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DIFFERENTIAL ACTIONS OF 5-HYDROXYTRYPTAMINE ON THE ISOLATED RAT RIGHT AND LEFT ATRIA

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Abstract

The positive chronotropic and inotropic effects of 5-hydroxytryptamine (5-HT) on the isolated rat right and left atria respectively have been investigated in the presence of 5-HT antagonists, i.e., methysergide and cyproheptadine, and beta-adrenergic blocking agent, i.e., propranolol. Methysergide (0.47 µg/ml) or cyproheptadine (0.02 µg/ml) reduced the positive chronotropic effect of 5-HT (2.0 µg/ml) on the right atria to about 50% of controls, but completely abolished the positive inotropic effect of 5-HT on the left atria. Propranolol (0.15 µg/ml) attenuated the positive chronotropic effect of 5-HT similar to those of methysergide and cyproheptadine. However, propranolol did not reduce the positive inotropic effect induced by 5-HT on the left atria. Combinations of 5-HT antagonists with beta blocker totally inhibited the positive chronotropic effect. The positive chronotropic response of the isolated right atria from reserpinized rats was also abolished by either methysergide or cyproheptadine alone.

It is concluded that in the rats the effect of 5-HT on the right atrial rates is mediated by the combination of the direct effect on 5-HT receptor and indirect effect through catecholamine release from the intra-cardiac stores. In contrast, the positive inotropic effect on the left atria is due to a direct action of 5-HT and does not involve endogenous catecholamine release.

5-HT or serotonin has a well-documented effect on the cardiovascular system (1). It has been proposed that 5-HT has different mode of actions on isolated cardiac tissues depending on animal species. In

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case of rabbit (2,3) and dog (4) the cardiac stimulating effect of 5-HT appears to be mediated by endogenous catecholamine release from intracardiac store. However, in the cat and guinea-pig, a different mechanism has been suggested. In these species the mechanism is believed to involve direct stimulation of the specific serotonergic receptors on the cardiac cells (2). The present study was undertaken to assess the mechanisms of the chronotropic and inotropic effects of 5-HT on the isolated rat right and left atria, respectively. The use of the right atria for chronotropic stuay and the left atria for inotropic study eliminate any possible interference that changes in rate may have on contractile force. To our knowledge there has been no reported study concerning the effect of 5-HT on the separated right and left atria isolated from the rats. The results presented below show that 5-HT acts differently to increase the rate and isometric force of the isolated rat right and left atria, respectively.

METHODS

PREPARATIONS OF ISOLATED RAT ATRIA

Male Wistar rats weighing 250-300 g were killed by blowing on the heads. The heart was quickly excised and placed in a petri-dish containing oxygenated Locke solution (of the following composition, in mM : NaCl 155.8; $CaCl_2$ 2.15; KCl 5.6; $NaHCO_3$ 1.8 and glucose 5.0). The atria were dissected out and cut into right and left sides. They were then transferred into the organ baths containing 25 ml Locke solution continuously bubbled with 100% oxygen and maintained at 37°C. Each preparation was applied a 1 g preload. The right atria which beated spontaneously were allowed to equilibrate until stable rate was obtained. The left atria were electrically stimulated with square wave pulse (5 V strength and 5 msec duration) to beat at a constant rate of 250/min. The tissues were allowed to equilibrate until the contractile force was stable. The rate and isometric tension were recorded with isometric

force transducer (Grass FT 03 C) connected to a recorder (Beckman Dynograph recorder type R).

RESERPINE PRETREATMENT

Rats were injected intraperitoneally with reserpine at the dose of 5mg/kg/day for two consecutive days. They were sacrificed on the third day and the left and right atria were isolated and prepared for experiments as described above.

EXPERIMENTAL PROCEDURES

After the tissues had been equilibrated until the spontaneous beating rate (right atria) and isometric force (left atria) were stable, 5-HT was administered to the organ bath chamber by a microsyringe and the responses were recorded for 15 min. The same preparation was washed repeatedly with Locke solution and allowed to recover for at least 15 min before the effects of the blockers on the action of 5-HT were tested, and then the preparation was discarded. Control experiments revealed that there was no tachyphylaxis to the second addition of 5-HT.

CHEMICALS

Drugs used were as follow : 5-hydroxytryptamine creatinine sulfate (Sigma), cyproheptadine hydrochloride (Merck, Sharp & Dohm), methysergide hemimaleate (Sandoz), propranolol hydrochloride (Inderal inj., I.C.I.) and reserpine (Serpasil inj., Ciba Geigy). Cyproheptadine hydrochloride was dissolved in methanol; all other drugs were dissolved in double-distilled water.

RESULTS

POSITIVE CHRONOTROPIC EFFECT ON THE RAT ISOLATED RIGHT ATRIA

The effects of 5-HT (2 µg/ml), methysergide (0.47 µg/ml) and 5-HT plus methysergide on the spontaneous rate of isolated rat right

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atria are recorded in Figure 1. 5-HT produced a 10-15% increase in rate over controls and the positive chronotropic effect was well sustained during the 15 min experimental period. When methysergide, a 5-HT antagonist, was also present the positive chronotropic response to 5-HT was approximately 50% attenuated. Similar results were also obtained when either another 5-HT antagonist, cyproheptadine (0.02 µg/ml), or a beta-adrenergic blocking agent, propranolol (0.15 µg/ml), was used instead of methysergide. It must be pointed out that in order to avoid any non-specific effect, the dose of individual blocking agent employed in this study exerted only minimum (2-3%) or no inhibition on atrial activity by itself.

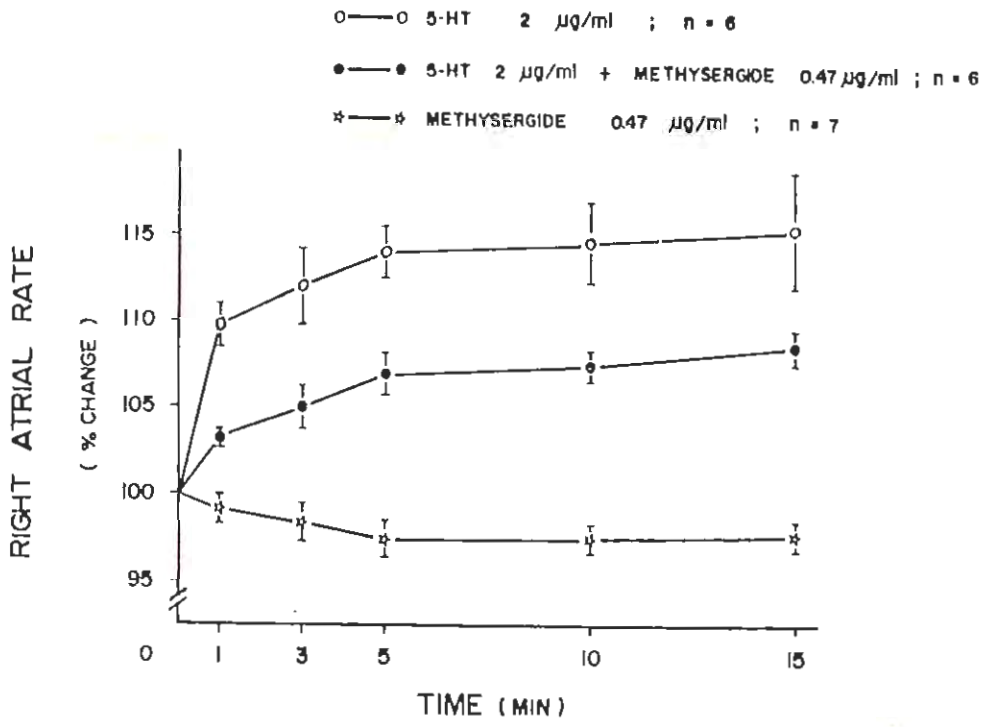

Figure 1. Effect of methysergide on the positive chronotropic action of 5-HT on the isolated rat right atria. Methysergide was added 5 min before 5-HT. Each point represents a mean ± S.E.M.

Figure 2. reports the effect of two antagonists combined, propranolol and methysergide, on the right atrial response to 5-HT. It is seen that in the presence of both 5-HT antagonist and beta blocker, the positive chronotropic effect of 5-HT was completely abolished. Similar but somewhat less striking results were obtained when cyproheptadine (0.02 µg/ml) replaced methysergide (results not shown). Further experiments were then performed with isolated right atria from reserpinized rats. The control right atrial rate collected from 32 reserpine-pretreated rats was 263 ± 18 beats/min which was similar to non-treated rats (266 ± 22, n = 75). However, the body weight of reserpinized rats was decreased by 43.72 ± 1.49 g (n = 32).

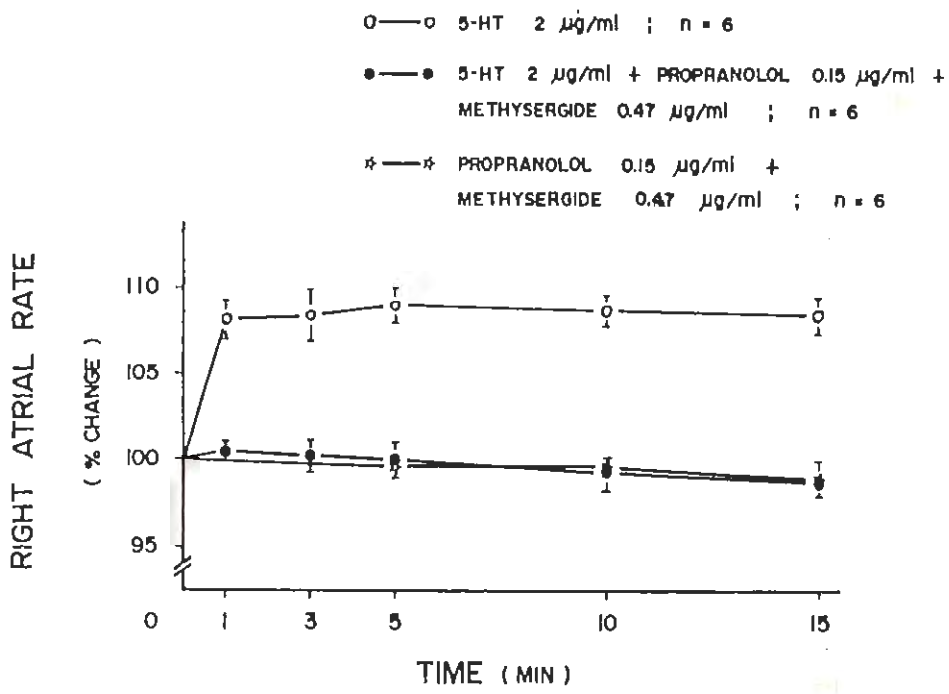

Figure 2. Blockade by propranolol plus methysergide of the positive chronotropic action of 5-HT on the isolated rat right atria. Both antagonists were added 5 min before 5-HT. Each point represents a mean ± S.E.M.

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As shown in Figure 3, in the reserpinized right atria, the positive chronotropic effect of 5-HT was almost completely blocked by methysergide alone (compared with Figure 1). Similar results were also found with cyproheptadine (0.02 µg/ml).

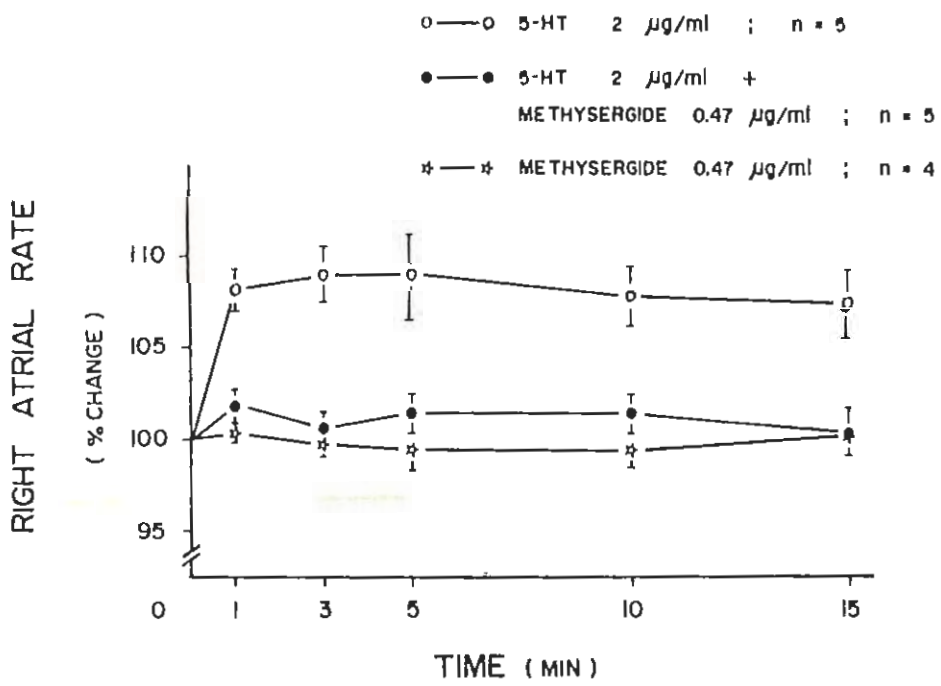

Figure 3. Inhibition by methysergide of the positive chronotropic action of 5-HT on the isolated right atria from reserpinized rats. Methysergide was added 5 min before 5-HT. Each point represents a mean ± S.E.M.

POSITIVE INOTROPIC EFFECT ON THE RAT ISOLATED LEFT ATRIA

Figure 4. records the effects of 5-HT (2 µg/ml), cyproheptadine (0.02 µg/ml) and 5-HT plus cyproheptadine on the isometric force of the electrically driven isolated rat left atria. 5-HT produced a 10-15% increment of contractile force and the effect was well sustained for at least 10 min. Prior addition of cyproheptadine practically caused complete inhibition of the positive inotropic response. Methyser-

gide (0.47 µg/ml) was found to be slightly less effective than cyproheptadine. In contrast to the effect on the rate, propranolol (0.15 µg/ml) was found totally inert against the action of 5-HT on the left atrial isometric force (Figure 5).

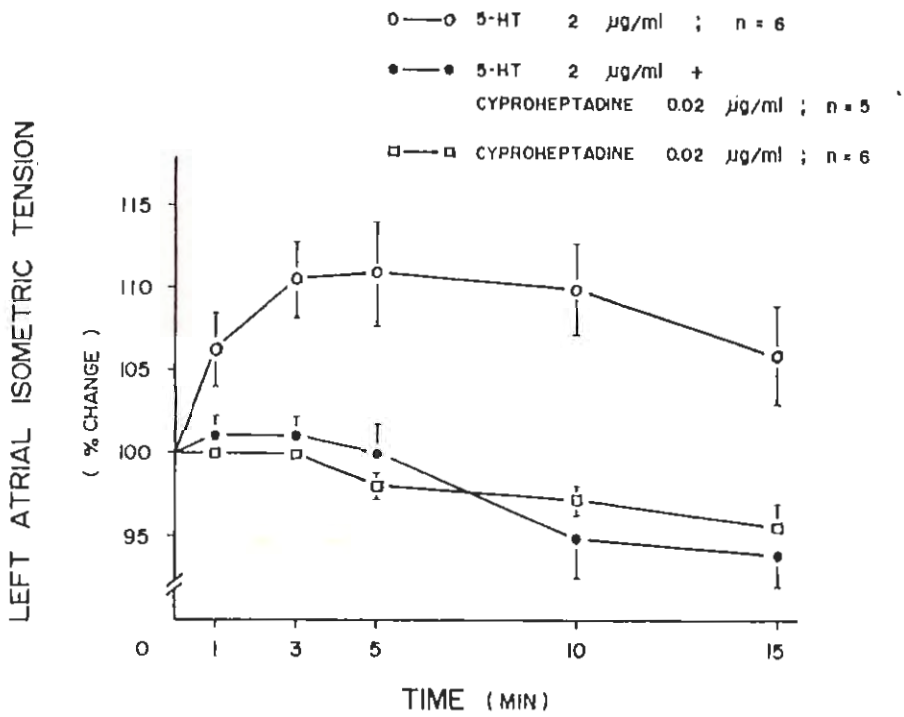

Figure 4. Abolition by cyproheptadine of the positive inotropic effect of 5-HT on the electrically paced isolated rat left atria. Cyproheptadine was added 5 min before 5-HT. Each point represents a mean ± S.E.M.

DISCUSSION

The results presented in this paper have shown the stimulatory effect of 5-HT on the right atrial rate and the left atrial isometric tension of the rats. The positive chronotropic effect is partially antagonized by 5-HT antagonists (methysergide and cyproheptadine) or beta-adrenergic blocking drug (propranolol). However, severe or com-

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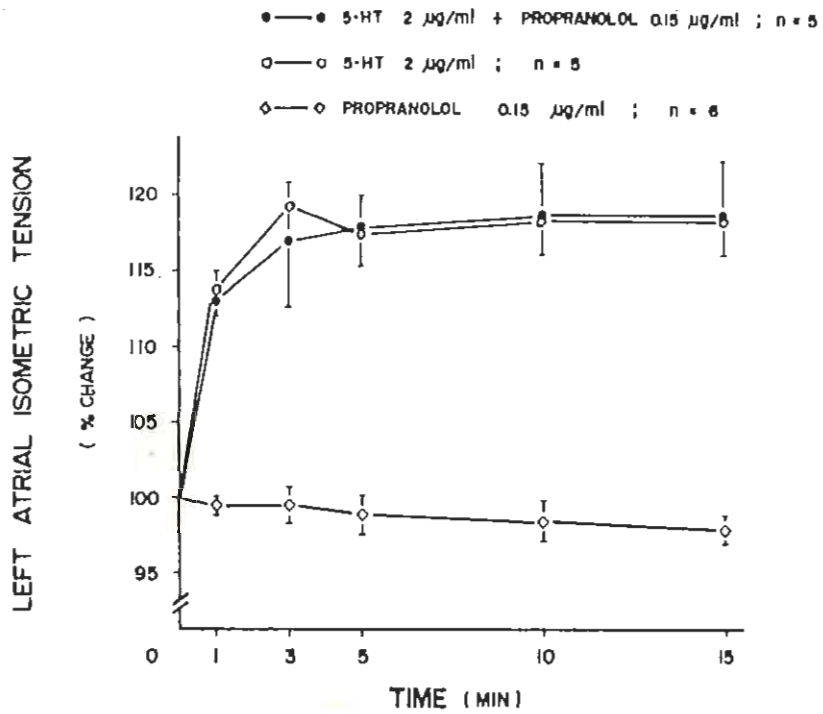

Figure 5. Effect of propranolol on the positive inotropic action of 5-HT on the electrically paced isolated rat left atria. Propranolol was added 5 min before 5-HT. Each point represents a mean ± S.E.M.

plete inhibition can be achieved by combining 5-HT antagonist with beta blocker. In contrast, the positive inotropic effect is completely abolished by 5-HT antagonists, particularly cyproheptadine; and propranolol is totally ineffective at the same dose. These observations indicate that 5-HT stimulates rat right and left atria by different mechanisms. The effect on rate appears to have both direct and indirect components. The indirect part is most certainly resulted from endogenous catecholamine.

This conclusion is also supported by the experiments in which reserpine is used to deplete neuronal storage of catecholamine. Thus, in the reserpinized right atria, 5-HT antagonists alone strongly or completely block the positive chronotropic effect of 5-HT. On the

contrary, the effect on force seems to have only direct component and mediates solely through specific interaction with serotonergic receptors on myocardial cells. It has recently been proposed that there are at least two distinct populations of 5-HT receptors, the 5-HT_1 or S_1 and 5-HT_2 or S_2 receptors. Methysergide and, particularly, cyproheptadine have a higher affinity for the S_2 than for the S_1 receptor (5). Since low doses of methysergide and cyproheptadine were found to reduce the rate and abolish the contractile response, this may suggest the presence of S_2 receptor subtype in the rat atria.

The apparent inability of 5-HT to induce endogenous catecholamine release in the left atria may not be related to the different norepinephrine contents in the right and left sides. Histochemical study has shown that in rabbit and guinea-pig hearts norepinephrine is more concentrated in the right atrium and ventricle than in the left ones (6). However, the mode of actions of 5-HT on cardiac tissues isolated from rabbit and guinea-pig are different. At present, the mechanism of the endogenous catecholamine liberation evoked by 5-HT in the right atria is not known. There are at least two possibilities : (a) stimulation of serotonergic receptor on nerve terminal, which triggers the release of the adrenergic neurotransmitter (7); and (b) a tyramine-like indirect sympathomimetic action (8). Alternatively, 5-HT may inhibit norepinephrine reuptake by sympathetic nerve terminal leading to local accumulation of the amine (9). Further experiments are needed to clarify this point.

Recent experimental results reported by other investigators are in agreement with our data. For example, Sakai and Akima (10) show that in isolated, blood-perfused rat heart, single injections of 5-HT (0.1-3 µg) into the coronary perfusion produced the dose-dependent increases in left vertricular dP/dt max and perfusion pressure. These effects are not significantly affected by treatment with propranolol but are abolished by methysergide. Similarly, Higgins and co-workers (11) have shown that low concentration of 5-HT (10^{-5}M) increases contractile activity of the isolated perfused working rat heart. This

effect is blocked by methysergide but not by atenolol, a cardioselective beta blocker. Thus these studies show that the positive inotropic action of 5-HT on isolated rat heart is a direct effect and does not involve the release of endogenous catecholamine. Our results with isolated rat left atria also point to the same conclusion.

CONCLUSION

The present results indicate differential actions of 5-HT in increasing the rate and contractile force by isolated rat right and left atria, respectively. Our results also question the validity of previous studies on the stimulatory effect of 5-HT and the alteration of this effect by certain blocking agents which had been performed with the isolated whole atria.

ACKNOWLEDGEMENT

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