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BIOCHEMICAL CHARACTERIZATION OF THE MECHANISMS INVOLVED IN THE 5-HYDROXYTRYPTAMINE-INDUCED INCREASE IN RAT ATRIAL RATE

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Abstract—Several possible mechanisms for 5-hydroxytryptamine (5-HT)-induced tachycardia in rat have been suggested: an activation of 5-HT_{1C} or 5-HT_2 receptors, an indirect sympathomimetic effect or a mechanism independent of 5-HT_2 receptor stimulation. The aim of this study was to investigate the involvement of these mechanisms in the 5-HT-induced increase in rat atrial rate using biochemical methods. Indeed, the 5-HT_{1C} and 5-HT_2 receptors are linked to phosphoinositide hydrolysis and the noradrenaline (NA) released by 5-HT can stimulate the β_1-adrenergic receptors linked to adenylate cyclase stimulation. The effect of varying concentrations of 5-HT on inositol phospholipid hydrolysis and adenylate cyclase activity of the rat isolated atria were measured. 5-HT (2 μM) did not modify total inositol phosphate (IP) production, while 5-HT 10 and 50 μM increased it 2-fold. The 5-HT_2 antagonist ketanserin (1 μM) abolished IP accumulation induced by 5-HT (50 μM), which indicates that this accumulation is 5-HT_2 and not 5-HT_{1C} receptor-mediated. Moreover, cyclic AMP (cAMP) formation was enhanced by 5-HT (5, 10, 20 and 50 μM). When atria were incubated 10 min with the β-adrenergic receptor antagonist nadolol (1 μM), the increase in the cAMP level induced by 5-HT, whatever its concentration (10, 20 or 50 μM), was inhibited. Treating rats with reserpine (2.5 mg/kg, i.p., 48 and 24 hr before experimentation), which caused NA depletion in the heart, seemed to reduce the stimulating effect of 5-HT 10 and 50 μM on adenylate cyclase activity. Thus, the 5-HT-induced increase in cAMP is indirectly due to the activation of the β-adrenergic receptors by the NA released by 5-HT. It is concluded that 5-HT stimulates both phosphoinositide turnover and adenylate cyclase activity in the rat isolated atria by activation of 5-HT_2 receptors and by an indirect sympathomimetic effect.

Key words: 5-HT; rat atrial rate; inositol phosphate; cyclic AMP; indirect sympathomimetic effect; 5-HT_2 receptors

In the rat, several possible mechanisms for 5-HT†-induced tachycardia have been described: the indirect sympathomimetic effect [1–3], the activation of 5-HT_{1C} receptors [3] and the stimulation of 5-HT_2 receptors [2–4]. In contrast, other investigators using the 5-HT_2 receptor antagonist ketanserin concluded that these receptors are not involved in the tachycardiac response to 5-HT [5].

This group previously reported the chronotropic effect of 5-HT in the rat isolated atria [7]. The mean values of the maximal increases in atrial rate reached 15–25% in the presence of 5-HT, 5, 10, 20 and 50 μM. This group recently demonstrated that, at 50 μM, 5-HT is taken up into the NA storage vesicles within the sympathetic nerves of the rat isolated atria [6]. In addition, this group has also reported that, at the same concentration, the increase in atrial rate was associated with NA release [7]. Thus, the tyramine-like indirect sympathomimetic effect is a mechanism of the 5-HT-induced chronotropic effect at this concentration. The purpose of the present study, using a biochemical approach, was to confirm this mechanism and investigate if 5-HT_{1C} or 5-HT_2 receptors are also involved in the 5-HT effect. The 5-HT_{1C} and 5-HT_2 receptors are linked to phosphoinositide hydrolysis [8] and the NA released by 5-HT can stimulate the β-adrenergic receptors linked to adenylate cyclase stimulation [9]. Therefore, the effect of different concentrations of 5-HT on the formation of IP and adenylate cyclase activity in the rat isolated atria were measured. The inhibition of the 5-HT effects by antagonists was also investigated. Ketanserin and nadolol were used to block the 5-HT_2 and β-adrenergic receptors, respectively, while reserpine, which causes NA depletion in the heart, was used to inhibit the tyramine-like effect.

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 KHS of the following composition (mmol/L): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.45, $NaHCO_3$ 25, KH_2PO_4

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† Abbreviations: 5-HT, 5-hydroxytryptamine; NA, noradrenaline; IP, inositolphosphates; IP_1, inositolmonophosphate; IP_2, inositoldiphosphate; IP_3, inositoltriphosphate; cAMP, cyclic AMP; KHS, Krebs–Henseleit solution.

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 rest of 30 min preceded any experimentation to allow atria equilibration under a tension of 1 g.

Labelling and accumulation of [^{3}H]*IP*. Atria were incubated with *myo*-[2-^{3}H]inositol (122 kBq/mL, 0.175 μM) for 30 min. Labelled atria were subsequently washed four times with fresh KHS without [^{3}H]inositol. In the control group, LiCl (final concentration 10 mM) was added to the organ bath fluid for a 30 min incubation in order to inhibit myoinositol phosphatase [10]. In the 5-HT groups, 5-HT (final concentration 2, 10 or 50 μM) was added to the organ bath fluid 10 min after LiCl affusion, for a 20 min incubation. In the ketanserin group, the experimental procedure was the same as in the 5-HT (50 μM) group but ketanserin (final concentration 1 μM) was added at the same time as LiCl. At the end of the incubation period atria were washed in ice-cold KHS, weighed and transferred into tubes containing 675 μL of chloroform–methanol–10 M HCl (100:200:1, by vol.).

Extraction and chromatographic separation of [^{3}H]*IP* [11]. Atria were homogenized after addition of 180 μL of 5 mM EDTA to the chloroform–methanol mixture. The homogenizer tip was rinsed with 570 μL of another mixture of chloroform–methanol–10 M HCl–5 mM EDTA (100:200:1:80). The rinsing fluid, chloroform (750 μL) and 5 mM EDTA (75 μL), was added to the homogenate. After centrifugation (1600 *g*, 20 min) an aliquot (650 μL) of the supernatant was neutralized with 2.2 mL of 6.25 mM sodium borate and applied to a 1 mL AG1-X8 column (formate form, 200–400 mesh, Bio-Rad Laboratories). The column was then washed with 20 mL of 0.1 M formic acid. The IP were eluted sequentially by addition of 10 mL of 0.2 M ammonium formate–0.1 M formic acid (for IP_1), 10 mL of 0.5 M ammonium formate–0.1 M formic acid (for IP_2) and 10 mL of 1 M ammonium formate–0.1 M formic acid (for IP_3). The radioactivity of the three eluent samples was counted.

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. Nadolol (1 μM) was added for 15 min in the nadolol group, while 5-HT (10, 20 or 50 μM) was added 10 min after nadolol (1 μM) for a 5 min incubation in the nadolol + 5-HT group. In reserpine-treated rats (2.5 mg/kg, i.p., 48 and 24 hr before experimentation) the incubation conditions were the same as in the groups of untreated rats. In all experiments atria were immediately dipped into liquid nitrogen at the end of the incubation period and stored frozen at −80°. Before the assay the still frozen atria were weighed, mixed with 1 mL of perchloric acid 1.1 N and then ground (4°, 30 sec) and centrifuged (4000 *g*, 4°, 10 min). Five hundred microlitres of the supernatant were neutralized using a solution of potassium hydroxide 1 N and centrifuged again (4000 *g*, 4°, 10 min), to discard the potassium perchlorate precipitate. The supernatant was collected, appropriately diluted and assayed for cAMP using a radioimmunoassay kit (Immunotech, France).

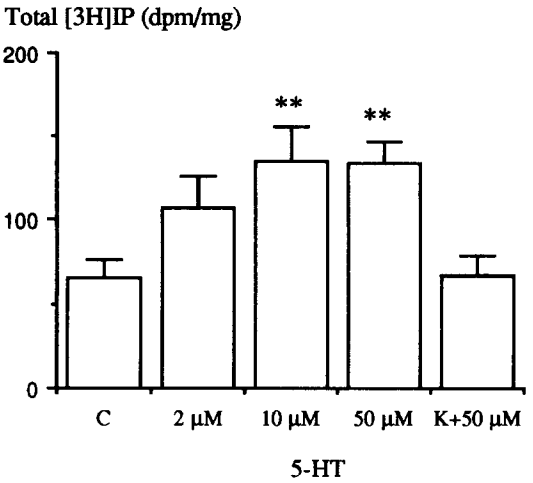

Fig. 1. Antagonism by ketanserin of the 5-HT-induced accumulation of [^{3}H]IP in rat atria. In the control group (C) LiCl (10 mM) was added to the organ bath fluid for a 30 min incubation. In the 5-HT groups, 5-HT (2, 10 or 50 μM) was added 10 min after LiCl affusion, for a 20 min incubation. For the ketanserin group (K +50 μM), ketanserin (1 μM) and LiCl (10 mM) were added 10 min before 5-HT (50 μM). Data are means ±SEM, N = 5–7. **P < 0.01 significantly different compared to control group values.

Chemicals. *Myo*-[2-^{3}H]inositol (7×10^8 kBq/mmol, Amersham, U.K.). 5-Hydroxytryptamine creatinine sulphate (Sigma Chemical Co., St Louis, MO, U.S.A.), ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium), nadolol (Bristol-Myers Squibb, Paris-la Defeuse, France), reserpine (Ciba-Geigy AG, Basel, Switzerland).

Statistical analysis. Results are expressed as mean values ± SEM. Analysis of variance followed by the Scheffé test was used for multiple comparisons. The results were analysed according to the Student's *t*-test when only two groups were compared.

RESULTS

5-*HT-stimulated phosphoinositide metabolism*

The effect of a 20 min incubation of atria with three different concentrations of 5-HT (2, 10 and 50 μM) on IP formation was examined. 5-HT (2 μM, N = 5) did not modify the total [^{3}H]IP values compared with the control group (N = 7), while 5-HT (10 μM, N = 5) and 5-HT (50 μM, N = 5) increased it 2-fold (P < 0.01). When ketanserin (1 μM, N = 5) was added 10 min before 5-HT (50 μM) no modification in the total [^{3}H]IP level was observed compared with the control group (Fig. 1).

In all groups, [^{3}H]IP_3 as compared to [^{3}H]IP_2 and [^{3}H]IP_1, represented a small fraction of the total [^{3}H]IP accumulated in atria. Thus, the [^{3}H]IP_3 values ranged between 4.3 and 7.9% of total [^{3}H]IP, while the [^{3}H]IP_2 and [^{3}H]IP_1 values reached 21.1–29.9% and 62.1–74.1%, respectively.

5-*HT-stimulated cAMP production*

The resting content of cAMP in atria (control, N = 5) was 0.69 ± 0.03 nmol/g. A 5 min period of incubation with 5-HT stimulated cAMP production.

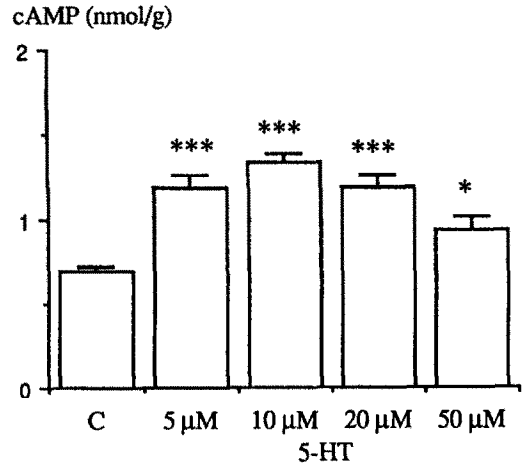

Fig. 2. Stimulation of adenylate cyclase activity by 5-HT. Rat atria were incubated 5 min with 5-HT (5, 10, 20 or 50 μM). Results are mean values ±SEM, N = 5–8. *P < 0.05, **P < 0.001 compared to control group (C).

cAMP content reached 1.18 ± 0.07 nmol/g with 5-HT 5 μM (N = 5, P < 0.001), 1.33 ± 0.06 nmol/g with 5-HT 10 μM (N = 6, P < 0.001), 1.18 ± 0.07 nmol/g with 5-HT 20 μM (N = 6, P < 0.001) and 0.93 ± 0.08 nmol/g with 5-HT 50 μM (N = 8, P < 0.05). Thus, the 5-HT-induced increase in cAMP was 71, 93, 71 and 35% with 5, 10, 20 and 50 μM, respectively (Fig. 2).

Nadolol (1 μM) did not modify the resting content of cAMP (0.75 ± 0.04 nmol/g, no significant difference compared with the control group values, N = 7). When atria were pre-incubated with nadolol (1 μM), 5-HT (10, 20 or 50 μM, N = 7) failed to increase cAMP production. Thus, cAMP values in the nadolol +5-HT group were not significantly different from those in the nadolol group (Fig. 3).

The treatment with reserpine (2.5 mg/kg, i.p., 48 and 24 hr before experiments) increased the cAMP level in atria from 0.69 ± 0.03 nmol/g in the control group (N = 5) to 1.22 ± 0.07 nmol/g in the reserpine group (N = 6) (P < 0.001) (Fig. 4a). In atria from reserpine-treated rats a 5 min incubation with 5-HT (10 μM, N = 6) and with 5-HT (50 μM, N = 6) increased cAMP values to 1.36 ± 0.02 nmol/g (P < 0.05 when compared with reserpine group values) and 1.48 ± 0.03 nmol/g (P < 0.01), respectively (Fig. 4b). This increase was less important than in atria from untreated rats: 11 and 21%, respectively, at 10 and 50 μM in the reserpine-treated groups vs 93 and 35% in the untreated groups, as indicated above (see Fig. 2).

DISCUSSION

5-HT stimulates inositol phospholipid hydrolysis in many tissues. This effect is mediated by 5-HT_2 receptor stimulation in rat aorta [12–15], rat jugular vein [16], rat cerebral cortex [17–19], dog trachea [20] and human and rabbit platelets [21, 22]; it is mediated by 5-HT_{1C} receptor in the choroid plexus [23]. The effect of varying concentrations of 5-HT on the [^{3}H]IP formation were measured in the rat isolated atria. Under these experimental conditions, accumulation of IP_3 compared to that of IP_2, and in particular IP_1, constituted a small fraction of total IP. However, this does not reflect the total amount of the IP_3 generated because IP_3 is rapidly hydrolysed into IP_2 and then into IP_1. Further hydrolysis of IP_1 was inhibited by LiCl [10]. Thus, it was considered that the values of total [^{3}H]IP instead of [^{3}H]-IP_3 should be used to discuss the current results [24]. Under these conditions, 5-HT 2 μM failed to increase

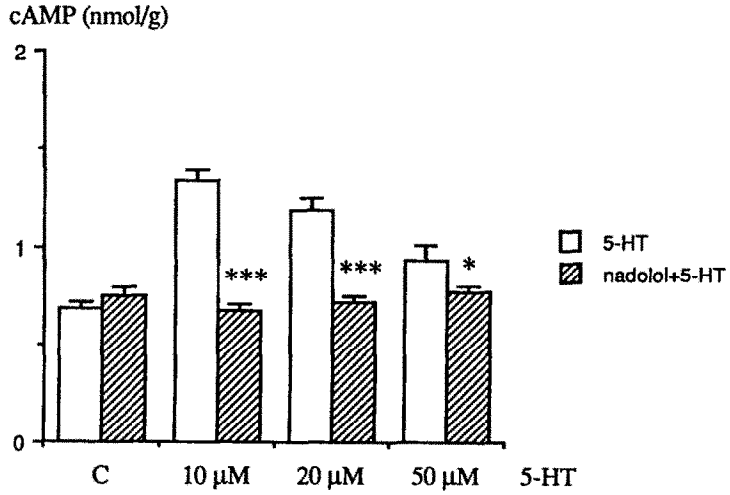

Fig. 3. Effect of nadolol on 5-HT-stimulated cAMP production. Nadolol (1 μM) or KHS was added to the rat atria bathing fluid for 15 min in the control groups (C). Nadolol (1 μM) was added for 10 min followed by 5-HT (10, 20 or 50 μM) for an additional 5 min incubation. Data are means ±SEM, N = 7. *P < 0.05, ***P < 0.001 compared with the corresponding 5-HT group value.

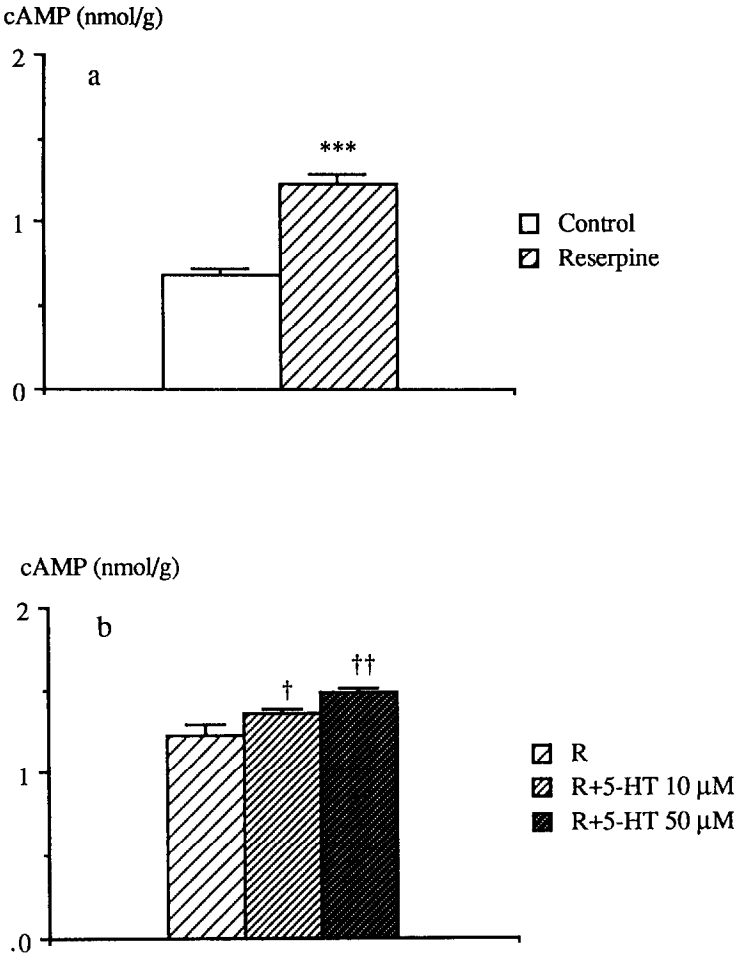

Fig. 4. Effect of reserpine on resting cAMP (Panel a) and on 5-HT-stimulated cAMP production (panel b). The cAMP level was measured in atria from control rats and in atria from reserpine-treated rats (2.5 mg/kg, i.p., 48 and 24 hr before experiments) (R). The effect of a 5 min incubation of atria from reserpine-treated rats with 5-HT (10 μM) or 5-HT (50 μM) was measured. Values are means ±SEM, N = 5 or 6. ***P < 0.001 compared to C group values. †P < 0.05, ††P < 0.01 compared to R group values.

total [^{3}H]IP production, while 5-HT 10 μM and 50 μM increased it by the same amount. The 5-HT$_2$ antagonist ketanserin completely blocked the total [^{3}H]IP accumulation induced by 5-HT 50 μM. The affinity values of ketanserin for 5-HT$_{1C}$ and 5-HT$_2$ recognition sites, as expressed by pK_D, were 7 and 8.9, respectively [25]. These results indicate that activation of the inositol phospholipid hydrolysis by 5-HT in rat isolated atria is 5-HT$_2$ and not 5-HT$_{1C}$ receptor mediated. Thus, 5-HT$_2$ receptors are activated by 5-HT at the concentration of 50 μM, known to induce an increase in atrial rate and in NA release [7]. α_1-adrenoceptors, like 5-HT$_2$ receptors, promote an acceleration of phosphoinositide metabolism in the cardiac muscles [26–28] and are blocked by ketanserin [29, 30]. This compound is also an α_1-adrenergic antagonist [29, 30], capable of blocking α_1-adrenoceptors at 1 μM [31]. However, the possibility of activation by NA released by 5-HT can be ruled out, because they are not functional when β-adrenoceptors are activated [32–35].

5-HT induces a small rise in cAMP in rat heart slices, indirectly mediated by the increase in NA release [36]. In contrast, 5-HT does not increase cAMP production in isolated cardiac myocytes [37]. In the present study, 5-HT 5, 10, 20 and 50 μM stimulated adenylate cyclase activity in rat isolated atria: this effect was less important at the concentration of 50 μM. This decrease in response with increasing concentration has been reported with several amines, such as 5-HT, NA and tyramine, on rat heart slices [36]. It may be suggested that a large quantity of released NA can activate α_2-adrenoceptors and/or that 5-HT activates 5-HT$_1$-like receptors, which are both negatively linked to adenylate cyclase. The activation of adenylate cyclase by any concentration of 5-HT was abolished when the atria were pre-incubated with the $\beta_1\beta_2$-adrenergic receptor antagonist nadolol (1 μM). These results

demonstrate that the increase in cAMP production is indirectly induced by 5-HT via the release of NA and the subsequent stimulation of the β-adrenergic receptors coupled to adenylate cyclase. The results obtained in atria from rats pre-treated with reserpine, which depletes NA from neuronal terminals, are somewhat difficult to discuss due to the large increase in cAMP after administration of this drug. None the less, it may be observed that, if not reduced in absolute value, the stimulating effect of 5-HT 10 and 50 μM on adenylate cyclase activity is relatively weaker in reserpinized rats than in control rats. The remaining effect of 5-HT could be related to the small amount of NA (~0.40 pmol/mg of tissue) which remains in atria of reserpine-treated rats [6]. This NA could be released by 5-HT and activate the β-adrenergic receptors.

In summary, the results of the present study show that 5-HT activates phosphoinositide metabolism. This activation is exclusively 5-HT_2 mediated at a concentration of 50 μM. Moreover 5-HT_2 induces an increase in cAMP production which is indirectly caused by the interaction between the NA released by 5-HT and the β-adrenergic receptors.

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