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Receptor mechanisms involved in the 5-HT-induced inotropic action in the rat isolated atrium

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1 The effects of 5-hydroxytryptamine (5-HT) in rat cardiac preparations were studied. 5-HT up to 10 μM failed to affect contractility in papillary muscles. However, in electrically driven (1 Hz) left atria 5-HT exerted a positive inotropic effect that started at 1 μM and attained its maximum at 10 μM ($312 \pm 50\%$ of predrug value, $n = 8$).

2 5-HT 10 μM stimulated the content of inositol-1,4,5-trisphosphate but not of cyclic AMP in rat left atria.

3 Plasma and serum levels of 5-HT amounted to about 0.3 μM and 15 μM, respectively.

4 The selective 5-HT_4 receptor antagonists GR 125487 (10 nM and 1 μM) and SB 203186 (1 μM) did not attenuate the positive inotropic effect of 5-HT in rat left atria. In contrast, the 5-HT_2 receptor antagonist ketanserin (5 nM, 50 nM, 1 μM) resulted in a concentration-dependent diminution of the positive inotropic effect of 5-HT in rat left atria.

5 Reverse transcriptase polymerase chain reaction with specific primers detected mRNA of the 5-HT_{2A} receptor in rat atria and ventricles, while expression of the 5-HT_4 receptor was confined to atria.

6 It is suggested that the positive inotropic effect of 5-HT in electrically driven rat left atria is mediated by ketanserin-sensitive 5-HT_{2A} receptors and not through 5-HT_4 receptors.

Keywords: 5-HT-receptors; inotropy; ketanserin; GR 125487; SB 203186

Introduction

5-Hydroxytryptamine (5-HT) receptors have been divided into 7 classes, based upon their pharmacological profile, their cDNA-deduced primary sequences and their signal transduction mechanisms (Hoyer *et al*., 1994). With the exception of the ligand gated channel 5-HT_3 receptor, all 5-HT receptors belong to the superfamily of G-protein coupled receptors containing seven membrane spanning regions. The 5-HT_1 receptor class is divided into five subtypes all of which inhibit adenylyl cyclase activity. The three 5-HT_2 receptor subtypes, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, stimulate phosphatidylinositol-4,5-bisphosphate hydrolysis. 5-HT_4, 5-HT_6, and 5-HT_7 receptors stimulate adenylyl cyclase activity (Hoyer *et al*., 1994). The second messenger pathway of the 5-HT_5 receptors is presently unknown.

Contraction experiments indicate that a 5-HT_4 receptor mediates the positive inotropic, positive chronotropic and proarrhythmic effects of 5-HT in both human and porcine atria and therefore the porcine atrium has been suggested to be an appropriate model for man (Kaumann, 1990; 1991; 1994; Kaumann *et al*., 1990; 1991; 1995; Villalon *et al*., 1990; Kaumann & Sanders, 1992; 1994). Controversy exists about the receptor involved in contractility in the rat: for tachycardia (in pithed rats), an indirect sympathomimetic effect (Göthert *et al*., 1986), the activation of 5-HT_{1C} receptors (Dabiré *et al*., 1992) or the stimulation of 5-HT_2 receptors have been implied (pithed hypertensive rats: Docherty, 1988; Krstic & Katusic, 1989). However, there is a controversy about whether 5-HT mediates a positive inotropic effect in the rat isolated cardiac preparations. Whereas Ouadid *et al*. (1991) failed to detect any positive inotropic effect by 5-HT in rat left atrial strips, Benfey *et al*. (1974) demonstrated a pronounced 5-HT-induced positive inotropic effect in the rat isolated left atrium. Moreover, an antagonist based classification of the positive inotropic effect in rat isolated cardiac preparations is currently lacking. We hypothesized that a 5-HT_4 receptor might mediate a positive inotropic effect in rat atrial or ventricular preparations based on the following observations: cloning and sequencing studies have shown that a 5-HT_4 receptor is expressed in the rat heart (Gerald *et al*., 1995). It exhibits 89% nucleotide sequence and 95% protein sequence homology to the partially cloned human 5-HT_4 receptor (Ullmer *et al*., 1995) and one might speculate that rat heart is a useful model for 5-HT derivatives intended for human used. Therefore, we investigated whether or not a 5-HT_4 receptor might mediate a positive inotropic effect in the rat, like in human hearts by using an integrative approach.

Methods

Contraction experiments

Contraction experiments were performed as described previously (Böhm *et al*., 1984). Papillary muscles and left atria were isolated from reserpine-treated male Wistar rats weighing between 180 and 250 g, with 6–8 animals for each experimental condition. Reserpine-treated rats were used in order to exclude any indirect effect resulting from endogenous catecholamines (Trendelenburg, 1960). The bathing solution contained (in mM) NaCl 119.8, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 22.6, Na_2EDTA 0.05, ascorbic acid 0.28, glucose 5.05 and was continuously gassed with 95% O_2 and 5% CO_2 maintained at 35°C and pH 7.4.

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Force of contraction was measured in an isometric setup after preloading each muscle to optimal length. Papillary muscles and left atria were electrically stimulated at 1 Hz with rectangular pulses of 5 ms duration (Grass stimulator SD9; Grass, Quincy, MA, U.S.A.), the voltage was about 10 to 20% above threshold. Following a 30 min period of stabilization GR 125487 (10 nM and 1 μM, Gale *et al*. 1994) SB 203186 (1 μM, Parker, 1995) and ketanserin (5 nM, 50 nM, 1 μM, Van Nueten *et al*., 1981) were added to the bathing solution and equilibrated for 45 min. 5-HT (0.1 – 100 μM) was administered cumulatively (equilibration time 5 to 10 min for each concentration) followed by a washout period of a minimum of 15 min. Thereafter, a second concentration-response curve for isoprenaline (1 nM – 10 μM) and after wash out a third concentration-response curve for Ca^{2+} (1.8 mM – 14.4 mM) was carried out, in order to test the maximum positive inotropic effect of each preparation. Time-course of contraction was evaluated from twitches recorded at high chart speed. The time from peak force to 90% relaxation was termed relaxation time and the time from 10% of generated force to peak force regarded as time to peak tension. Their sum was regarded as total contraction time in the present study.

Plasma and serum levels of 5-HT

Rats were killed by a blow to the neck and bled from the carotid arteries. Blood was collected from the carotid arteries in tubes without heparin (referred to as 'serum') and centrifuged at 14000 *g* for 10 min at 4°C. The supernatant was stored at −80°C. Alternatively, blood was drawn from the vena cava inferior of ether-anaesthetized rats into plastic tubes containing heparin and centrifuged. The supernatant was collected (referred to as 'plasma') and kept at −80°C. Determination of 5-HT in plasma and serum was performed by means of high performance liquid chromatography (h.p.l.c.) with electrochemical detection, according to Hajos *et al*. (1986) with modifications. In brief, 200 μl of precipitant (Recipe, Munich, FRG), 200 μl of plasma (or serum) and 10 pg ml^{-1} N-methyl-5-HT as internal standard were mixed and centrifuged at 14,000 *g* for 5 min. Forty microlitres of the resulting supernatant were injected into the h.p.l.c. system, which consisted of a LC Workstation Class LC10 (Shimadzu, Kyoto, Japan) with a SIL-10A auto injector, a LC-10AT liquid chromatograph, a Waters model 410 electrochemical detector (Waters Millipore, Eschborn, FRG) with a glassy carbon working electrode set at a potential of 0.7 V versus Ag/AgCl and software provided by the manufacturer. Chromatographic separation was performed on a reversed phase C18 column (5 μm, 150 mm × 4.6 mm i.d., Recipe) at ambient temperature. The mobile phase consisted of 50 mM citric acid, 27 mM sodium dihydrogen phosphate buffer, 0.27 mM sodium EDTA, 1.5 mM sodium 1-octane-sulphonate (Sigma, Munich, F.R.G.) dissolved in distilled water/methanol 85/15% v/v. Flow-rate was 1 ml min^{-1}.

Determination of inositol-1,4,5-trisphosphate

Inositol-1,4,5-trisphosphate (IP_3) was determined in freeze-clamped left atria. Isolated left atria were mounted in the organ bath as described above and incubated with 10 μM 5-HT, with 1 μM isoprenaline or buffer. After seven minutes when the inotropic effect was stable, atria were freeze-clamped. Frozen atria were transferred into liquid nitrogen cooled teflon tubes. To each sample 500 μl of a freshly mixed solution containing 8% trichloracetic acid (TCA), 1 μmol EDTA per 20 mg wet weight and 1 μmol NaF per 20 mg wet weight according to Mayr and Thieleczek (1991). Frozen samples were homogenized in a micro-dismembrator (Braun, Melsungen, Germany) and transferred into polypropylene tubes. After centrifugation at 14000 *g* for 5 min the supernatant was incubated at 30°C for 20 min and extracted with diethylether. Ten to twenty microlitres of the aqueous phase were used to measure IP_3 by radioligand assay, according to the instructions of the manufacturer (BIOTRAK, Amersham, Life Science, Buckinghamshire, U.K.). Recoveries of IP_3 run with each experiment were 95 ± 8% (n = 8). Protein was determined according to Bradford (1976).

Determination of cyclic AMP content

Adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels (cyclic AMP) were determined in freeze-clamped left atria as previously described (Böhm *et al*., 1984). Rat left atria were mounted in the organ bath as described above and incubated with 10 μM 5-HT, with 1 μM isoprenaline or buffer. After seven minutes when the inotropic effect was stable, atria were freeze-clamped and cyclic AMP was measured by radio-immunoassay. Recoveries of cyclic AMP run with each experiment were 89 ± 5% (n = 8). Protein was determined according to Bradford (1976).

Reverse transcription polymerase chain reaction

Total RNA was prepared with RNAzol BTM (Cinna/Biotecs, Houston, Texas, U.S.A.). cDNA was prepared from total RNA with random hexanucleotide primers by use of reverse transcriptase (Superscript II, Gibco BRL, F.R.G.). An aliquot of the first strand cDNA (500 ng) was amplified in a 50 μl PCR mixture (200 μM dNTPs final concentration) containing 2.5 u of *Taq* polymerase in the buffer supplied by the manufacturer (Gibco BRL, F.R.G.) and 0.4 μM of primers for 5-HT_{4S} and 5-HT_{4L} (Gerald *et al*., 1995), or for 5-HT_{2A} (according to Ullmer *et al*., 1995), with 1 cycle of 94°C for 4 min, 35 cycles of 91°C for 90 s, 64°C for 90 s and 72°C for 3 min, with a post-incubation of 72°C for 10 min, respectively. The PCR products were run on an ethidium bromide gel.

Southern blot analysis

5-HT_4 PCR products were initially identified by Southern blotting. PCR products were transferred to nylon membranes (Hybond N, Amersham, F.R.G.) in 20 × SSC solution. A 45 bp 5-HT_{4S} and a 5-HT_{4L} cDNA (Gerald *et al*., 1995) were labelled with fluorescein-ATP by random primer labelling (Renaissance Kit, Du Pont, F.R.G.). Membranes were hybridized overnight in a 60°C water bath with 5 × SSC, 0.1% (w/v) sodium dodecyl sulphate (SDS), 0.5% (w/v) blocking reagent and 5% (w/v) dextrane sulphate and the labelled probe. Hybridized membranes were washed twice at room temperature with 2 × SSC, 0.1% SDS for 15 min and once with 0.2 × SSC, 0.1% SDS for 15 min. Membranes were incubated with antifluorescein HRP conjugate, chemiluminescence reagent (Renaissance Kit, Du Pont, F.R.G.).

Sequencing

The identity of the PCR-products was confirmed by sequencing with the AmpliTaq-FS DNA polymerase (Dye Deoxy Terminator Cycle Sequencing Kit, Applied Biosystems, Weiterstadt, F.R.G.) and an ABI PRISM-310 automated sequencer (Applied Biosystems, Weiterstadt, F.R.G.).

Data analysis

Data shown are means ± s.e.mean. Statistical significance was estimated by analysis of variance followed by Bonferroni's *t* test as appropriate. $P<0.05$ was considered to be significant.

Drugs and materials

GR 125487 (1-[2-[(methyl-sulphonyl)amino]ethyl]-4-piperidinyl-methyl-5-fluoro-2-methoxy-1H-indole-3-carboxylate) and SB 203186 (1-piperidinyl)ethyl-1H-indole-3-carboxylate) were kindly provided by Glaxo (Ware, U.K.) and Smith Kline Beecham (Welwyn, U.K.), respectively. Ketanserin was from Sigma (Munich, FRG). All other chemicals were of analytical or best commercially available grade. Twice distilled water was used throughout. Stock solutions were prepared fresh daily.

Results

Inotropic effect of 5-HT in isolated papillary muscle and left atrium

In electrically driven (1 Hz) rat left atrium 5-HT exerted a concentration-dependent (0.1 – 100 μM) positive inotropic effect (Figure 1). The positive inotropic effect started at 1 μM 5-HT with a maximum effect at 10 μM 5-HT and amounted to 312% of predrug value. The maximum response to 5-HT was 42 ± 8% of the maximum effect to Ca^{2+} (10.8 mM) in the same preparations. 5-HT was less potent and less effective than isoprenaline in rat atria (Figure 1). In papillary muscles where isoprenaline was a potent and effective positive inotropic agent, 5-HT did not exert a positive inotropic effect (Figure 1). 5-HT had no effect on the time-course of the contraction in isolated, electrically-driven left atrium of the rat. For instance, predrug value of total contraction time was 67.7 ± 2.29 ms and 68.2 ± 1.99 ms ($P>0.05$, $n=7$) in the presence of 10 μM 5-HT where the inotropic effect was maximum. In contrast, the β-adrenoceptor agonist isoprenaline at 10 μM reduced total contraction time in the same preparations by 10.5% (from 64.9 ± 1.54 ms to 58.1 ± 0.95 ms, $n=7$, $P<0.05$).

To identify the 5-HT receptor that mediates the positive inotropic effective in atria we used subtype selective antagonists. GR125487 is a selective 5-HT_4 antagonist with an $pK_B=10.0$ for the 5-HT_4-mediated relaxation of rat oesophagus (Gale *et al*., 1994). Assuming a competitive antagonism and a pA_2 of 10, one can calculate (MacKay, 1978) that 10 nM GR 125487 should increase the EC_{50} of 5-HT by a factor of about 100 and 1 μM by a factor of about 10000. Likewise, based on the $pK_B=8.7$ for the selective 5-HT_4 antagonist SB 203186 (Kaumann, 1994; Parker, 1995), one can predict that 1 μM SB 203186 should increase the EC_{50} for 5-HT in the rat atrium by a factor of about 500. However, neither antagonist increased the EC_{50} for 5-HT (Table 1).

The potent 5-HT_2 receptor antagonist ketanserin concentration-dependently blocked the positive inotropic effect of 5-HT (Figure 2, Table 1). Addition of 5 nM and 50 nM ketanserin shifted the concentration-effect curve to higher EC_{50} values (from 0.77 ± 0.10 μM to 5.50 ± 0.96 μM and 87.0 ± 13.5 μM, respectively; Table 1). The calculated pK_B value from Schild plot analysis was 8.9.

Effects of 5-HT on IP_3 and cyclic AMP content

In rat isolated, electrically-driven atria, 5-HT increased IP_3 levels by 188% vs control. Under the same conditions isoprenaline was ineffective at altering tissue IP_3 content (Figure 3). Cyclic AMP levels were increased by 230% vs control during isoprenaline stimulation whereas 5-HT under the same conditions had no effect on cyclic AMP content (Figure 4).

Table 1 EC_{50} values of the positive inotropic effect of agonists and antagonists in rat left atrium

Treatment	n	*EC_{50}* (μM)
5-HT	8	0.77 ± 0.10
GR125487 (10 nM)	7	0.66 ± 0.11
GR125487 (1 μM)	7	0.76 ± 0.12
SB 203186 (1 μM)	5	2.76 ± 0.86
Ketanserin (5 nM)	8	5.50 ± 0.96*
Ketanserin (50 nM)	8	87.0 ± 13.5*

Data are mean ± s.e.mean, *$P<0.05$ vs 5-HT.

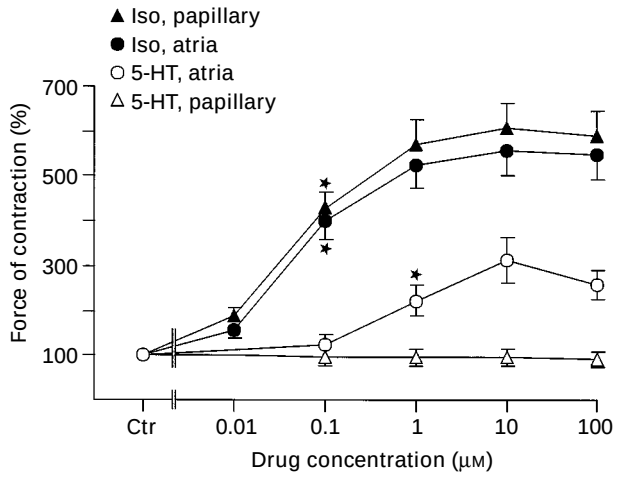

Figure 1 Concentration-responsive curve for the effect of 5-HT and isoprenaline (Iso) on force of contraction in rat isolated, electrically-driven left atria ($n=8$) and papillary muscles. ($n=5$). Predrug values amounted to 5.05 ± 0.54 mN in atria and 4.96 ± 0.68 mN in papillary muscles. *Indicates the first significant differences vs predrug values (Ctr), $P<0.05$.

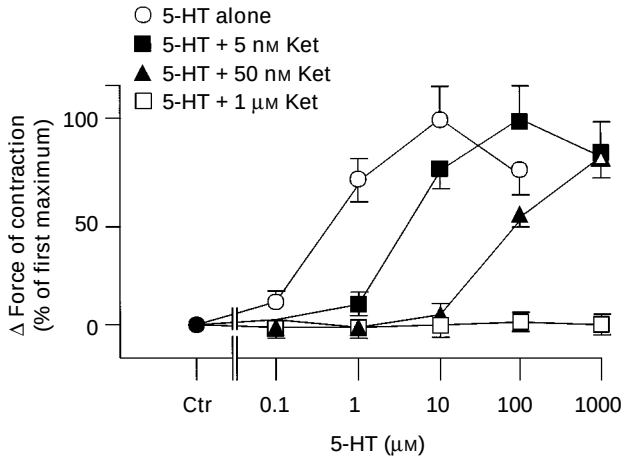

Figure 2 Concentration-response curve for the effect of 5-HT alone ($n=8$) and in the additional presence of ketanserin (Ket; 5 nM, $n=8$; 50 nM, $n=8$ and 1 μM, $n=7$). Maximum force of the concentration-response curve of 5-HT alone was 10.41 ± 2.47 mN. The developed force of the subsequent curves is expressed as a percentage of the maximum force of the 5-HT concentration-response curve. Ctr: predrug value.

5-HT plasma and serum levels

In rat, 5-HT plasma levels amounted to 0.29 ± 0.07 μM ($n=5$) and 5-HT serum levels were 14.7 ± 1.97 μM ($n=8$). For comparison, EC_{50} values for the positive inotropic effect of 5-HT are included (Figure 5).

Expression of 5-HT$_{2A}$ and 5-HT$_4$ receptor mRNA

The distribution of mRNA of 5-HT$_{2A}$, 5-HT$_{4L}$ and 5-HT$_{4S}$ receptors was examined by PCR amplification of tissue specific cDNA from reverse transcription of mRNA. PCR products corresponding to the expected size of the 5-HT$_{2A}$ (265 bp) receptor were found in rat atria and ventricles (Figure 6). In contrast, PCR products corresponding to the 5-HT$_{4L}$ (468 bp) and 5-HT$_{4S}$ (416 bp) receptors were confined to rat atria (Figure 6). Contamination with genomic DNA was excluded by absence of any signals in the gel without addition of the reverse transcriptase to the reaction (data not shown).

First PCR products were identified by Southern blots. Hybridization of 5-HT$_{4L}$ and 5-HT$_{4S}$ PCR products was detected with specific oligonucleotide probes (Figure 7). These hybridization signals were specific for each splice variant since cross-hybridization of the 5-HT$_{4L}$ PCR product with the 5-HT$_{4S}$ internal probe and the 5-HT$_{4L}$ PCR product with the 5-HT$_{4S}$ internal probe revealed no hybridization signal (data not shown). Gerald *et al*. (1995) showed that only the 5-HT$_{4S}$ splice variant is expressed in atrium. We found both splice variants, 5-HT$_{4S}$ and 5-HT$_{4L}$ in rat atrium. This is most probably due to our altered PCR protocol which enabled us to detect low level expression of mRNAs, e.g. longer cycle length and lower denaturing temperatures which prolongs half-life of the Taq polymerase. Finally, the identity of the PCR products was confirmed by sequencing analysis (data not shown).

Discussion

Controversial findings have been presented on the inotropic effect of 5-HT in the rat heart. Some failed to detect any

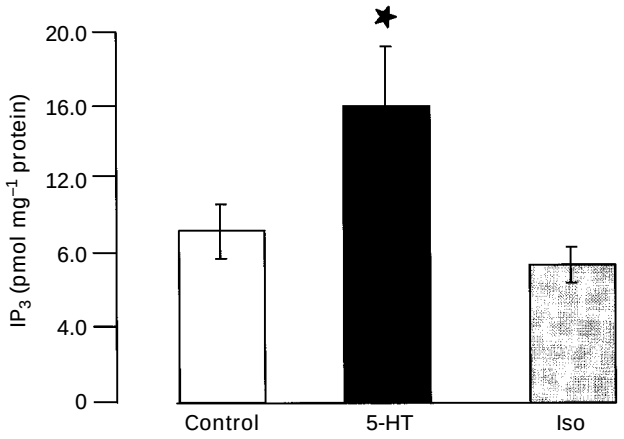

Figure 3 Effect of 10 μM 5-HT ($n=8$) and 1 μM isoprenaline ($n=7$) on IP_3 content in rat isolated, electrically-driven left atria. $^*P<0.05$ vs control ($n=8$).

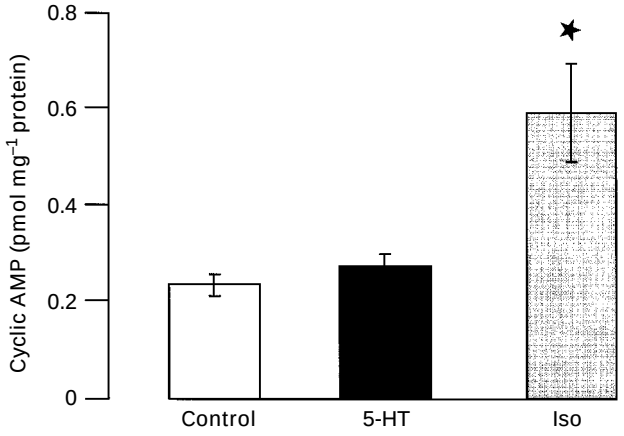

Figure 4 Effect of 10 μM 5-HT ($n=8$), 1 μM isoprenaline ($n=8$) and buffer (control, $n=8$) on cyclic AMP content in rat isolated, electrically-driven left atria. $^*P<0.05$ vs control.

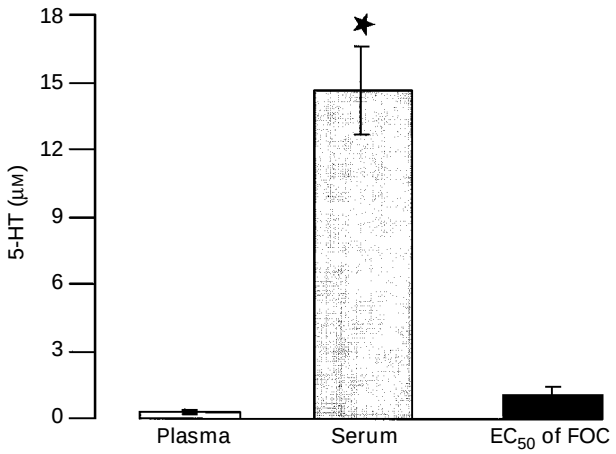

Figure 5 Rat plasma ($n=5$) and rat serum ($n=8$) concentrations of 5-HT. EC_{50} values for the positive inotropic effects of 5-HT (0.1–100 μM) on rat isolated, electrically-driven left atria are included. $^*P<0.05$ vs plasma.

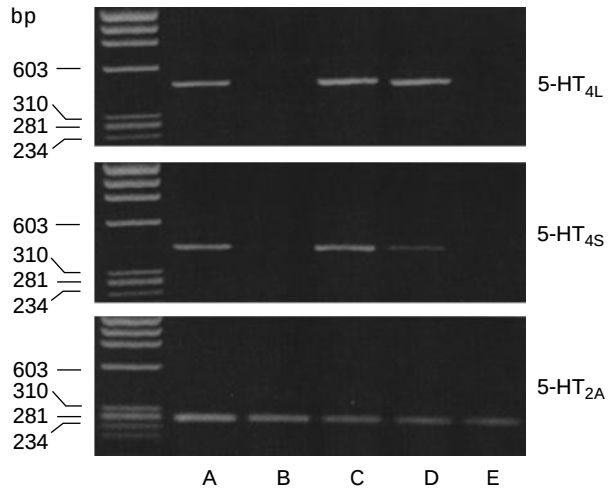

Figure 6 Photograph of an ethidium bromide stained agarose gel. Agarose gel electrophoresis of PCR products amplified from rat cDNA derived from various rat tissues (A: atrium, B: ventricle, C: colon, D: brain, E: liver). 5-HT$_{4S}$, 5-HT$_{4L}$ and 5-HT$_{2A}$ receptor primers gave rise to PCR products of 416 bp, 468 bp and 265 bp, respectively. Similar results were obtained in five independent experiments.

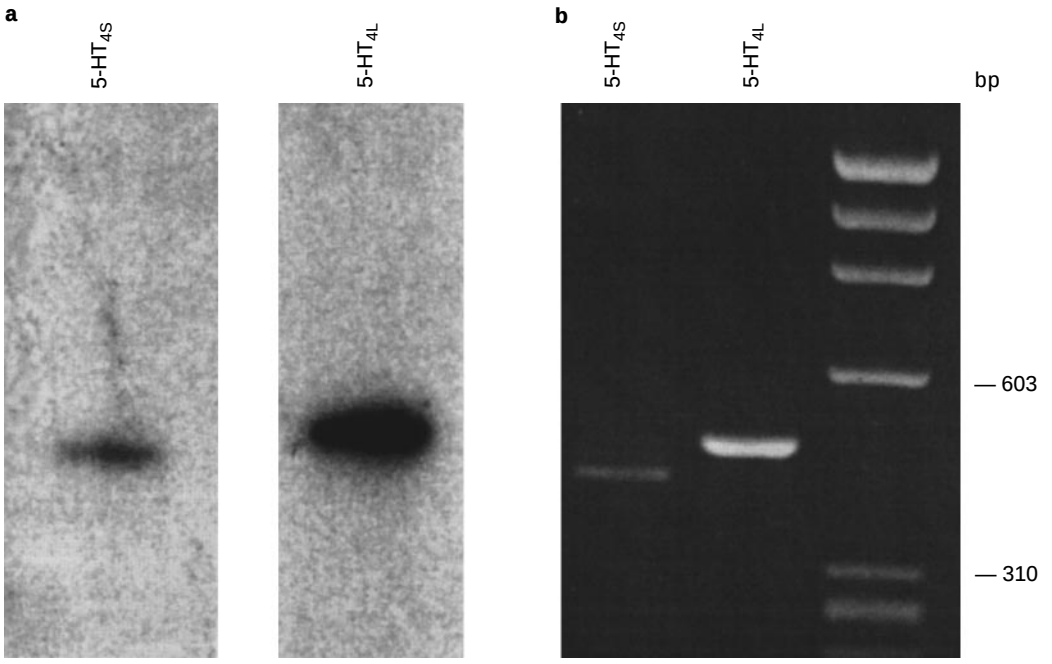

Figure 7 (b) Photograph of an ethidium bromide stained agarose gel. Electrophoresis of PCR-amplified products derived from rat atrium cDNA with 5-HT_{4S} and 5-HT_{4L} receptor primers. PCR products of 468 bp for 5-HT_{4L} and 416 bp for 5-HT_{4S} were obtained and transferred to nitrocellulose. The membrane was hybridized with fluorescein-labelled probes (from outside the PCR primer region) specific for the 5-HT_{4S} and 5-HT_{4L} receptor. (a) Specific signals after detection with a chemofluorescence generating antibody raised against fluorescein (see Methods for further details).

inotropic effect of 5-HT in rat atrial preparations (Ouadid *et al*., 1991). In agreement with Benfey *et al*. (1974) we noted a substantial positive inotropic effect of 5-HT in the rat. However, the positive inotropic effect of 5-HT was confined to atria and absent in papillary muscles; this had not been previously shown by Benfey *et al*. (1974). The reason why Ouadid and coworkers (1991) failed to detect a positive inotropic effect is presently unknown. The same regional difference was found in human cardiac preparations (Jahnel *et al*., 1992): the positive inotropic effect was present in atria but absent in ventricles. As in human atria, the positive inotropic effect of 5-HT was smaller than that of the β-adrenoceptor agonist isoprenaline (Kaumann *et al*., 1990).

Apparently, there seems to be a species difference in the inotropic effect of 5-HT. Previous work on rodents clearly indicated that there is a positive inotropic effect in guinea-pig left atrium (Kaumann, 1991; Tramontana *et al*., 1993). The present work detected a positive inotropic effect in rat atrium. However, in another rodent species, namely mouse, we failed to detect a positive inotropic effect in isolated left atrium (data not shown).

Whether humoral 5-HT levels are sufficient to increase cardiac contractility in the rat *in vivo* is questionable. Indeed rat 5-HT plasma concentrations were low and amounted to 0.29 μM, whereas serum concentrations were much higher (14.7 μM). Most 5-HT in blood is stored in platelets. Platelets remove 5-HT from the blood extracellular space through a high affinity transporter for 5-HT (Verbeuren, 1992). Consequently, the concentration of 5-HT in the coagulated serum is much higher than in uncoagulated serum (Celada *et al*., 1994). Thus, rat 5-HT plasma concentrations were below the EC_{50} values (0.77 ± 0.10 μM) for the positive inotropic effect of 5-HT, whereas serum concentrations were above the EC_{50} values. Thus, 5-HT after being released from aggregating platelets in the atrium could be inotropically active and might e.g. facilitate the dislodging of a new thrombus by enhancing atrial contractility (Kaumann, 1994). These contractile data prompted our initial hypothesis that the same receptors, namely the 5-HT_4 receptor, mediate the positive inotropic effect in the atrium.

5-HT was inotropically less potent in rat atrial preparations than in human atrium (Kaumann *et al*., 1990; EC_{50}: 0.20 μM vs 0.77 μM this study). This may raise some doubt as to the validity of our initial hypothesis that the same receptor is operational in the rat and human atrium.

Moreover, 5-HT did not shorten time-course of contraction in rat atria in contrast to human atria (Kaumann *et al*., 1990), suggesting a cyclic AMP-independent positive inotropic effect. Indeed, 5-HT did not increase cyclic AMP in rat atria (this study) but did in human atria (Kaumann *et al*., 1990) or after transient expression of the rat cloned 5-HT_4 receptor in eukaryotic COS-7 cells (Gerald *et al*., 1995). In neonatal rat ventricular myocytes 5-HT reduced cyclic AMP content (Higgins *et al*., 1980). Hence, one could speculate that 5-HT exerts a – negative – inotropic effect in neonatal preparations which is postnatally lost. However, while we noted a positive inotropic effect of 5-HT in neonatal rat atria, in isolated ventricular preparations from the same neonatal (1d) rats 5-HT up to 100 μM failed to elicit a positive inotropic effect (unpublished observations).

While 5-HT failed to affect cyclic AMP content in rat atria, it induced an increase in IP_3 content. This was initial evidence that the rat atrial-5-HT receptor might belong to the family of 5-HT_2 receptors that typically activate phospholipase C.

Likewise, contractile data with receptor antagonists cast doubt on the involvement of 5-HT_4 receptors in the positive inotropic effect in rat atria. The positive inotropic effect could not be attenuated by a high concentration of SB 203186. However, SB 203186 has not been tested on rat tissues known to possess functionally active 5-HT_4 receptors like the rat oesophagus. But those data are available for another selective

5-HT_4 receptor antagonist, namely GR 125487 (Gale *et al*., 1994). GR 125487 at 1 μM saturates 5-HT_4 receptors. Still, GR 125487 at the concentrations tested did not reduce the positive inotropic effect of 5-HT. As a result, 5-HT_{4L} and 5-HT_{4S} receptors are unlikely to mediate atrial inotropy. This raises the question as to the meaning and function of the mRNA for 5-HT_{4L} and 5-HT_{4S} receptors. First, it is puzzling why 5-HT in COS cells transfected with 5-HT_{4L} and 5-HT_{4S} stimulates cyclic AMP levels, whereas we failed to detect any increase in rat atrial cyclic AMP levels by 5-HT. Perhaps the cellular environment in COS cells which are immortalized from monkey kidney cells, is very different from native conditions. Thus, it is conceivable that 5-HT_4 receptors couple to other signal transduction systems – e.g. ion channels – or are even mute in the rat heart. Finally, one can speculate that they derive from nonmuscle cells like nerve cells which are more abundant in atria than in ventricle or that their translation from RNA to protein is very ineffective.

In contrast to the selective 5-HT_4 antagonists studied, the 5-HT_2 antagonist ketanserin concentration-dependently antagonized the positive inotropic effect of 5-HT in rat left atrium with a pK_B value of 8.9 comparable to that obtained in the rat tail artery (Frenken & Kaumann, 1987). Moreover, the present data fit well with results obtained in pithed spontaneously hypertensive rats where ketanserin antagonized the 5-HT-evoked tachycardia (Docherty, 1988). Ketanserin is a well characterized antagonist at 5-HT_{2A} receptors (Hoyer *et al*., 1994: pA_2 about 9). Thus, our contraction experiments strongly imply that 5-HT_{2A} receptors were involved. These receptors have been shown previously in rat aorta (Ullmer *et al*., 1995). But the present work provides the first evidence for their presence in the rat heart. Others failed to detect 5-HT_{2A} receptors in the rat heart using the less sensitive radioligand binding technique (Pazos *et al*., 1985).

It could be argued that ketanserin might block other 5-HT receptors. Ketanserin has a pK_i of 5.45 for rat 5-HT_{2B} receptors (Wainscott *et al*., 1993) and a pK_i of 7.32 for rat 5-HT_{2C} receptors (Leonhardt *et al*., 1992). However, the concentration of 5 nM ketanserin used in some of the present experiments shifted the 5-HT concentration-effect curve to the right. Because 5 nM ketanserin would not be expected to block 5-HT_{2C} receptors the experiments provide functional evidence that 5-HT_{2A} receptors are involved in the 5-HT-induced positive inotropic effect of rat left atria. Moreover, Foguet *et al*. (1992) and Julius *et al*. (1988) were unable to detect the 5-HT_{2B} or 5-HT_{2C} receptor mRNA, respectively, in the rat heart.

The mRNA for 5-HT_3 receptors could be detected in mouse cardiac tissue (Maricq *et al*., 1991). Whether these receptors couple to inotropy is at least doubtful: we failed to detect any positive inotropic effect of up to 10 μM 5-HT in isolated mice left atria, whereas isoprenaline was effective in the same preparations (unpublished observations). In addition 5-HT_3 receptors have been defined by their insensivity to ketanserin and their high sensitivity to the antagonist tropisetron (Boess & Martin, 1994). However, 10 nM tropisetron (pK_i = 11 at 5-HT_3 receptors; rat vagus see Hoyer *et al*., 1994), which should increase the EC_{50} of 5-HT by a factor of about 1000 (MacKay, 1978), failed to increase the EC_{50} of 5-HT in rat atria (unpublished observations), whereas ketanserin completely blocked the effect (this study). Hence, it is unlikely that 5-HT_3 receptors are involved in the positive inotropic effect of 5-HT in rat atria. 5-HT_1 receptor mRNAs have not been demonstrated in the rat heart. However, we cannot exclude the involvement of as yet unidentified ketanserin 5-HT receptors in the positive inotropic effect of 5-HT.

The present work indicates that in contrast to pig and man (both 5-HT_4) there is a broad variability in the receptors which mediate the positive inotropic effect of 5-HT in inferior species. For instance, in rat this inotropic effect is mediated by 5-HT_{2A} receptors (this work), but in guinea-pig 5-HT_3 receptors have been found to be involved (Tramontana *et al*., 1993).

Another finding in the present study may cast doubt on the functional role of 5-HT_{2A} receptors: the expression of the 5-HT_{2A} receptor mRNA was not confined to the atria but was detectable in atria and ventricles. How this correlates with the absence of an inotropic effect of 5-HT in the ventricle is questionable. However, similar apparent discrepancies have been found before: like 5-HT, angiotensin II, endothelin and calcitonin gene-related peptide exert positive inotropic effects in human atria but not human ventricles (Du *et al*., 1994), although receptors have been detected on human ventricular myocytes (Molenaar, 1993). One could speculate that 5-HT_{2A} receptors might alter functions other than inotropy e.g. metabolism in the rat ventricle (Booz & Baker, 1996).

In conclusion, 5-HT exerts a positive inotropic effect in the rat atrium but not in ventricle. It is suggested that, in contrast to the human atrium, the positive inotropic effect in the rat atrium is not mediated via 5-HT_4 receptors but via a ketanserin-sensitive receptor likely to be the 5-HT_{2A} receptor.

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