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DISSOCIATION OF 5-HYDROXYTRYPTAMINE EFFECTS ON MYOCARDIAL CONTRACTILITY AND CYCLIC AMP ACCUMULATION

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1 5-Hydroxytryptamine increased the contractility of the isolated rat atrium and produced a small rise in cyclic 3′,5′-adenosine monophosphate (cyclic AMP) accumulation in rat heart slices. These effects were similar to those of tyramine.

2 Incubation with phenoxybenzamine prevented the inotropic effect of 5-hydroxytryptamine but did not inhibit the rise in cyclic AMP accumulation.

3 A single injection of reserpine 24 h before the experiment did not inhibit the inotropic effect of 5-hydroxytryptamine but prevented the rise in cyclic AMP accumulation.

4 It is concluded that in the rat 5-hydroxytryptamine increases cardiac contractility chiefly by a direct effect which is blocked by phenoxybenzamine, whereas it increases myocardial cyclic AMP accumulation indirectly by releasing endogenous catecholamines. Cardiac contractility may thus be increased without a rise in cyclic AMP accumulation, and cyclic AMP accumulation may be increased without a rise in contractility.

Introduction

The effects of 5-hydroxytryptamine on contractility and rate of the mammalian heart have received little attention, and data in the literature are scanty. The intravenous infusion of 5-hydroxytryptamine in man directly increases the heart rate (LeMessurier, Schwartz & Whelan, 1959). Species variations have been noted in the cardiac responses to 5-hydroxytryptamine of the dog, cat, guinea-pig and rabbit (Schneider & Yonkman, 1954; Trendelenburg, 1960). The rat heart has not been studied.

We have found that 5-hydroxytryptamine is an effective positive inotropic agent in the rat isolated atrium, and we have investigated the correlation of the inotropic effect with the stimulation of the enzyme adenylate cyclase. Effects of 5-hydroxytryptamine on myocardial adenylate cyclase have not been studied before. 5-Hydroxytryptamine increases the level of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) in certain tissues and some positive inotropic agents are said to owe their effect to the stimulation of myocardial adenylate cyclase (Robison, Butcher & Sutherland, 1971).

In continuation of previous studies on the correlation of inotropic drug effects and cyclic AMP accumulation (Benfey, 1971; Benfey, Kunos & Nickerson, 1973), we have found that effects of 5-hydroxytryptamine on cardiac contractility and cyclic AMP accumulation can be separated.

Methods

Contractility

Male Sprague-Dawley rats of 180-300 g weight were anaesthetized with ether, the hearts were quickly removed and the left atria were suspended in a solution containing (mM) NaCl 115.3, KCl 4.6, $CaCl_2$ 1.8, $MgSO_4$ 1.1, $NaHCO_3$ 22.1, KH_2PO_4 1.1 and glucose 11.1 which was kept at 31°C and aerated with 5% CO_2 in oxygen. The atria were driven by a Grass stimulator (model SD5) at 1 Hz with 3 ms square-wave pulses at a voltage slightly above threshold (3-6 V). Isometric contractions were recorded with a force-displacement transducer (Grass FT 83C) and a polygraph (Grass model 5). The positive inotropic agents were added cumulatively.

Reserpine was injected intraperitoneally in a single dose of 5 mg/kg 24 h before the experiment or in a daily dose of 0.5 mg/kg on seven days. 6-Hydroxydopamine was injected intravenously twice within 24 h in a dose of 50 mg/kg and a week later twice within 24 h in a dose of

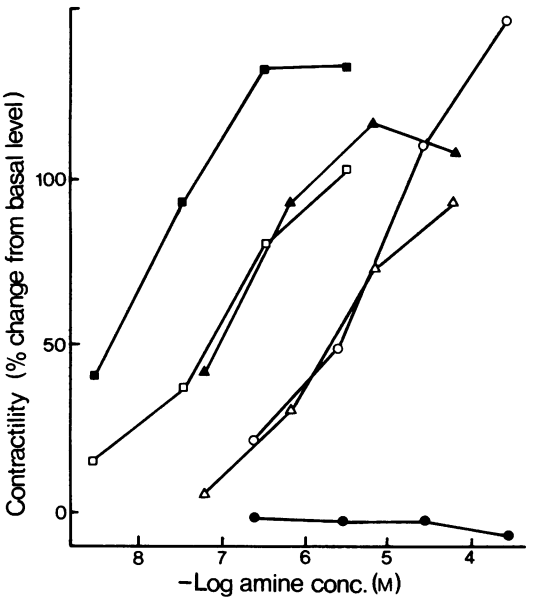

Fig. 1 Effects of 5-hydroxytryptamine (○), noradrenaline (□) and tyramine (△) on contractility of the rat isolated artrium. Solid symbols: after 7.4 μM phenoxybenzamine treatment. Means of 4-17 experiments.

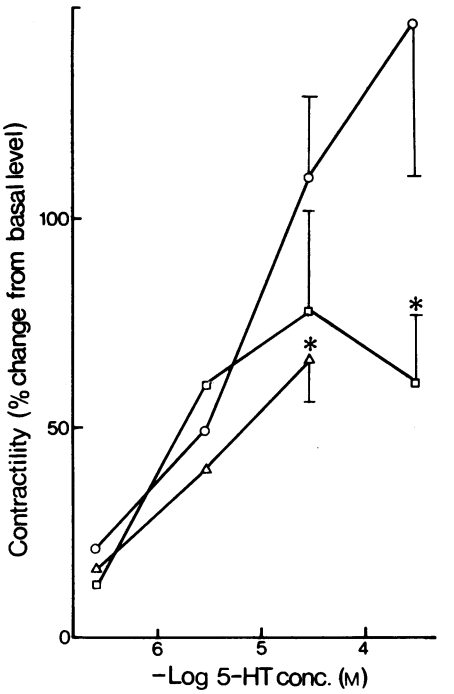

Fig. 2 Effects of 5-hydroxytryptamine (5-HT) on contractility of the rat isolated atrium before (○) and after treatment with reserpine (△; seven daily injections) and 6-hydroxydopamine (□). Means ± s.e. of 4-16 experiments. * $P < 0.05$, compared to control (no pretreatment).

100 mg/kg; the experiment was done 4-8 days after the last injection. Exposure to phenoxybenzamine was for 40 min, the solution being changed every 10 min, and the preparation was then washed repeatedly for 1-2 h before the inotropic agent was added. Exposure to the other drugs was for 10 min and they remained in the bath.

Cyclic AMP accumulation

Ventricle slices from male Sprague-Dawley rats anaesthetized with ether and from guinea-pigs killed by a blow on the neck were prepared with a Stadie-Riggs tissue slicer and suspended in a solution containing (mM) NaCl 137, $CaCl_2$ 1.8, KCl 2.68, $NaHCO_3$ 11.9, NaH_2PO_4 0.362, glucose 5.55, and disodium edetate (EDTA) 0.01 which was kept at 37° C and aerated with 5% CO_2 in oxygen.

After preincubation with 2 μCi [^{14}C]-adenine in a metabolic shaker for 60 min, the medium was replaced four times with fresh medium containing 6.7 mM theophylline and the inotropic agents were then added for 15 minutes. Exposure to phenoxybenzamine and the other drugs was for 60 minutes. Phenoxybenzamine was removed from the bath before addition of the inotropic agents and the other drugs remained in the bath. The reserpine and 6-hydroxydopamine pretreatment was the same as that for the contractility experiments.

After incubation with the inotropic agents the slices were placed in 1 ml 6% trichloroacetic acid (TCA) containing 50 μg carrier cyclic AMP and 0.1 μCi [^{3}H]-cyclic AMP, homogenized with a tissue grinder, and the homogenate centrifuged for 10 min at 2,000 *g*. The supernatant was shaken once with benzene and twice with ether, the organic phase discarded and the aqueous phase purified by column chromatography on Dowex 50 and negative adsorption on nascent $BaSO_4$ (Krishna, Weiss & Brodie, 1968). Radioactivity was counted in a Picker 330 liquid scintillation counter using standard methods of double-isotope counting with external standardization. The counting data were processed by a PDP 8L computer (Digital Equipment Corp.) which was programmed to compute d/min for both isotopes. All

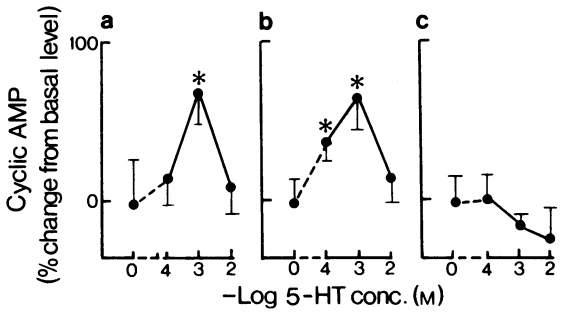

Fig. 3 Effects of 5-hydroxytryptamine (5-HT) on cyclic AMP accumulation in rat heart slices. (a) Control; (b) following treatment with 74 μM phenoxybenzamine; (c) following reserpine treatment (5 mg/kg injected 24 h earlier). Means ± s.e. of 4-18 incubations. * $P < 0.05$, compared to control (no 5-hydroxytryptamine).

^{14}C counts were corrected to 100% on the basis of the tritium recovered. Mean recovery was 40%.

We have used the prelabelling method for the study of changes in myocardial cyclic AMP levels before (Benfey, 1971; Benfey *et al.*, 1973) and its validity has been confirmed. Lee, Kuo & Greengard (1971) compared the prelabelling method in rat heart slices with the protein kinase method which measures total cyclic AMP and concluded that the ATP newly synthesized from the radioactive adenine was in equilibrium with the existing pool used for the production of cyclic AMP.

Protein was determined by the biuret method (Kabat & Mayer, 1961). One rat or guinea-pig ventricle slice (mean protein content 14.3 and 19.7 mg, respectively) was used for each incubation.

The mean basal levels of cyclic AMP were: rat; 29.5 d/min per mg protein, and guinea-pig; 56.4 d/min per mg protein. Drug treatment did not significantly change these values.

The statistical calculations were made according to conventional procedures (Mainland, 1952).

Drugs and chemicals

These included (−)-noradrenaline bitartrate (Winthrop), tyramine hydrochloride (Eastman), 5-hydroxytryptamine creatine sulphate (Sigma), 6-hydroxydopamine hydrobromide (Regis), reserpine (Serpasil, Ciba), phenoxybenzamine hydrochloride (Smith, Kline & French), dihydroergotamine methane sulphonate (Sandoz), morphine sulphate, cocaine hydrochloride, AG 50W-X4 (200-400 mesh, Bio-Rad Labs.), cyclic 3′,5′-AMP, cyclic [^{3}H]-AMP (sp. act. 16.3 Ci/mmol) and [^{14}C]-adenine (sp. act. 52-58 mCi/mmol, Schwarz BioResearch).

Results

Contractility

5-Hydroxytryptamine increased the contractility of the rat isolated atrium (Figure 1). The efficacy of 5-hydroxytryptamine was similar to that of noradrenaline and tyramine and its potency was approximately four times lower than that of tyramine and 100 times lower than that of noradrenaline (Figure 1). Phenoxybenzamine (7.4 μM) completely inhibited the inotropic effect of 5-hydroxytryptamine and potentiated the effects of noradrenaline and tyramine (Figure 1).

Reserpine (seven daily injections) and 6-hydroxydopamine pretreatment significantly reduced the inotropic effect of 5-hydroxytryptamine (Figure 2). A single injection of reserpine, 5 mg/kg, 24 h earlier did not inhibit the inotropic effect of 5-hydroxytryptamine. In additional experiments it was found that cocaine (59 μM) and dihydroergotamine (15 μM), but not morphine (266 μM), inhibited the inotropic effect of 5-hydroxytryptamine.

Cyclic AMP accumulation

5-Hydroxytryptamine increased cyclic AMP accumulation in rat heart slices, and this effect was inhibited by a single injection of reserpine 24 h earlier, but not by phenoxybenzamine (74 μM) pretreatment (Figure 3).

The effect of tyramine on cyclic AMP accumulation was similar to that of 5-hydroxytryptamine and much smaller than that of noradrenaline; phenoxybenzamine did not inhibit the effect of tyramine on cyclic AMP accumulation (Figure 4). In additional experiments it was found that 6-hydroxydopamine pretreatment, cocaine (59 μM) and dihydroergotamine (15 μM), but not morphine (266 μM), inhibited the effect of 5-hydroxytryptamine on cyclic AMP accumulation.

In guinea-pig heart slices, 5-hydroxytryptamine did not significantly increase myocardial cyclic AMP accumulation and tyramine produced a small rise which was inhibited by phenoxybenzamine (Figure 5).

Discussion

The inotropic efficacy of 5-hydroxytryptamine in the rat atrium was similar to that of noradrenaline.

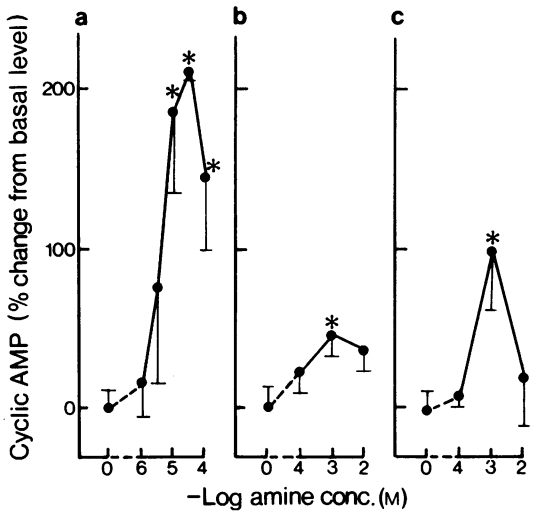

Fig. 4 Effects of noradrenaline (a), tyramine (b), and tyramine following phenoxybenzamine (74 μM) treatment (c) on cyclic AMP accumulation in rat heart slices. Means ± s.e. of 4-18 incubations. * $P < 0.05$, compared to control (no inotropic agent).

The same result was obtained in the atrium of the guinea-pig (Greeff, Benfey & Bokelmann, 1959; Trendelenburg, 1960), cat and rabbit (Trendelenburg, 1960).

Repeated injections of reserpine were needed for a partial inhibition of the inotropic effect of 5-hydroxytryptamine which indicates that a minor part of the inotropic effect of 5-hydroxytryptamine is due to the release of endogenous catecholamines. The interaction of reserpine and 5-hydroxytryptamine is species dependent: reserpine pretreatment inhibits the inotropic effect of 5-hydroxytryptamine on the rabbit atrium, but not on the guinea-pig atrium (Trendelenburg, 1960).

Phenoxybenzamine treatment completely inhibited the inotropic effect of 5-hydroxytryptamine on the rat atrium. As phenoxybenzamine inhibits the inotropic effect of tyramine on the guinea-pig atrium (Benfey & Greeff, 1961), we studied tyramine in the rat heart. Unlike 5-hydroxytryptamine, tyramine is known to exert its inotropic effect in all animal species studied, chiefly through the release of endogenous catecholamines.

The effect of tyramine on contractility of the rat atrium was similar to that of 5-hydroxytryptamine but was not inhibited by phenoxybenzamine. Thus, despite the fact that it did not inhibit the inotropic effect of tyramine which chiefly acts by releasing catecholamines, phenoxybenzamine blocked the inotropic effect of 5-hydroxytryptamine, although a minor part of the inotropic effect of 5-hydroxytryptamine appeared to be due to the release of endogenous catecholamines.

In contrast to the inotropic effect, the effect of 5-hydroxytryptamine on cyclic AMP accumulation in rat heart slices appeared to be due entirely to the release of endogenous catecholamines, as it was absent after a single injection of reserpine. Furthermore, phenoxybenzamine treatment did not inhibit the effect of 5-hydroxytryptamine on cyclic AMP accumulation.

It may be concluded from these results in the rat heart that following reserpine treatment

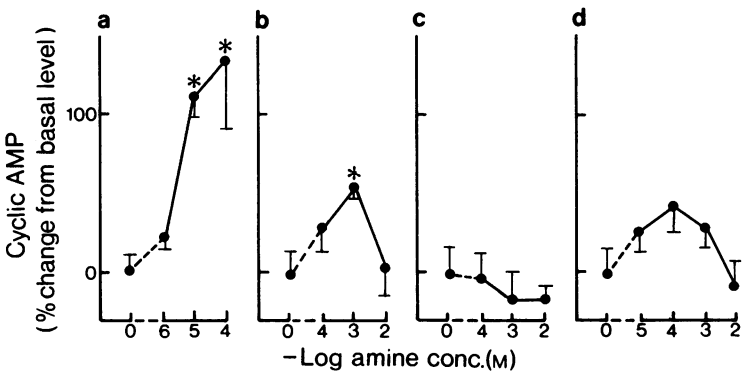

Fig. 5 Effects of noradrenaline (a), tyramine (b), tyramine after phenoxybenzamine (74 μM) treatment (c), and 5-hydroxytryptamine (d) on cyclic AMP accumulation in guinea-pig heart slices. Means ± s.e. of 4-15 incubations. * $P < 0.05$, compared to control (no inotropic agent).

5-hydroxytryptamine can raise cardiac contractility without increasing cyclic AMP accumulation, and that following phenoxybenzamine treatment 5-hydroxytryptamine can increase cyclic AMP accumulation without increasing contractility.

Cocaine and dihydroergotamine inhibited the effects of 5-hydroxytryptamine on contractility and cyclic AMP accumulation of the rat heart. Similarly, cocaine and lysergic acid diethylamide inhibit the inotropic effect of 5-hydroxytryptamine on the cat, guinea-pig and rabbit atrium (Trendelenburg, 1960). Morphine was ineffective in the rat heart; it inhibits the inotropic effect of 5-hydroxytryptamine on the guinea-pig and rabbit atrium, but not the cat atrium (Trendelenburg, 1960).

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