A 5-hydroxytryptamine receptor in human atrium

¹*†Alberto J. Kaumann, †Louise Sanders, *Anthony M. Brown, *Kenneth J. Murray & †Morris J. Brown

*Smith Kline Beecham Pharmaceuticals, The Frythe, Welwyn, Hertfordshire AL6 9AR and †Clinical Pharmacology Unit, Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QQ

- 1 The effects of 5-hydroxytryptamine (5-HT) were investigated on right atrial appendages obtained from patients treated with β -adrenoceptor blocking agents who were undergoing open heart surgery. Atrial strips were paced under isometric conditions.
- 2 5-HT increased contractile force to approximately one half of the force produced by a saturating concentration of (–)-isoprenaline. Both 5-HT and (–)-isoprenaline accelerated the onset of relaxation, as indicated by an abbreviation of time to peak force.
- 3 The effects of 5-HT were resistant to blockade by 0.4 μ M ($\pm$)-propranolol, 1 μ M (–)-pindolol, 0.4 μ M methiothepin, 4 μ M yohimbine, 0.4 μ M ketanserin, 10 μ M phenoxybenzamine, 1 μ M methysergide, 2 μ M MDL 72222 and 20 μ M granisetron.
- 4 Cocaine 6 μ M potentiated the effects of 5-HT, increasing the pEC₅₀ from 6.6 to 7.4. The inotropic potency of 5-HT is five times greater than that of (–)-noradrenaline.
- 5 ICS 205930 antagonized competitively the effects of 5-HT with a pK_B of 6.7.
- 6 In the presence of 0.4 μ M ($\pm$)-propranolol, 10 μ M 5-HT increased both adenosine 3':5' cyclic-monophosphate (cyclic AMP) levels and cyclic AMP-dependent protein kinase activity by approximately one half and two thirds respectively, of the corresponding effects of 200 μ M (–)-isoprenaline.
- 7 Both the increase in cyclic AMP levels and the stimulation of protein kinase activity are consistent with the inotropic effects of 5-HT being mediated by cyclic AMP-dependent phosphorylation of Ca²⁺ channels and of proteins involved in contraction and relaxation.
- 8 The human atrial 5-HT receptor resembles the neuronal 'so called' 5-HT₄ receptor of rodents both in increasing cyclic AMP levels and in its affinity for ICS 205930.

Introduction

There is a bewildering heterogeneity of heart 5-hydroxytryptamine (5-HT) receptors among species (see Kaumann *et al.*, 1990). 5-HT increases myocardial contractions of molluscs (Greenberg, 1960) through receptors possibly positively coupled to adenylyl cyclase (Sawada *et al.*, 1984). 5-HT increases ventricular contractile force and prolongs action potentials in feline (Kaumann 1983; 1985) and guinea-pig (Kaumann *et al.*, 1990) hearts through unknown receptors. 5-HT increases the contractile force of cat atria through non-classified receptors (Kaumann, 1983; 1985). In rabbits 5-HT causes positive inotropic effects mainly indirectly through a release of noradrenaline (Trendelenburg, 1960; Fozard, 1984). 5-HT causes tachycardia perhaps through 5-HT₂ receptors (Peroutka & Snyder, 1979) in rat (Docherty, 1987) or 5-HT₁-like receptors (Bradley *et al.*, 1986) in cat (Saxena *et al.*, 1985—but see Kaumann, 1986) and through unknown receptors in pig (Bom *et al.*, 1988) and guinea-pig (Walter *et al.*, 1984).

We now describe some characteristics of a 5-HT receptor in human atrium. Progress reports of this work have been presented (Kaumann *et al.*, 1989b; 1990).

Methods

Patients

Right atrial appendages were obtained from 49 patients (39 males, 10 females), undergoing open heart surgery, 47 with coronary heart disease receiving coronary artery bypass grafts and 2 with aortic valve disease. The average age of the patients was 59 $\pm$ 1 years for the whole group, 58 $\pm$ 1 years

for the males and 63 $\pm$ 2 years for the females. All the patients had been receiving β -adrenoceptor blocking agents daily for more than 2 months, up to and including the day of operation. The β -blockers were primarily atenolol (28) and metoprolol (10); on occasion individual patients were taking acebutolol (3), propranolol (2), bisoprolol (1), sotalol (1), nadolol (1), oxprenolol (1), timolol (1), labetalol (1). Some patients had also been receiving diuretics, nitrates, calcium channel antagonists, antacids, captopril, enalapril, bezafibrate, clofibrate. Anaesthesia was induced with thiopentone and midazolam and maintained with fentanyl and trichloroethylene; atracurium was used as muscle relaxant.

Isolated atrial strips

The tissues were dissected and set up as described by Gille *et al.* (1985). After excision, the right atrial appendages were immediately placed in an oxygenated solution at room temperature containing (mm): Na⁺ 125, K⁺ 5, Ca²⁺ 2.25, Mg²⁺ 0.5, Cl[–] 98.5, SO₄^{2–} 0.5, HCO₃[–] 29, HPO₄^{2–} 1, EDTA 0.04. Tissues were continuously oxygenated until dissection. Dissection was at room temperature and started within 120 min of surgical removal. Each atrial appendage was usually cut into 4 strips. The strips were trimmed to a thickness $\leq$ 1 mm to facilitate diffusion of oxygen and drugs. The tissues were mounted, on occasion in pairs, (usually from different patients), in a 50 ml organ bath and apparatus described by Blinks (1965). The organ bath contained a solution at 37°C of the above composition supplemented with (mm): Na⁺ 15, fumarate 5, pyruvate 5, L-glutamate 5, glucose 10 and ascorbate 0.2. The solution was gassed with 95% O₂ and 5% CO₂. The water used was deionised and twice distilled in glass.

Each strip was attached to a strain-gauge transducer and driven at 2 s intervals with a square-wave pulse of 5 ms duration and of just over threshold voltage. After the determination of a length-force curve, the length of each strip was set

¹ Author for correspondence.

at 50% of the resting tension associated with maximum developed force (Kaumann, 1972). Contractile force was recorded on a chart recorder. Fast speed recordings (100 mm s^{-1}) were used to measure time to peak force (Figure 1).

Concentration-effect curves and calculations

The inotropic potency of 5-HT was estimated from cumulative concentration-effect curves. Only a single curve for 5-HT was determined per tissue. All experiments were terminated by the administration of a saturating concentration of (–)-isoprenaline ($200 \mu\text{M}$). The inotropic effects of 5-HT were expressed as percentages of the maximum effect of 5-HT. The maximum inotropic effect of 5-HT was estimated as a percentage of the (–)-isoprenaline-induced effect. $-\log \text{EC}_{10}$, EC_{30} , EC_{50} , EC_{70} and EC_{90} values were determined from the concentration-effect curve of each individual experiment by interpolation and the mean $\pm$ s.e.mean of the log values from all the experiments was calculated for each effect level.

Whenever antagonists were used, they were present for at least 45 min before the concentration-effect curve for 5-HT was determined. Phenoxybenzamine was an exception, being incubated for 30 min. Competitive antagonism of the inotropic effects of 5-HT was analysed by the equation of Arunlakshana & Schild (1959).

Biochemical assays

Adenosine 3':5'-cyclic monophosphate (cyclic AMP)-dependent protein kinase activity and cyclic AMP levels were determined in freeze-clamped atria. The tissues were homogenized in 20 volumes of buffer containing NaCl 100 mM, NaPO_4 10 mM, isobutyl methyl xanthine (IBMX) 0.5 mM, EDTA 10 mM (pH 6.8) with a Polytron-Brinckman (position 8, 5 s). The homogenate was centrifuged at 6000 *g* for 5 min at 4°C. Supernatants, containing soluble protein kinase, were incubated for 2 min at 30°C with $[\text{P}^{32}]\text{-}\gamma\text{-ATP}$ 300 μM , Na_2HPO_4 50 mM (pH 6.8), MgCl_2 10 mM, EGTA 1 mM and malantide 20 μM in the presence and absence of 2 μM cyclic AMP, as described by Murray *et al.* (1990). The activity ratio was obtained by dividing the counts measured in the absence of cyclic AMP by those measured in its presence. The activity ratio measures the proportion of cyclic AMP-dependent

protein kinase active in the atrium. Soluble cyclic AMP was determined in this sample by radioimmunoassay. Protein was determined by the method of Bradford (1976). Results are expressed as mean $\pm$ s.e.mean.

Materials

The following drugs were purchased: $\gamma\text{-}[\text{P}^{32}]\text{-ATP}$ and a cyclic AMP assay kit from the Radiochemical Centre, Amersham, U.K.; cocaine hydrochloride from Merck (Darmstadt, F.R.G.); 5-HT hydrochloride and IBMX from Sigma Chemical Co. (St. Louis, MO, U.S.A.); yohimbine hydrochloride from Simes (Milan, Italy). The following drugs were gifts: (–)-pindolol (prepared as a tartrate), ICS 205930 (3 α -tropanyl) 1H-indole-3-carboxylate and methysergide maleate from Sandoz (Basle, Switzerland); phenoxybenzamine hydrochloride from Smith Kline & French, Welwyn, Herts.; (±)-propranolol hydrochloride from ICI, Macclesfield, Cheshire; MDL 72222 (1 α H,3 α ,5 α H-tropan-3-yl-3,5-dichlorobenzoate) from Merrell Dow Research Institute, Strasbourg, France; methiothepin hydrochloride from Roche, Welwyn, Herts.; ketanserin tartrate from Janssen, Beerse, Belgium; granisetron (BRL 43694) hydrochloride from Beecham Pharmaceuticals, Harlow, Essex.

The 5-HT stock solution (0.1 M) and dilutions were made up in 0.2 mM ascorbate. ICS 205930 and MDL 72222 were dissolved in dimethylsulphoxide (DMSO). The largest volume of DMSO used (15 μl in 50 ml) did not alter basal contractile force or the response to 5-HT. Phenoxybenzamine was dissolved in ethanol. All other drugs were dissolved in twice distilled water.

Results

Inotropic effects of 5-hydroxytryptamine

Administration of 5-HT results in a rapid increase in atrial contractile force (Figure 1). 5-HT hastens the onset of atrial muscle relaxation, which is seen as an abbreviation of the time to peak force (Figures 1, 2). These effects resemble those produced by (–)-isoprenaline (Figure 1). The effects of 5-HT on contractile force (Figure 3) and time to peak force (Figure 2) were concentration-dependent with a pEC_{50} of 6.6 (Table 1).

Figure 1 Comparison of the inotropic effects of 5-hydroxytryptamine (5-HT) and (–)-isoprenaline on atrial strips obtained from a patient (male, 57 years of age) with coronary heart disease. The tissues were exposed to $0.4 \mu\text{M}$ (±)-propranolol throughout. Fast speed tracings are shown before and after steady state drug effects. The amplification was halved (5-HT) or reduced 5 fold ((–)-isoprenaline) during the development of drug effects. Five minutes after addition of the drugs the tissues were freeze clamped for biochemical assays.

Figure 2 Abbreviation of time to peak force by 5-hydroxytryptamine (5-HT, ●) in the absence (a) and presence (b) of 6 μM cocaine. Data obtained from fast speed tracings, as shown in Figure 1. Data for the time to peak force in the absence of 5-HT (○) and in the presence of (–)-isoprenaline (▲) are shown on the left. Numbers given indicate the number of patients.

The maximum inotropic effect of 5-HT was approximately one half of that of 200 μM (–)-isoprenaline (Table 1).

A series of blocking agents did not significantly modify the potency and maximum inotropic effects of 5-HT (Table 1). An exception was ICS 205930 (2 μM) which caused surmountable antagonism (Table 1 and see below). None of the antagonists modified basal contractile force.

We addressed the question of the specificity of the blocking effect of ICS 205930 by investigating whether ICS 205930

Figure 3 Potentiation of the positive inotropic effects of 5-hydroxytryptamine (5-HT) by cocaine in human atria (paced 0.5 Hz, 37°C). Results from pairs of atrial strips from the same patient, one in the absence (○), the other in the presence of 6 μM cocaine (●). (a) Untreated strips from 6 patients. (b) Strips exposed to (–)-pindolol 1 μM from 5 patients. Horizontal bars through symbols are s.e.mean. Columns represent the maximum inotropic effects of 5-HT expressed as a percentage of the effect of 200 μM (–)-isoprenaline.

blocked the positive inotropic responses to (–)-isoprenaline. Atrial appendages from 3 patients were each divided into 2 strips; one strip was exposed to 3 μM ICS 205930 for 1 h, the other to solvent. A cumulative concentration-effect curve for (–)-isoprenaline was then determined, starting at 0.2 nM and ending at the concentration producing a maximum inotropic effect. ICS 205930 did not affect the responses to (–)-isoprenaline. The pEC₅₀ ($\bar{x} \pm$ s.e.mean) for (–)-isoprenaline was 8.0 ± 0.1 and 8.1 ± 0.1 in the absence and presence of ICS 205930 respectively.

Table 1 Inotropic potency of 5-hydroxytryptamine (5-HT) and effects of antagonists

Antagonist	n	Max. response (% (–)-isoprenaline)	–log EC ₅₀ (M)	log CR ^a
<i>No cocaine</i>				
None	26 (26)	57 ± 4	6.64 ± 0.08	
(±)-Propranolol 0.4 μM	5 (5)	47.5 ± 12	6.88 ± 0.15	–0.22 ± 0.16
(–)-Pindolol 1 μM	7 (7)	62 ± 8	6.70 ± 0.18	–0.24 ± 0.18
Methiothepin 0.4 μM	1 (1)	55	6.84	0.10
Yohimbine 4 μM	1 (1)	53	6.53	0.09
Ketanserin 0.4 μM	3	63 ± 1	6.60 ± 0.21	0.08 ± 0.26
Phenoxybenzamine 10 μM	3 (6)	50 ± 15	6.85 ± 0.18	–0.17 ± 0.29
Methysergide 1 μM	2	66.5	6.91	0.02
MDL 72222 2 μM	2 (2)	53	6.55	–0.26
Granisetron 20 μM	2 (2)	65	6.53	0.32
ICS 205930 2 μM	5 (5)	51 ± 6	5.82 ± 0.16	1.14 ± 0.20
<i>Cocaine (6 μM)</i>				
None	6 (6)	50 ± 16	7.40 ± 0.20	
(–)-Pindolol 1 μM	15 (15)	67 ± 6	7.46 ± 0.08	0.13 ± 0.14
(–)-Pindolol 1 μM + ICS 205930 0.3 μM	6	73 ± 8	6.92 ± 0.12	0.40 ± 0.14
(–)-Pindolol 1 μM + ICS 205930 1 μM	6 (8)	73 ± 5	6.53 ± 0.14	0.80 ± 0.21
(–)-Pindolol 1 μM + ICS 205930 3 μM	6	70 ± 6	6.12 ± 0.06	1.22 ± 0.13

^a CR = the concentration-ratio of 5-HT in the presence and absence of antagonist calculated from the respective EC₅₀ values. EC₅₀s in the absence of antagonist were taken from a parallel atrial strip of the same patient.

n = number of atrial strips; number of patients in parentheses.

Potentialiation by cocaine

5-HT is taken up by tissues in a cocaine-sensitive manner (Thoa *et al.*, 1969; Fukuda *et al.*, 1986). Blockade of 5-HT uptake by cocaine may make more 5-HT available to the receptors and thus cause potentiation. We therefore investigated the effects of cocaine. Cocaine caused a six fold potentiation of the positive inotropic effects of 5-HT (Figure 3, Table 1). In some tissues cocaine slightly increased basal contractile force, an effect not observed in the presence of $1\text{ }\mu\text{M}$ (–)-pindolol. Potentiation of the 5-HT effects by cocaine was also observed in the presence of $1\text{ }\mu\text{M}$ (–)-pindolol (Figure 3, Table 1).

Competitive blockade by ICS 205930

The blocking effects of ICS 205930 were observed also in the presence of $6\text{ }\mu\text{M}$ cocaine and $1\text{ }\mu\text{M}$ (–)-pindolol (Figure 4, Table 1). The antagonism was concentration-dependent, yield-

ing a Schild-plot with a slope not significantly different from 1 (Figures 4, 5).

An equilibrium dissociation constant ($-\log M$ (pK_B)) of 6.7 was estimated for the ICS 205930/5-HT receptor complex. As can be seen from Figure 5 this was independent of the presence of $6\text{ }\mu\text{M}$ cocaine.

Increase in cyclic AMP levels and protein kinase activity

An untreated tissue, a (–)-isoprenaline-treated tissue and a 5-HT-treated tissue obtained from the same patient were freeze clamped. The tissues were frozen after equilibrium responses to the amines had been reached (Figure 1). Biochemical assays revealed that $10\text{ }\mu\text{M}$ 5-HT both increased cyclic AMP levels and stimulated cyclic AMP-dependent protein kinase activity (Figure 6). The effects of 5-HT were approximately one half (cyclic AMP) or two thirds (protein kinase) of the corresponding effects caused by $200\text{ }\mu\text{M}$ (–)-isoprenaline.

Figure 4 Competitive antagonism by ICS 205930 of the positive inotropic effects of 5-hydroxytryptamine (5-HT). Results from 4 atrial strips ($6\text{ }\mu\text{M}$ cocaine and $1\text{ }\mu\text{M}$ (–)-pindolol), of a patient (male, 62 years of age) with coronary heart disease. (a) Cumulative administration of 5-HT in the absence and presence of the indicated ICS 205930 concentration. Dots depict nanomolar 5-HT concentrations. (b) Concentration-effect curves for 5-HT from the results in (a); (○) control, (●) ICS 205930 $0.3\text{ }\mu\text{M}$, (△) ICS 205930 $1\text{ }\mu\text{M}$, (▲) ICS 205930 $3\text{ }\mu\text{M}$. (c) Schild plot with slope 0.87 from the results in (a) and (b); symbols correspond to the ICS 205930 concentrations used in (b).

Figure 5 Schild plot of the antagonism by ICS 205930 of the positive inotropic effects of 5-hydroxytryptamine (5-HT). Results obtained from 8 patients whose atrial strips were exposed to $6\mu\text{M}$ cocaine and $1\mu\text{M}$ (–)-pindolol (●), and 5 patients whose atrial strips were not exposed to either $6\mu\text{M}$ cocaine or $1\mu\text{M}$ (–)-pindolol (○). Bars through symbols are s.e.mean of 5 or 6 strips. The overall slope calculated by linear regression of all the data points was 0.87 ± 0.24 (s.e.mean calculated by the equation of Draper & Smith, 1966). In order to take account of pairing of some of the data points, the mean slope was also estimated as the mean of the slopes for the experiments in which 2 or more concentrations of ICS 205930 were used (6 patients). This gave a value of 0.98 ± 0.11 . By either method, the slope is not significantly different from 1.

Discussion

Effect of 5-hydroxytryptamine uptake blockers

We have described a potent positive inotropic effect of 5-HT on human atrium. Cocaine potentiated the inotropic effect of 5-HT, presumably by preventing neuronal (Thoa *et al.*, 1969; Paiva *et al.*, 1984) and/or extraneuronal (Paiva *et al.*, 1984; Fukuda *et al.*, 1986) uptake of 5-HT, thereby making more 5-HT available at the receptors. In some tissues cocaine slightly enhanced basal contractile force, probably by preventing reuptake of noradrenaline released from nerve endings (Iversen, 1967) during each pacing stimulus. The released noradrenaline appeared to enhance contractile force through β -adrenoceptors because cocaine failed to cause an inotropic effect in the presence of the β -adrenoceptor antagonist (–)

Figure 6 Increase in atrial adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels (a) and stimulation of cyclic AMP-dependent protein kinase activity (b) caused by 5-hydroxytryptamine (5-HT) and (–)-isoprenaline in the presence of $0.4\mu\text{M}$ (±)-propranolol. Results from 7 patients. Experiments carried out as in Figure 1. Columns represent mean with s.e.mean indicated by vertical bars.

pindolol. (–)-Pindolol did not prevent the potentiating effects of cocaine. The inotropic potency of 5-HT ($\text{pEC}_{50} = 7.4$, this paper) on human atria is five times greater than that of (–)-noradrenaline ($\text{pEC}_{50} = 6.6$) (Gille *et al.*, 1985; Kaumann *et al.*, 1989a) in the absence of tissue uptake of the amines. The difference in potency between 5-HT and (–)-noradrenaline was also observed when amine uptake was not blocked ($\text{pEC}_{50} = 6.6$ for 5-HT, this paper; and $\text{pEC}_{50} = 5.8$ for (–)-noradrenaline, Gille *et al.*, 1985).

It is likely that $6\mu\text{M}$ cocaine, used in our experiments, caused maximum potentiation of the inotropic effects of 5-HT by nearly saturating 5-HT uptake. Some evidence (Hyttel, 1982) supports our assumption that $6\mu\text{M}$ cocaine causes near maximum inhibition of 5-HT uptake. In rat brain, Hyttel (1982) showed that cocaine prevents 5-HT uptake with an IC_{50} of $0.26\mu\text{M}$. Assuming that cocaine acts by competing with 5-HT, this IC_{50} will be approximately equal to the K_i for cocaine since in the experiments of Hyttel the 5-HT concentration was well below its K_m . In the present experiments, the uptake carrier also appears not to be saturated by 5-HT even at the high concentrations used to surmount antagonism by ICS 205930 since the blocking potency was the same in the presence and absence of $6\mu\text{M}$ cocaine (Figure 5). Assuming a K_i of $0.26\mu\text{M}$, this concentration of cocaine would produce more than 95% inhibition of the neuronal uptake mechanism.

Phenoxylbenzamine has been reported to be a weak blocker ($\text{IC}_{50} = 13.5\mu\text{M}$) of 5-HT uptake into human platelets, a system which resembles neuronal uptake (Ahtee & Saarnivaara, 1971). We did not observe significant 5-HT potentiation with $10\mu\text{M}$ phenoxylbenzamine, presumably because under our conditions 5-HT uptake was insufficiently blocked.

Nature of the receptor

Which receptor mediates the positive inotropic effects of 5-HT? The participation of a variety of receptors can be excluded because appropriate blocking agents, at concentrations that nearly saturate these receptors, did not significantly modify the atrial 5-HT responses (Table 1).

- (1) β_1 , β_2 or β_3 -Adrenoceptors could be involved either through direct interaction with 5-HT or through interaction with noradrenaline released by 5-HT. However, we discard these possibilities because the β -adrenoceptor antagonists (±)-propranolol $0.4\mu\text{M}$ and (–)-pindolol $1\mu\text{M}$ were ineffective. pK_B values for (–)-propranolol of 8.6 and 9.0 have been estimated for β_1 - and β_2 -adrenoceptors respectively of human atrium (Gille *et al.*, 1985). pK_B values for (–)-pindolol of 9.3 and 8.9 have been reported for β_1 - and β_2 -adrenoceptors in human atrium (Kaumann & Lobnig, 1986). A pK_D of 8.0 has been estimated for pindolol in fat cell β_3 -adrenoceptors (Emorine *et al.*, 1989); these receptors could be involved in the cardiostimulant effects of certain indole amines (Kaumann, 1989).
- (2) 5-HT_{1A} receptors can be discounted because $1\mu\text{M}$ (–)-pindolol was ineffective. A pK_D value of 7.7 has been estimated for 5-HT_{1A} receptors in pig cortex (Hoyer, 1989).
- (3) 5-HT_{1C} receptors are not involved because $1\mu\text{M}$ methysergide was ineffective. A pK_D value of 8.6 has been estimated for 5-HT_{1C} receptors in pig choroid plexus (Hoyer, 1989).
- (4) 5-HT_{1D} receptors can be eliminated because neither $4\mu\text{M}$ yohimbine nor $1\mu\text{M}$ methysergide blocked the effects of 5-HT. pK_D values of 7.1 for yohimbine and 8.4 for methysergide have been estimated in calf caudate (Hoyer, 1989).
- (5) 5-HT₁-like receptors can be excluded because methysergide and methiothepin, known to block these receptors (Bradley *et al.*, 1986), were ineffective.
- (6) 5-HT₂ receptors are not involved because $0.4\mu\text{M}$ ketanserin, $4\mu\text{M}$ yohimbine, $1\mu\text{M}$ methysergide, $10\mu\text{M}$ phenoxylbenzamine (30 min) were ineffective. pK_B estimates for smooth muscle 5-HT₂ receptors are 9.4–9.6 for ketanserin (Frenken & Kaumann, 1984; Kaumann & Frenken, 1985; Lemoine & Kaumann, 1986), 7.2 for yohimbine

(Kaumann, 1983) and 9.0 for methysergide (Kaumann & Brown, 1990). Phenoxybenzamine, as used under our conditions, irreversibly blocks 5-HT₂ receptors (Clancy & Maayani, 1985; Frenken & Kaumann, 1987).

- (7) 5-HT₃ receptors are not involved because 2 μ M MDL 72222 did not block and 20 μ M granisetron caused only marginal blockade of the effects of 5-HT. From blockade of 5-HT₃ receptors high pA₂ values have been estimated for both MDL 72222 (7.9 vagus, 9.3 sympathetic nerves) and granisetron (9.9 vagus, 10.7 sympathetic nerves) (Fozard 1989).

The experiments with the series of antagonists that have been discussed were carried out in the absence of cocaine. It could, therefore, be possible that one or more of these antagonists did block the inotropic effects of 5-HT through receptors but that this was obscured by a simultaneous inhibition, by the antagonist, of 5-HT uptake by atrial nerves. This seems unlikely, as to obtain the results in Table 1, exactly equal effects on the receptor and 5-HT uptake carrier would be required; also none of the antagonists used are reported 5-HT uptake inhibitors.

Blockade of 5-HT uptake by an antagonist would have made more 5-HT available to 5-HT receptors and so potentiated the positive inotropic effect of 5-HT. The following argues against this. As discussed above it can be assumed that 6 μ M cocaine causes near maximal blockade of 5-HT uptake. Hence, the maximum potentiating effect of any uptake inhibitor is the same as that of cocaine (i.e. 6 fold, Figure 3). The concentrations of the antagonists used are many more than 6 times their respective reciprocal affinities. The high antagonist concentrations used should therefore still have caused blockade if the respective receptors were involved. This was not observed.

ICS 205930 was the only antagonist found to block competitively the positive inotropic effects of 5-HT. This blockade was specific to 5-HT as neither basal force nor β -adrenoceptor-mediated responses to (–)-isoprenaline were changed. The blockade by ICS 205930 was not different in the presence or absence of cocaine suggesting that the neuronal capture of 5-HT did not distort antagonism at least in the 5-HT concentration range used. The blocking potency of ICS 205930 on human atrium is inconsistent with its high affinity for 5-HT₃ receptors (pA₂ values 10.2–10.6; Fozard, 1989). Our estimated pK_B value of 6.7 is similar to pK_B values of ICS 205930 for rodent brain 'so called' 5-HT₄ receptors positively coupled to adenylyl cyclase (pK_B = 6.2, Dumuis *et al.*, 1988; 1989) and for intestinal 5-HT receptors that mediate release of acetylcholine (pK_B = 6.4, Craig & Clarke, 1989). This evi-

dence, taken together with the increase in cyclic AMP levels we found, suggests, but does not prove, that human atrial 5-HT receptors resemble rodent cerebral 'so called' 5-HT₄ receptors and perhaps some intestinal 5-HT receptors.

Nature of the transduction mechanism

We have shown that 5-HT enhances contractile force and hastens relaxation. These effects are characteristic of agents that increase myocardial cyclic AMP and indeed we have provided evidence that 5-HT increases cyclic AMP levels and stimulates cyclic AMP-dependent protein kinase activity. Similar inotropic and biochemical effects are also caused by (–)-isoprenaline and other catecholamines. Both effects can be mediated either through β_1 -adrenoceptors ((–)-noradrenaline) or through β_2 -adrenoceptors ((–)-adrenaline) in human atrium (Kaumann *et al.*, 1989a; Hall *et al.*, 1990). We therefore suggest that 5-HT and catecholamines share a common biochemical pathway in human atrium. The pathway is likely to consist of a G_s transducer protein that is activated to stimulate adenylyl cyclase by a receptor-ligand complex with 5-HT or by a complex of a catecholamine with a β_1 - or β_2 -adrenoceptor. The cyclic AMP formed specifically activates the cyclic AMP-dependent protein kinase which subsequently phosphorylates proteins involved in the contraction and relaxation processes (England, 1983). Phosphorylation of membrane Ca²⁺ channels increases the Ca²⁺ influx which may enhance contraction either directly or indirectly by inducing Ca²⁺ release from internal stores (Reuter, 1983). Phosphorylation of phospholamban causes faster pumping back of Ca²⁺ into stores, hastening relaxation (Tada & Katz, 1982). Phosphorylation of troponin I decreases the affinity of troponin C for Ca²⁺, thereby also contributing to the acceleration of the onset of relaxation (England, 1983).

Conclusion

The transduction mechanism of human atrial 5-HT receptors probably resembles that of rodent cerebral 'so called' 5-HT₄ receptors (Dumuis *et al.*, 1988; 1989) because both receptors increase cyclic AMP levels upon activation by 5-HT, presumably through a G_s protein. This property and the similar affinity of ICS 205930 suggests that rodent cerebral 'so called' 5-HT₄ receptors and human atrial 5-HT receptors are similar.

We are grateful to Messrs. T.A.H. English, S. Large, J. Wallwork and F.C. Wells of Papworth Hospital for the reliable supply of atrial appendages and to Dr K.-H. Graefe, University of Würzburg for discussions about 5-HT uptake.

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(Received January 22, 1990

Revised April 10, 1990

Accepted April 20, 1990)