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MECHANISM OF THE INDIRECT SYMPATHOMIMETIC EFFECT OF 5-HYDROXYTRYPTAMINE ON THE ISOLATED HEART OF THE RABBIT

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1 Rabbit isolated hearts, perfused by the Langendorff technique, were used to investigate the indirect sympathomimetic effects of 5-hydroxytryptamine (5-HT). Comparisons were made with noradrenaline and with two indirectly acting sympathomimetic agents with entirely different mechanisms of action, tyramine and dimethylphenylpiperazinium (DMPP).

2 The cardiac stimulant effects of 5-HT, tyramine and DMPP were inhibited by propranolol and practolol and the pA_2 values obtained were similar to those obtained with noradrenaline as the agonist.

3 Responses to 5-HT, tyramine and DMPP were greatly reduced on hearts from rabbits pretreated with 6-hydroxydopamine. Such hearts had less than 7% of their normal catecholamine concentration and no fluorescence characteristic of noradrenaline in the cardiac sympathetic nerves could be demonstrated.

4 Rapid, reversible and selective tachyphylaxis to 5-HT was demonstrated during perfusion with 5-HT. In hearts desensitized to DMPP by perfusion with DMPP, responses to 5-HT were also reduced.

5 Perfusion of hearts with colchicine inhibited stimulant responses to 5-HT and DMPP but had little effect on responses to noradrenaline or tyramine.

6 Desmethylimipramine enhanced cardiac stimulant responses to noradrenaline and to a lesser extent, those to 5-HT and DMPP. Responses to tyramine were consistently inhibited by desmethylimipramine.

7 Tetrodotoxin abolished responses of the heart to electrical nerve stimulation but left responses to noradrenaline, 5-HT and DMPP unaffected.

8 5-HT, tyramine and DMPP evoked ^{3}H-release from hearts whose neuronal noradrenaline stores had been labelled by perfusion with [^{3}H]-(−)-noradrenaline. The pattern of release evoked by 5-HT was similar to that of DMPP but differed from that of tyramine.

9 Reducing the calcium concentration in the Tyrode solution from 3.6 to 0.2 mEq/l did not affect ^{3}H-overflow after tyramine but greatly inhibited that evoked by 5-HT and DMPP.

10 The results confirm that the stimulant effects of 5-HT on the rabbit isolated heart are the result of noradrenaline release. They further suggest that the site of the release is the terminal sympathetic nerve network. The mechanism of release shows more similarities to that of DMPP (calcium-dependent depolarization and exocytosis) than to that of tyramine (neuronal uptake and stoichiometric displacement).

Introduction

5-Hydroxytryptamine (5-HT) stimulates the rate and force of contraction of the rabbit isolated heart and the effects are abolished by reserpine suggesting mediation by catecholamine release (Jacob & Poite-Beviérre, 1960). This action has now been further investigated. In particular, 6-hydroxydopamine, which selectively destroys sympathetic nerves (Thoenen, 1972), has been used to define more precisely the source of the catecholamine released by 5-HT. Further, and in order to clarify the mechanism of its releasing action, 5-HT has been compared with two compounds, both indirect sympathomimetics, but with entirely different mechanisms of action. First, tyramine, which releases noradrenaline by stoichiometric displacement after being taken up into the nerve fibres by active transport (Trendelenburg, 1972). Second, dimethylphenylpiperazinium (DMPP), which releases noradrenaline by depolarization of the nerve

terminals as a result of activation of specific receptor sites (Muscholl, 1970).

A preliminary account of some of these findings has already appeared (Fozard & Mwaluko, 1975a).

Methods

Perfusion of the heart

Rabbits of either sex weighing 1.6 to 2.8 kg were given heparin (500 u/kg) into a marginal ear vein. Two to 5 min later they were stunned by a blow to the head and bled. Hearts were rapidly removed some with the right sympathetic nerves attached (Huković & Muscholl, 1962) and perfused according to the Langendorff technique with modified Tyrode solution at 35.5°C. The Tyrode solution (concentrations in g/l: NaCl 8.0, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, $NaHCO_3$ 1.0, NaH_2PO_4 0.05, glucose 1.0 and ascorbic acid 0.01) was gassed with a mixture of 95% O_2 and 5% CO_2. Perfusion pressure was maintained at 60 cm water. In some experiments the concentration of calcium chloride was reduced to 0.011 g/l (Lindmar, Löffelholz & Muscholl, 1967). Right ventricular tension and rate and right atrial tension were recorded as described by Fozard & Muscholl (1971).

Design of the experiments

Dose-effect curves to 5-HT, noradrenaline, tyramine and DMPP on atrial and ventricular tension development and ventricular rate were established on separate hearts by bolus injections of each compound. In general 5 min was the interval between doses, but if the effects of an earlier dose were still in evidence, this interval was extended. In most hearts, after a 15 min interval, a second dose-effect curve was obtained for a given agonist. Thus, two quantitative means of comparison were possible. First, assessment could be made of the effects of pretreatment with 6-hydroxydopamine by comparison of the initial dose-effect curves obtained on hearts from untreated and pretreated animals. Second, by including a modifying drug in the perfusion solution immediately after the first dose-effect curve had been established, a comparison could be made between the changes produced in the second dose-effect curve by the modifying drug and the effects in the control situation where no modifying drug was present.

Values for pA_2 were estimated using cardiac rate as the index of the response by the method of Arunlakshana & Schild (1959).

In order to investigate selective and cross-tachyphylaxis between the agonist drugs, hearts were set up as described above and a response to an ED_{50} bolus injection of either 5-HT (8 μg), noradrenaline (0.64 μg), tyramine (40 μg) or DMPP (40 μg) was established. In control experiments, the agonist ED_{50}'s were repeated twice with a 15 min interval between each challenge making three responses in all. In test experiments, the hearts were perfused with various concentrations of the agonist drugs immediately after the first ED_{50} response had been obtained and during the second ED_{50} injection. The perfusion fluid was changed back to control Tyrode solution immediately after the second ED_{50} response had been obtained and the third ED_{50} was injected 15 min later. Quantitative comparisons were made between the second responses in the control series and the equivalent responses obtained during perfusion with agonist drugs in the test experiments.

Experiments with [^{3}H]-(−)-noradrenaline

The endogenous cardiac neuronal noradrenaline stores were labelled with [^{3}H]-noradrenaline by the procedure described by Starke (1971). Chromatographically pure (−)-noradrenaline-[7-^{3}H] (sp. act. 7.2 Ci/mmol) was diluted freshly each day with unlabelled noradrenaline to give the working stock solution. This was infused at the rate of 0.1 ml/min (with a Palmer slow injection apparatus) into the arterial inflow to give a final concentration in the medium of 10 ng/ml and radioactive concentration of 43 nCi/ml. In these experiments the flow rate was maintained at 25 ml/min by means of a roller pump (Watson-Marlowe Type MHRE 200), and the duration of the loading period was 12 minutes.

In control experiments, the whole of the venous outflow was collected in 20 three min aliquots during a 60 min washout period. Test doses of 5-HT, tyramine and DMPP were introduced by bolus injections into the arterial inflow, at times 30, 42 and 54 min respectively after the end of the loading period. Samples of perfusate were collected at various time intervals before, during and after drug challenge as detailed in the Results section.

The total radioactivity contained in each sample of perfusate was measured by dispersing a 1 ml aliquot in 10 ml of scintillation fluid (0.1 g dimethyl POPOP, 5.5 g diphenyloxazole, 333 ml Triton X-100, 667 ml toluene) and counting in a Packard Tri-Carb Scintillation Spectrometer (Model 3320). Counting efficiency and quenching were monitored by external standardization and the results corrected accordingly.

Pretreatment with 6-hydroxydopamine

Rabbits were pretreated with 6-hydroxydopamine after the method of Fozard, Kelly & Small (1973). The degree of destruction of the cardiac sympathetic nerves was assessed by fluorimetric assay of the endogenous cardiac noradrenaline concentrations (Welch & Welch, 1969) and by histochemical

fluorescence microscopy (Spriggs, Lever, Rees & Graham, 1966).

Statistical analysis

All measurements of variations of means quoted are standard errors. Student's *t* test was used to assess the significance of differences between mean values. The number of observations is indicated by *n*.

Drugs

The drugs used were atropine sulphate (Macfarlan Smith), colchicine (Koch-Light), dimethylphenylpiperazinium iodide (Emmanuel), heparin (Evans), 5-hydroxytryptamine creatinine sulphate (Koch-Light), 6-hydroxydopamine hydrobromide (Whatman Biochemicals), (−)-noradrenaline bitartrate (Koch-Light), practolol and propranolol hydrochloride (ICI), tetrodotoxin (Sankyo), tyramine hydrochloride (Koch-Light).

Results

Responses of the rabbit heart to 5-hydroxytryptamine; use of Tyrode solution containing atropine

In untreated hearts, responses to 5-HT over the dose range used (0.5 to 512 μg) were usually biphasic consisting of an initial negative chronotropic and inotropic phase followed by a stimulant response. Responses to DMPP were similarly routinely biphasic. The initial inhibitory responses obtained with 5-HT and DMPP could be abolished by perfusion with low concentrations of atropine (Fozard & Muscholl, 1971; Mwaluko, 1975). Since this work was concerned solely with the mechanism of the stimulant response to 5-HT, all experiments have been carried out in the presence of atropine, 0.5 μg/ml. This concentration of atropine is devoid of nicotinic receptor blocking activity (Lindmar, Löffelholz & Muscholl, 1968) and can be considered to be selectively anti-muscarinic.

Effects of propranolol and practolol on cardiac responses to noradrenaline, 5-hydroxytryptamine, tyramine and dimethylphenylpiperazinium

Responses to all the agonists were inhibited by low concentrations of propranolol and practolol. The pA_2 values obtained for the antagonism are presented in Table 1. For each antagonist, the values obtained with 5-HT were similar to those obtained with the indirectly acting sympathomimetic amines, tyramine and DMPP. They were also similar to those obtained with noradrenaline which stimulates the β-adrenoceptors directly.

Effects of pretreatment with 6-hydroxydopamine on cardiac responses to 5-hydroxytryptamine, dimethylphenylpiperazinium, tyramine and noradrenaline

Pretreatment with 6-hydroxydopamine resulted in a fall in the cardiac noradrenaline concentration from 842 ± 112 ng/g ($n=9$) to 62.5 ± 29.0 ng/g ($n=6$) which represents a highly significant ($P<0.001$) reduction of 93% from control values. In normal atria and ventricles processed for histochemical fluorescence microscopy, the specific fluorescence attributable to noradrenaline in the cardiac sympathetic nerves could be seen. After pretreatment with 6-hydroxydopamine the fluorescence was greatly reduced and in most fields examined it was completely absent (Mwaluko, 1975).

Figure 1 shows that stimulation of cardiac rate evoked by 5-HT, tyramine and DMPP was greatly reduced in hearts from animals pretreated with 6-hydroxydopamine. In contrast, the sensitivity of these hearts to noradrenaline was somewhat increased compared to the equivalent controls.

Experiments demonstrating tachyphylaxis to 5-hydroxytryptamine and dimethylphenylpiperazinium, its reversibility and selectivity

In the control experiments shown as the upper histograms in Figure 2, responses to ED_{50} doses of 5-HT, DMPP, noradrenaline and tyramine were

Table 1 pA_2 values (± s.e. mean) for the antagonism by propranolol and practolol of the stimulant responses on cardiac rate produced by noradrenaline, 5-hydroxytryptamine (5-HT), tyramine and dimethylphenylpiperazinium (DMPP)

	Noradrenaline	n	*5-HT*	n	*Tyramine*	n	*DMPP*	n
Propranolol	8.42 ± 0.08	8	8.43 ± 0.24	5	8.29 ± 0.12	3	8.45 ± 0.16	3
Practolol	6.04 ± 0.26	3	6.16 ± 0.14	4	6.09 ± 0.11	3	5.92 (6.31) (5.52)	2

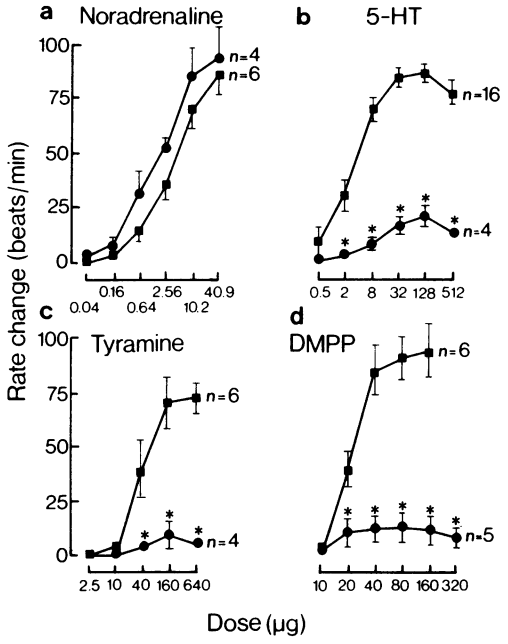

Figure 1 Effects of 6-hydroxydopamine on cardiac rate responses to bolus injections of (a) noradrenaline, (b) 5-hydroxytryptamine (5-HT), (c) tyramine and (d) dimethylphenylpiperazinium (DMPP). Atropine, 0.5 μg/ml was present throughout the experiments. (■) Hearts from untreated animals; (●) hearts from animals pretreated with 6-hydroxydopamine.
* Indicates a significant difference ($P < 0.05$) between response magnitudes at the same dose levels.

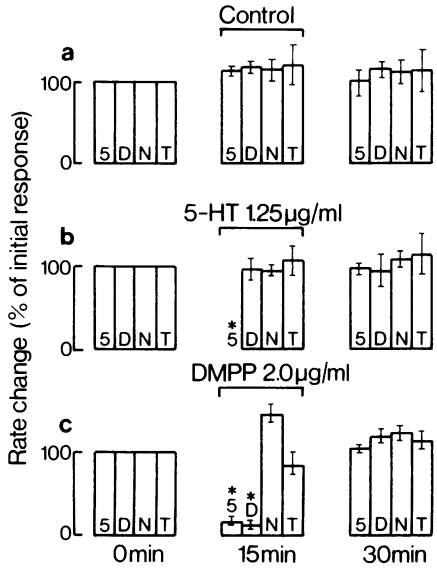

Figure 2 Tachyphylaxis and cross-tachyphylaxis during perfusion with 5-hydroxytryptamine (5-HT) and dimethylphenylpiperazinium (DMPP). Atropine, 0.5 μg/ml, was present throughout the experiments. The histograms represent the rate responses to bolus injections of ED_{50} doses of 5-HT (5), DMPP (D), noradrenaline (N) and tyramine (T) established at time zero (left hand histograms) and repeated twice at 15 min intervals. Responses are expressed as percentages of the initial rate response which was taken as 100%, and the vertical lines indicate the standard errors of the mean values calculated from 4 to 7 individual experiments. (a) Upper series: drug-free Tyrode solution throughout. (b) Middle series: 5-HT, 1.25 μg/ml present in perfusion fluid during establishment of second responses. (c) Lower series: DMPP, 2.0 μg/ml, present in perfusion fluid during establishment of second responses.
* Indicates a significant ($P < 0.05$) difference from the equivalent value in the control series.

established and repeated twice at 15 min intervals. When expressed in terms of the initial responses, no significant variation was detectable. In the test experiments, the second responses in the series were elicited during perfusion with either 5-HT, 1.25 μg/ml, or DMPP, 2.0 μg/ml, concentrations found in preliminary experiments just to abolish responses to the respective agonists. Although perfusion with each compound produced an initial sympathomimetic effect, these were not sustained and at the time of retesting the bolus injections, the baseline cardiac rates were not significantly different from those recorded immediately before the first responses in the series (Mwaluko, 1975).

During perfusion with 5-HT, 1.25 μg/ml, the responses to bolus injections of 5-HT were abolished, yet those to DMPP, noradrenaline and tyramine were little changed. Fifteen min after changing back to drug-free Tyrode solution, responses to 5-HT were back to control levels. With DMPP, 2.0 μg/ml, present in the perfusion solution, responses to bolus injections of 5-HT and DMPP were significantly reduced, those to noradrenaline were increased and those to tyramine were not significantly altered. After 15 min perfusion in drug-free Tyrode solution responses to all the agonists had returned to control levels.

Effects of colchicine on cardiac responses to 5-hydroxytryptamine, noradrenaline, tyramine and dimethylphenylpiperazinium

Figure 3 shows the effects of colchicine on the change in cardiac rate produced by bolus injections of 5-HT, noradrenaline, tyramine, and DMPP. In the control experiments in the upper part of the figure, two dose-response curves were established with a 15 min

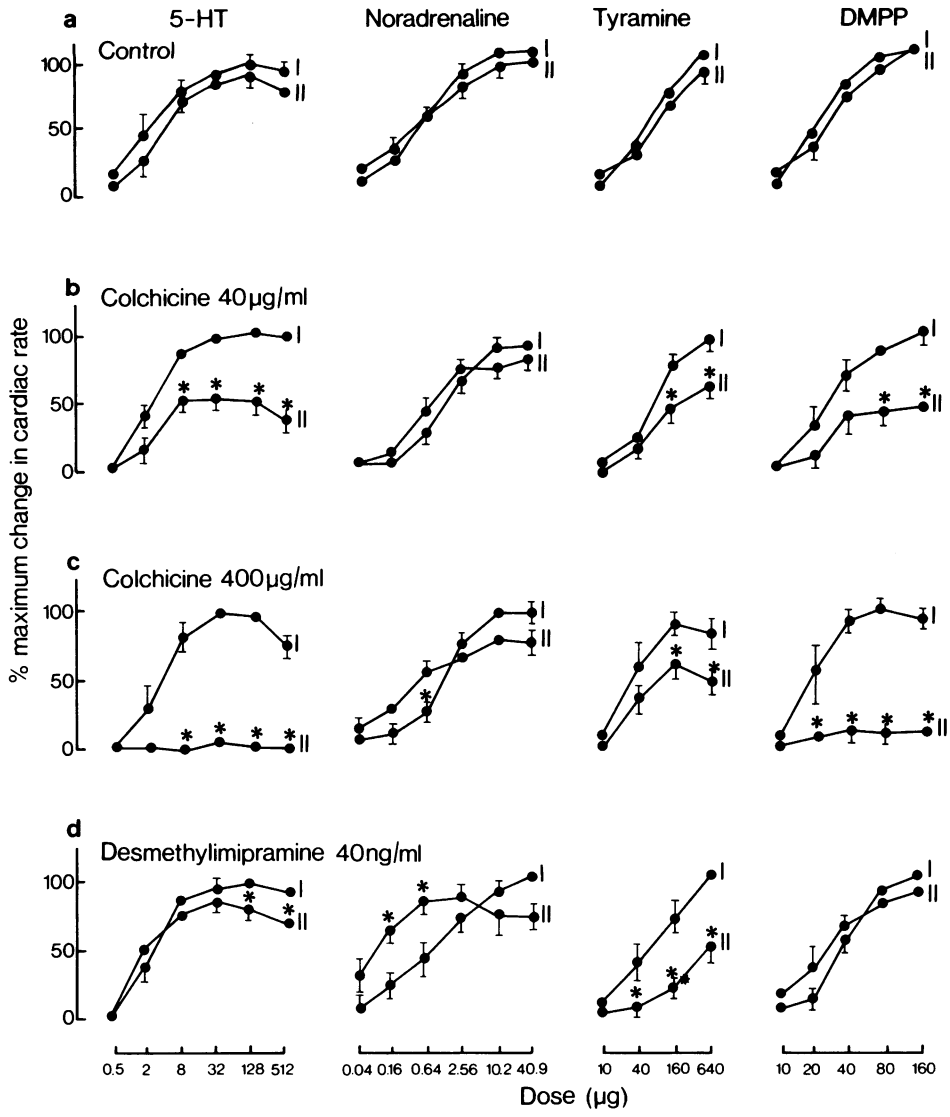

Figure 3 Effects of colchicine and desmethylimipramine on cardiac rate responses to bolus injections of 5-hydroxytryptamine (5-HT), noradrenaline, tyramine and dimethylphenylpiperazinium (DMPP). Atropine, 0.5 μg/ml was present throughout the experiments. (a) Upper graphs: control experiments, agonist dose-response curves established (I) and repeated after a 15 min interval (II). Lower graphs: (b) colchicine, 40 μg/ml and (c) 400 μg/ml, (d) desipramine, 40 ng/ml included in perfusion fluid after the first dose-response curve (I) and throughout the second dose-response curve (II). The points represent the mean values from 3 to 5 individual experiments with standard errors.
* Indicates a significant difference ($P < 0.05$) between response magnitudes at the same dose levels.

interval between them and for each agonist, responses proved to be reproducible. In the test experiments, hearts were perfused with Tyrode solution containing colchicine immediately after the first dose-response curve had been established. Colchicine, 40 and 400 μg/ml produced a concentration-dependent inhibition of responses to 5-HT and DMPP. In each case, the antagonism could not be surmounted by increasing the doses of either 5-HT or DMPP. In contrast, stimulation of cardiac rate evoked by

noradrenaline and tyramine was not inhibited by colchicine in a concentration-dependent fashion, although as Figure 3 shows, small but significant depression of the responses to tyramine was observed at each concentration of colchicine used.

Effects of desmethylimipramine on the cardiac responses to 5-hydroxytryptamine, noradrenaline, tyramine and dimethylphenylpiperazinium

The experimental design of these experiments was identical to that described for colchicine outlined above. In the presence of 40 ng/ml desmethylimipramine, the stimulant effects of 5-HT and DMPP were modified similarly. First, the magnitudes of the responses were little changed compared to control values (Figure 3). Second, in each case there was a considerable prolongation of the duration of the cardiac rate response. Responses to noradrenaline were clearly enhanced during perfusion of the hearts with desmethylimipramine (Figure 3) and as with 5-HT and DMPP, the duration of the responses was markedly prolonged. In direct contrast, the cardiac stimulant response to tyramine was inhibited over the whole dose-range by desmethylimipramine (Figure 3).

Effects of tetrodotoxin on cardiac responses evoked by electrical stimulation of the cardiac sympathetic nerves and by bolus injections of noradrenaline, 5-hydroxytryptamine and dimethylphenylpiperazinium

Electrical stimulation of the cardiac sympathetic nerves by the method of Huković & Muscholl (1962) evoked frequency-dependent increases in ventricular tension development and ventricular rate. Tetrodotoxin, 0.5 μg/ml, abolished responses to electrical nerve stimulation but did not affect the response to a bolus injection of noradrenaline (Mwaluko, 1975). Responses to 5-HT and DMPP were completely unaffected by perfusion with tetrodotoxin (Figure 4).

Drug-evoked release of ^3H from hearts whose noradrenaline stores had been labelled by perfusion with [^3H]-(−)-noradrenaline; effects of lowered extracellular Ca^{2+} concentration

The cardiac neuronal noradrenaline stores were labelled by perfusion with [^{3}H]-noradrenaline (10 ng/ml and 43 nCi/ml) for 12 min (Starke, 1971). In contrast to tyramine and DMPP, bolus injection of a dose of 5-HT representing an ED_{50} on mechanical performance (8 μg) proved only weakly effective as a stimulant of ^{3}H-release from the heart. This observation, which may reflect a restricted locus of transmitter releasing action of 5-HT (Mwaluko, 1975), is being further investigated. However, in order to make quantitative comparisons in the present experiments, a higher dose of 5-HT (512 μg) was

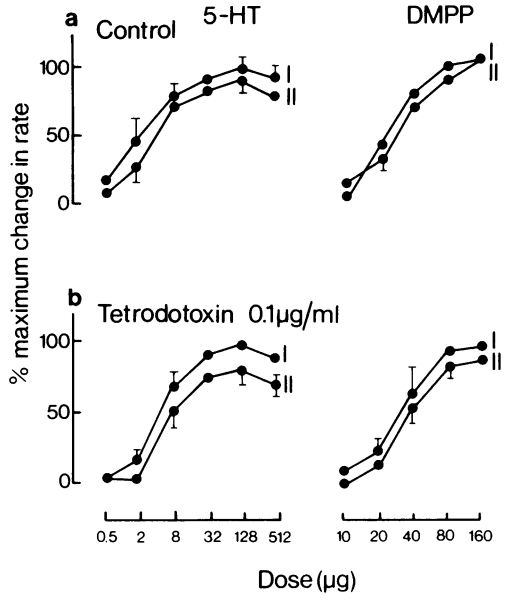

Figure 4 Effects of tetrodotoxin on cardiac rate responses to bolus injections of 5-hydroxytryptamine (5-HT) and dimethylphenylpiperazinium (DMPP). Atropine, 0.5 μg/ml was present throughout the experiments. (a) Control experiments, agonist dose-response curves established (I) and repeated after a 15 min interval (II). (b) Tetrodotoxin, 0.1 μg/ml included in the perfusion fluid after the first dose-response curve (I) and throughout the second dose-response curve (II). Other details as in Figure 3.

chosen to stimulate ^{3}H-release. Qualitatively identical results to those presented below have been obtained in separate experiments with lower doses of 5-HT.

In control experiments, where the total venous outflow was collected over a 60 min period, there was an initial sharp fall in the rate of ^{3}H-washout during the first 10 to 12 min, which was followed by a slow but constant decline over the remaining perfusion period (Mwaluko, 1975). 5-HT (512 μg), tyramine (40 μg) and DMPP (40 μg) were administered during this period as bolus injections, 30, 42 and 54 min after the end of the loading period respectively. The venous outflow was sampled as follows: 1 min before and 1 min after the injection of agonist, the outflow was collected in 10 s aliquots. For a 2 min period before and after the 10 s collection period, 1 min samples were collected for assay. At all remaining times, venous outflow was collected in 3 min samples for radioassay.

The results are expressed as nCi/10 s in Figure 5a and nCi/min in Figure 5b. 5-HT (512 μg) evoked a marked overflow of ^{3}H into the perfusion fluid. The peak of this overflow was reached within 10 to 30 s of

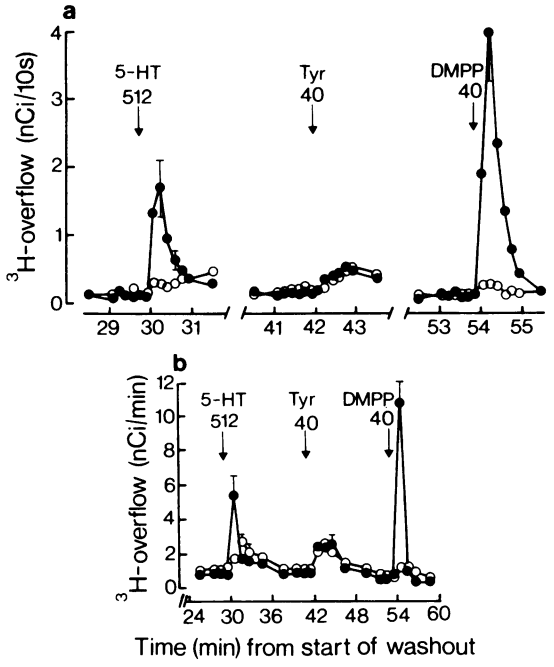

Figure 5 Spontaneous and evoked ^{3}H-overflow from hearts whose neuronal transmitter stores had been labelled by perfusion with [^{3}H]-(−)-noradrenaline (10 ng/ml, 43 nCi/ml) for 12 minutes. Atropine 0.5 μg/ml was present throughout the experiments. Zero time marks the end of the loading period with [^{3}H]-noradrenaline. Washout was with either Tyrode solution containing its normal calcium ion concentration (●), or Tyrode solution containing 6% of its normal calcium ion concentration (○). The points represent the means of 4 observations; vertical lines show s.e. mean. (a) Effect of bolus injections of 5-HT (512 μg), tyramine 40 μg (Tyr), and DMPP (40 μg), results presented as nCi/10 s. (b) Results presented as nCi/minute.

the injection and little release was evident after 1 to 1.5 min (Figure 5a). However, there was a secondary, low level, increase in ^{3}H-overflow, apparent 3 to 5 min after the injection which can be seen when the data are expressed as nCi/min (Figure 5b). DMPP (40 μg) also evoked a marked increase in ^{3}H-overflow and the pattern of the release was similar to that of 5-HT. Thus, the peak effect was reached between 10 and 30 s after administration and the ^{3}H concentration was close to pre-injection levels after 1 to 1.5 min (Figures 5a and b). Tyramine (40 μg) also caused a significant increase in ^{3}H-overflow although, in contrast to the pattern of release seen with 5-HT and DMPP, the peak of ^{3}H-overflow was not reached until 50 to 60 s after the injection (Figure 5a), and the overflow was still elevated 3 to 5 min after the injection (Figure 5b).

When the calcium ion concentration of the Tyrode solution was lowered from 3.6 to 0.2 mEq/l immediately after the loading period, the heart stopped beating and remained quiescent for the duration of the perfusion. The initial peak of ^{3}H-overflow evoked both by 5-HT and DMPP was greatly and significantly reduced by lowering the calcium ion concentration (Figures 5a and b). In contrast, lowering the extracellular calcium ion concentration had no significant effect on the ^{3}H-overflow evoked by tyramine. It was also apparent that the secondary component of the ^{3}H-release evoked by 5-HT persisted under conditions of lowered calcium ion concentration (Figure 5b).

Discussion

There are several pieces of evidence suggesting that the stimulant action of 5-HT on rabbit isolated hearts or atria is both sympathomimetic and indirect. For instance, the prototype β-adrenoceptor antagonist drug, dichloroisoprenaline, was used by Trendelenburg (1960) to antagonize successfully the stimulant response to 5-HT on rabbit atria. Further, reserpine pretreatment was used by both Jacob & Poite-Bevière (1960) and Trendelenburg (1960) to reduce effectively the cardiac stimulant response to 5-HT. The present data extend these earlier observations by using the newer, more selective β-adrenoceptor antagonists, propranolol and practolol, by adopting a more quantitative approach to the assessment of antagonism and by using a catecholamine depleting agent, 6-hydroxydopamine, whose action is selective for the sympathetic nerves.

Thus the fact that the pA_2 values for the antagonism by propranolol and practolol of the cardiac stimulant effects of 5-HT, noradrenaline, tyramine and DMPP are similar to each other and to the values found by other workers for the antagonism of cardiac β-adrenoceptors by the two drugs (Furchgott, 1972), is convincing evidence that stimulant responses to all these drugs are the result of activation of β-adrenoceptors. Further, the mode of action of 5-HT, tyramine and DMPP can be described as indirect, the result of catecholamine release from the cardiac sympathetic nerves, since their effects were reduced by pretreatment with 6-hydroxydopamine which, unlike reserpine, does not deplete amines from chromaffin tissue (Thoenen, Tranzer & Haeusler, 1970). This latter conclusion is further supported by the observations presented in Figure 5 where 5-HT along with tyramine and DMPP evoked a release of ^{3}H from hearts whose neuronal noradrenaline stores had been labelled by prior perfusion with [^{3}H]-(−)-noradrenaline (Starke, 1971).

When explanations are sought for the mechanism of

the indirect sympathomimetic actions of 5-HT, there are two principal possibilities. First, there is the well known excitatory effect of 5-HT on both parasympathetic (Rocha e Silva, Valle & Picarelli, 1953; Gaddum & Picarelli, 1957) and sympathetic (Trendelenburg, 1956; Haefely, 1974b) nervous tissue. Detailed analysis of the phenomenon using isolated sympathetic ganglia suggests that transmitter release results from depolarization of the nerve cells as a result of activation of receptors specific for tryptamines (Haefely, 1974b). With the exception of the receptor sites involved, this action is analogous to that of stimulants at nicotinic receptors such as DMPP (Haefely, 1974a;b). The other possibility which may be referred to as 'tyramine-like' is supported by the observations that 5-HT is accumulated by cardiac sympathetic nerves by active transport (Burgen & Iversen, 1965; Fillion, Lluch & Uvnas, 1971; Jester & Horst, 1972), and that 5-HT can displace endogenous noradrenaline from its storage sites within such nerves (Andeń, 1964; Gillis, 1964). Such a mechanism is claimed to operate in the indirect sympathomimetic response to 5-HT which has been demonstrated in the cat isolated spleen strip (Innes, 1962; Pluchino, 1972), nictitating membrane (Pluchino, 1972) and rat anococcygeous muscle (McGrath, 1973). The evidence from the present work allows the firm conclusion to be drawn that the indirect sympathomimetic response to 5-HT in rabbit hearts is, like DMPP, the result of neuronal depolarization and not, like tyramine, dependent on neuronal uptake and stoichiometric displacement of the stored transmitter.

First, complete and reversible tachyphylaxis to bolus injections of 5-HT was readily produced by perfusion with 1.25 μg/ml 5-HT (Figure 2). In this respect the stimulant effect of 5-HT on cardiac sympathetic nerves is similar to its actions on the parasympathetic neurones of the guinea-pig ileum (Gaddum & Picarelli, 1957) and the cells of the superior cervical ganglion of the cat (Trendelenburg, 1956; Haefely, 1974a). Tachyphylaxis to 5-HT was selective in that it was not extended to DMPP or tyramine (Figure 2), which suggests its site of action is not the nicotinic receptor and that 5-HT and tyramine have basically different mechanisms of action. In contrast, during perfusion with DMPP, 2 μg/ml, responses to both DMPP and 5-HT were considerably and similarly reduced, although those to tyramine were still very much in evidence. The possibility of an interaction at the level of the nicotinic receptors as an explanation of the phenomenon can be discounted; first, because of the selectivity of tachyphylaxis to 5-HT, and secondly because hexamethonium, in concentrations that abolish responses to DMPP, leaves responses to 5-HT unaffected (Fozard & Mwaluko, 1975b). The explanation might however be related to the non depolarizing 'adrenergic neurone blocking activity' of DMPP described for the rabbit heart by Löffelholz (1970a; b). It is easy to envisage that such a property, which is demonstrably able to inhibit the powerful depolarizing stimulus arising from electrical nerve stimulation, would also inhibit a relatively weak, chemical stimulus effected by 5-HT. However, perhaps the most important point in this context is not to establish the exact mechanism of the inhibition produced by DMPP but to highlight the differential effects of DMPP in inhibiting 5-HT more readily than tyramine. This fact once again links the mechanism of action of 5-HT with that of DMPP rather than with that of tyramine.

The use of colchicine further amplifies the association of the mechanism of action of 5-HT with that of DMPP rather than tyramine. Colchicine interferes with the exocytotic secretion of many hormones (Lacy, Howell, Young & Fink, 1968; Kraicer & Milligan, 1971; Douglas & Sorimachi, 1972; Sorimachi, Oesch & Thoenen, 1973) possibly by causing disintegration of microtubules (Borisy & Taylor, 1967). In the present experiments, responses to 5-HT and DMPP, but not those to noradrenaline or tyramine, were inhibited by colchicine (Figure 3). In the case of nicotinic agonists colchicine has been specifically suggested to interfere with some step(s) in an excitation-secretion coupling mechanism which is calcium ion-dependent and which results in the release of noradrenaline, probably by exocytosis from the noradrenergic nerve terminals (Smith & Winkler, 1972; Sorimachi *et al.*, 1973). The present results suggest a similar conclusion would be justified with 5-HT as the stimulus to release, especially as responses to tyramine, whose action is independent of both extracellular calcium ion and the process of exocytosis (Lindmar *et al.*, 1967; Thoenen, Huerlimann & Haefely, 1969; Chubb, De Potter & De Schaepdryver, 1972; Thoa, Wooten, Axelrod & Kopin, 1975) remained largely unaffected by perfusion with colchicine (Figure 3).

The reasoning behind the experiments with desmethylimipramine was that if 5-HT were releasing noradrenaline by a 'tyramine-like' mechanism then its action should be inhibited by drugs which interfere with the neuronal monoamine uptake pathway. Desipramine (40 ng/ml) has previously been shown to block noradrenaline uptake in the rabbit isolated heart (Wennmalm, 1971; Barth, Manns & Muscholl, 1975) and in the present experiments, potentiation of noradrenaline coupled with inhibition of tyramine (Figure 3) are a predictable consequence of such an action (Trendelenburg, 1972). In practice, responses to 5-HT, like those to DMPP, were not inhibited by perfusion with desipramine (Figure 3) and their durations were markedly prolonged. Thus, transmitter release evoked both by 5-HT and DMPP appears to take place independently of the neuronal amine transport mechanism. However, inhibition of reuptake

of the released transmitter would be a significant factor contributing to the enhanced responses observed.

The results obtained in the experiments where endogenous neuronal noradrenaline stores were labelled with [^{3}H]-noradrenaline add further weight to the argument that the mechanism by which 5-HT stimulates the cardiac sympathetic nerves is more similar to DMPP than to tyramine. Thus, whereas 5-HT mimicked DMPP in causing a sharp peak of ^{3}H-release which was virtually complete within 1 min, tyramine showed a slow onset and decline of release with the rate of ^{3}H-overflow, expressed as nCi/min, being constant over the 3 min period immediately following the injection (Figure 5). These characteristic time courses cannot have as their bases the particular sequence of agonist injections employed in Figure 5, since identical release patterns were observed in separate preliminary experiments where the agonists were injected either alone or in entirely random sequences. DMPP has been shown to cause an 'explosive' release of noradrenaline from cardiac sympathetic nerves by a brief depolarization of the neuronal membrane which is terminated rapidly by 'autoinhibition' (Haeusler, Thoenen, Haefely & Huerlimann, 1968; Löffelholz, 1970a; b). The present data support this concept for DMPP and suggest that a similar mechanism may operate with 5-HT. Tyramine, on the other hand, must first be taken up by the neuronal membrane (Commarato, Brody & McNeill, 1969), then by the storage vesicles (Musacchio, Kopin & Weise, 1965), before displacing the stored transmitter. The relatively slow increase in the perfusate ^{3}H concentration presumably reflects these movements. Similarly, since termination of the releasing action of tyramine depends on enzymatic metabolism (Musacchio *et al.*, 1965; Smith, 1966), the decline of its releasing effect will be a more gradual phenomenon than, say, the decline of the releasing action of DMPP brought about by autoinhibition.

When the calcium concentration of the perfusion fluid was reduced to 6% of normal, the initial peak of ^{3}H-release caused by DMPP and 5-HT was abolished whereas ^{3}H-release by tyramine remained unaffected (Figure 5). These results confirm previous reports that sympathetic transmitter release evoked by DMPP is calcium-dependent (Lindmar *et al.*, 1967; Haeusler *et al.*, 1969; Chubb *et al.*, 1972; Thoa *et al.*, 1975). The demonstration that release by 5-HT is, like DMPP, dependent on extracellular calcium ion has its counterpart in the perfused cat adrenal gland, where catecholamine release evoked both by nicotinic agonists and 5-HT was abolished by omission of calcium from the perfusion medium (Douglas & Rubin, 1963; Poisner & Douglas, 1966). The results clearly distinguish between the mechanisms of action of 5-HT and tyramine and emphasize again the basic similarity between the mechanisms of action of 5-HT and DMPP.

The experiments with calcium-depleted Tyrode solution do, however, disclose that the small secondary peak of ^{3}H-overflow normally observed 2 to 5 min after the injection of 5-HT persists unchanged during calcium deprivation (Figure 5b). The simplest explanation for the observation is that at the high dose used to elicit ^{3}H-release, 5-HT has properties characteristic of DMPP (the initial peak) and tyramine (the secondary release). Such a conclusion might have been anticipated from the literature where, at high doses, 5-HT has been shown to be taken up by the sympathetic nerves and to release endogenous noradrenaline from its vesicular storage sites (see above). The possibility that the 'tyramine-like' action of 5-HT is a complicating factor in the interpretation of the data obtained using end organ response measurements can be discounted; first, because the time course of the response on mechanical performance and the 'tyramine-like' release (Figure 5b) are quite different; second, because the phenomenon is not observed with doses of 5-HT less than 512 μg, which is 64 times the ED_{50} for stimulation of mechanical performance (Mwaluko, 1975) and finally, because a 'tyramine-like' release mechanism would be incompatible with the data obtained with colchicine (Figure 3), desmethylimipramine (Figure 3) and the demonstration of selective tachyphylaxis to 5-HT (Figure 2).

Tetrodotoxin blocks propagated action potentials in nerve and muscle by preventing the rapid inward sodium current (Kuryama, Osa & Toida, 1966) and, predictably, the effects of electrical nerve stimulation were abolished during perfusion with the drug. In contrast, responses to 5-HT and DMPP remained unaltered (Figure 4). Sympathetic transmitter release by nicotinic agonists has previously been shown to be resistant to tetrodotoxin, despite action potential generation in the nerves being abolished (Haeusler *et al.*, 1968; Krauss, Carpenter & Kopin, 1970; Su & Bevan, 1970; Westfall & Brasted, 1972; Thoa *et al.*, 1975). Krauss *et al.* (1970) described the mechanism of action of nicotinic agonists as resulting from propagated depolarization and an increased calcium permeability of the nerve terminal that is independent of a propagated action potential. The present results suggest a similar description would be appropriate to the action of 5-HT.

In conclusion, the mechanism of the indirect sympathomimetic effect of 5-HT on the rabbit isolated heart has been shown to be fundamentally different from that of tyramine. In only one respect, however, has it been shown to differ from that of DMPP, and that is in the nature of the sites which trigger the response (Figure 3). By analogy with data from the superior cervical ganglion where receptor sites specific for tryptamines appear to exist on the cell bodies of the sympathetic neurones (Haefely, 1974b), the

likelihood is that transmitter release by 5-HT is mediated through similar sites on the terminal sympathetic fibres. To establish this as a fact is the subject of our continuing investigations (Fozard & Mwaluko, 1975b).

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