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# MECHANISMS OF STIMULATION OF RAT CARDIAC MUSCLE BY 5-HYDROXYTRYPTAMINE

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**Abstract**—Administration of 5-hydroxytryptamine to isolated perfused working rat heart preparations caused an increase in contractile activity. The increase in activity due to low concentrations of 5-hydroxytryptamine ($10^{-5}$ M) could be blocked by either methysergide or propanolol but not by atenolol. At high concentrations of 5-hydroxytryptamine ($10^{-3}$ M) no increase in tissue cyclic AMP was found but a significant rise in the value of the cyclic AMP-dependent protein kinase activation ratio was observed, suggesting a slight increase in intracellular cyclic AMP had occurred. It was also observed that while low concentrations of 5-hydroxytryptamine (up to $10^{-5}$ M) did not provoke catecholamine release from the intra-cardiac stores higher concentrations ($10^{-4}$ M) did. It is suggested that low concentrations of 5-hydroxytryptamine have a direct action on cardiac muscle but that at high concentrations a direct action cannot be separated from an action by which catecholamines are released from intra-cardiac stores.

5-Hydroxytryptamine has been shown to stimulate cardiac function in several species [1]. Two alternative mechanisms by which this stimulation is achieved have been suggested. The first is an indirect action of 5-hydroxytryptamine on presynaptic receptors which lie on the sympathetic neurones innervating the heart. Such stimulation results in the local release of catecholamines. This mechanism has been demonstrated in the rabbit [2, 3] and the dog [4, 5]. In the cat and guinea-pig a different mechanism obtains (2). In these species the mechanism appears to be by direct stimulation of receptors on the cardiac cells. Recently Sakai and Akima [6] showed that the inotropic stimulation of the blood perfused rat heart was antagonised by methysergide but not by the $\beta$-adrenergic antagonist propranolol, suggesting that in this species the mechanism is unlikely to involve catecholamine action at the cardiac $\beta$-adrenergic receptor.

Von Hungen, Roberts and Hill [7] and others [8, 9] have shown that the activity of brain adenylate cyclase is enhanced by 5-hydroxytryptamine. Incubation of slices of brain tissue with 5-hydroxytryptamine causes an increase in the tissue cyclic AMP content. This effect appears to be antagonised by methysergide but not by propranolol [7]. Hence data based on studies of neural tissue suggest that 5-hydroxytryptamine has a direct action which is probably mediated by cyclic AMP.

The aim of the work reported here was to determine the nature of the stimulatory effects of 5-HT on rat cardiac tissue and to ascertain whether or not this stimulation was dependent on activation of cardiac adenylate cyclase.

## MATERIALS AND METHODS

*Working heart perfusions.* Rats (male, Alderley Park strain, 300 g body wt) were injected with 250 units of heparin i.p. 20 min before the induction of anaesthesia with Sagatal (100 mg/kg i.p.). After deep anaesthesia had been achieved hearts were removed by cutting along the line of the pericardium and dropped into ice cold saline. Hearts were perfused as working preparations after the method of Neely *et al.* [10]. Each heart was quickly cannulated via the aorta and initially perfused as a Langendorff preparation with Krebs–Henseleit buffer at 37° from a preperfusion reservoir exerting a hydrostatic pressure equivalent to 60 mm Hg on the heart. Perfusion in this way was carried out for 10 min to clear the coronary bed of blood and to permit the cannulation of the left atrium. During this period the perfusate passing through the heart was discarded. At the end of this preperfusion period the retrograde perfusate flow was discontinued and antegrade perfusion of the heart via the left atrium was commenced with 60 ml of Krebs–Henseleit buffer recirculated through a large oxygenation chamber and perfusate reservoir at a hydrostatic pressure of 5 cm $H_2O$ supplied from a bubble trap. From the left atrium, ventricular contraction pumped the perfusate into a small pressure chamber located immediately above the heart. This chamber contained 2.5 ml of air which provided compliance in the aortic outflow tract. From the compliance chamber perfusate was forced by the action of the heart into the recirculating buffer reservoir against an afterload of 60 mm Hg hydrostatic pressure. The heart was perfused for 15 min to stabilize the working preparation.

The Krebs–Henseleit buffer contained 142.20 mmoles $Na^+$, 5.87 mmoles $K^+$, 1.17 mmoles $Mg^{2+}$, 3.02 mmoles $Ca^{2+}$, 128.14 mmoles $Cl^-$, 1.17 mmoles $SO_4^{2-}$, 1.17 mmoles $H_2PO_4^-$, 24.80 mmoles

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$HCO_3^-$, 0.50 mmoles EDTA, and 11.00 mmoles glucose/litre and the solution was kept continuously equilibrated with a 95% $O_2$/5% $CO_2$ gas mixture.

The preparation was monitored using a pressure transducer (Bell and Howell) attached to a short side arm on the aortic cannula. The differential of the pressure function and heart rate were monitored and all parameters were recorded on a Devices M19 recorder.

After 15 min as a working preparation had elapsed, drugs as concentrated solutions were added to the recirculating perfusate in the reservoir. Before, during and after treatment with drugs the contractile performance of the heart preparations was constantly monitored. For biochemical studies of the 5-hydroxytryptamine-treated tissue, hearts were perfused as described above except that as the peak stimulated effect was attained the perfused hearts were rapidly crushed between blocks of aluminium precooled in liquid nitrogen. The frozen tissue wafers were subsequently powdered in a Braun Mikro-dismembrator at the temperature of liquid nitrogen. The frozen powders were used for subsequent estimations of tissue cyclic AMP and protein kinase activation ratio.

*Perfusion studies of radioactive noradrenaline release.* Hearts were removed from heparinized and anaesthetized rats as described above. The aorta was cannulated and the hearts perfused at a hydrostatic pressure equivalent to 60 mm Hg as Langendorff preparations [11] with Krebs–Henseleit buffer containing 200 $\mu$g of l-noradrenaline and 60 $\mu$Ci [7,8-$^3$H]L-noradrenaline (Radiochemical Centre, Amersham, U.K.) and 5 mmoles ascorbic acid/litre for 20 min. Buffer was not recirculated in this experiment. Iverson [12] has previously shown that this technique labels the endogenous noradrenaline stores in the rat heart.

At the end of the labelling period the perfusate was switched to ordinary Krebs–Henseleit buffer which contained no noradrenaline and washout of the radiolabelled catecholamine from the heart was commenced. After 10 min of washout the perfusate was again changed to one which contained a varied concentration of 5-hydroxytryptamine. Throughout the washout phase of perfusion the total coronary effluent was collected every 0.5 min in a fraction collector (LKB instruments). A sample of effluent from each fraction was taken for analysis of radioactivity.

*Extraction and determination of cyclic AMP.* Weighed aliquots of frozen heart powders were extracted with boiling HCl (50 mM) and assayed as described previously [13].

*Estimation of the protein kinase activation ratio.* Frozen heart powders were homogenised in 3 vols. of ice-cold 10 mM potassium phosphate buffer, pH 6.8, containing 0.5 mM methylisobutylxanthine (an inhibitor of phosphodiesterase) using a motor-driven Teflon–glass homogeniser. The estimation of protein kinase activity present in the homogenate in the presence and absence of added cyclic AMP was performed according to the method of Kuo and Greengard [14].

*Statistical evaluation.* The difference between means of treatment groups was assessed using Student's *t*-test. Results were only considered to be statistically significant if a value of $P < 0.05$ was obtained.

## RESULTS AND DISCUSSION

When 5-HT was added to the perfusate of isolated working rat hearts stimulation of beating activity was observed. Figure 1 shows the percentage change in heart rate, peak systolic pressure development, $dP/dt_{max}$ and the value calculated for the pressure-rate product which is a simple index of cardiac work output [10]. The largest change in heart rate was observed when $10^{-4}$ M 5-hydroxytryptamine was present in the perfusate. However peak systolic pressure development was increased maximally at a lower ($10^{-5}$ M) concentration of 5-hydroxytryptamine.

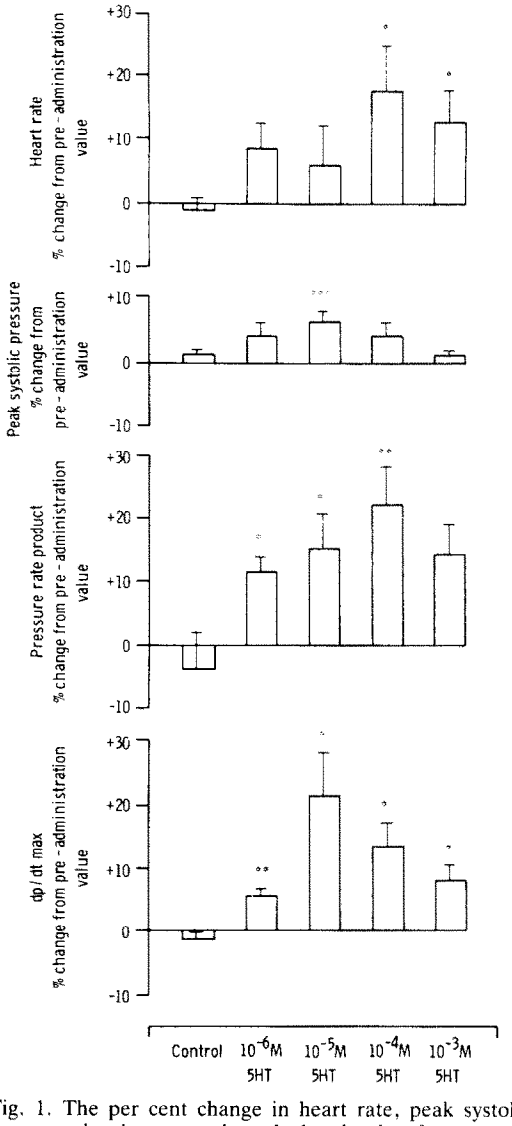


Fig. 1. The per cent change in heart rate, peak systolic pressure development, the calculated value for pressure rate product and $dP/dt_{max}$ provoked by administration of various concentrations of 5-hydroxytryptamine (5-HT). Data are presented as means + S.E.M. for 4 hearts. *:$P < 0.05$, **:$P < 0.02$, ***:$P < 0.002$, vs corresponding control value.

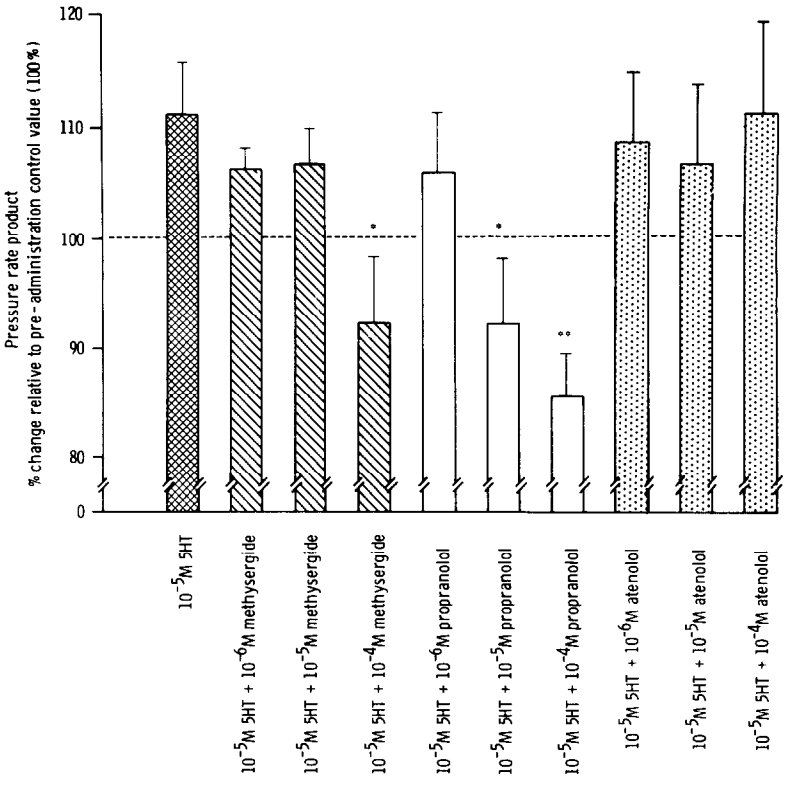


Fig. 2. The effect of increasing concentrations of methysergide (single hatched bars) propranolol (empty bars) and atenolol (shaded bars) on 5-hydroxytryptamine stimulated (double hatched bars) increase in pressure rate product. Results are expressed as the per cent change in pressure rate product from the pre-administration control value. Data are presented as means + S.E.M. for 4 hearts. *$P < 0.05$, **:$P < 0.01$ vs effect of 5-hydroxytryptamine alone.

Pressure-rate product followed heart rate and a 20% increase in this parameter was observed at a 5-hydroxytryptamine concentration of $10^{-4}$ M. Significant increases in dP/dt max were observed at all concentrations of 5-hydroxytryptamine tested although the peak effect was observed at $10^{-5}$ M.

The beating rate of neonatal heart cells in culture has been shown to increase on exposure to 5-hydroxytryptamine [15]. The peak of this effect is achieved at $10^{-4}$ M and interestingly a similar decline in overall stimulant activity was observed as the concentration of 5-hydroxytryptamine was raised above this [15]. In these respects the two preparations appear to be quite similar in their responses to this compound.

The stimulation of the perfused cardiac preparations by 5-hydroxytryptamine could be antagonised by the addition of methysergide or propranolol

Table 1. The effect of various concentrations of 5-hydroxytryptamine on tissue content of cyclic AMP and myocardial cyclic AMP dependent protein kinase activation ratio

| Conditions | Myocardial cyclic AMP content (n moles/g dry tissue). | Protein Kinase activation ratio (- cAMP/ +cAMP). |
|---|---|---|
| Control | 1.044 ± 0.116 | 0.518 ± 0.009 |
| + $10^{-6}$M 5 hydroxy-tryptamine | 1.192 ± 0.180 | 0.537 ± 0.003 |
| + $10^{-5}$M 5 hydroxy-tryptamine | 0.812 ± 0.095 | 0.517 ± 0.009 |
| + $10^{-4}$M 5 hydroxy-tryptamine | 1.043 ± 0.064 | 0.540 ± 0.007 |
| + $10^{-3}$M 5 hydroxy-tryptamine | 0.956 ± 0.140 | 0.549 ± 0.005 * |

Data are presented as means ± S.E.M. for 4 hearts. *:$P < 0.01$ vs corresponding control value.

Results are not significantly different from control value unless otherwise indicated.

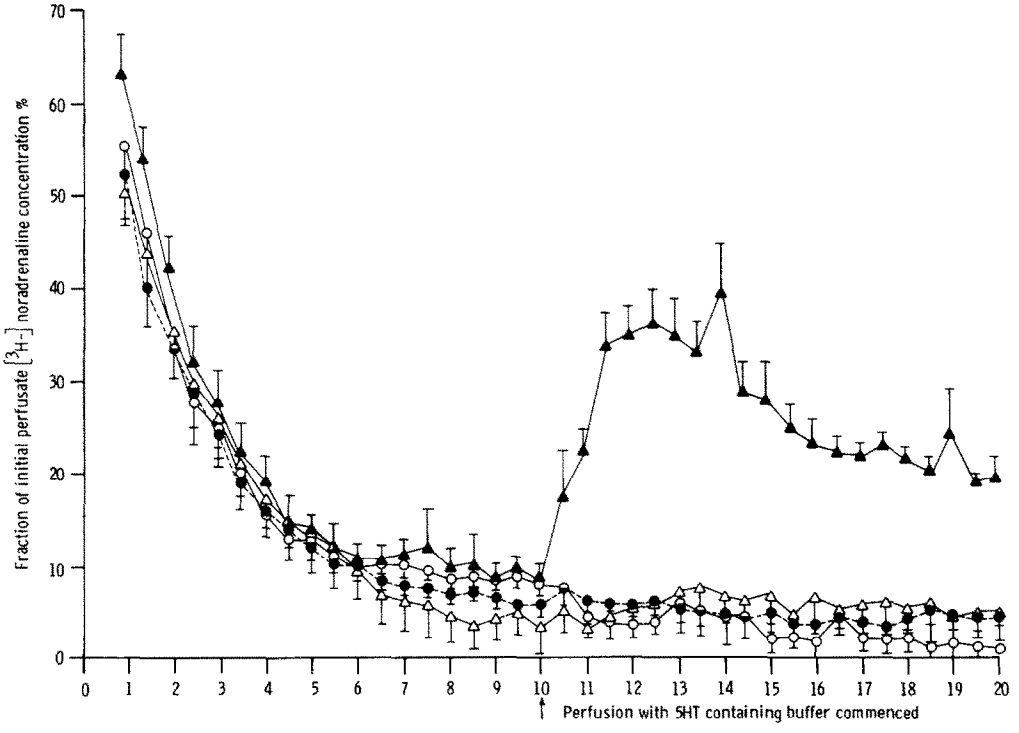


Fig. 3. The effect of various 5-hydroxytryptamine concentrations on catecholamine release from the Langendorff perfused rat heart. ●: control, ○: $10^{-6}$ M, △: $10^{-5}$ M, ▲: $10^{-4}$ M 5-hydroxytryptamine. Perfusion with buffer containing $^{3}$H-noradrenaline was stopped and perfusion with buffer containing no noradrenaline started at 0 min. The perfusate was changed to one containing 5-hydroxytryptamine (5-HT) at time 10 min. Data are presented as means + S.E.M. of 4 hearts.

to the perfusate (Fig. 2). The cardioselective β-adrenergic blocker atenolol was without antagonist activity towards 5-hydroxytryptamine. These observations are consistent with those made in the neonatal cardiac cell cultures [15], but are at variance with those of Sakai and Akima [6] who showed that methysergide but not propranolol blocked the stimulant activity of 5-hydroxytryptamine. Middlemiss, Blakeborough and Leather [16] have reported that propranolol has a stereospecific affinity for the 5-hydroxytryptamine receptor from rat brain which is similar in magnitude to that of methysergide. The observations reported here and elsewhere [15, 16] suggest that propanolol possesses some 5-hydroxytryptamine antagonist activity, a property which is not shared by other β-adrenergic antagonists.

For studies involving the effect of 5-hydroxytryptamine on cardiac cyclic AMP levels, hearts were perfused as described above and stimulation was achieved by adding various concentrations of 5-hydroxytryptamine to the perfusate. As the peak stimulation of the tissue was observed the tissue was rapidly frozen and treated as described above. Table 1 shows the effect of various concentrations of added 5-hydroxytryptamine on tissue cyclic AMP content and the protein kinase activation ratio [14]. No changes were seen in tissue cyclic AMP on stimulation of contractile activity with 5-hydroxytryptamine. It is possible that small changes in tissue cyclic AMP might not be seen against a background of basal adenylate cyclase activity and thus although the cardiotonic effect of 5-hydroxytryptamine may be mediated by cyclic AMP, gross changes in this metabolite are not easily observed. The effect of slight changes in tissue cyclic AMP have dramatic effects on the activity of cyclic AMP-dependent protein kinase [14]. In the experiments described here a significant change in the ratio of activities (−cyclic AMP/+cyclic AMP) was observed only in the presence of $10^{-3}$ M 5-hydroxytryptamine. On the basis of these data it seems reasonable to conclude that 5-hydroxytryptamine exerts at least part of its cardiotonic action without mediation by cyclic AMP.

The ability of 5-hydroxytryptamine to provoke catecholamine release from the rat heart was assessed by labelling the endogenous catecholamine stores with [7,8-$^{3}$H]L-noradrenaline as described above. Figure 3 shows the effect of various concentrations of 5-hydroxytryptamine in the perfusate on the outflow of tritium from the perfused heart. When 5-hydroxytryptamine was present in the perfusate at concentrations up to $10^{-5}$ M no catecholamine release was observed. However when 5-hydroxytryptamine was present at a concentration of $10^{-4}$ M catecholamine release was pronounced.

The blockade of the stimulation provoked by $10^{-5}$ M 5-hydroxytryptamine by methysergide and propanol but not by atenolol clearly suggests that at low concentrations the cardiotonic activity of 5-hydroxytryptamine is not mediated by stimulation of the β-adrenergic receptor. However, the data obtained on protein kinase activation ratio suggest

that at high concentrations the presence of 5-hydroxytryptamine caused a small rise in cardiac cyclic AMP levels. This observation is supported by the catecholamine-release data (Fig. 3) showing that 5-hydroxytryptamine at concentrations around $10^{-4}$ M can provoke release of $^{3}$H-noradrenaline from cardiac stores.

Sakai and Akima [6] concluded from their work in the blood perfused rat heart that 5-hydroxytryptamine produced its effects by a direct mechanism not involving catecholamines and in this respect most closely resembled the cat and guinea-pig rather than rabbit and dog. Data from studies of cardiac cell cultures [15] are in agreement with their observations. However, the data presented here would suggest that at high doses (above $10^{-5}$ M) 5-hydroxytryptamine may cause catecholamine release perhaps additional to its stimulant activity. The doses of 5-hydroxytryptamine used by Sakai and Akima were in the micromolar range and based on the observations made here would not be expected to release catecholamines. The cardiac cell cultures are devoid of endogenous adrenergic influences [15, 17] and thus no evidence of the catecholamine releasing properties of 5-HT would be expected from such a preparation.

Considered together the data obtained indicate that low concentrations of 5-hydroxytryptamine (up to $10^{-5}$ M) have a direct action on cardiac muscle but at high concentrations such an effect cannot be separated from an action by which catecholamines are released from intracardiac stores.

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