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ON THE MECHANISM OF TACHYPHYLAXIS TO TYRAMINE IN THE ISOLATED RAT HEART

BY

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Tyramine was shown to release [^{3}H]-catecholamines from an isolated rat heart previously perfused with [^{3}H]-noradrenaline. With successive injections of tyramine the amount of [^{3}H]-catecholamine released fell progressively and there was a parallel decrease in the increment of amplitude and rate of contraction of the heart. Reserpinized hearts were shown to take up less [^{3}H]-noradrenaline than normal hearts. Release of radioactivity and loss of responsiveness to tyramine occurred more rapidly in the reserpinized heart. In the same preparation the uptake of [^{14}C]-tyramine exceeded the quantity of the noradrenaline released.

Burn & Rand (1958) suggested that tyramine and certain sympathomimetic amines act by releasing catecholamines from a store in the walls of arteries or from the heart. Support for this hypothesis has come from several laboratories. Lockett & Eakins (1960) showed an increase in the noradrenaline content of aortic blood following tyramine injection; Schümann & Weigmann (1960) and Euler & Lishajko (1960) found that tyramine accelerated the release of noradrenaline from granules isolated from sympathetic nerves; Burn & Burn (1961) and Hertting, Axelrod & Patrick (1961) showed that radioactive noradrenaline taken up by the heart was released by tyramine *in vitro* and *in vivo*; Lindmar & Muscholl (1961) demonstrated an increase in the rate of release of noradrenaline from the isolated perfused rabbit heart with tyramine; and Potter, Axelrod & Kopin (1962) observed a partial and limited depletion of noradrenaline in the hearts of rats treated with repeated doses of tyramine. The reversal and prevention of the development of tachyphylaxis by noradrenaline infusions (Cowan, Cannon, Koppanyi & Maengwyn-Davies, 1961) has also been interpreted to support the hypothesis of Burn & Rand.

An alternative hypothesis for the action of tyramine was presented by Carlsson, Rosengren, Bertler & Nilsson (1957), who suggested that tyramine was fully active only in the presence of an intact adrenergic system. Muscholl (1960), who did not find an increase in the noradrenaline content of the hearts of reserpinized rats following an infusion of noradrenaline, concluded that the restoration of the effect

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of tyramine in these animals did not depend on the replenishment of the noradrenaline stores. Nasmyth (1960) could not demonstrate depletion of pressor amines in the heart following tyramine perfusion of isolated hearts. These reports have not been consistent with the hypothesis of Burn & Rand (1958).

The availability of [^{3}H]-noradrenaline of high specific activity (1,300 μc/μmole) and the demonstration that the isolated perfused rat heart takes up and releases infused [^{3}H]-noradrenaline (Hertting, Kopin & Gordon, 1962) permitted a study of the effects of tyramine on the outflow of [^{3}H]-noradrenaline from the isolated perfused hearts of normal and reserpinized rats. It will be shown that there is a direct relationship between the release of [^{3}H]-noradrenaline and the response to tyramine in isolated hearts from normal and reserpinized rats. The development of tachyphylaxis to tyramine appears to be a result of the depletion of easily releasable stores.

METHODS

Sprague-Dawley female rats weighing 200 to 300 g were killed by a blow on the head; the hearts were immediately removed and perfused by the Langendorff technique with warm (38° C) oxygenated (95% oxygen, 5% carbon dioxide) Krebs-Ringer bicarbonate solution containing one-half the recommended (Umbreit, Burris & Stauffer, 1957) calcium concentration and 1 g glucose/l. The perfusion rate was 3 to 5 ml./min at a pressure of 60 cm water. A transducer (Statham Strain Gauge) was attached to the apex of the heart to record amplitude and rate of contraction.

Five min after starting the perfusion, a continuous infusion of high specific activity (1,300 μc/μmole) [^{3}H]-7-(±)-noradrenaline (0.14 μg/min) was begun through a side arm in the perfusion cannula. The concentration in the cannula varied from 0.03 to 0.05 μg/ml., depending on the rate of flow of the perfusing fluid through the individual hearts. The infusion was continued for 2 to 4 min. Timed collections of the perfusate were made in graduated tubes containing 0.2 ml. 6 N hydrochloric acid. At the end of the experiment the total radioactivity and the [^{3}H]-noradrenaline were determined in the perfusate and in the heart (Whitby, Axelrod & Weil-Malherbe, 1961). Tyramine (10 μg in 0.2 ml. Krebs-Ringer bicarbonate solution) was injected into the perfusion cannula.

Six rats were treated with reserpine (Serpasil, Ciba), 2.5 mg/kg intraperitoneally 24 and 5 hr prior to removal of the hearts for perfusion. In other experiments [^{14}C]-2-tyramine (4.25 μc/μmole, California Corporation for Biochemical Research) was infused at a constant rate into the perfusion cannula: the concentration in the cannula varied from 0.75 to 1.5 μg tyramine/ml., depending on the rate of perfusion through the heart. After allowing the infusions to run for 2, 4, 6, or 8 min, the hearts were immediately removed and assayed for [^{14}C]-tyramine by the following method. The heart was homogenized with 9 ml. 0.4 N perchloric acid, and centrifuged. An aliquot of the supernatant solution was adjusted to *p*H 6.5 with potassium carbonate using a glass electrode *p*H meter. The solution was passed through a Dowex 50 column, the amine eluted with 3 N ammonium hydroxide and an aliquot of the eluate assayed for ^{14}C in a liquid scintillation spectrometer. The concentration of [^{14}C]-tyramine in the perfusion fluid was determined from the concentration of ^{14}C in the effluent.

RESULTS

Release of [^{3}H]-noradrenaline following the repeated administration of tyramine. The effect of tyramine on the outflow of radioactivity from the perfused heart is shown in Fig. 1. About 85% of the radioactivity in the perfusate after tyramine injections was found to be catecholamine and the total radioactivity could be used

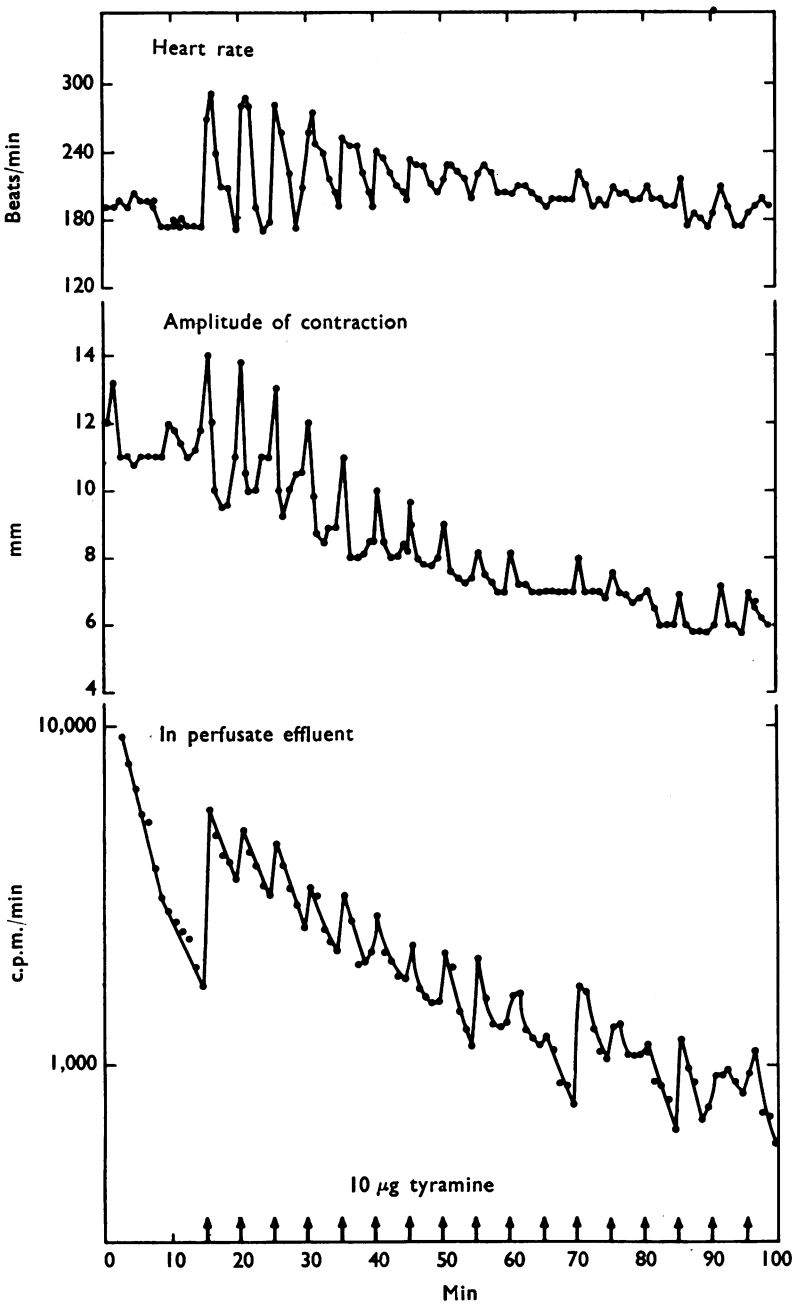

Fig. 1. The rate and amplitude of contraction in response to tyramine and the rate of release of ^3H in the isolated perfused rat heart. Ordinates: upper, beats/min; middle, amplitude of contraction; lower, radioactivity. Abscissa: time in min.

as an index of the released catecholamines. The responses of the heart rate and amplitude of contraction were also measured. The amount of [^{3}H]-catecholamine released paralleled the effect on the rate and amplitude of cardiac contraction. The response to each injection of tyramine became progressively smaller and was accompanied by a corresponding fall in the increment of the rate of release of radioactivity.

It was possible to calculate the amount of [^{3}H]-noradrenaline remaining in the heart at any given time by taking the sum of the amount found in the heart at

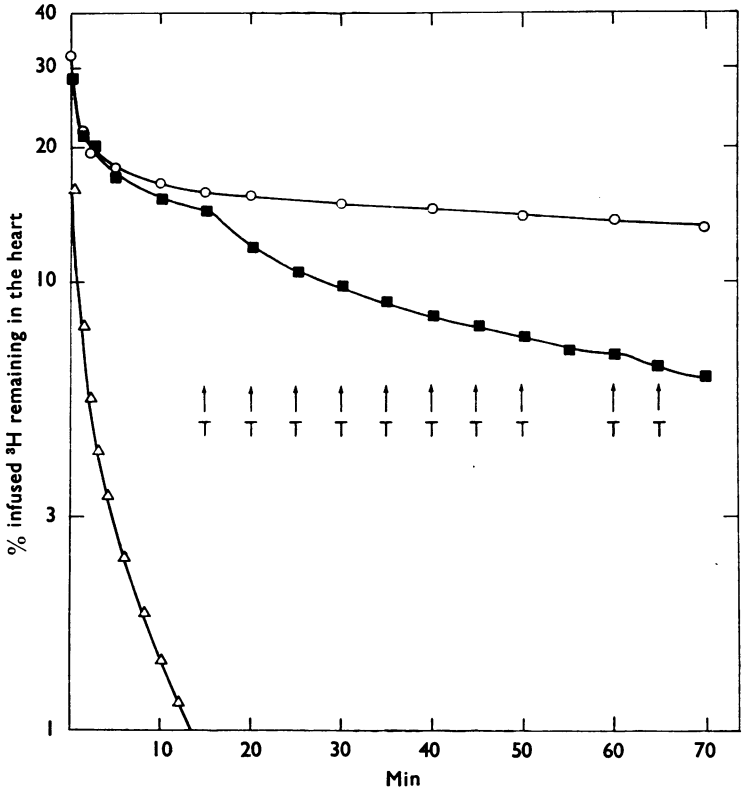

Fig. 2. ^{3}H-Noradrenaline in the isolated perfused rat heart. Normal (○——○), reserpinized (△——△) and tyramine treated (■——■). Reserpinized hearts were obtained from rats pretreated with reserpine. Tyramine, 10 μg each injection, was administered into the perfusion cannula. Ordinate: % infused ^{3}H remaining in the heart. Abscissa: time in min.

the end of the experiment and the amount of [^{3}H] released in the interval between that time and the end of the experiment. The course of release of radioactivity from the heart is shown (Fig. 2).

Effect of tyramine on the release of [^{3}H]-noradrenaline from the isolated reserpinized heart. The net uptake of [^{3}H]-noradrenaline by the reserpinized heart was less than that of the normal heart; the release of radioactivity was much more rapid (Fig. 2). Tyramine quickened the rate of release of [^{3}H]-noradrenaline even

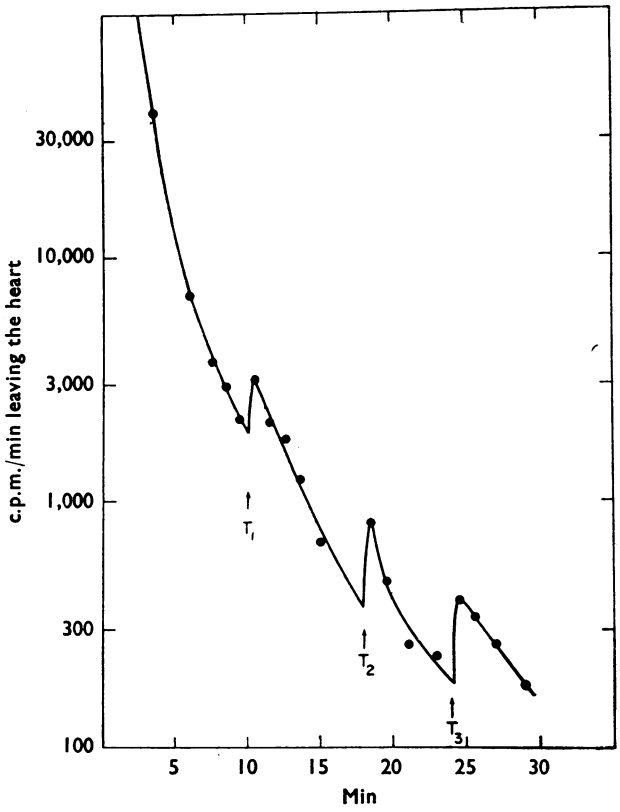

Fig. 3. Effect of tyramine on the rate of release of [^{3}H] from the isolated perfused heart of a reserpinized rat. Ordinate: radioactivity in the perfusate leaving the heart. Abscissa: time in min.

in the reserpinized heart (Fig. 3). Following the administration of noradrenaline, the responsiveness of the reserpinized heart to tyramine was restored, and the responses to tyramine paralleled the release of [^{3}H]-noradrenaline (Fig. 4). In the reserpinized hearts the more rapid release of catecholamine was accompanied by a more rapid development of tachyphylaxis to tyramine (Fig. 4).

Uptake of [^{14}*C*]*-2-tyramine by the isolated heart.* Schümann & Philippu (1961) concluded that catecholamine released by tyramine from chromaffin granules of the adrenals was replaced stoichiometrically. The possibility that the release of noradrenaline by tyramine is the result of molecular displacement was investigated by measuring the [^{14}C]-tyramine content of the isolated heart following infusion of the labelled amine. The concentration of [^{14}C]-tyramine in the heart water would not be expected to exceed that of the perfusion fluid unless a mechanism for concentration of the amine was present. In effect, the concentration of [^{14}C]-tyramine (Fig. 5) rose above that present in the perfusion fluid. It was calculated from the specific activity (4.25 μc/μmole) of tyramine that, after 8 min, the heart contained about three times the molar equivalent of the noradrenaline usually present in the heart.

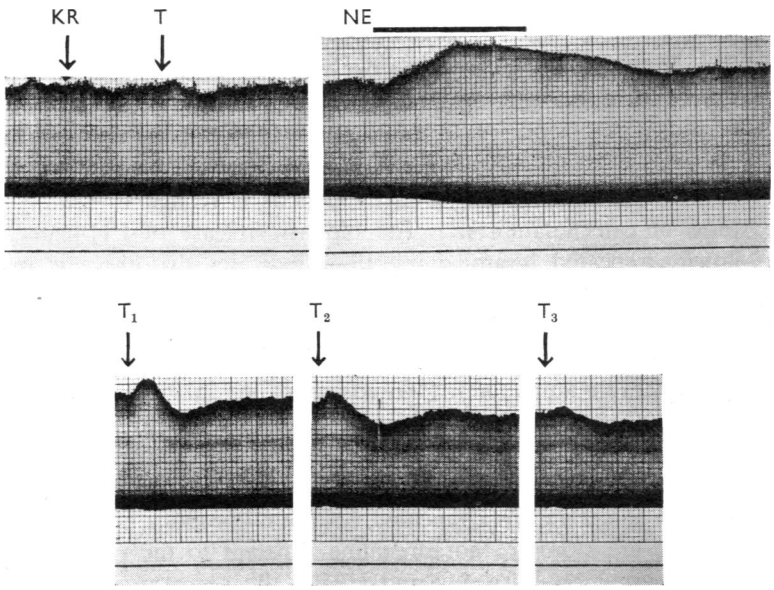

Fig. 4. Effect of tyramine on the response of the isolated perfused heart from a reserpinized rat. At KR an injection of 0.2 ml. Krebs-Ringer bicarbonate solution was made into the perfusion cannula. This was followed by an injection of 10 μg tyramine (T). Two min later (0·28 μg) [^{3}H]-noradrenaline (NE) was infused during an interval of 2 min. Tyramine (10 μg) was then injected 10 min (T_1), 18 min (T_2), and 24 min (T_3) after the noradrenaline infusion. Each small division represents 4 sec.

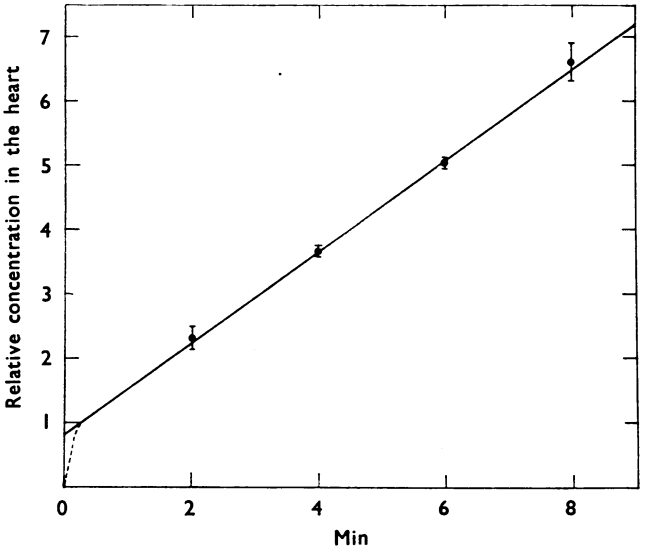

Fig. 5. The uptake of [^{14}C]-tyramine by the isolated perfused heart. The relative concentration of tyramine was determined by the ratio of tyramine concentration in the heart to the concentration in the perfusion fluid. Each point represents the mean (±s.e.) of six rat hearts. Ordinate: relative concentration of tyramine in the heart. Abscissa: time in min.

DISCUSSION

The experiments described here support the hypothesis that the action of tyramine is mediated by the release of catecholamines. Successive injections of tyramine caused a progressive fall in the release of catecholamine and the rate and amplitude of contraction of the isolated heart were correspondingly reduced. These observations suggest that the development of tachyphylaxis is related to the depletion of available stores of catecholamines. The biphasic release of [^{3}H]-catecholamine (Fig. 2) following repeated tyramine injections indicates that there is more than one store from which tyramine may liberate noradrenaline. The difference in rate of release of radioactivity may be a consequence of a difference in avidity of the storage site for the D and L forms of the administered [^{3}H]-noradrenaline. The presence of considerable amounts of [^{3}H]-noradrenaline in the heart after repeated tyramine injections (Fig. 2), the ability of an organ to respond to sympathetic nerve stimulation after the development of tachyphylaxis to a sympathomimetic amine (Cowan *et al.*, 1961), and the decreasing fraction of noradrenaline released from the heart of intact animals during repeated tyramine injections (Potter *et al.*, 1962) indicate that there are stores of noradrenaline resistant to the releasing action of tyramine. As reported by Burn & Rand (1958), the reserpinized heart does not respond to tyramine, but an infusion of noradrenaline was shown to be taken up by the reserpinized heart. Radioactivity was rapidly released from this preparation, and tyramine further increased the rate of release. These findings indicate that tyramine does, indeed, enhance release of noradrenaline in the reserpinized heart provided the stores of noradrenaline have been partially replenished. The more rapid development of tachyphylaxis in the reserpinized heart is probably related to the rapidity with which stored noradrenaline is released.

The amount of [^{14}C]-tyramine found in the heart was greater than the expected amount based on heart water content and concentration of tyramine in the perfusion fluid ; it also exceeded the amount of noradrenaline normally extractable from the heart. This may explain the findings of Nasmyth (1960), who could not show a decrease in the " pressor amine " content of the heart following tyramine perfusion. The additive effects of residual noradrenaline and tyramine taken up by the heart during the perfusions may have been measured in his bioassay. Our data are compatible with displacement by tyramine of noradrenaline from a portion of its stores. The capacity for amine uptake may exceed the normal amine content and binding may provide an effective mechanism for the termination of the action of physiologically active amines.

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