants (see Hoyer *et al.*, 1985; Richardson & Engel, 1986).

Consideration of non-5-HT mechanisms

Since the chronotropic response to 5-HT could not be classified into any of the above categories, some common non-5-HT mechanisms were considered. The involvement of baroreceptors was ruled out by the fact that the changes in blood pressure were inconsistent and that instillation of 5-HT, though admittedly in a high concentration, on the right atrium also raised heart rate. Moreover, tachycardiac responses of similar magnitude were obtained in animals after complete ganglion (hexamethonium + atropine) or sympathetic and parasympathetic (propranolol + atropine) blockade. The use of atropine, hexamethonium, phentolamine and propranolol also precluded the involvement of the muscarinic and nicotinic receptors, α- and β-adrenoceptors and the release of catecholamines (by a tyramine-like action) in the mechanism of tachycardia produced by 5-HT in the pig.

Similarly, the increase in heart rate by 5-HT in the pig was not mediated by histamine H_1 and H_2 or dopamine receptors because the respective antagonists – mepyramine, cimetidine and haloperidol – were also incapable of modifying the responses to 5-HT.

Does a new type of 5-HT receptor mediate 5-HT-induced tachycardia in the pig?

The inability to find a specific mechanism responsible for the positive chronotropic action can suggest that 5-HT may be taken up into some neurone to displace a neurotransmitter agent (other than those mentioned above: noradrenaline, acetylcholine, histamine and dopamine). If an unidentified neurotransmitter is indeed displaced and released by 5-HT, this would involve an uptake process that is distinct from the one selectively inhibited (in the brain and blood platelets) by indalpine (Le Fur & Uzan, 1977; Ashkenazi *et al.*, 1983) and fluvoxamine (Claasen *et al.*, 1977) since these drugs increased (not reduced) the magnitude and duration of 5-HT-induced tachycardia. Though such a possibility cannot be entirely dismissed, it is perhaps more likely that the tachycardia elicited by 5-HT is mediated by a receptor type which is different from those characterized so far, i.e. '5-HT_1-like', 5-HT_2 or 5-HT_3 receptors (Bradley *et al.*, 1986; Saxena *et al.*, 1986b). This 5-HT receptor, apparently not much

dependent on extracellular calcium as indicated by the ineffectiveness of verapamil, may resemble the one that mediates either the positive inotropic effect of 5-HT in the kitten isolated papillary muscle (Kaumann, 1985; 1986) or the slow depolarization of myenteric type II/AH neurones in the guinea-pig small intestine (Mawe *et al.*, 1986). In the kitten isolated papillary muscle the responses to 5-HT, unlike that in the kitten isolated atria (Kaumann, 1985; 1986) or the cat heart *in vivo* (Saxena *et al.*, 1985a; Connor *et al.*, 1986), are neither mimicked by 5-CT nor antagonized by methysergide or phenoxybenzamine. Similarly, the above 5-HT receptor in the gut is insensitive to ICS 205-930 or lysergide, but is antagonized by dipeptides of 5-hydroxytryptophan and excited by hydroxylated indalpines (Mawe *et al.*, 1986). It is interesting to note that indalpine, which may be hydroxylated *in vivo* to stimulate the type of receptor being described by Mawe *et al.* (1986), caused tachycardia in our experiments. Alternatively, however, indalpine and fluvoxamine may be increasing the concentration of 5-HT at the receptor mediating the tachycardiac response to 5-HT, by blocking the uptake mechanism in the blood platelets or at some 5-HT neurones, possibly in the pig heart.

Lastly, it is still plausible that the stimulation of this new 5-HT receptor may lead to a release of a neurotransmitter substance. One such candidate can be calcitonin gene-related peptide which has recently been suggested as functioning as a neurotransmitter in the guinea-pig right atrium (Saito *et al.*, 1986).

References

ASHKENAZI, R., FINBERG, J.P.M. & YOUDIM, M.B.H. (1983). Effects of LM 5008, a selective inhibitor of 5-hydroxytryptamine uptake, on blood pressure and responses to sympathomimetic amines. *Br. J. Pharmacol.*, **79**, 915–922.

BRADLEY, P.B., ENGEL, G., FENIUK, W., FOZARD, J.R., HUMPHREY, P.P.A., MIDDLEMISS, D.N., MYLECHARANE, E.J., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology*, **25**, 563–576.

CLAASEN, V., DAVIES, J.E., HERTTING, G. & PLACHETA, P. (1977). Fluvoxamine, a specific 5-hydroxytryptamine uptake inhibitor. *Br. J. Pharmacol.*, **60**, 505–516.

CONNOR, H.E., FENIUK, W., HUMPHREY, P.P.A. & PERREN, M.J. (1986). 5-Carboxamidotryptamine is a selective agonist at 5-HT receptors mediating vasodilatation and tachycardia in anaesthetized cats. *Br. J. Pharmacol.*, **87**, 417–426.

DE BOER, H.J., DHASMANA, K.M. & SAXENA, P.R. (1986). The tachycardiac response to 5-hydroxytryptamine in the spinal guinea-pig. *Br. J. Pharmacol.*, **89**, P545.

DHASMANA, K.M., DE BOER, H.J., BANERJEE, A.K. & SAXENA, P.R. (1988). Analysis of the tachycardiac response to 5-hydroxytryptamine in the spinal guinea-pig. *Eur. J. Pharmacol.*, In press.

DUNCKER, D.J., SAXENA, P.R. & VERDOUW, P.D. (1985). 5-Hydroxytryptamine causes tachycardia in pigs by acting on receptors unrelated to 5-HT_1, 5-HT_2, or M-type. *Br. J. Pharmacol.*, **86**, 599P.

EGLEN, R.M., PARK, K.C. & WHITING, R.L. (1985). Analysis of the positive chronotropic action of 5-HT on the isolated guinea-pig atria. *Br. J. Pharmacol.*, **86**, P658.

FENIUK, W., HUMPHREY, P.P.A. & WATTS, A.D. (1981). Analysis of the mechanism of 5-hydroxytryptamine-induced vasopressor responses in ganglion-blocked anaesthetized dogs. *J. Pharm. Pharmacol.*, **33**, 155–160.

FOZARD, J.R. (1984). MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **326**, 36–44.

GADDUM, J.H. & PICARELLI, Z.P. (1957). Two kinds of tryptamine receptor. *Br. J. Pharmacol.*, **12**, 323–328.

GONZÁLEZ ALVAREZ, R. & GARCÍA RODRÍGUEZ, M. (1977). Sobre el mecanismo de acción de la serotonina en la auricula aislada de hamster. I. Serotonina: amina de acción mixta. *Acta Physiol. Lat. Am.*, **27**, 239–247.

GÖTHERT, M., SCHLICKER, E. & KOLLECKER, P. (1986). Receptor-mediated effects of serotonin and 5-methoxytryptamine on noradrenaline release in the rat vena cava and in the heart of the pithed rat. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **332**, 124–130.

HOLLANDER, W., MICHELSON, A.L. & WILKINS, R.W. (1957). Serotonin and antiserotonins. I. Their circulatory, respiratory, and renal effects in man. *Circulation*, **16**, 246–255.

HOYER, D., ENGEL, G. & KALKMAN, H.O. (1985). Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes: radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (−)-[^{125}I]iodocyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserin. *Eur. J. Pharmacol.*, **118**, 13–23.

KAUMANN, A.J. (1985). Two classes of myocardial 5-hydroxytryptamine receptors that are neither 5-HT_1 nor 5-HT_2. *J. Cardiovasc. Pharmacol.*, **7** (Suppl. 7), S76–S78.

KAUMANN, A.J. (1986). Further differences between 5-HT receptors of atrium and ventricle in cat heart. *Br. J. Pharmacol.*, **89**, 546P.

LE FUR, G. & UZAN, A. Effects of 4-(3-indolylalkyl)piperidine derivatives on uptake and release of noradrenaline, dopamine and 5-hydroxytryptamine in rat brain synaptosomes, rat heart and human blood platelets. *Biochem. Pharmacol.*, **26**, 497–503.

LEMESSURIER, D.H., SCHWARTZ, C.J. & WHELAN, R.F. (1959). Cardiovascular effects of intravenous infusions of 5-hydroxytryptamine in man. *Br. J. Pharmacol.*, **14**, 246–250.

LEYSEN, J.E., AWOUTERS, F., KENNIS, L., LADURON, P.M., VANDERBERK, J. & JANSSEN, P.A.J. (1981). Receptor binding profile of R 41468, a novel antagonist at 5-HT_2 receptors. *Life Sci.*, **28**, 1015–1022.

MAWE, G.M., BRANCHEK, T.A. & GERSHON, M.D. (1986). Peripheral neural serotonin receptors: Identification and characterization with specific antagonists and agonists. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 9799–9803.

OWEN, D.A.A. (1977). Histamine receptors in the cardiovascular system. *Gen. Pharmacol.*, **8**, 141–156.

PAGE, I.H. (1958). Serotonin (5-hydroxytryptamine); the last four years. *Physiol. Rev.*, **38**, 277–335.

PAINTAL, A.S. (1973). Vagal sensory receptors and their reflex effects. *Physiol. Rev.*, **53**, 159–227.

PEROUTKA, S.J. & SNYDER, S.H. (1979). Multiple serotonin receptors differential binding of [^{3}H]-5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]-spiroperidol. *Mol. Pharmacol.*, **16**, 687–699.

RICHARDSON, B.P. & ENGEL, G. (1986). The pharmacology and function of 5-HT_3 receptors. *Trends Neurol. Sci.*, **5**, 424–428.

RICHARDSON, B.P., ENGEL, G., DONATSCH, P. & STADLER, P.A. (1985). Identification of M-receptor subtypes and their specific blockade by a new class of drugs. *Nature*, **316**, 126–131.

SAITO, A., KIMURA, S. & GOTO, K. (1986). Calcitonin gene-related peptide as potential neurotransmitter in guinea pig right atrium. *Am. J. Physiol.*, **250**, H693–H698.

SAXENA, P.R. (1985). An interactive computer program for data management and parametric and non-parametric statistical analysis. *Br. J. Pharmacol.*, **86**, 818P.

SAXENA, P.R. (1986). Nature of the 5-hydroxytryptamine receptors in mammalian heart. *Prog. Pharmacol.*, **6**, 173–185.

SAXENA, P.R. & LAWANG, A. (1985). A comparison of cardiovascular and smooth muscle effects of 5-hydroxytryptamine and 5-carboxamidotryptamine, a selective agonist of 5-HT_1 receptors. *Arch. Int. Pharmacodyn.*, **277**, 235–252.

SAXENA, P.R. & VERDOUW, P.D. (1982). Redistribution by 5-hydroxytryptamine of carotid blood at the expense of arteriovenous anastomotic blood flow. *J. Physiol.*, **332**, 501–520.

SAXENA, P.R. & VERDOUW, P.D. (1984). Effects of methysergide and 5-hydroxytryptamine on carotid blood distribution in pigs: further evidence for the presence of atypical 5-HT receptors. *Br. J. Pharmacol.*, **82**, 817–826.

SAXENA, P.R. & VERDOUW, P.D. (1985). 5-Carboxamide tryptamine, a compound with high affinity for 5-hydroxytryptamine$_1$ binding sites, dilates arterioles and constricts arteriovenous anastomoses. *Br. J. Pharmacol.*, **84**, 533–544.

SAXENA, P.R., MYLECHARANE, E.J. & HEILIGERS, J. (1985a). Analysis of the heart rate effects of 5-hydroxytryptamine in the cat; mediation by '5-HT_1-like' receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **330**, 121–129.

SAXENA, P.R., HEILIGERS, J., MYLECHARANE, E.J. & TIO, R. (1985b). Excitatory 5-hydroxytryptamine receptors in the cat urinary bladder are of the 5-HT_2 and 'M' type. *J. Autonom. Pharmacol.*, **5**, 101–107.

SAXENA, P.R., DUNCKER, D.J. BOM, A.H., HEILIGERS, J. & VERDOUW, P.D. (1986a). Effects of MDL 72222 and methiothepin on carotid vascular responses to 5-hydroxytryptamine in the pig: Evidence for the presence of '5-hydroxytryptamine$_1$-like' receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **333**, 198–204.

SAXENA, P.R., RICHARDSON, B.P., MYLECHARANE, E.J., MIDDLEMISS, D.N., HUMPHREY, P.P.A., FOZARD, J.R., FENIUK, W., ENGEL, G. & BRADLEY, P.B. (1986b). Functional receptors for 5-hydroxytryptamine (5-HT). *Trends Pharmacol. Sci.*, 7(7), Centre-fold.

TRENDELENBURG, U. (1960). The action of histamine and 5-hydroxytryptamine on isolated mammalian atria. *J. Pharmacol. Exp. Ther.*, **130**, 450–460.

VERDOUW, P.D., JENNEWEIN, H.M., HEILIGERS, J., DUNCKER, D.J. & SAXENA, P.R. (1984). Redistribution of carotid artery blood flow by 5-HT: effects of the 5-HT_2 receptor antagonist ketanserin and WAL 1307. *Eur. J. Pharmacol.*, **102**, 499–509.

VERDOUW, P.D., JENNEWEIN, H.M., MIERAU, J. & SAXENA, P.R. (1985). N-(3-acetylaminophenyl)- piperazine hydrochloride (BEA 1654), a putative 5-HT_1 agonist, causes constriction of arteriovenous anastomoses and dilatation of arterioles. *Eur. J. Pharmacol.*, **107**, 337–346.

(Received June 16, 1987.
Revised October 19, 1987.
Accepted November 3, 1987.)