Effects of 5-Hydroxytryptamine and Related Compounds on the Sympathetic Nerves of the Rabbit Heart

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Summary. In isolated rabbit hearts perfused with Tyrode solution we investigated the effects of various mono- and dihydroxytryptamines on the noradrenaline release from (and uptake into) the terminal sympathetic nerve fibres. The noradrenaline in the perfusate was estimated spectrofluorimetrically. Alterations of heart rate were also determined.

1. 5-Hydroxytryptamine (5-HT), 6-hydroxytryptamine (6-HT) and 5,7-dihydroxytryptamine (5,7-DHT) inhibited the uptake of exogenous noradrenaline from the perfusion fluid into the sympathetic nerves. Continuous perfusion of hearts with 6-HT caused a gradual increase in noradrenaline release and heart rate. Desipramine abolished both the noradrenaline-releasing and the positive chronotropic effects, whereas pindolol inhibited only the increase in heart rate. Perfusion of hearts with 5-HT before and during bolus injection of 6-HT did not alter the positive chronotropic effect of 6-HT.

2. Continuous perfusion of hearts with 5-HT, 5,7-DHT or 5,6-dihydroxytryptamine (5,6-DHT) rapidly induced a release of high amounts of noradrenaline and/or a pronounced increase in heart rate which returned to slightly elevated levels within the first 4 min of perfusion with these indolethylamines.

4. Bolus injection of tryptamine, 5-HT, 7-hydroxytryptamine (7-HT), 5,6-DHT or 5,7-DHT caused a noradrenaline release and a positive chronotropic effect which attained a maximum within the first 2 min after injection, whereas 4-hydroxytryptamine and 6,7-dihydroxytryptamine were ineffective.

5. The 5-HT- or 5,7-DHT-induced noradrenaline release was not decreased by desipramine, methiothepin, methysergide and pindolol. However, the latter compound inhibited the positive chronotropic effects evoked by the indolethylamines.

6. Reserpine pretreatment, cocaine, mianserin, verapamil or 5-HT (infused into the aortic cannula before and during injection of 5-HT or 5,7-DHT) decreased the noradrenaline-releasing effect of the indolethylamines.

7. Dose-response curves were established for the positive chronotropic effects induced by tryptamine, 5-HT, 7-HT, 5,6-DHT and 5,7-DHT. In addition, concentration-response curves were determined for the inhibitory effects of these compounds on the 5-HT-induced increase in heart rate. There was a good correlation between the negative logarithms of the ED50 and IC50 values of the indolethylamines (r = 0.99); the slope of the regression line was about 1.

These findings confirm that the positive chronotropic effects of indolethylamines on the rabbit heart are due to noradrenaline release. Whereas 6-HT appears to cause its indirect sympathomimetic effect by a tyramine-like mechanism, the other compounds induce noradrenaline release by activation of presynaptic 5-HT receptors on the terminal sympathetic nerve fibres.

Key words: 5-Hydroxytryptamine — Indolethylamines — Presynaptic receptors — Cardiac sympathetic nerves — Noradrenaline release.

Introduction

The positive chronotropic and inotropic effects of 5-hydroxytryptamine (5-HT) on rabbit atria and the perfused rabbit heart are due to an indirect sympathomimetic action (Trendelenburg, 1960; Jacob and Poite-Bevierre, 1960; Fozard and Mwaluko, 1976). Evidence has been presented that the release of noradrenaline is probably caused by an activation of presynaptic 5-HT receptors on the terminal sympathetic nerve fibres.
The effects of these compounds on noradrenaline overflow were also capable of stimulating noradrenaline release (Fozard and Mobarok Ali, 1978). Several analogues of 5-HT were also capable of stimulating noradrenaline release (Fozard and Mobarok Ali, 1978). Perfusion of hearts with low concentrations of 5-HT or its analogues inhibited the cardiac stimulant responses to bolus injections of 5-HT; the rank order of potency as antagonists of 5-HT was similar to that as agonists (Fozard and Mobarok Ali, 1978).

The suggestions concerning the site and mechanism of action of these compounds were mainly based on indirect evidence, since in almost all experiments only the positive chronotropic and inotropic effects of the tryptamine derivatives were measured. Therefore, we decided to perform additional experiments in which the effects of these compounds on noradrenaline overflow from the isolated rabbit heart were determined. Furthermore, we compared the potencies of several mono- and dihydroxytryptamines, and we studied to what extent a tyramine-like component contributes to the indirect sympathomimetic effects of these compounds.

Some of the results were reported at the Conference on Serotonin Neurotoxins, New York, 1977 (Göttert and Klupp, 1978) and at the Joint Meeting of German and Italian Pharmacologists, Venezia, 1977 (Göttert and Dührsen, 1977).

**Methods**

*Perfusion of Rabbit Hearts.* The experiments were done on isolated hearts of mongrel rabbits (either sex) weighing 1.4 - 2.9 kg. All details of isolation and perfusion of the hearts and of all other experimental procedures have been described previously (Göttert, 1974). Briefly, the hearts were perfused with Tyrode solution (33°C) at a constant flow rate of 25 ml/min. The composition of the solution was as follows (mM): NaCl 137; KCl 2.7; CaCl₂ 1.8; MgCl₂ 1.1; NaHCO₃ 11.9; NaH₂PO₄ 0.4; glucose 5.5; ascorbic acid 0.06 (aeration with 95% oxygen and 5% carbon dioxide). The perfusion fluid contained 1.8 μM atropine in order to abolish initial negative chronotropic and inotropic effects of 5-HT (Fozard and Mwaluko, 1976) and its analogues. The apex of the heart was connected to a strain gauge (diastolic tension 10 g) and the contractions triggered a rate meter. Tension developed by the hearts and rate of contractions were displayed on a Hellige recorder.

The experiments began after an equilibration period of 20 min. The compounds investigated were dissolved in Tyrode solution; they were either infused into the aortic cannula at a rate of 0.5 - 1.5 ml/min or administered by bolus injection. The solutions of the various indolethylamines always contained ascorbic acid (ratio between molar concentrations of the indolethylamines and those of ascorbic acid 3:5).

*Infusion of Various Indolethylamines.* The indolethylamines were infused into the aortic cannula for 10 min. As a rule the perfusate was collected in 2-min fractions during the initial 4 and the last 2 min of infusion; in some of the experiments with 6-hydroxytryptamine, we also collected the perfusate between the 4th and 8th min of infusion.

In further series of experiments with 6-hydroxytryptamine, 2 periods of infusion of this compound (10 min duration) were applied at an interval of 30 min (from onset to onset); the perfusate was collected only during the last 2 min of each infusion period. Ten minutes before and during the second infusion, desipramine or pindolol were present in the perfusion fluid.

*Bolus Injections of Indolethylamines.* In most of the experiments in which the influence of drugs on indolethylamine-induced noradrenaline output and positive chronotropic effect was studied, 2 or 3 bolus injections (S₁ - S₅) were delivered to each heart every 15 min (sampling of perfusates during the 2 min subsequent to each injection). The drugs investigated were present in the perfusion fluid from 10 min before until 5 min after S₂. The noradrenaline output at any stimulation period was calculated as the fraction of that evoked by S₁.

For comparison of the potencies of various indolethylamines, tryptamine and six mono- or dihydroxytryptamines were injected into the aortic cannula at intervals of 10 min (sampling of perfusates during the 2 min subsequent to the injections). In 50% of the experiments the sequence of injections was as shown in Table 2, in the remaining 50%, it was reversed. Ten minutes after the last injection of an indolethylamine, a bolus injection of noradrenaline was delivered. In order to investigate the influence of reserpine pretreatment on the indolethylamine-induced effects, reserpine 5 mg/kg was administered to rabbits 48 and 24 h before the hearts were removed (i.p. injections). In the experiments on these hearts, some of the indolethylamines and noradrenaline were injected according to the same experimental protocol as previously described.

*Experiments with Pargyline.* Two bolus injections of 5-HT or 5,7-dihydroxytryptamine were delivered into the aortic cannula at an interval of 47 min and the perfusate was collected during the 2 min subsequent to the injections. Seven minutes after the first stimulation, the 30 min infusion of pargyline 625 μM was initiated.

The influence of pargyline on the tyramine-induced noradrenaline release was also investigated. For this purpose, tyramine was infused into the aortic cannula for 6 min. Five minutes after the end of the first administration of tyramine, pargyline was added to the perfusion fluid for 30 min. After another 10 min-interval, a second infusion of tyramine (6 min duration) was initiated. The perfusate was sampled throughout the tyramine infusions.

*Stimulation with Acetylcholine.* In these experiments the atropine concentration in the Tyrode solution amounted to 3.5 μM throughout the perfusion. Acetylcholine was infused 3 times at intervals of 15 min (final concentration 180 μM, duration 40 s). Sampling of perfusate started with the onset of stimulation and lasted for 2 min. Mianserin was present in the perfusion fluid from 10 min before until 5 min after the second stimulation.

*Dose-Response Curves for the Stimulant Effect of Indolethylamines.* In these experiments only the increase in heart rate induced by bolus injection of indolethylamines was determined. In each heart only one dose-response curve was established. The dose of the indolethylamine studied was increased by a factor of 4. The interval between the injections usually amounted to 5 min; this interval was extended, if the heart rate had not yet reached the initial value within this time period (as described by Fozard and Mwaluko, 1976). The E₅₀ value of the indolethylamine was determined for each individual experiment.

*Concentration-Response Curves of Indolethylamines for the Inhibition of 5-HT-Induced Effect on Heart Rate.* In each heart 5 injections of 11.2 umol 5-HT (S₁ - S₅) were applied at intervals of 11 min and the positive chronotropic effects were recorded. Various indolethylamines (in each heart only one compound was tested) were present in the perfusion fluid from 5 min before until 2 min after S₂, S₃, and S₄, respectively (the indolethylamine concentration infused was increased by a factor of 3.2 from stimulation period to stimulation period). S₁ and S₅ served as controls (the increase in heart rate