CMLS, Cell. mol. life sci. 53 (1997) 263–266
1420-682X/97/030263-04 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

5-Hydroxytryptamine and β-adrenoceptors in rat isolated atria

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Received 30 September 1996; received after revision 15 November 1996; accepted 5 December 1996

Abstract. The positive chronotropic effect of a high concentration of 5-hydroxytryptamine (5-HT) in rat isolated atria results mainly from a tyramine-like mechanism and is linked to an increase in cAMP production by an indirect stimulation of β-adrenoceptors. Using this preparation, we have compared the action of tyramine and 5-HT. The tyramine (0.15 μM)-induced increase in atrial rate was suppressed by atenolol (a β_1-blocking drug) and by nadolol (a $\beta_1\beta_2$-blocker), while the positive chronotropic effect of 5-HT was reduced by atenolol and suppressed by nadolol. The 5-HT-induced elevation in cAMP was unchanged in the presence of atenolol and abolished by nadolol. The involvement of β_2-adrenoceptors in the effects of 5-HT could result from competition between 5-HT and noradrenaline at the β_1-adrenoceptors that results in a fixation of noradrenaline on β_2-adrenoceptors.
Key words. 5-Hydroxytryptamine; tyramine; rat isolated atria; β-adrenoceptors; cAMP.

The mechanisms of the 5-HT-induced increase in rat atrial rate in vitro have been recently studied and reported in several papers. When added to rat isolated atria, 5-HT induced a dose-dependent increase in atrial rate. Using ^{14}C-5-HT, we showed that at a high concentration (50 μM) 5-HT is taken up into sympathetic nerve terminals, since the uptake of the labelled amine was inhibited after nerve destruction by 6-hydroxydopamine and after the treatment of rats by reserpine, which inhibits the uptake of noradrenaline into its storage vesicles [1]. The 5-HT-induced increase in atrial rate was associated with a noradrenaline release measured in atria preloaded with ^{3}H-noradrenaline. Both effects were enhanced after MAO blockade by pargyline [2]. Lastly, the 5-HT-induced release in noradrenaline resulted in an increase in cAMP in atria following β-adrenoceptor stimulation [3]. Thus these findings as a whole demonstrate the tyramine-like mechanism of action of 5-HT in rat isolated atria.

This observation led us to go on with the comparison between the effects of tyramine and 5-HT. Their mechanism of action was studied using two β-blocking drugs, atenolol and nadolol, which are devoid of sympathomimetic activity and stabilizing membrane activity. They differ because atenolol is a selective β_1-adrenergic blocking drug, while nadolol is a nonselective $\beta_1\beta_2$-blocker. We measured their antagonistic activity against the chronotropic effect of tyramine and 5-HT and the 5-HT-induced increase in cAMP production.

Materials and methods

Rat isolated atria. Male Sprague-Dawley rats (Charles River, France), weighing 250–350 g, were killed by a blow on the head and exsanguinated.

Both atria were rapidly dissected out and set up in an organ bath which contained 4 ml of Krebs-Henseleit solution (KHS) with the following composition (mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.45, $NaHCO_3$ 25, KH_2PO_4 1, glucose 11.1, Na_2-EDTA 0.07, ascorbic acid 0.07 and atropine sulphate 0.7 μmol/l. The organ bath was kept at 37° and was constantly gassed with 5% CO_2 in O_2. A pause of 30 min preceded any experiment to allow atria to equilibrate under a tension of 1 g.

The isometric contractions of atria were recorded via a force transducer (Celaster) coupled with an ink recorder (Linseis L6012). Atrial rate was measured every min over a 10 min period in the absence or in the presence of different drugs.

cAMP assay. In the 5-HT groups, 5-HT (5, 10, 20 or 50 μM) was added to the organ bath fluid for a 5 min incubation.

Atenolol (1 μM) or nadolol (1 μM) was added for 15 min in the control groups, while 5-HT (10, 20 or 50 μM) was added 10 min after the β-blocking drug for a 5 min incubation in the treated groups. In all experiments, atria were immediately dipped into liquid nitrogen at the end of the incubation period, and stored frozen at −80°C. Before the assay the atria, still frozen, were weighed, mixed with 1 ml of perchloric acid 1.1 N and then ground (4°C, 30 s) and centrifuged (4000*g*, 4°C, 10 min). 500 μl of the supernatant were neutralized using a solution of potassium hydroxide 1 N and centrifuged again (4000*g*, 4°C, 10 min) to remove the potassium perchlorate precipitate. The supernatant was collected, appropriately diluted and assayed for cAMP using a radioimmunoassay kit (Immunotech, France).

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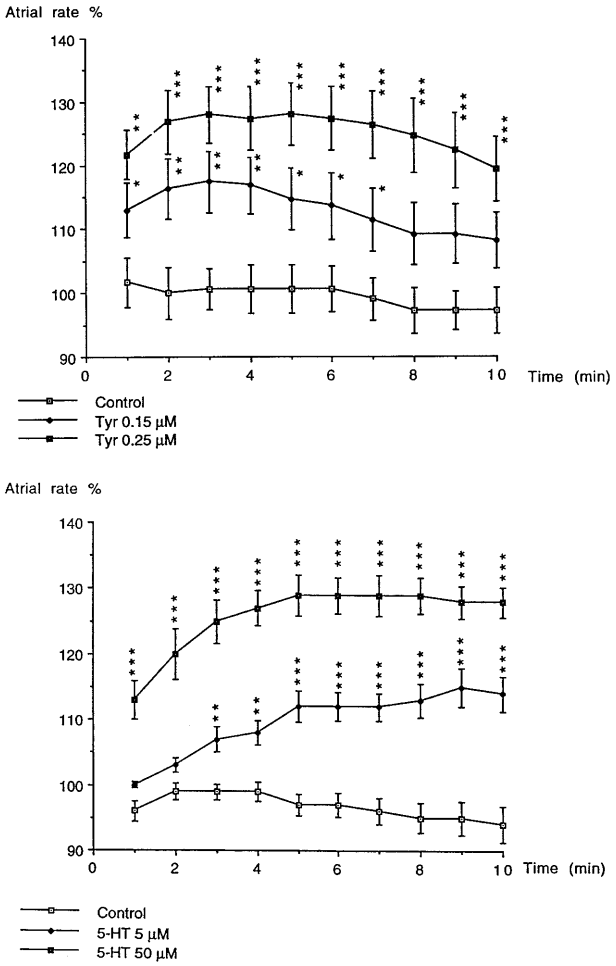

Figure 1. Time-course of the effect of tyramine (Tyr) (0.15 and 0.25 μM) and 5-HT (5 and 50 μM) on the rat atrial rate. Results are per cent of initial values. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control.

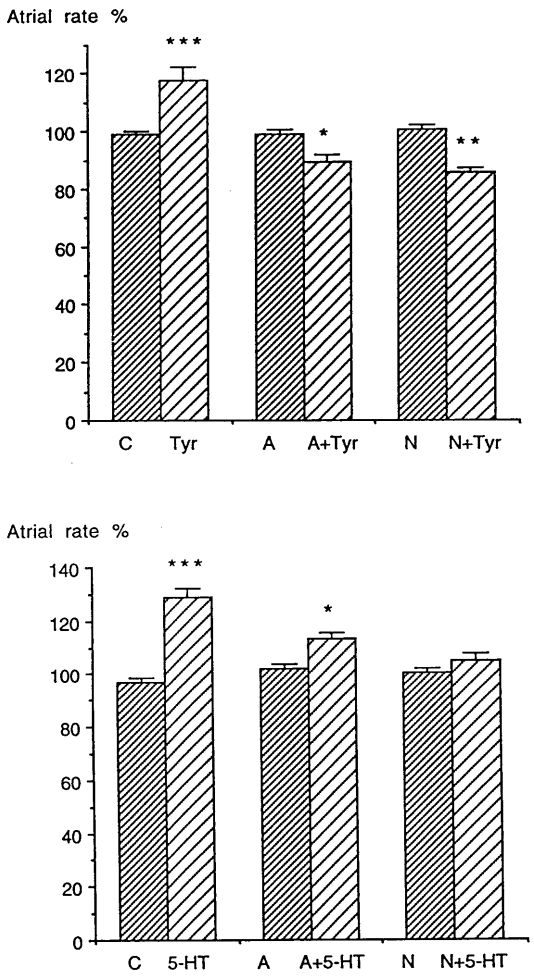

Figure 2. Effect of atenolol (A, 0.5 μM) and nadolol (N, 0.5 μM) on the maximal chronotropic effect of tyramine (Tyr, 0.15 μM) (upper panel) and of atenolol (A, 1 μM) and nadolol (N, 1 μM) on the maximal chronotropic effect of 5-HT (50 μM) (lower panel). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to the corresponding control without amine.

Chemicals. 5-hydroxytryptamine creatinine sulphate (Sigma), atenolol (I.C.I.), nadolol (Squibb).

Statistical analysis. Results are expressed as mean values ± SEM. Statistical significance was determined by Student's t-test for unpaired and paired data.

Results

Effects of β-blocking drugs on the chronotropic effect of tyramine and 5-HT. The basal atrial rate of contraction, when measured every min for 10 min in the absence of any drug, did not show any significant variation. The positive chronotropic effect of 0.15 μM and 0.25 μM tyramine was measured in the same way. The maximum increase appeared 3 min after tyramine addition and it reached 17% and 28% respectively ($n = 6$, $p < 0.01$), then the effect lessened. Likewise, the effect of 5-HT on the same preparation reached a plateau 5 min after 5-HT addition. The mean values of the maximum increases were 15% and 25% after 5 μM and 50 μM respectively ($n = 6$, $p < 0.001$) (fig. 1).

Atenolol (0.5 μM and 1 μM) or nadolol (0.5 μM and 1 μM) did not change basal atrial rate during the 5 min following their application.

Tyramine (0.15 μM) added 5 min after either β-blocking drug (0.5 μM, $n = 6$) exhibited no more tachycardic effect, and even a weak decrease in atrial rate was observed. By contrast, the chronotropic effect of 5-HT (50 μM) was reduced by about 55% after atenolol (1 μM) application and by 86% after nadolol (1 μM, $n = 6$) (fig. 2).

Effects of β-blocking drugs on the 5-HT-stimulated cAMP production. A 5 min period of incubation with 5-HT stimulated the cAMP production. The cAMP content increased by 71% with 5-HT 5 μM ($n = 5$, $P < 0.001$), by 93% with 5-HT 10 μM ($n = 6$, $p < 0.001$), by 71% with 5-HT 20 μM ($n = 6$, $p < 0.001$) and by 35% with 5-HT 50 μM ($n = 8$, $p < 0.05$). Atenolol (1 μM) and nadolol (1 μM) did not modify the resting content of

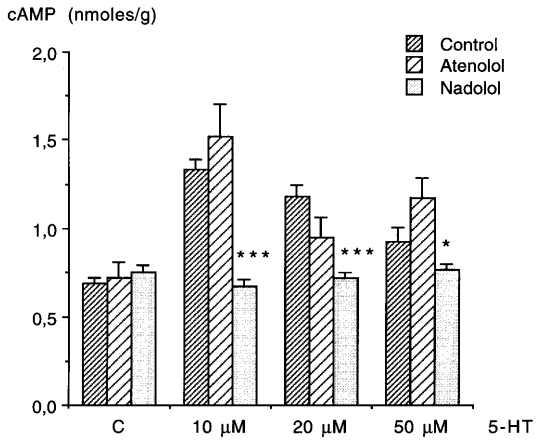

Figure 3. Effect of atenolol and nadolol on the 5-HT-stimulated cAMP production. In the C groups, KHS or atenolol (1 µM) or nadolol (1 µM) were added to the bathing fluid for 15 min. In the other groups , 5-HT (10, 20 or 50 µM) was added for 5 min after KHS, atenolol or nadolol. *$p < 0.05$, ***$p < 0.001$ compared to the corresponding 5-HT value.

cAMP (0.72 ± 0.03 nmol/g and 0.75 ± 0.04 nmol/g, respectively, no significant difference compared with the control group values, $n = 7$).

When atria were preincubated with atenolol (1 µM), the 5-HT (10, 20 or 50 µM)-induced increase in cAMP production was unchanged ($n = 7$). After nadolol (1 µM), 5-HT (10, 20 or 50 µM, $n = 7$) failed to increase the cAMP production (fig. 3).

Discussion

We have shown that 5-HT, like tyramine, induced a positive chronotropic effect in rat isolated atria. Much larger concentrations of 5-HT than of tyramine are needed and the 5-HT effect appears more slowly than tyramine's. The releasing action of tyramine on catecholamines was first reported many years ago [4]. An increase in contractility and in cAMP content in the rat isolated heart which results from this releasing action was also described [5]. The tyramine-like effect of 5-HT in the pithed rat was first suggested by Göthert et al. [6]; Docherty [7] then described it after high dose administration. Our studies in rat isolated atria have shown that 5-HT is taken up in the noradrenaline storage of this preparation [1], that high concentrations can release noradrenaline by a tyramine-like mechanism [2], and in this way can increase cAMP production [3].

However, after β-blockade, a difference appears between the two amines: while the effect of tyramine was abolished by a β_1-blocking drug, atenolol, and by a $\beta_1\beta_2$-blocking drug, nadolol, the chronotropic effect of 5-HT, suppressed by nadolol, was only reduced by atenolol. The observation that the 5-HT-induced increase in cAMP content of the atria was suppressed only by nadolol and not by atenolol supports this result. These two β-blocking drugs were chosen because they lack membrane stabilizing properties and partial agonist activity. Thus, 5-HT increases atrial rate and cAMP production by a mechanism different from β_1-adrenoceptor activation. The 5-HT chronotropic activity could result, beyond its tyramine-like effect, from an activation of postsynaptic receptors. However, among 5-HT receptors, only 5-HT_4, 5-HT_6 and 5-HT_7 are able to stimulate cAMP production, but they have never been detected in the heart of rats [8]. β_2-adrenoceptors are also linked to adenylate cyclase activation, and in this study catecholamines released by 5-HT seem to bind preferentially to β_2-adrenoceptors. This could be the consequence of the fixation of 5-HT to β_1-adrenoceptors. A fixation of 5-HT on β-adrenoceptors inducing blockade in a competitive manner has been shown by Fujita et al. [9]. The binding affinity of 5-HT at β-adrenoceptors is about 200 times lower than that of noradrenaline. However, taking into account the high concentration of 5-HT we used (50 µM), which is within the range used in Fujita's experiments, this binding can occur.

The relative density of β_1- and β_2-adrenoceptors in the rat heart has been investigated by some authors [10–13]. The β_2/β_1-adrenoceptor ratio reaches about 20% in the adult rat [14] and proportion of β_2 would be higher in atria than in ventricles [11, 12, 15] so that their effect on the chronotropic properties of the heart would be significant and greater than on its inotropism.

Our results suggest that in rat isolated atria, a high concentration of 5-HT can bind to β_1-adrenoceptors and inhibit their activation by noradrenaline. This leads to the preferential fixation on β_2-adrenoceptors of the noradrenaline released by a tyramine-like mechanism.

1 El Rawadi C., Heimburger M., Davy M., Midol-Monnet M. and Cohen Y. (1992) Uptake of 5-hydroxytryptamine in rat isolated atria. Gen. Pharmac. **23:** 613–617
2 El Rawadi C., Glondu M., Davy M., Midol-Monnet M. and Cohen Y. (1993) Mechanism of the chronotropic action and noradrenaline release induced by a high concentration of 5-hydroxytryptamine in the rat isolated atria. J. Auton. Pharmacol. **13:** 329–339
3 El Rawadi C., Davy M., Midol-Monnet M. and Cohen Y. (1994) Biochemical characterization of the mechanisms involved in the 5-hydroxytryptamine-induced increase in rat atrial rate. Biochem. Pharmacol. **48:** 683–688
4 Burn J. H. and Rand M. J. (1958) The action of sympathomimetic amines in animals treated with reserpine. J. Physiol. **144:** 314–346
5 Young B. A. and McNeill J. H. (1974) The effect of noradrenaline and tyramine on cardiac contractility, cyclic AMP, and phosphorylase a in normal and hyperthyroid rats. Can. J. Physiol. Pharmacol. **52:** 375–383
6 Göthert M., Schlicker E. and Kollecker P. (1986) Receptor-mediated effects of serotonin and 5-methoxytryptamine on noradrenaline release in the rat vena cava and in the heart of the pithed rat. Naunyn-Schiemedeberg's Arch. Pharmacol. **332:** 124–130
7 Docherty J. R. (1988) Investigations of cardiovascular 5-hydroxytryptamine receptor subtypes in the rat. Naunyn-Schiemedeberg's Arch. Pharmacol. **337:** 1–8
8 Hoyer D., Clarke D. E., Fozard J. R., Hartig P. R., Martin G. R., Mylecharane E. J. et al. (1994) International union of pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol. Rev. **46:** 157–203

9 Fujita R., Tamazawa Y., Barnard E. A. and Matsumoto M. (1993) Blocking effect of serotonin on β-adrenoceptor activity in follicle-enclosed *Xenopus* oocytes. Eur. J. Pharmacol. **240:** 213–217

10 Minneman K. P., Hedberg A. and Molinoff P. B. (1979) Comparison of *beta* adrenergic receptor subtypes in mammalian tissues. J. Pharmacol. Exp. Ther. **211:** 502–508

11 Hedberg A., Minneman K. P. and Molinoff P. B. (1980) Differential distribution of *beta*-1 and *beta*-2 adrenergic receptors in cat and guinea-pig heart. J. Pharmacol. Exp. Ther. **212:** 503–508

12 Molenaar P., Canale E. and Summers R. J. (1987) Autoradiographic localization of *beta*-1 and *beta*-2 adrenoceptors in guinea pig atrium and regions of the conducting system. J. Pharmacol. Exp. Ther. **241:** 1048–1064

13 Hedberg A., Kempf F. Jr., Josephson M. E. and Molinoff P. B. (1985) Coexistence of *beta*-1 and *beta*-2 adrenergic receptors in the human heart: effects of treatment with receptor antagonists or calcium entry blockers. J. Pharmacol. Exp. Ther. **234:** 561–568

14 Michel M. C., Wang X. L., Schlicker E., Göthert M., Beckeringh J. J. and Brodde O. E. (1987) Increased β_2-adrenoceptor density in heart, kidney and lung of spontaneously hypertensive rats. J. Autonom. Pharmacol. **7:** 41–51

15 Brodde O. E. (1987) Cardiac beta-adrenergic receptors. I. S. I. Atlas of Science: Pharmacol. **1:** 107–112

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