Naunyn-Schmiedeberg's Arch Pharmacol (1994) 349:331–337

Naunyn-Schmiedeberg's
Archives of
Pharmacology

5-Hydroxytryptamine causes rate-dependent arrhythmias through 5-HT_4 receptors in human atrium: facilitation by chronic β-adrenoceptor blockade

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Received: 7 September 1993/Accepted: 22 November 1993

Abstract. We have investigated the ability of 5-hydroxytryptamine (5-HT) to elicit arrhythmic contractions in isolated human atrial strips as a function of pacing rate (0.1–2 Hz) using a method recently introduced by us (Kaumann and Sanders, this journal, 1993b) and examined the nature of the 5-HT receptors involved. Right atrial appendage tissue was obtained from 14 patients undergoing cardiac surgery. None of the patients had advanced heart failure. 5-HT (0.6–20 µmol/l) induced arrhythmic contractions during pacing in 4/11 atrial strips from 3/4 patients who had not received β blockers and in 21/27 atrial strips from 9/10 patients who had been chronically treated with β blockers (primarily β_1-selective). The incidence of arrhythmic contractions evoked by 5-HT did not reach statistical significance in the atrial tissue from the non-β blocked patients but was highly significant in the atrial tissue from the chronically β blocked patients. The arrhythmic contractions usually occurred more frequently at low than at high pacing rates and were observed at the physiological frequency of 1 Hz in 1/4 atrial strips from 1/4 of the non-β blocked patients and 6/11 strips from 5/10 of the β blocked patients. The 5-HT-evoked arrhythmic contractions were observed during blockade of β_1-adrenoceptors, β_2-adrenoceptors and 5-HT_3 receptors, ruling out the participation of these receptors. The 5-HT-evoked arrhythmic contractions were totally inhibited within 30 min by the selective 5-HT_4 receptor antagonist SB 203186 ((1-piperidinyl)ethyl 1H-indole 3-carboxylate) 100 nmol/l whereas they persisted in time-matched controls. The blockade of 5-HT-evoked arrhythmic contractions by SB 203186 was surmounted by high concentrations (400–1800 µmol/l) of 5-HT. Our results demonstrate that 5-HT elicits rate-dependent arrhythmic contractions in isolated human atrium through the 5-HT_4 receptor and that they are facilitated in atrial tissue from patients treated with β blockers. Our results suggest that endogenous, platelet-derived 5-HT may cause atrial arrhythmias and that exogenous 5-HT_4 agonists/partial agonists may be arrhythmogenic.

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Key words: 5-HT-evoked arrhythmias – 5-HT_4 receptors – Human atrium – SB 203186 – 5-HT_4 receptor antagonist – Chronic β-adrenoceptor blockade

Introduction

5-HT_4 receptors (Bockaert et al. 1992) mediate increases in contractile force, adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels and cyclic AMP-dependent protein kinase (PKA) activity in human right (Kaumann et al. 1990a, 1991) and left (Sanders and Kaumann 1992) atrium. It has been proposed that 5-hydroxytryptamine-(5-HT) activated PKA phosphorylates sarcolemmal Ca^{2+} channels, thereby increasing their permeability to Ca^{2+} and making more Ca^{2+} available for contraction (Kaumann et al. 1990a, 1991). This proposal has been verified in human atrial myocytes, in which 5-HT causes a marked increase in Ca^{2+} current (Ouadid et al. 1991; Jahnel et al. 1992) with the obligatory involvement of PKA (Ouadid et al. 1991).

Pronounced increases in Ca^{2+} current can overload heart cells with Ca^{2+}, a condition associated with the generation of arrhythmias (Berlin et al. 1989; Thandroyen et al. 1991). These arrhythmias may be due to the generation of Ca^{2+}-dependent afterdepolarisations and triggered action potentials (Wit and Rosen 1986; January and Fozzard 1988). The triggered action potentials may be associated with or cause triggered arrhythmic contractions. While studying the effects of 5-HT on the contractile force of human right atrial strips we observed that 5-HT evokes arrhythmic contractions in some strips paced at 0.5 Hz and attributed this to Ca^{2+} overload caused by 5-HT_4 receptor stimulation (Kaumann and Sanders 1993a). We now demonstrate the production of arrhythmic contractions by 5-HT using an experimental model that assesses the incidence of arrhythmic contractions as a function of heart rate (pacing) (Kaumann and Sanders 1993b). We also demonstrate that the 5-HT-evoked arrhythmic contractions are mediated through

5-HT_4 receptors by using the potent and selective 5-HT_4 receptor antagonist SB 203186 (Parker et al. 1993). Progress reports of this work have been presented (Kaumann and Sanders 1993 c, d).

Methods

Patients. Pieces of right atrial appendage were obtained from 14 patients (11 males, 3 females, average age 63 ± 3 years (mean ± SEM)) undergoing coronary artery bypass surgery (13) or aortic valve repair. None of the patients had advanced heart failure. Up to and including the day of operation, 10 of the patients had been treated with β blockers (βB) (8 with β_1-selective atenolol, 2 with timolol); eight of these and one patient who had not been treated with a β blocker (non-βB) had also been taking nifedipine, diltiazem or amlodipine. One of the non-βB patients was receiving enalapril. Nine of the patients had also taken aspirin until a few days before the day of operation. Some of the patients were also variously receiving nitrates, diuretics, lipid-lowering drugs, benzodiazepines, anti-ulcer drugs and/or antibiotics.

Atrial appendages. On excision the atrial pieces were immediately placed in an oxygenated modified Krebs solution (mmol/l: Na^+ 125, K^+ 5, Ca^{2+} 2.25, Mg^{2+} 0.5, Cl^- 98.5, SO_4^{2-} 0.5, HCO_3^- 29, HPO_4^{2-} 1, EDTA 0.04) at room temperature and maintained in this solution until their dissection into 1–4 strips, which was carried out within 1 h of excision. The atrial strips were set up to contract isometrically at 37 °C, often in pairs, under a pacing rate of 1 Hz, in a 50 ml organ-bath containing the solution above supplemented with (mmol/l) Na^+ 15, fumarate 5, pyruvate 5, L-glutamate 5, glucose 10, ascorbate 0.2 and continuously gassed with 95% O_2/5% CO_2, as described (Kaumann et al. 1991). To obviate possible activation of β_1- and β_2-adrenoceptors, both of which mediate catecholamine-evoked arrhythmic contractions (Kaumann and Sanders 1993 b), each strip was incubated either with CGP 20712A 300 nmol/l, to block β_1-adrenoceptors (25 strips), or with ICI 118551 50 nmol/l, to block β_2-adrenoceptors (15 strips) (Kaumann and Lemoine 1987).

Backward staircases. Once the contractions were stable the method of Kaumann and Sanders (1993 b) was used to detect arrhythmic contractions. An interval-force relationship (staircase) was established for rates of 0.1, 0.2, 0.5, 1 and 2 Hz, the rate being increased every 2 min (Fig. 1 a). The staircase was then determined in the backward mode (i.e. 2–0.1 Hz) with a rest interval of 2 min following each 2 min pacing period (Run 1) (Fig. 1 b). The pacing rate was then set to 0.5 Hz or 1 Hz and 5-HT was added, either cumulatively to a maximum 5-HT concentration of 6–20 µmol/l or as a single addition to give a final concentration of 10 µmol/l. For some patients one strip was left without 5-HT, to act as a time-matched control for the effects of 5-HT. Once 5-HT had caused equilibrium responses (Figs. 1 c, 2 a, c) the pacing rate was increased to 2 Hz and the backward staircase with 2 min rest periods was repeated in the presence of 5-HT (Run 2). The tissues were then paced at the highest pacing rate (usually 0.2 or 0.5 Hz) at which 5-HT-evoked arrhythmic contractions were maintained in all the strips from one patient during Run 2. The 5-HT_4 receptor antagonist SB 203186 (100 nmol/l) was then administered to at least 1 atrial strip from each patient and where available another strip from the same patient served as a time-matched tissue with arrhythmic contractions in the absence of SB 203186. A saturating concentration of 5-HT (400–1800 µmol/l) was later administered to the strips that had received SB 203186. The tissues were then washed and exposed to catecholamines, either to activate β_1-adrenoceptors ((−)-noradrenaline in the presence of ICI 118551 50 nmol/l) or to activate β_2-adrenoceptors ((−)-adrenaline in the presence of CGP 20712A 300 nmol/l), to determine the ability of the β-adrenoceptor subtypes to mediate arrhythmic contractions. The effect of SB 203186 100 nmol/l on catecholamine-evoked arrhythmic contractions was then investigated. The pacing rates (Hz) at which and following which arrhythmic contractions occurred were noted. The half-time ($t_{1/2}$, min) of onset of blockade of 5-HT-evoked arrhythmic contractions by SB 203186 was computed.

Statistics. The significance of differences between the incidence of spontaneous arrhythmic contractions and 5-HT-evoked arrhythmic contractions and between non-βB and βB groups was assessed with the Fisher exact probability test (Siegel 1956) using Statgraphics PLUS (STSC, Rockville, MD, USA). Differences discernible at a P value equal to or less than 0.05 were considered to be statistically significant.

Drugs. 5-Hydroxytryptamine hydrochloride, (−)-noradrenaline bitartrate and (−)-adrenaline bitartrate were purchased from Sigma (Poole, UK). The following drugs were gifts: (1-piperidinyl)ethyl 1 H-indole 3-carboxylate (SB 203186) and granisetron from SmithKline Beecham (Harlow, UK); 1-[2(3-carbamoyl-4-hydroxy phenoxy)-ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-2-propanol (CGP 20712A) from CIBA Geigy (Basel, Switzerland) and erythro-(β)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol). HCl (ICI 118551) from ICI (Macclesfield, Cheshire, UK). The 5-HT stock solution (200 mmol/l) and dilutions were made up in 0.2 mmol/l ascorbate; the catecholamine stock solutions (100 mmol/l) were made up in acidified (pH 3–4) 0.04 mmol/l EDTA, the dilutions in 0.04 mmol/l EDTA. The water used was softened and twice-distilled.

Results

The staircase exhibited by the atrial strips was usually positive in both the forward mode (Fig. 1 a) and the backward mode (Fig. 1 b). The staircase observed in the backward mode (i.e. with rest periods) mirrored the staircase in the forward mode (i.e. without rest periods) (Fig. 1 b) (see also Kaumann and Sanders 1993 b). Before the administration of 5-HT there was a complete absence of spontaneous arrhythmic contractions during the control backward staircase (Run 1) in 11/11 atrial strips from 4 non-βB patients and in 22/27 atrial strips from 10 βB patients (Table 1, Fig. 1 b). In the 5 atrial strips from the βB patients showing spontaneous arrhythmic contractions during Run 1 they were apparent in both the presence (Paced) and absence (Rest) of electrical stimulation (Table 1). The incidence of spontaneous arrhythmic contractions in atrial tissue from βB patients was, however, not statistically significant with respect to atrial tissue from non-βB patients (Table 1). 5-HT elicited arrhythmic contractions during pacing in 4/11 strips from 3/4 non-βB patients and in 21/27 strips from 9/10 βB patients, with a higher incidence at low than at high pacing rates (Table 1, Figs. 1–3). The incidence of 5-HT-evoked arrhythmic contractions did not reach significance in the atrial strips from the non-βB patients but was highly significant in the atrial strips from the βB patients (Table 1). For the 4 strips from the non-βB patients showing 5-HT-evoked arrhythmic contractions CGP 20712A 300 nmol/l was present in the bath. For the 21 atrial strips from the βB patients showing arrhythmic contractions, CGP 20712A 300 nmol/l was present in the bath for 12 strips (from 9 patients) while for the remaining 9 strips (from 7 patients) ICI 118551 50 nmol/l was present. In the 5 strips from the 4 βB patients where there were pre-existing arrhythmic contractions in the control backward staircase (Run 1) 5-HT tended to shift the appearance of arrhythmic contractions in the backward staircase to higher pacing rates than were seen in the absence of 5-HT (not shown). Once arrhythmic contractions had appeared at a particular pacing rate during the backward staircase they were nearly always present at the lower frequencies.

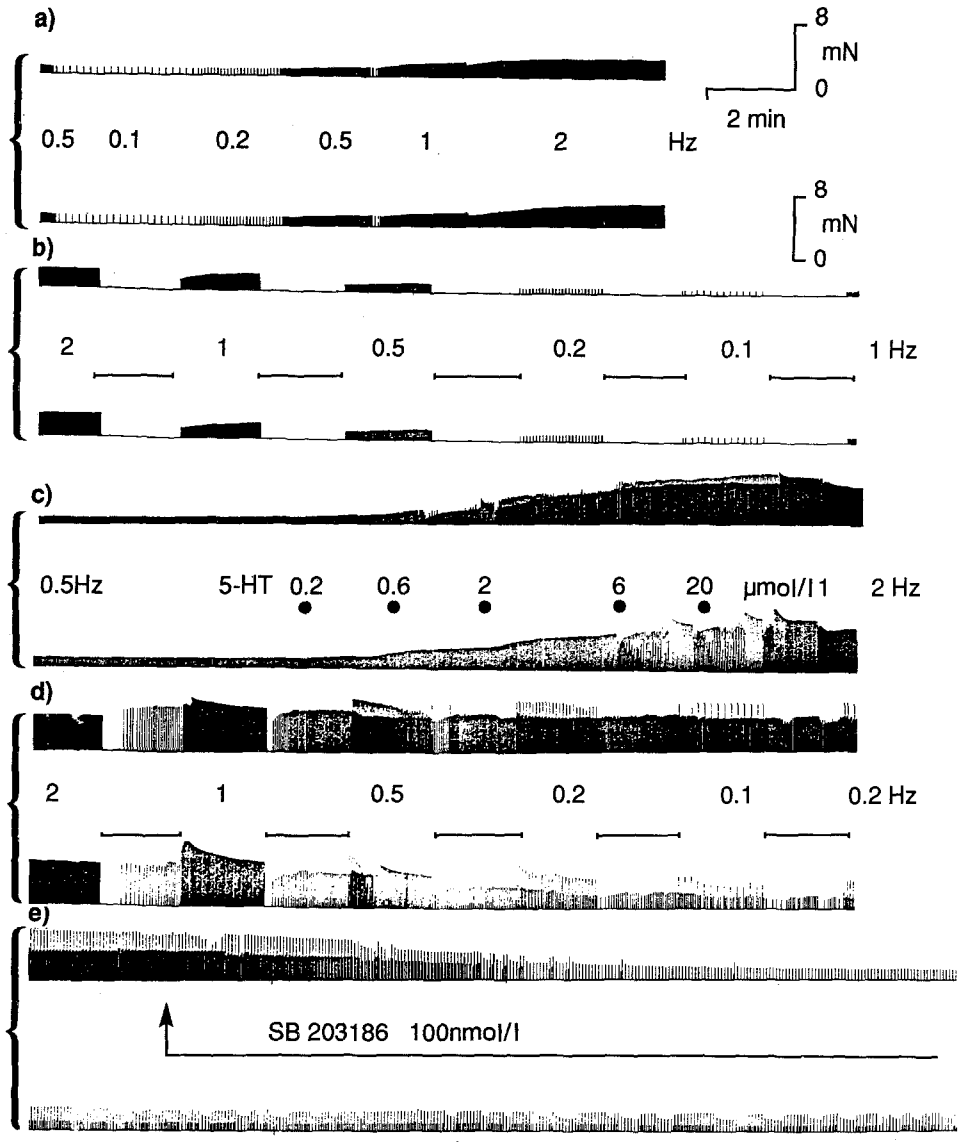

Fig. 1a–e. 5-HT-evoked arrhythmic contractions in paced tissue and in the absence of electrical stimulation and their blockade by SB 203186 100 nmol/l. *Upper and lower traces* in **a–e** show a continuous tracing for each of two time-matched right atrial strips from a 58 year-old male with coronary artery disease who had been treated with timolol. CGP 20712A 300 nmol/l was present throughout the experiment. **a** Interval-force relationship for both strips. Figures show the pacing rate (Hz). **b** Backward staircase for both strips in the absence of 5-HT (Run 1). Note the absence of arrhythmic contractions. *Figures* indicate the pacing rate; *bars* indicate the rest periods. **c** Cumulative 5-HT concentration-effect curve in both strips carried out at a pacing frequency of 0.5 Hz. *Dots* indicate the time of addition of 5-HT; *figures* indicate the cumulative concentration of 5-HT. Note the appearance of arrhythmic contractions at 0.6 µmol/l 5-HT for the upper trace and 6 µmol/l 5-HT for the lower trace. Following an increase of the 5-HT concentration to 20 µmol/l the pacing rate was increased to 1 Hz and then 2 Hz, as shown. **d** Backward staircase in the presence of 20 µmol/l 5-HT (Run 2). *Figures* and *bars* as in **b**. Note the appearance of marked arrhythmic contractions in both strips in the rest period following a pacing rate of 2 Hz and the maintenance of these following lower pacing rates. Note also that arrhythmic contractions appear during pacing at a pacing rate of 0.5 Hz, and that they are maintained at the lower pacing rates. At the end of Run 2 the pacing rate was returned to 0.2 Hz, the highest pacing rate at which arrhythmic contractions were maintained in both strips. **e** The blocking effects of SB 203186, added to the strip represented by the *upper trace* and present as indicated. The *strip* represented by the lower trace provided time-matched arrhythmic contractions. Note the rapid disappearance of arrhythmic contractions in the presence of SB 203186 ($t_{1/2}$ of blockade by SB 203186 = 8 min), while the arrhythmic contractions persisted at 0.2 Hz in the absence of SB 203186 (*lower trace*)

Unlike the 5-HT-evoked arrhythmic contractions that appeared during pacing, the 5-HT-evoked arrhythmic contractions in the rest periods showed little frequency dependence (Table 1). During the rest periods arrhythmic contractions first appeared in the backward staircase following pacing at 2 Hz [in 3/11 strips from 3/4 non-βB patients and in 15/23 strips from 8/10 βB patients showing no spontaneous arrhythmic contractions at 2 Hz in the control run (Fig. 1d, Table 1)]. On the other hand, during pacing only 1 strip (from a non-βB patient) displayed arrhythmic contractions at 2 Hz (Table 1) and it was not until the physiological frequency of 1 Hz that

Table 1. Incidence of arrhythmias as a function of pacing rate

Pacing rate (Hz)	Control				5-HT (6–20 μmol/l)				*P* value	
	Paced	%	Rest	%	Paced	%	Rest	%	Paced	Rest
Non-βB (4 patients, 11 strips)										
2	0/11	0	0/11	0	1/11	9	3/11	27	1	0.21
1	0/11	0	0/11	0	1/11	9	3/11	27	1	0.21
0.5	0/11	0	0/11	0	3/11	27	2/11	18	0.21	0.48
0.2	0/11	0	0/11	0	3/11	27	2/11	18	0.21	0.48
0.1	0/11	0	0/11	0	3/11	27	2/11	18	0.21	0.48
Total	0/11	0	0/11	0	3/11	27	3/11	27	0.21	0.21
βB (10 patients, 27 strips)										
2	0/27	0	3/27	11	0/27	0	18/27	67	1	0.00006
1	0/27	0	3/27	11	6/27	22	22/27	81	0.023	0.00001
0.5	2/27	7	5/27	19	15/27	56	22/27	81	0.0003	0.00001
0.2	5/27	19	4/27	15	19/27	70	21/27	78	0.0003	0.00001
0.1	4/27	15	4/27	15	21/27	78	21/27	78	0.00001	0.00001
Total	5/27	19	5/27	19	21/27	78	22/27	81	0.00003	0.00001
P totals	0.29		0.29		0.008		0.003			

Data expressed as both the number and percentage (%) of atrial strips showing spontaneous (control (Run 1)) and 5-HT-evoked (Run 2) arrhythmic contractions at each pacing rate (Paced) and during the rest period (Rest) following each pacing rate
Non-βB, Non-β blocker treated patients; βB, β blocker treated patients; total, total number of strips showing arrhythmic contractions; *P* totals, *P* values between totals

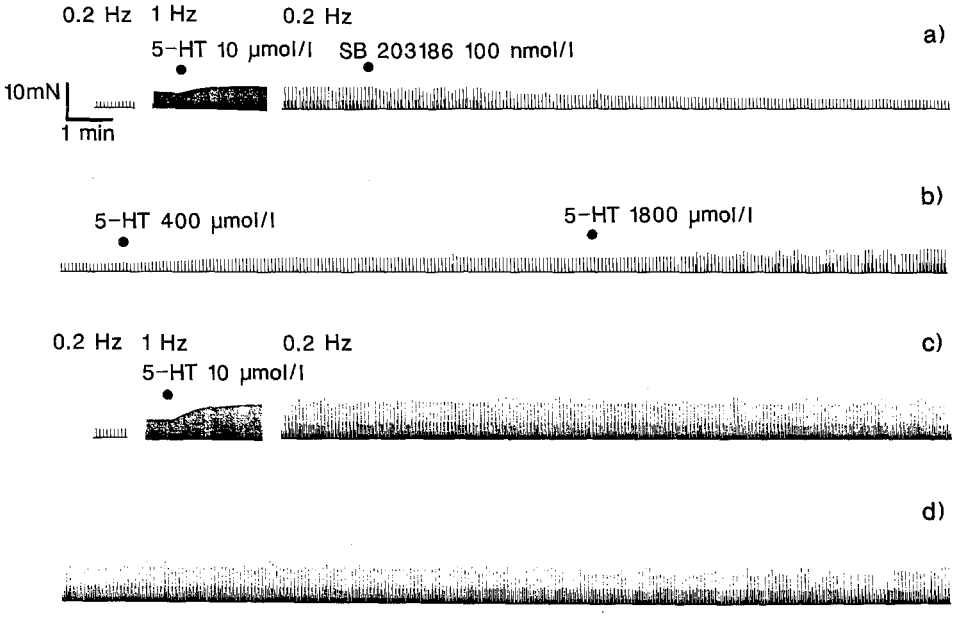

Fig. 2a–d. Surmountability by 5-HT of blockade by SB 203186 of 5-HT-evoked arrhythmic contractions. **a**, **b** and **c**, **d** show parts of a continuous tracing for each of two time-matched right atrial strips from a 59 year-old male patient with coronary artery disease treated with atenolol. CGP 20712A 300 nmol/l was present throughout the experiment in the organ-bath containing the strip whose tracing is shown in **a** and **b**; ICI 118551 50 nmol/l was present throughout the experiment in the organ-bath containing the strip whose tracing is shown in **c** and **d**. **a** Induction of arrhythmic contractions by 5-HT followed by blockade by SB 203186 100 nmol/l. During the backward staircase in the presence of 5-HT (Run 2, not shown) the highest pacing rate at which sustained 5-HT-evoked arrhythmic contractions were maintained in both strips was 0.2 Hz. The *left-hand part* of **a** shows the response of the tissue to stimulation at 0.2 Hz during the backward staircase in the absence of 5-HT (Run 1). Note the absence of arrhythmic contractions. The *middle part* of **a** shows the response of the tissue to the administration of 5-HT 10 μmol/l at a pacing rate of 1 Hz. Note the absence of arrhythmic contractions at 1 Hz. When the response to 5-HT at 1 Hz had reached equilibrium, the pacing rate was adjusted to 0.2 Hz (*right-hand trace*), at which frequency arrhythmic contractions became apparent. These arrhythmic contractions at 0.2 Hz were rapidly abolished by SB 203186 ($t_{1/2}$ of blockade by SB 203186 = 3 min). **b** Following the increase of the 5-HT concentration to 400 μmol/l in the presence of 100 nmol/l SB 203186 only a single extrasystole occurred but a further raising of the 5-HT concentration to 1800 μmol/l in the presence of SB 203186 restored the 5-HT-evoked arrhythmic contractions. **c**, **d** Time-matched recording of the response of the second atrial strip from the same patient showing the sustainability of the 5-HT-evoked arrhythmic contractions in the absence of SB 203186. The arrhythmic contractions persisted for 36 min from initiation of pacing at 0.2 Hz in the portion of the experiment illustrated and continued for a further 20 min (not shown), after which time the tissues were washed. The left-hand and middle tracings shown in **c** correspond to the *left-hand and middle tracings* shown in **a**

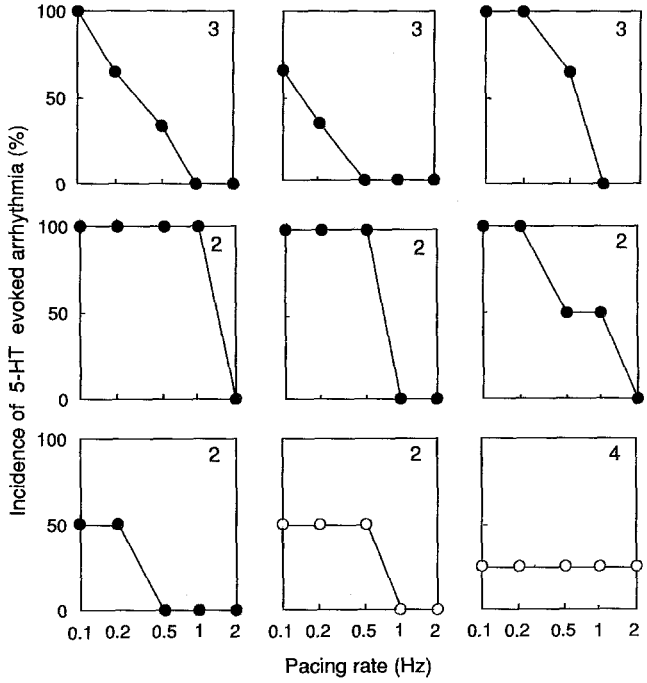

Fig. 3. Frequency dependence of 5-HT-evoked arrhythmic contractions. *Each graph* represents the data for an individual patient and shows the incidence of 5-HT-evoked arrhythmic contractions as a function of pacing rate across the atrial strips cut from the tissue obtained from that patient. Data from strips with spontaneous arrhythmic contractions in the control backward staircase (Run 1) were excluded from this analysis. Data not shown for patients where only one strip was exposed to 5-HT or only one strip remained for the analysis after exclusion of the strips with spontaneous arrhythmic contractions. *Numbers in top right-hand corner* indicate the number of strips used in the analysis. *Filled symbols* indicate βB patients, *open symbols* indicate non-βB patients. Patients and β blockers (*parentheses*) were, from top left: 54 year-old female (timolol); 67 year-old male (atenolol); 67 year-old female (atenolol); 42 year-old male (atenolol); 58 year-old male (timolol); 59 year-old male (atenolol); 55 year-old male (atenolol); 81 year-old female; 65 year-old male. All the patients were suffering from coronary artery disease except the 81 year-old female, who had aortic valve disease

5-HT-evoked arrhythmic contractions really started to appear [in 1/4 strips from 1/4 of the non-βB patients and 6/11 strips from 5/10 of the βB patients (Table 1)]. The 5-HT-induced arrhythmic contractions consisted of extrasystoles and/or pacemaker activity during both pacing and rest. Where 5-HT was not added (2 patients), the second backward staircase (Run 2) remained completely free of arrhythmic contractions, in agreement with previous data from 6 patients (Kaumann and Sanders 1993b). The 5-HT-evoked arrhythmic contractions did not fade in intensity for up to 1 h (Fig. 2c, d).

SB 203186 100 nmol/l consistently abolished the 5-HT-evoked arrhythmic contractions in 1/1 strips from 1/4 non-βB patients and 11/11 strips from 8/10 βB patients (Figs. 1e, 2a). The half-time of onset of blockade ($t_{1/2}$) ranged from 0.5 to 15.0 min (mean ± SEM, 5.2 ± 1.2 min) for the βB patients and was 5 min for the non-βB patient. The large variety in the $t_{1/2}$ values probably reflects variable sensitivities of the tissues to 5-HT. In 7 time-matched atrial strips from 6 βB patients not exposed to SB 203186 the 5-HT-evoked arrhythmic contractions consistently persisted at the time that SB 203186 had abolished the 5-HT-evoked arrhythmic contractions in the parallel strips (Figs. 1e, 2c, d). A backward staircase run in the presence of SB 203186 100 nmol/l following blockade of the arrhythmic contractions at the set pacing rate was completely free of arrhythmic contractions whilst these persisted in the corresponding time-matched strips (data of 4 strips from 3 of the βB patients, not shown). In the 1 strip from 1 non-βB patient and 11 strips from 8 βB patients where 5-HT-evoked arrhythmic contractions had been blocked by SB 203186, a high concentration of 5-HT (400–1800 µmol/l) restored the arrhythmic contractions in the presence of SB 203186 100 nmol/l (Fig. 2b) in 11 of these strips at the pacing frequency at which arrhythmic contractions had been blocked. A backward staircase run in the presence of both SB 203186 100 nmol/l and 5-HT 400–1800 µmol/l in 3 strips from 3 patients (all βB) confirmed that the high concentration of 5-HT also restored arrhythmic contractions previously blocked by SB 203186 at other pacing frequencies and during the rest periods (not shown).

The 5-HT_3 receptor antagonist granisetron (1 µmol/l) failed to block 5-HT-evoked arrhythmic contractions (not shown) in all cases where it was used (5 strips from 3 βB patients).

Washout of 5-HT at the end of the experiment completely abolished the arrhythmic contractions. Where added after washout of 5-HT both (−)-noradrenaline (3 strips from 2 non-βB patients, 11 strips from 8 βB patients) and (−)-adrenaline (4 strips from 3 non-βB patients, 15 strips from 8 βB patients) consistently elicited arrhythmic contractions except in 1 strip from 1 non-βB patient ((−)-noradrenaline). All the catecholamine-evoked arrhythmic contractions were resistant to blockade by SB 203186 100 nmol/l where it was added ((−)-noradrenaline: 7 strips from 6 βB patients; (−)-adrenaline: 1 strip from 1 non-βB patient, 11 strips from 6 βB patients) (not shown).

Discussion

We have confirmed our initial observation (Kaumann and Sanders 1993a) that 5-HT evokes atrial arrhythmic contractions and have now demonstrated their rate dependence, observing a higher incidence at low than at high pacing rates, and that their appearance is facilitated by chronic β-adrenoceptor blockade (Table 1, Fig. 3). Our results are consistent with mediation of the 5-HT-evoked arrhythmic contractions by 5-HT_4 receptors. The 5-HT-evoked arrhythmic contractions were observed in the presence of CGP 20712A or ICI 118551 at concentrations that saturate β_1- and β_2-adrenoceptors respectively (Kaumann and Lemoine 1987), ruling out both the participation of endogenous (−)-noradrenaline acting through these receptors and direct activation of these receptors by 5-HT. The 5-HT-evoked arrhythmic contractions were also resistant to blockade by 1 µmol/l granisetron, a concentration that saturates 5-HT_3 receptors (Fozard 1989), thus excluding 5-HT_3 receptors as mediators of the 5-HT-evoked arrhythmic contractions.

The selective 5-HT_4 receptor antagonist SB 203186 (Parker et al. 1993), on the other hand, consistently abolished the 5-HT-evoked arrhythmic contractions at a concentration (100 nmol/l) that saturates human atrial 5-HT_4 receptors ($pK_B = 8.7$). The involvement of 5-HT_4 receptors is further supported by the finding that in the presence of 100 nmol/l SB 203186 concentrations of 5-HT (400–1800 µmol/l) around 100 times higher than those used in its absence (6–20 µmol/l) surmounted the blockade of 5-HT-evoked arrhythmic contractions in 11 of the 12 strips from 9 patients to which SB 203186 had been administered. If the 5-HT-evoked arrhythmic contractions are mediated by 5-HT_4 receptors one would expect 5-HT concentrations 50 times higher than those eliciting the arrhythmic contractions in the absence of the blocker to surmount the blockade caused by 100 nmol/l SB 203186 ($100\ nmol/l = 50 \times K_B$) and cause the reappearance of arrhythmic contractions. This was indeed observed.

The blocking effect of SB 203186 100 nmol/l appears to be due only to 5-HT_4 receptor blockade and not to other anti-arrhythmic effects. SB 203186 100 nmol/l failed both to affect arrhythmic contractions elicited by catecholamines (this work) and to block spontaneous arrhythmic contractions observed in the absence of 5-HT in atrial strips from 5 patients paced at 0.5 Hz (S. G. Parker and A. J. Kaumann, unpublished observations).

Although the number of non-βB patients from whom we obtained atrial tissue for this study was low, our data so far show that tissues from βB patients are more susceptible to 5-HT-evoked arrhythmic contractions than are tissues from non-βB patients (Table 1). The incidence of 5-HT-evoked arrhythmic contractions compared to control conditions was not significant in the low number of non-βB patients studied but became highly significant in the βB patients (Table 1). Such sensitisation of the human atrial 5-HT_4 receptor system by chronic β-adrenoceptor blockade has previously been noticed in studies of the positive inotropic effects of 5-HT in intact isolated human atrial strips (Kaumann et al. 1990b) and recently found also in human atrial myocytes (F. DelMonte, S. E. Harding and A. J. Kaumann, unpublished experiments). In addition to sensitisation of the human atrial 5-HT_4 receptor system by chronic β-adrenoceptor blockade, we have also observed a greater incidence of catecholamine-evoked arrhythmic contractions mediated through both β_1- and β_2-adrenoceptors in atrial tissue from βB patients than in atrial tissue from non-βB patients (Kaumann and Sanders 1993b). It thus seems as though chronic β-adrenoceptor blockade may sensitise receptor systems that are probably acting via a cyclic AMP-dependent pathway to generate arrhythmic contractions and inotropic responses. It should be noted, however, that β_1-adrenoceptor-selective blockade sensitises β_2-adrenoceptor-mediated, but not β_1-adrenoceptor-mediated inotropic responses to catecholamines (Hall et al. 1990). Thus the β_1-adrenoceptor-mediated hyperarrhythmic responsiveness observed in atria from βB patients (Kaumann and Sanders 1993b) appears to be due to a mechanism that is independent of inotropic responses mediated through β_1-adrenoceptors.

One remarkable aspect of 5-HT-evoked arrhythmic contractions is the lack of 5-HT-induced desensitisation (Figs. 1, 2). Exposure of the tissues to 10 µmol/l 5-HT caused maintained arrhythmic contractions for up to one hour. This is in marked contrast to the fast desensitisation of 5-HT_4 receptors caused by 10 µmol/l 5-HT in mouse embryonic colliculi neurones, in which 5-HT-stimulated adenylyl cyclase activity faded to less than one-third in 10 min (Ansanay et al. 1992). The benzamides cisapride and renzapride, which are potent 5-HT_4 receptor agonists in mouse colliculi neurones, also cause fast and pronounced desensitisation of 5-HT-stimulated adenylyl cyclase activity, whilst metoclopramide, a weak agonist at these receptors, does not. Based on a direct correlation between agonist potency (presumably affinity) and the degree of desensitisation, Ansanay et al. (1992) proposed that desensitisation is a function of the duration of the 5-HT_4 receptor-agonist complex. Our observations that 5-HT-evoked arrhythmic contractions are not desensitised up to 1 h after exposure to 5-HT suggest that human atrial 5-HT_4 receptors function in a different way from mouse colliculi 5-HT_4 receptors.

To account for the different susceptibility to desensitisation by 5-HT of the murine neuronal and human atrial 5-HT_4 receptors it is possible that 5-HT_4 receptors differ substantially between the species or that the desensitisation mechanisms differ between murine brain cells and human myocardial cells, or both. The former hypothesis has already been put forward by Kaumann et al. (1991) to explain differences in the potencies and efficacies of cisapride and renzapride as positive inotropic agonists at human atrial 5-HT_4 receptors and as agonists of adenylyl cyclase activity at the mouse colliculi neuronal 5-HT_4 receptors. Both compounds are full agonists and nearly equipotent to 5-HT at mouse colliculi neuronal 5-HT_4 receptors (Dumuis et al. 1989; Ansanay et al. 1992) but are only partial agonists and less potent than 5-HT at human atrial 5-HT_4 receptors (Kaumann et al. 1991). In support of the second hypothesis Ansanay et al. (1992) were able to demonstrate that desensitisation of mouse neuronal 5-HT_4 receptors is independent of cyclic AMP but totally prevented by Zn^{2+} and partially prevented by heparin, consistent with the involvement of β-adrenoceptor kinase (βARK) or a similar enzyme. It is not known whether human atrial 5-HT_4 receptors are phosphorylated by βARK or a similar enzyme. This awaits investigation.

Our evidence is consistent with our earlier proposals that endogenous 5-HT derived from aggregating platelets may cause atrial arrhythmias through 5-HT_4 receptors and that these arrhythmias could then degenerate into atrial fibrillation (Kaumann 1991a; Kaumann and Sanders 1993a). In addition, both 5-HT (Le Messurier et al. 1959) and intravenously administered cisapride (Bateman 1986) cause tachycardia in man. Since cisapride is a partial agonist at human atrial 5-HT_4 receptors (Kaumann et al. 1991) it has been suggested that both 5-HT and cisapride-evoked tachycardia in man occur through sinoatrial 5-HT_4 receptors (Kaumann 1991b). Furthermore, it has also recently been reported that cisapride can not only cause tachycardia but also generate supraven-

tricular arrhythmias in man (Olssen and Edwards 1992). The possibility that cisapride-evoked supraventricular arrhythmias are mediated through atrial 5-HT_4 receptors will be tested in our current model of rate-dependent arrhythmic contractions.

Why are 5-HT-evoked arrhythmic contractions more often observed at low than at high pacing rates? One possible explanation is that the increased voltage-dependent Ca^{2+} inward current caused by 5-HT (Ouadid et al. 1991; Jahnel et al. 1992) is overcome by overdrive suppression (Vassalle 1977), a phenomenon that is caused by gain of myocyte Na^+ at high pacing rates. The high intracellular Na^+ turns on the Na^+/K^+-dependent ATPase, which generates electrogenic hyperpolarisation that would prevent voltage-dependent flow of Ca^{2+} current during pacing at high rates but which would not be operant during low pacing rates and the rest periods. This hypothesis needs to be tested on human atrial cells.

Acknowledgements. We thank the surgeons of Papworth Hospital for the supply of atrial tissues and Mr. David Brown of AFRC Babraham Institute for help with the statistics.

References

Ansanay H, Sebben M, Bockaert J, Dumuis A (1992) Characterization of homologous 5-hydroxytryptamine$_4$ receptor desensitization in colliculi neurones. Mol Pharmacol 42:808–816

Bateman DN (1986) The action of cisapride on gastric emptying and the pharmacodynamics and pharmacokinetics of oral diazepam. Eur J Clin Pharmacol 30:205–208

Berlin JR, Cannell MB, Lederer WS (1989) Cellular origin of the transient inward current in cardiac myocytes. Role of fluctuations and waves of elevated intracellular calcium. Circ Res 65:115–126

Bockaert J, Fozard JR, Dumuis A, Clarke DE (1992) The 5-HT_4 receptor: a place in the sun. Trends Pharmacol Sci 13:141–145

Dumuis A, Sebben M, Bockaert J (1989) The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT_4) positively coupled to adenylate cyclase. Naunyn Schmiedebergs Arch Pharmacol 340:403–410

Fozard JR (1989) The development and early clinical evaluation of selective 5-HT_3 receptor antagonists. In: Fozard J (ed) The peripheral actions of 5-hydroxytryptamine. Oxford University Press, Oxford, pp 354–376

Hall JA, Kaumann AJ, Brown MJ (1990) Selective β_1-adrenoceptor blockade enhances positive inotropic responses to endogenous catecholamines mediated through β_2-adrenoceptors in human atrial myocardium. Circ Res 66:1610–1623

Jahnel U, Rupp J, Ertl R, Nawrath H (1992) Positive inotropic responses to 5-HT in human atrial but not in ventricular heart muscle. Naunyn Schmiedebergs Arch Pharmacol 346:482–485

January CT, Fozzard HA (1988) Delayed afterdepolarizations in heart muscle: mechanisms and relevance. Pharmacol Rev 40:219–227

Kaumann AJ (1991a) Use of 5-HT_4 receptor antagonists in the treatment of arrhythmias and stroke. International Patent Classification, A61K 31/00, 31/46

Kaumann AJ (1991b) 5-Hydroxytryptamine and the human heart. In: Fozard J, Saxena PR (eds) Serotonin: Molecular biology, receptors and functional effects. Birkhäuser, Basel, Switzerland, pp 365–373

Kaumann AJ, Lemoine H (1987) β_2-Adrenoceptor-mediated positive inotropic effect of adrenaline in human ventricular myocardium. Quantitative discrepancies with binding and adenylate cyclase stimulation. Naunyn Schmiedebergs Arch Pharmacol 335:403–411

Kaumann AJ, Sanders L (1993a) 5-Hydroxytryptamine causes arrhythmias in isolated human atrium. Br J Pharmacol 108:245P

Kaumann AJ, Sanders L (1993b) Both β_1- and β_2-adrenoceptors mediate catecholamine-evoked arrhythmias in isolated human right atrium. Naunyn Schmiedebergs Arch Pharmacol 348:536–540

Kaumann AJ, Sanders L (1993c) 5-Hydroxytryptamine (5-HT) evokes beat rate-dependent arrhythmias in isolated human atrium. J Mol Cell Cardiol 25(Suppl 1):S 78

Kaumann AJ, Sanders L (1993d) 5-HT_4 receptor-mediated arrhythmias in isolated human atrium. Meeting of the British and Italian Pharmacological Societies, Rome, September 1993

Kaumann AJ, Sanders L, Brown AM, Murray KJ, Brown MJ (1990a) A 5-hydroxytryptamine receptor in human atrium. Br J Pharmacol 100:879–885

Kaumann AJ, Sanders L, Brown MJ (1990b) Chronic β_1-adrenoceptor blockade enhances positive inotropic responses to 5-hydroxytryptamine in human atrium. J Mol Cell Cardiol 22(Suppl III):S 2

Kaumann AJ, Sanders L, Brown AM, Murray KJ, Brown MJ (1991) A 5-HT_4-like receptor in human right atrium. Naunyn Schmiedebergs Arch Pharmacol 344:150–159

Le Messurier DH, Schwartz CJ, Whelan RF (1959) Cardiovascular effects of intravenous infusions of 5-hydroxytryptamine in man. Br J Pharmacol 14:246–250

Olssen S, Edwards IR (1992) Tachycardia during cisapride treatment. Br Med J 305:748–749

Ouadid H, Seguin J, Dumuis A, Bockaert J, Nargeot J (1991) Serotonin increases calcium current in human atrial myocytes via the newly described 5-hydroxytryptamine$_4$ receptors. Mol Pharmacol 41: 346–351

Parker SG, Hamburger S, Taylor EM, Kaumann AJ (1993) SB 203186, a potent 5-HT_4 receptor antagonist, in porcine sinoatrial node and human and porcine atrium. Br J Pharmacol 108:68P

Sanders L, Kaumann AJ (1992) A 5-HT_4-like receptor in human left atrium. Naunyn Schmiedebergs Arch Pharmacol 345:382–386

Siegel S (1956) Nonparametric statistics for the behavioral sciences. McGraw Hill, New York, pp 95–110

Thandroyen FT, Morris AC, Hagler HK, Ziman B, Pai L, Willerson JT, Buja LM (1991) Intracellular calcium transients and arrhythmia in isolated heart cells. Circ Res 69:810–819

Vassalle M (1977) The relationship among cardiac pacemakers. Overdrive suppression. Circ Res 41:269–277

Wit AL, Rosen MR (1986) Afterdepolarizations and triggered activity. In: Fozzard HA (ed) The heart and cardiovascular system. Raven Press, New York, pp 1449–1490