THE MODE OF ACTION OF TYRAMINE

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The repeated administration of tyramine to the isolated perfused guinea-pig heart gradually decreased the positive inotropic response which was paralleled by a decrease in the noradrenaline content of the heart and an increase in the noradrenaline content of the perfusate. Phenethylamine, ephedrine, amphetamine, guanethidine and bretylium were administered to the isolated heart until no further positive inotropic effect was obtained. The absence of an inotropic response with phenethylamine and guanethidine was associated with a decrease in the noradrenaline content of the heart. The absence of an inotropic effect with amphetamine, bretylium and ephedrine was not associated with a decrease in the amount of noradrenaline in the heart.

Burn and Rand (1958) proposed that tyramine exerts its sympathomimetic effect by effecting a release of noradrenaline from tissue stores, since postganglionic sympathetic denervation of a tissue greatly reduces its responsiveness to tyramine (Burn and Tainter, 1931; Burn, 1932; Fleckenstein and Burn, 1953). Prior treatment with reserpine reduces the pressor action of tyramine (Carlsson, Rosengren, Bertler and Nilsson, 1957) and also depletes endogenous stores of noradrenaline (Burn and Rand, 1957), and the pressor action of tyramine may be re-established, after treatment with reserpine, by infusion of noradrenaline (Burn and Rand, 1958).

Tyramine increases the concentration of adrenaline and noradrenaline in the aortic plasma of cats (Lockett and Eakins, 1960); and adrenalectomised dogs pretreated with phenoxybenzamine (Weiner, Draskoczy and Burack, 1962) and elevates the level of noradrenaline in venous effluents of cat spleen (Stjärne, 1961). Tyramine releases noradrenaline from isolated chromaffin and adrenergic neurone granules. (Schüman, 1960; von Euler and Lishajko, 1960). In addition tyramine causes the release of radioactivity from tissue equilibrated with (^{3}H)-noradrenaline (Hertting, Axelrod and Patrick 1961; Burn and Burn, 1961). Weiner (1962) and others observed that large subcutaneous doses of tyramine reduce the amount of noradrenaline in the heart, spleen and brown fat of the rat.

Experiments in this laboratory (Davey, Farmer and Reinert, 1962) have shown that the noradrenaline content of the isolated guinea-pig heart is markedly reduced if successive doses of tyramine are given until no positive inotropic effect is obtained. The object of the present work was to determine whether the noradrenaline content of the isolated perfused guinea-pig heart and its perfusate, as well as the magnitude of the positive inotropic effect, are related to the total amount of tyramine administered. Some observations have also been made with guanethidine, phenethylamine, amphetamine, bretylium and ephedrine. Since the completion of this work, Axelrod and his colleagues have published the observation that tyramine releases (^{3}H)-noradrenaline from isolated rat hearts previously perfused with (^{3}H)-noradrenaline and that with successive injections of

tyramine the amount of (^{3}H)-noradrenaline released is reduced progressively and there is a parallel decrease in the increment of amplitude and rate of contraction of the heart.

METHODS

Guinea-pig hearts were perfused by the method of Langendorff with a warm (36°) oxygenated (95 per cent O_2, 5 per cent CO_2) Krebs's solution which was of the following composition (g./litre NaCl, 7·1; KCl, 0·35; $CaCl_2$, 0·28; $MgSO_4 \cdot 7H_2O$, 0·28; $NaHCO_3$, 2·09; KH_2PO_4, 0·16; glucose 1·0); the perfusate was collected in containers immersed in ice; 100 μg. of the amine under investigation was injected into a cannula close to the heart every 5–10 min. until no positive inotropic effect was observed,

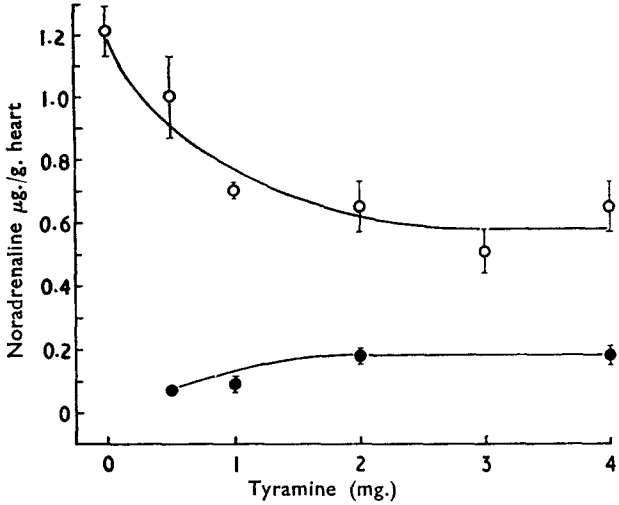

FIG. 1 The effect of tyramine (1, 2, 3 and 4 mg.) on the noradrenaline content (○) and output (●) of the isolated guinea-pig heart. The hearts were given 100 μg. every 5-10 min. Results expressed as μg. noradrenaline per g. heart.

except with guanethidine where it was found that 200 μg. was necessary to produce a positive inotropic effect. The noradrenaline content of the whole heart and perfusate was determined by the method of Merrills (1962). The method in outline involved the homogenisation of the heart in 0·3M perchloric acid, adsorption of the noradrenaline contained in a neutralised aliquot of this perchloric acid extract on alumina, elution by means of relevant pH adjustment and finally fluorimetric estimation of noradrenaline in the eluate. The perfusate from the heart was initially acidified with hydrochloric acid, and then neutralised and adsorbed onto alumina as above. The use of thioglycollic acid as a stabilising agent makes this method more specific for the estimation of noradrenaline. Tyramine, adrenaline, isoprenaline and 3,4-dihydroxyphenylalanine produced no interference with the estimation of noradrenaline and the recovery of noradrenaline added to tissues was 90 per cent.

RESULTS

The effects of graded doses of tyramine on the content and output of noradrenaline from the isolated guinea-pig heart are shown in Fig. 1. Fig. 2 shows two typical tracings obtained during the continued administration of tyramine to the isolated heart. A total of 3 mg. was needed before the tyramine failed to produce a positive inotropic effect, but the

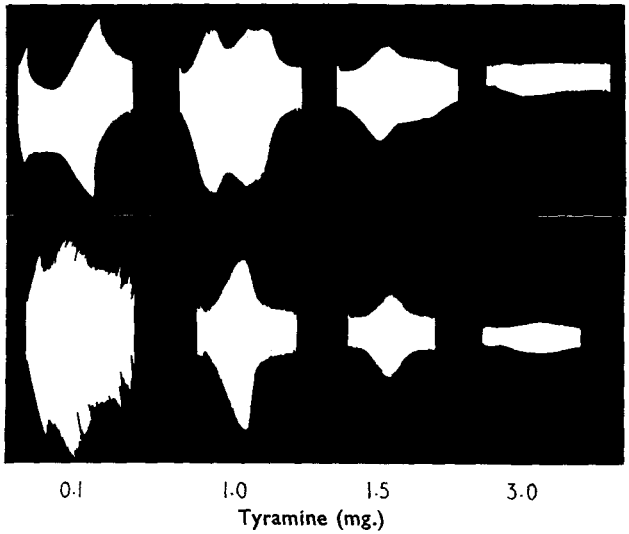

FIG. 2. The effect of successive doses of tyramine on the force of contraction of the isolated guinea-pig heart. The upper and lower records are two representative tracings. The hearts were given 100 μg. tyramine every 5-10 min. The response of the hearts at an accummulated dose of 0.1, 1.0, 1.5 and 3.0 mg. tyramine are shown. Note the marked decrease in response between 1.0 and 1.5 mg. tyramine.

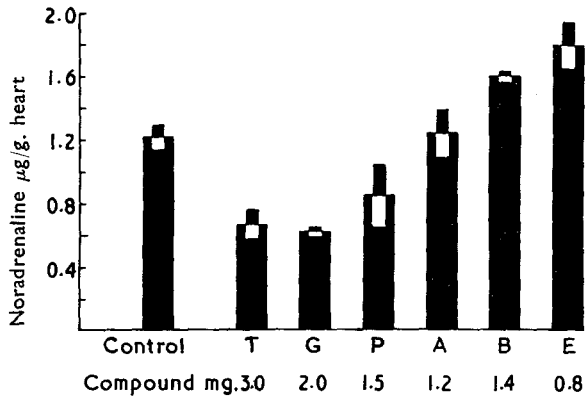

FIG. 3. The effect of tyramine (T), guanethidine (G), phenethylamine (P), amphetamine (A), bretylium (B), and ephedrine (E) on the noradrenaline content of the isolated guinea-pig heart. The columns represent the mean result for 5 hearts $\pm$ s.e.

response was much reduced after 1·5 mg. (accummulative dose) (see Fig. 2). The depletion of noradrenaline in the heart by tyramine was accompanied by the release of noradrenaline into the perfusate.

Guanethidine, phenethylamine, amphetamine, bretylium and ephedrine produced sympathomimetic effects on the isolated guinea-pig heart. The repeated administration of these agents caused successively smaller responses and finally no response. With guanethidine and phenethylamine there was a decrease in the amount of noradrenaline in the heart. Amphetamine produced no change in the heart noradrenaline concentration; with ephedrine and bretylium there was an increase in heart noradrenaline concentration (Fig. 3).

Discussion

Our results support the hypothesis of Burn and Rand (1958) that tyramine mediates its sympathomimetic effects by the release of catecholamines. The evidence for this is that the reduction of the noradrenaline level in the isolated guinea-pig heart is proportional to the amount of tyramine administered to the heart and that the depletion is associated with the appearance of noradrenaline in the perfusate. When tyramine no longer produces an inotropic effect there is a depletion of noradrenaline; further injections of tyramine do not produce any greater decrease in the noradrenaline levels of the heart. These results are in agreement with those of Axelrod and others (1962) who studied the effect of tyramine on the content and release of (^{3}H)-noradrenaline from isolated rat hearts equilibrated with (^{3}H)-noradrenaline.

The response to tyramine disappears at a time when the noradrenaline stores of the heart are approximately 45 per cent. Von Euler and Lishajko (1960) observed that tyramine reduced the noradrenaline content of isolated adrenergic granules to a maximum of 40–50 per cent of that of the controls. This indicated that noradrenaline in the heart is stored in two forms, one, a "bound" form and the other, a "free" form. The amine in the "free" form appears to be readily available to tyramine.

There was a depression of the spontaneous heart rate when tyramine no longer produced a positive inotropic effect. This may indicate that the noradrenaline stores through which tyramine acts are those concerned with controlling heart rate by spontaneous liberation of noradrenaline and possibly are those acted upon by impulses from postganglionic sympathetic nerve fibres.

Lockett and Eakins (1960) observed an increase in plasma adrenaline as well as noradrenaline in the aorta of cats injected with tyramine. Adrenaline was not determined in experiments as we found it to constitute less than 5 per cent of the total catecholamines in the guinea-pig heart. Therefore, our results do not preclude the possibility that tyramine liberates adrenaline as well as noradrenaline.

Burn and Rand (1958) proposed that certain sympathomimetic amines in addition to tyramine, namely, phenethylamine, amphetamine, and ephedrine depended upon the presence of stores of noradrenaline in the tissues to exert their sympathomimetic action. Similar observations have

been made with guanethidine and bretylium (Bein, 1960; Furchgott, 1960). The continued administration of single doses of these compounds to the isolated guinea-pig heart produced successively smaller positive inotropic effects and finally no response. The estimation of the noradrenaline content of the whole heart showed that the loss of a positive inotropic effect after phenethylamine or guanethidine could be ascribed to a decrease of the heart noradrenaline concentration, that is, the positive inotropic effect was limited by the amount of noradrenaline available in the heart itself. The responses to amphetamine, ephedrine and bretylium disappeared when the heart amine levels were either normal as with amphetamine or raised as for ephedrine and bretylium. Therefore, failure of amphetamine, ephedrine and bretylium to elicit an inotropic effect is not due to lack of noradrenaline *per se*. The increase in noradrenaline concentration produced by ephedrine and bretylium indicates some interference with the release or destruction of noradrenaline.

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