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EFFECT OF TYRAMINE ON ISOLATED GUINEA-PIG ATRIA IN RELATION TO THEIR NORADRENALINE STORES

BY

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The relation between the noradrenaline content of isolated guinea-pig atria and the rate-increasing action of tyramine was studied by the use of pretreatment with reserpine as a pharmacological tool for graded depletion of the noradrenaline stores. Reserpine was more potent in depleting the stores than in reducing the biological response to tyramine; 50% depletion had little effect on the response to tyramine; 50% reduction of the response to tyramine occurred when the noradrenaline content fell to about 10% of normal. Depletion of the stores of guinea-pig atria did not result in supersensitivity to noradrenaline. Exposure of heavily pretreated atria to 3×10^{-6} noradrenaline for 10 min (followed by repeated washing for 45 min) restored the response to tyramine to 70% of normal; it failed, however, to restore the noradrenaline content to the level expected from the experiments with reserpine alone. Restoration of the response to tyramine was accompanied by a small but significant increase in the noradrenaline content of the atria; a change in sensitivity to added noradrenaline did not occur. The results are consistent with the view that (a) the noradrenaline stores consist of two compartments the smaller of which is important for the action of tyramine, that (b) this smaller compartment can be at least partially refilled by exposure of the atria to noradrenaline, and that (c) there is no direct relationship between the noradrenaline content and the sensitivity to noradrenaline in guinea-pig atria.

It is well known that reserpine depletes the noradrenaline stores of peripheral organs (Carlsson, Rosengren, Bertler & Nilsson, 1957; Paasonen & Krayer, 1958; Burn & Rand, 1959) and abolishes the response of such tissues to tyramine and to stimulation of adrenergic nerves (Carlsson *et al.*, 1957; Burn & Rand, 1958; Trendelenburg & Gravenstein, 1958). Tyramine is therefore generally assumed to exert its sympathomimetic effects through the liberation of noradrenaline from peripheral noradrenaline stores. Although various studies deal with the effects of pretreatment with graded doses of reserpine, they have been restricted either to the observation of biological responses believed to be mediated through the liberation of noradrenaline (Fleming & Trendelenburg, 1961; Trendelenburg, 1961; Liebman, 1961; Waud, 1961; Liebman, Muskus & Waud, 1962) or to the determination of catechol amine content (Carlsson *et al.*, 1957). Since no attempt has yet been made to correlate these two phenomena quantitatively, it is not known how

much the noradrenaline stores must be depleted to reduce the sympathomimetic action of tyramine.

Although it is well established that noradrenaline restores the response of reserpine-pretreated preparations to tyramine (Burn & Rand, 1958, 1960) and to nerve stimulation (Burn & Rand, 1958; Gillespie & Mackenna, 1961), restoration of the noradrenaline stores has been observed by some authors (Pennefather & Rand, 1960) but not by others (Muscholl, 1960).

It was thus of interest to study the effect of pretreatment with graded doses of reserpine both on the response of isolated guinea-pig atria to tyramine and on their noradrenaline content. Furthermore, the restoration of the pharmacological response to tyramine by exposing heavily pretreated atria to noradrenaline has also been examined.

METHODS

The atria were dissected from the hearts of freshly killed guinea-pigs (350 to 520 g body weight) and suspended in Locke solution at 30° C. This solution contained 9 g sodium chloride, 0.42 g potassium chloride, 0.24 g calcium chloride, 0.5 g sodium bicarbonate and 2 g dextrose per litre, and was saturated with oxygen. At the beginning of the experiment the *p*H was about 7.5 and did not change appreciably during the experiment. The capacity of the organ baths was either 10 or 15 ml.; drug concentrations refer to the final concentration in the bath.

The spontaneous beat of the preparations was recorded by attaching the atria to the moving coil of a potentiometer, and the potential set up in the coil with each beat was recorded with a Grass ink-writing oscillograph. Before any drugs were tested, the preparations were left in the bath until their spontaneous resting rate did not change by more than 5 beats/min during a 10 min period of observation; this took about 1 hr. During resting periods, records were obtained every 5 or 10 min; during the exposure to drugs the rate was recorded every 30 sec. Drugs were kept in the bath for 10 min; the difference between the resting rate (determined just before the administration of the drug) and the maximal rate obtained during this 10 min period was taken as response. When, however, the drug failed to cause any increase in rate, the difference between resting rate and the rate recorded just before the removal of the drug was taken as response.

The following substances were used: l-noradrenaline bitartrate monohydrate (expressed as base), tyramine hydrochloride, ethylene-diaminetetra-acetic acid disodium salt (edetic acid), and reserpine phosphate (the weights refer to the salts). Reserpine phosphate (10 mg/ml.) was dissolved in 20% ascorbic acid and, if required, diluted with distilled water. Pretreatment with reserpine was carried out by a single intraperitoneal injection 24 hr before the experiment.

The tissue concentration of noradrenaline and adrenaline was determined by a modification of the fluorimetric trihydroxyindole procedure, the details of which have been described previously (Crout, 1961; Crout, Creveling & Udenfriend, 1961). Atria were removed from the organ bath, blotted with filter paper, weighed, and ground in cold 5% trichloroacetic acid. The homogenate was centrifuged, and catechol amines were extracted from the clear supernatant solution by adsorption to and elution from alumina. Aliquots of the eluate were assayed fluorimetrically; iodine was used as oxidizing agent. The average recovery of known amounts of noradrenaline carried through the procedure was 91%. All values are corrected for this loss and reported as μg/g wet tissue. The limit of sensitivity of the method is considered to be 0.06 μg/g for determinations performed on single atria and 0.015 μg/g for assays of 4 pooled atria.

Statistical calculations were made according to conventional procedures (Snedecor, 1946).

RESULTS

Effect of pretreatment with graded doses of reserpine on the response to tyramine. Preliminary experiments with isolated guinea-pig atria showed that the dose-response curve for rate-increasing action of tyramine has an ascending limb, a distinct maximum, and a descending limb; pretreatment with reserpine depresses the maximum of the dose-response curve without causing any horizontal shift. Similar observations on the heart-lung preparation of the dog have recently been

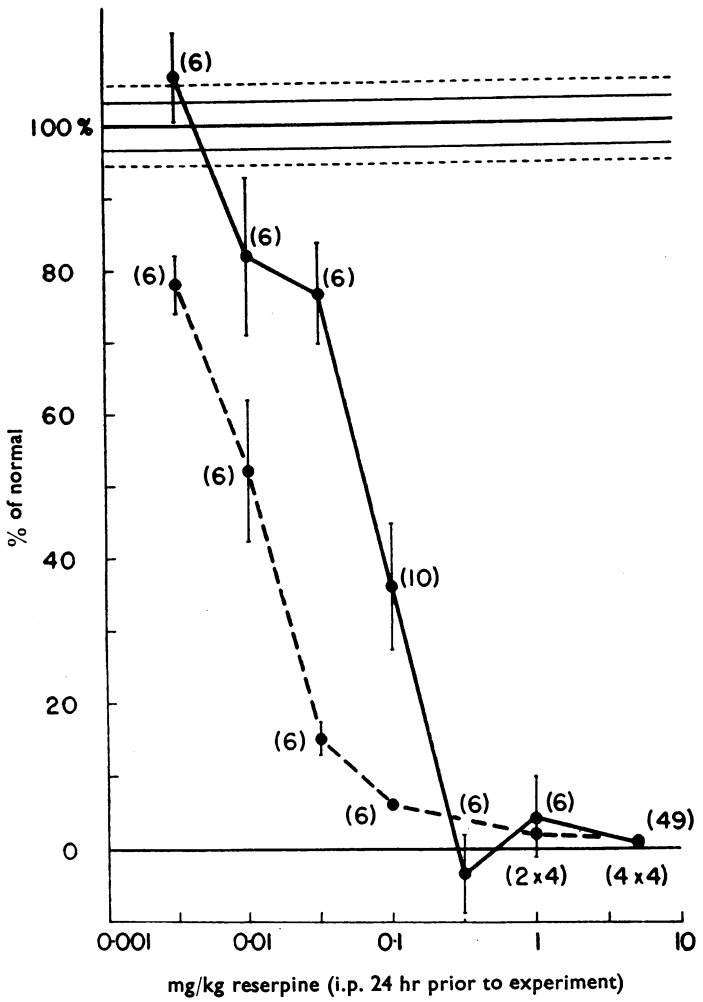

Fig. 1. Effect of pretreatment with graded doses of reserpine (given intraperitoneally 24 hr prior to experiment) on the maximal response of isolated guinea-pig atria to tyramine and on their noradrenaline content. Solid line: response of rate of beat to 10^{-5} tyramine expressed as % of control observations. Broken line: noradrenaline content in % of control observations. The horizontal lines near the 100% level indicate the standard error of the mean of the control observations (16 for response to tyramine, 8 for noradrenaline content); standard errors of means are indicated by vertical bars, numbers of observations in brackets.

reported by Liebman (1961). Under our experimental conditions the maximum of the dose-response curve for tyramine was obtained with a final concentration of 10^{-5} (w/v) tyramine, which increased the rate of beat of normal control preparations by 58.1 ± 2.0 beats/min (mean $\pm$ its standard error, 16 preparations). Pretreatment with reserpine, given 24 hr before the experiment, reduced or abolished the response to tyramine ; Fig. 1 demonstrates that this effect of pretreatment is clearly related to the dose of reserpine.

If it is assumed that tyramine exerts its sympathomimetic effects through the release of noradrenaline, it follows that at least two factors influence the response of any organ to tyramine: (a) the amount of noradrenaline available for liberation, and (b) the sensitivity of the organ to noradrenaline. To exclude the possibility that the reduction of the response to tyramine after pretreatment with reserpine was due to changes in sensitivity rather than to the depletion of the noradrenaline

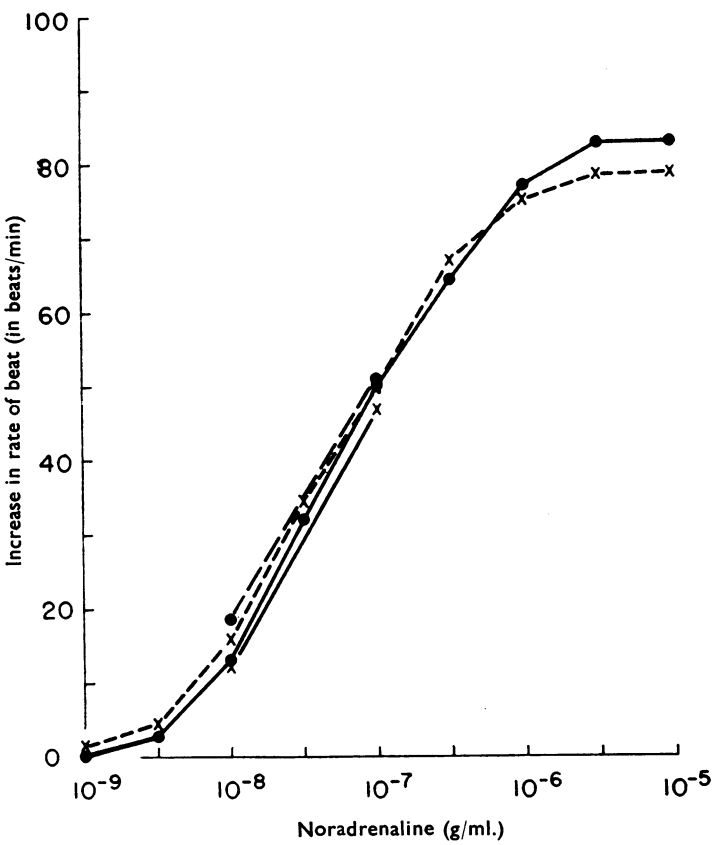

Fig. 2. Response to noradrenaline of 6 normal and 7 reserpine-pretreated guinea-pig atria (1 mg/kg, 24 hr prior to experiment). The sensitivity was first tested with two single exposures to 10^{-8} and 10^{-7} noradrenaline (short pair of curves) and then with full cumulative dose-response curve. Solid line: controls; broken line: pretreated with reserpine. Response is expressed as increase in beats/min.

stores, the sensitivity to noradrenaline was determined in atria from normal and from heavily pretreated guinea-pigs (1 mg/kg reserpine). No differences in sensitivity were detected between the two groups of atria, either when two test doses of noradrenaline were applied (with intermediate removal of the substance), or when full cumulative dose-response curves were obtained (by adding increasing amounts of noradrenaline to the bath without intervening changes of bath fluid) (Fig. 2). Pretreatment of the guinea-pig with 1 mg/kg reserpine thus did not affect the sensitivity of isolated atria to added noradrenaline.

Effect of pretreatment with graded doses of reserpine on the noradrenaline content. To ensure strict comparability of the results, the experimental conditions were identical with those of the preceding section. Atria from normal and from pretreated animals were isolated and left in the bath for about 1 hr (see Methods). At the point at which, in the preceding experiment, they would have been tested with tyramine, they were removed and assayed for noradrenaline and adrenaline. Eight atria from normal guinea-pigs were found to contain 4.6 ± 0.26 μg/g noradrenaline and 0.11 ± 0.018 μg/g adrenaline when assayed under these conditions. Accurate determinations of the catechol amine content in individual atria could not be performed in guinea-pigs pretreated with 1 mg/kg or more reserpine because of the severe depletion of tissue stores; 4 atria were therefore pooled for determinations in these animals.

Fig. 1 illustrates that any given dose of reserpine produced a proportionately greater reduction of noradrenaline stores than of the response to tyramine. When the noradrenaline stores were 50% of normal, the response to tyramine was only slightly reduced, and 50% reduction of the response to tyramine did not occur until the noradrenaline content was approximately 10% of normal. The adrenaline content of these atria was not reduced by doses of reserpine between 0.003 and 0.1 mg/kg; however, after reserpine doses of 1 mg/kg and higher no adrenaline was detectable.

Restoration by noradrenaline of the response to tyramine. For this series of experiments guinea-pigs were pretreated with 5 mg/kg reserpine (given intraperitoneally 24 hr prior to the experiment) in order to deplete their noradrenaline stores as much as possible (see Fig. 1). After the atria were isolated and their rate had become stable, their response to 10^{-5} tyramine was recorded for 10 min. The tyramine was washed out and 10 min later the atria were exposed for 10 min to noradrenaline. Since it was desirable to prevent oxidation of noradrenaline during the 10 min period of exposure, edetic acid was added to the bath (1.5×10^{-5}). This concentration of edetic acid binds a negligible proportion of the calcium of the solution (less than 2%) and presumably binds a substantial proportion of those heavy metal ions that catalyse the oxidation of noradrenaline. In separate experiments the half-life of added noradrenaline was determined under these conditions; it was found to be about 6 min. After the 10 min exposure to noradrenaline the bath fluid was changed repeatedly during an additional period of 45 min; the new bathing solution contained no edetic acid, since it was desirable to remove all free noradrenaline as quickly and as completely as possible. In the absence of edetic acid added noradrenaline had a half-life of only 50 sec. It can thus be assumed

that under these experimental conditions no free noradrenaline was left in the bath after the 45 min period. The test concentration of tyramine was then applied again.

The exposure of heavily pretreated atria to 3×10^{-6} noradrenaline partly restored their response to tyramine; a mean increase in rate of 40.4 ± 4.8 beats/min was observed (Fig. 3C). As an after-effect of the exposure to the high concentration of noradrenaline the resting rate decreased. However, this change in base-line activity did not account for the restoration of the response to tyramine, since the maximal absolute rate, observed during the second response to tyramine, exceeded that observed during the first response by about 20 beats/min. Hence, whether the results are expressed as "increase in beats/min," as "maximal absolute rate of beat" or as "increase in % of resting rate," partial restoration of the response to

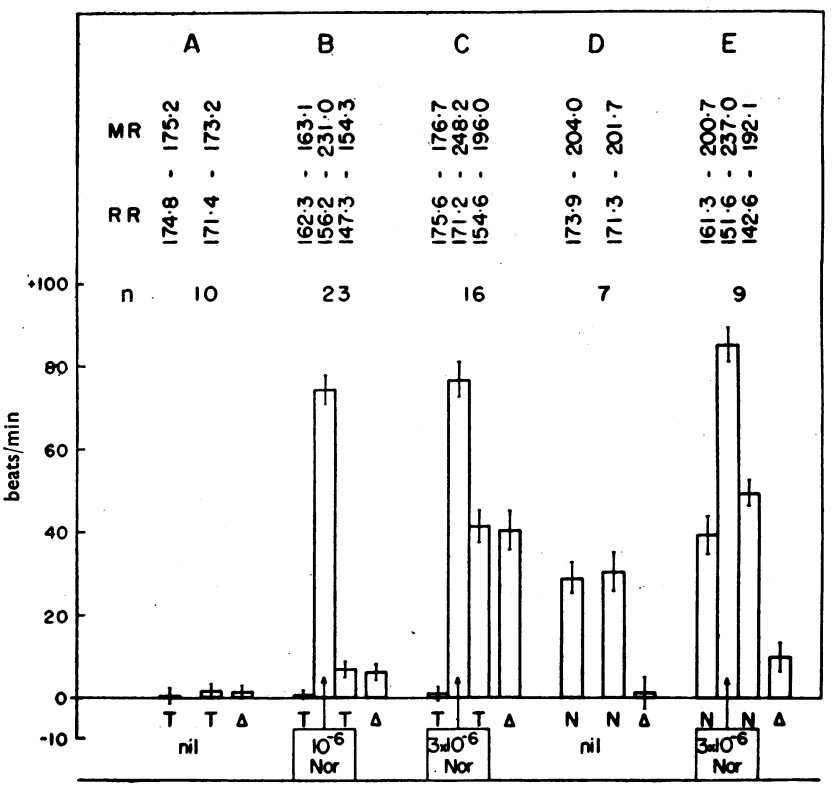

Fig. 3. Effect of exposure to noradrenaline (for 10 min) on the response of isolated guinea-pig atria to tyramine (A–C) and to noradrenaline (D–E) (after pretreatment with reserpine 5 mg/kg, 24 hr prior to experiment). A and D: controls (no exposure to noradrenaline); B: exposure to 10^{-6}, and C and E: exposure to 3×10^{-6} noradrenaline. Columns indicate mean responses (increase in beats/min over resting rate) to 10^{-5} tyramine hydrochloride (T) and to 10^{-7} noradrenaline base (N) before and after the exposure to noradrenaline (Nor), as well as the means of the individual differences between the two responses (Δ). Vertical bars indicate standard errors of means. RR=mean of resting rate prior to administration of active substance; MR=mean of maximal absolute rate of beat observed at height of response; n=number of observations.

tyramine was observed after the exposure of atria to 3×10^{-6} noradrenaline. A lower concentration of noradrenaline (10^{-6}) was less effective (Fig. 3B). As a control, the effect of tyramine was also tested on atria which had not been exposed to noradrenaline (but with identical time intervals); in this group the second response to tyramine was not significantly different from the first (Fig. 3A).

Further evidence that changes in resting rate did not account for the restoration of the response to tyramine was obtained by correlating change in resting rate of individual atria with change in response to tyramine; in the individual control preparations (that is, those which were not exposed to noradrenaline) the correlation coefficient was very low and not significant ($r=0.10$; $P>0.7$). Fig. 4A shows the regression line of this relationship for the control observations (broken line) and

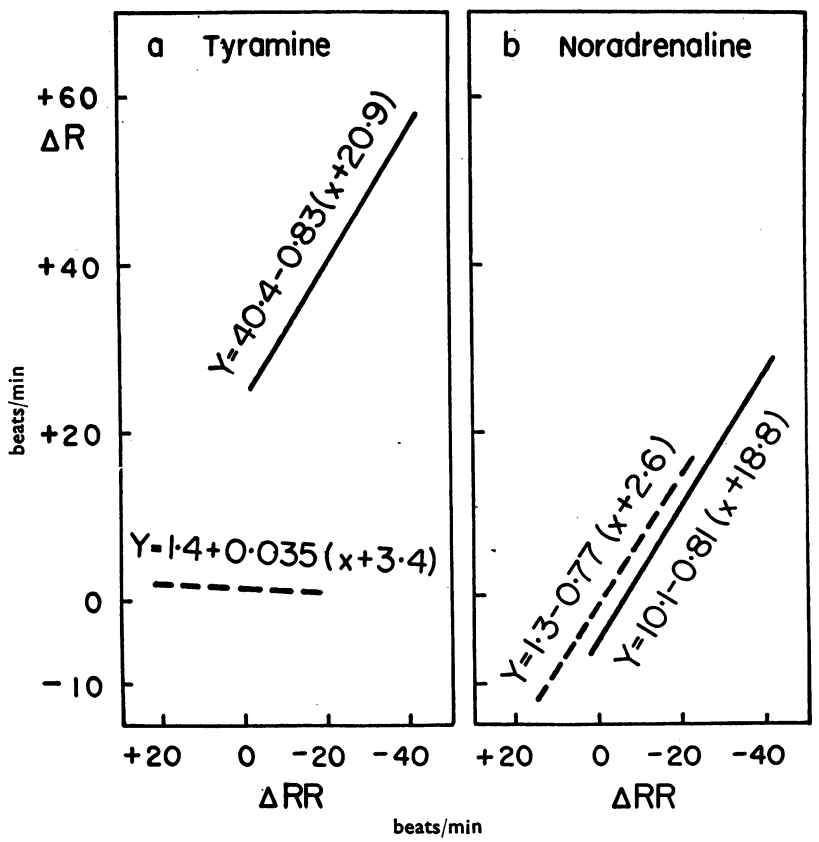

Fig. 4. Regression lines relating changes in resting rate to changes in response to tyramine (a) and to noradrenaline (b). Same preparations as in Fig. 3A, C, D and E. ΔR=difference between first and second response of individual atria; ΔRR=change in resting rate determined just before first and just before second response. Broken lines: control observations (with no exposure to noradrenaline); solid lines: with intervening exposure to 3×10^{-6} noradrenaline. The length of the regression lines indicates the range of variation in resting rate observed in that particular group. The equations of the regression lines ($Y=\bar{y}+b(x-\bar{x})$) are given; for details see text.

the regression line for the group exposed to 3×10^{-6} noradrenaline (solid line). It is evident that a shift along the control curve to the right (that is, a fall in resting rate) cannot account for the increased response to tyramine.

To determine whether the exposure to noradrenaline had any effect on the sensitivity of the atria to noradrenaline, two additional series of observations were made. Under otherwise identical experimental conditions the response of heavily pretreated atria to a test concentration of 10^{-7} noradrenaline was determined. The control group, which was not exposed to the high concentration of 3×10^{-6} noradrenaline, showed no difference between the two responses to the test concentration of 10^{-7} noradrenaline (Fig. 3D). However, the second response to the test concentration of 10^{-7} noradrenaline was increased by 10 beats/min in the group which was exposed to 3×10^{-6} noradrenaline (Fig. 3E). This seems to indicate that the exposure to a high concentration of noradrenaline increased the sensitivity of atria to subsequently added test concentrations of noradrenaline. Further analysis, however, shows that this is not true.

The mean resting rate of the control group remained nearly constant throughout the experiment (Fig. 3D), but individual atria within this group varied considerably (from +15 to −23 beats/min). Differences between the first and the second response to the test concentration of noradrenaline were highly significantly correlated to changes in resting rate ($r=0.93$; $P<0.01$). A similar correlation was found for the second group of atria, which had been exposed to 3×10^{-6} noradrenaline between the two tests (Fig. 3E) ($r=0.99$; $P<0.001$). The regression lines of these relationships are shown in Fig. 4B. As an after-effect of the exposure to the high concentration of noradrenaline, the resting rate of this group of preparations was decreased. The fact that this curve (solid line) is partly superimposed on and partly a continuation of the control curve (broken line) indicates that changes in resting rate account fully for the observed changes in response. Changes in sensitivity should have caused a vertical shift of the curves ; this was not observed.

The same calculations were carried out for those preparations which were tested with tyramine before and after the exposure to 3×10^{-6} noradrenaline (group C of Fig. 3). There was a correlation between the changes in resting rate and the changes in response to tyramine of individual preparations ($r=0.55$; $P<0.05$). Furthermore, Fig. 4A (solid line) shows that after restoration of the response to tyramine the slope of the regression line of this relationship was very similar to that observed with noradrenaline (Fig. 4B).

Effect of exposure to noradrenaline on the noradrenaline content of depleted atria. The procedure was the same as that described in the preceding section for the experiments illustrated in Fig. 3C. Atria from guinea-pigs pretreated with reserpine 5 mg/kg were isolated and kept in the bath for about 1 hr (see Methods). They were then tested with 10^{-5} tyramine for 10 min, washed for 10 min, and exposed to 3×10^{-6} noradrenaline for 10 min in the presence of edetic acid. The atria were then washed repeatedly for 45 min (without edetic acid) and removed from the bath for determination of the noradrenaline content ; 4 such atria were pooled for each assay to increase the sensitivity of the determination. These atria were designated as " refilled " and were comparable to those described previously which

showed an increase in rate by 40 beats/min when tested with a second dose of 10^{-5} tyramine (Fig. 3C). Control atria were treated in a similar manner except that they were removed from the bath immediately before the exposure to 3×10^{-6} noradrenaline. Four control and 4 "refilling" experiments were performed; the results are shown in Table 1. Control atria contained 0.051 ± 0.009 μg/g noradrena-

TABLE 1

IN VITRO UPTAKE OF NORADRENALINE BY ISOLATED ATRIA FROM GUINEA-PIGS PRETREATED WITH RESERPINE 5 MG/KG

Each value represents the noradrenaline content of a pool of 4 atria. See text for details of experiment. Atria were exposed for 10 min to 3×10^{-6} noradrenaline in the presence of edetic acid and subsequently washed repeatedly during a period of 45 min

Before exposure μg/g	After exposure μg/g
0·024	0·089
0·059	0·068
0·062	0·092
0·057	0·146
Mean ± s.e. 0·051 ± 0·009	0·099 ± 0·017

line (=1.1% of normal) while the "refilled" atria contained 0.099 ± 0.017 μg/g noradrenaline (=2.2% of normal). The difference between the means is significant ($P<0.05$). The noradrenaline isolated from both groups of atria had a fluorescence emission spectrum identical to that of authentic noradrenaline.

DISCUSSION

This study was undertaken in order to obtain information about the quantitative relation between the noradrenaline stores of cardiac tissue and the response of this tissue to tyramine. Pretreatment with reserpine was used as a pharmacological tool for depletion of the noradrenaline stores. The results obtained from pretreatment with graded doses of reserpine (Fig. 1) clearly indicated that a substantial depletion of the noradrenaline stores had to be achieved before the response to tyramine began to decline. Furthermore, very severe depletion of the noradrenaline stores was necessary in order to abolish the response to tyramine.

In full agreement with various reports based on experiments with intact animals or isolated organs (Burn & Rand, 1958, 1960; Kuschinsky, Lindmar, Lüllmann & Muscholl, 1960; Gillespie & Mackenna, 1961), exposure to noradrenaline of isolated atria of reserpine-pretreated guinea-pigs was found to restore their response to tyramine. If this restoration of the response to tyramine were due to a simple refilling of the noradrenaline stores, then, according to Fig. 1, a noradrenaline content of 10 to 15% of normal should be expected with the response to tyramine of about 40 beats/min, or about 70% of normal, that was observed after the exposure of the atria to 3×10^{-6} noradrenaline. However, the observed content of noradrenaline following "refilling" was only 2.2% of normal. If the noradrenaline stores comprised only one compartment, this discrepancy between the expected and the observed noradrenaline content would render untenable the concept of an indirect action of tyramine. Kuschinsky *et al.* (1960) and Muscholl (1960) found no uptake of noradrenaline into the heart of rats pretreated with reserpine; they therefore postulated that tyramine has a direct effect on cardiac tissue and that this direct action is

dependent on the presence at the receptors of very small concentrations of noradrenaline.

It is, however, not necessary to abandon the concept of an indirect action of tyramine if the assumption is made that the noradrenaline stores consist of two compartments. Such a working hypothesis has been proposed recently (Trendelenburg, 1961), and the present results are consistent with it. According to this hypothesis the noradrenaline stores consist of a small (A—" available " noradrenaline) and a large compartment (B—" bound " noradrenaline), which are in slow equilibrium with each other. Reserpine is assumed to uncouple the tightly bound noradrenaline of compartment B with the consequence that the transmitter escapes ; this leads to the well-known rate increase of the heart-lung preparation (Krayer & Fuentes, 1958), the rise of blood pressure in unanaesthetized dogs (Domino & Rech, 1957) and to the appearance of catechol amines in the urine (Carlsson *et al.*, 1957). Due to the large size of compartment B, this loss of noradrenaline from the tissue is easily detectable. Since the " bound " noradrenaline acts as a reservoir for compartment A, pretreatment with reserpine eventually causes (indirectly) the loss of " available " noradrenaline and only then reduces the effect of tyramine (Fig. 1) and of nerve stimulation (Trendelenburg & Gravenstein, 1958).

Exposure of normal tissues to noradrenaline leads to an increase in their noradrenaline content (Muscholl, 1960), but after pretreatment with reserpine the uptake of noradrenaline is very much reduced. Most observers emphasize the inhibition by reserpine of the uptake of noradrenaline into the tissue (Muscholl, 1960 ; Dengler, Spiegel & Titus, 1961). The present experiments, however, demonstrate that uptake of noradrenaline by heavily pretreated atria was detectable after their exposure to noradrenaline ; furthermore, significant uptake was found with the smallest noradrenaline concentration, which, under our experimental conditions, caused a clear restoration of the response to tyramine. It should be emphasized that future efforts to relate restoration of the response to tyramine to tissue uptake of noradrenaline must consider quantitatively the small amount of noradrenaline which is taken up rather than the larger amount which is not taken up.

The apparent discrepancy between the expected and the observed uptake can be resolved by the assumption that added noradrenaline is taken up by compartment A and that, in reserpine-pretreated tissues, it is not transferred into the " bound " form of compartment B. Because of the small size of compartment A, its refilling is difficult to detect, although tyramine (which is assumed to act on compartment A only) is quite effective again. The present experiments showed that the regression lines relating changes in resting rate to changes in response to tyramine and to noradrenaline had dissimilar slopes when depleted preparations were used (Fig. 4, broken lines) ; the regression coefficients were, however, very similar after refilling of the stores (Fig. 4, solid lines). This observation is consistent with the view that the restored effect of tyramine was due to the release of restored noradrenaline. Moreover, since any added noradrenaline was found to have a very short half-life in the absence of edetic acid (50 sec), it is extremely unlikely that, after a period of 45 min of repeated washing, there was any free noradrenaline left in the bath.

The present experiments do not give any indication as to the functional importance of the store into which the noradrenaline was taken up. However, supporting

evidence from other experiments indicates that the postulated compartment A is of high functional importance. Radioactive noradrenaline is known to be taken up by normal tissues, from where it is released by tyramine (isolated rabbit atria, Burn & Burn, 1961) or by nerve stimulation (cat spleen, Hertting & Axelrod, 1961). After pretreatment with graded doses of reserpine, the reduction of the response to tyramine is significantly correlated with the reduction of the response to nerve stimulation (cat nictitating membrane, Trendelenburg, 1961). Finally, exposure to noradrenaline restores the response of originally depleted tissues to both tyramine (Burn & Rand, 1958, 1960) and to nerve stimulation (Burn & Rand, 1960 ; Gillespie & Mackenna, 1961). It is therefore very likely that tyramine and nerve impulses act on the same store, and that it is this store (compartment A) which, in reserpine-pretreated preparations, takes up infused or added noradrenaline.

It is thus concluded (a) that the observations of the present series of experiments are compatible with the proposed theory of two compartments of noradrenaline stores and (b) that there is no need to abandon the concept that tyramine exerts its sympathomimetic effects through the liberation of endogenous noradrenaline. Whenever such an indirect biological response is related to the noradrenaline content of the same tissue, care has to be taken to eliminate the possibility that changes in sensitivity to noradrenaline distort the biological response to tyramine. The sensitivity to noradrenaline of isolated atria obtained from reserpine-pretreated guinea-pigs was found to be normal, although their noradrenaline content was very low (less than 2% of normal). The sensitivity to noradrenaline was tested by intermittent administration of two test doses and also with cumulative dose-response curves of noradrenaline. The two different test methods were chosen, since it can be argued that during the determination of a cumulative dose-response curve the originally depleted noradrenaline stores are gradually refilled ; according to Burn & Rand (1959) this gradual refilling would restore normal sensitivity to a tissue which was originally supersensitive. Both test methods failed to detect any signs of supersensitivity to noradrenaline in atria from animals pretreated with a large dose of reserpine. In addition, the exposure to noradrenaline of atria of heavily pretreated animals resulted in the restoration of the response to tyramine to 70% of normal, but there was no concomitant reduction in sensitivity to noradrenaline ; this should have been observed if the sensitivity to noradrenaline were inversely related to the noradrenaline content of the tissue. These observations give further support to the view that there is no direct relationship between the noradrenaline content of an organ and its sensitivity to this substance (Fleming & Trendelenburg, 1961 ; Trendelenburg & Weiner, 1962). This statement should not be interpreted as implying that supersensitivity to noradrenaline cannot be observed together with depletion of the noradrenaline stores. On the contrary, there is good evidence that depletion of the stores frequently goes hand in hand with supersensitivity to noradrenaline ; this does not seem to be a general rule, however. Organ and species differences may be of importance, since the heart of the spinal cat develops supersensitivity to noradrenaline more quickly than the nictitating membrane (Fleming & Trendelenburg, 1961), and since isolated atria from reserpine-pretreated rabbits are supersensitive to noradrenaline (Macmillan, 1959) whereas those from reserpine-pretreated guinea-pigs are not. This latter difference may reflect a difference in the schedule of

pretreatment with reserpine rather than a true species difference, since the rabbits received intraperitoneal injections 48 and 24 hr prior to the experiment, whereas in the present experiments on guinea-pig atria, reserpine was injected only once (24 hr prior to the experiment).

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REFERENCES

BURN, G. P. & BURN, J. H. (1961). Uptake of labelled noradrenaline by isolated atria. *Brit. J. Pharmacol.*, **16**, 344–351.

BURN, J. H. & RAND, M. J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol. (Lond.)*, **144**, 314–336.

BURN, J. H. & RAND, M. J. (1959). The cause of the supersensitivity of smooth muscle to noradrenaline after sympathetic degeneration. *J. Physiol. (Lond.)*, **147**, 135–143.

BURN, J. H. & RAND, M. J. (1960). The effect of precursors of noradrenaline on the response to tyramine and sympathetic stimulation. *Brit. J. Pharmacol.*, **15**, 47–55.

CARLSSON, A., ROSENGREN, E., BERTLER, Å. & NILSSON, J. (1957). Effect of reserpine on the metabolism of catecholamines. *Psychotropic Drugs*, pp. 363–372. Amsterdam: Elsevier Publishing Co.

CROUT, J. R. (1961). Catecholamines in urine. *Standard Methods of Clinical Chemistry*, vol. III, pp. 62–80. New York: Academic Press.

CROUT, J. R., CREVELING, C. R. & UDENFRIEND, S. (1961). Norepinephrine metabolism in rat brain and heart. *J. Pharmacol. exp. Ther.*, **132**, 269–277.

DENGLER, H. J., SPIEGEL, H. E. & TITUS, E. O. (1961). Uptake of tritium-labeled norepinephrine in brain and other tissues of cat *in vitro*. *Science*, **133**, 1072–1073.

DOMINO, E. F. & RECH, R. H. (1957). Observations on the initial hypertensive response to reserpine. *J. Pharmacol. exp. Ther.*, **121**, 171–182.

FLEMING, W. W. & TRENDELENBURG, U. (1961). Development of supersensitivity to norepinephrine after pretreatment with reserpine. *J. Pharmacol. exp. Ther.*, **133**, 41–51.

GILLESPIE, J. S. & MACKENNA, B. R. (1961). The inhibitory action of the sympathetic nerves on the smooth muscle of the rabbit gut, its reversal by reserpine and restoration by catecholamines and by DOPA. *J. Physiol. (Lond.)*, **156**, 17–34.

HERTTING, G. & AXELROD, J. (1961). Fate of titriated noradrenaline at the sympathetic nerve endings. *Nature (Lond.)*, **192**, 172–173.

KRAYER, O. & FUENTES, J. (1958). Changes of heart rate caused by direct cardiac action of reserpine. *J. Pharmacol. exp. Ther.*, **123**, 145–152.

KUSCHINSKY, G., LINDMAR, R., LÜLLMANN, H. & MUSCHOLL, E. (1960). Der Einfluss von Reserpin auf die Wirkung der " Neuro-Sympathomimetica." *Arch. exp. Path. Pharmak.*, **240**, 242–252.

LIEBMAN, J. (1961). Modification of the chronotropic action of sympathomimetic amines by reserpine in the heart-lung preparation of the dog. *J. Pharmacol. exp. Ther.*, **133**, 63–69.

LIEBMAN, J., MUSKUS, A. J. & WAUD, D. R. (1962). The depletion of norepinephrine stores in the heart of the dog by reserpine-type alkaloids. *J. Pharmacol. exp. Ther.*, **136**, 75–79.

MACMILLAN, W. H. (1959). A hypothesis concerning the effect of cocaine on the action of sympathomimetic amines. *Brit. J. Pharmacol.*, **14**, 385–391.

MUSCHOLL, E. (1960). Die Hemmung der Noradrenalinaufnahme des Herzens durch Reserpin und die Wirkung von Tyramin. *Arch. exp. Path. Pharmak.*, **240**, 234–241.

PAASONEN, M. & KRAYER, O. (1958). The release of norepinephrine from the mammalian heart by reserpine. *J. Pharmacol. exp. Ther.*, **123**, 153–160.

PENNEFATHER, J. N. & RAND, M. J. (1960). Increase in noradrenaline content of tissues after infusion of noradrenaline, dopamine and l-dopa. *J. Physiol. (Lond.)*, **154**, 277–287.

SNEDECOR, G. W. (1946). *Statistical Methods*. The Iowa State College Press.

TRENDELENBURG, U. (1961). Modification of the effect of tyramine by various agents and procedures. *J. Pharmacol. exp. Ther.*, **134**, 8–17.

TRENDELENBURG, U. & GRAVENSTEIN, J. S. (1958). Effect of reserpine pretreatment on stimulation of the accelerans nerve of the dog. *Science*, **128**, 901–903.

TRENDELENBURG, U. & WEINER, N. (1962). Sensitivity of the nictitating membrane after various procedures and agents. *J. Pharmacol. exp. Ther.*, **136**, 152–161.

WAUD, D. R. (1961). The influence of reserpine upon the changes in femoral blood flow produced by stimulation of the lumbar sympathetic chain. *Experientia*, **17**, 234.