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EFFECT OF NORADRENALINE ON THE ACTION OF NICOTINE AND TYRAMINE ON ISOLATED ATRIA

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Both nicotine (in the presence of atropine) and tyramine cause a rise in the rate of isolated rabbit atria. When noradrenaline is allowed to act on the atria for 20 to 30 min and then removed from the bath by repeated changes of the bath fluid, the action of nicotine and of tyramine is greatly increased. The first addition of noradrenaline to the bath often has a much smaller effect on the response to nicotine or to tyramine than have later additions. Sometimes the greater effect after the addition of nicotine persists for 2 or 3 hr. When reserpine is added to the bath and left in contact with the atria for 1 to 2 hr, and is then removed from the bath, the effect of noradrenaline on the atrial rate is much reduced in size and duration. Bretylium abolishes the action of nicotine but increases the action of tyramine.

The first suggestion that noradrenaline, when infused slowly into the blood stream, was taken up by tissues with a sympathetic innervation was made by Burn & Rand (1958). They observed that when the pressor and constrictor action of tyramine, and the constrictor action of sympathetic stimulation, were absent in cats and dogs previously treated with reserpine, these actions could be restored by the infusion of noradrenaline, and could be observed after the infusion of noradrenaline had ceased and its direct effect had passed away. They also observed that in normal animals which had not been treated with reserpine the constrictor action of tyramine and of sympathetic stimulation was increased after an infusion of noradrenaline (Burn & Rand, 1960a). Positive evidence of the uptake of noradrenaline was obtained by Pennefather & Rand (1960), who observed that after an infusion of noradrenaline the kidney and the horn of the uterus of one side each contained more noradrenaline than the organ of the opposite side removed before the infusion began. Positive evidence was also obtained by Whitby, Hertting & Axelrod (1960), who injected DL-β-^{3}H noradrenaline into cats intravenously, and saw that the ^{3}H noradrenaline was taken up by the adrenal gland, heart and spleen when these tissues were examined 1 hr after the injection of the noradrenaline. Small amounts were also taken up by the liver and muscle. Evidence has also been obtained by Muscholl (1960) that infusion of noradrenaline into the spinal rat increased the content of noradrenaline in the heart.

Evidence of uptake has also been obtained in isolated tissues. Gillespie & Mackenna (1959) suspended a piece of rabbit colon in a bath together with the

sympathetic nerves. Stimulation of these nerves caused inhibition of the contractions. When, however, the preparation was made from a rabbit which had been treated with reserpine, stimulation of the sympathetic nerves caused contraction of the colon. When noradrenaline was added to the bath and allowed to remain there for 15 min, stimulation of the nerves once more produced its usual inhibitory effect. This observation suggested that even in an organ bath the tissue could still take up noradrenaline, and hold it in such a way that it could be released by stimulation of sympathetic nerves. Huković (1961) has observed the contractions of the isolated vas deferens in response to hypogastric stimulation, and has seen that the contractions were increased after noradrenaline was added to the bath for 30 min and then washed out.

Observations have now been made on isolated atria from normal rabbits, not treated with reserpine, to study the effect of noradrenaline on the action of nicotine and of tyramine in causing an increase of atrial rate.

METHODS

The heart was excised from a freshly killed rabbit of about 2 kg. The atria were dissected at room temperature in a solution of the following composition, which was also used in the isolated organ bath: sodium chloride 8.5 g, potassium chloride 0.42 g, calcium chloride 0.24 g, dextrose 2.0 g, sodium bicarbonate 0.5 g, distilled water to 1 litre. This solution was bubbled with 95% oxygen and 5% carbon dioxide. The atria were attached to a light metal lever kept horizontal by a spring suspended vertically above the lever. When the atria contracted, a silver wire attached to the lever dipped into a salt solution in a small perspex bath and completed an electrical circuit. The current was supplied by two 45-volt batteries in series with a Post Office counter. Thus the atrial rate was recorded automatically on the counter. The organ bath was made of double-walled perspex and the atria were fully immersed in 5 ml. of solution. A stream of water at 32° C was maintained through the jacket surrounding the inner compartment. The solution was vigorously aerated through a sintered glass capsule placed below the atria.

Nicotine was used as the acid tartrate. The dose of nicotine is given in terms of the base (one-third of the weight of the salt). Tyramine was used in the form of the hydrochloride, and the dose of tyramine is given in terms of this salt. Reserpine was taken from ampoules of Serpasil supplied by Ciba. Bretylium was used in the form of bromide, and the dose is given in terms of this salt. Noradrenaline was used as L-arterenol bitartrate, and the dose is expressed in terms of the base. All observations made with nicotine were made in the presence of atropine sulphate (1 μg/ml.), which was added to the bath at least 1 min before the nicotine. The rate of beating was determined several times during a period of 5 or 10 min before nicotine or tyramine was added. When they were added, the rate was determined during six half-minute periods beginning at 30 sec, 90 sec, etc., after which the bath fluid was changed. The effect was recorded as the maximum increase in rate over the initial rate.

RESULTS

Effect of noradrenaline on the response to nicotine. In determining the effect of adding noradrenaline to the bath, a series of observations was first made on the effect of nicotine. Care was taken to see that the temperature at which the observations were made was approximately the same (± 0.2° C), and nicotine was added to the bath at fairly long intervals. Thus, in the experiment which is illustrated in Table 1, the interval was 45 to 53 min between the first five additions of nicotine, was 29 min between the fifth and sixth, and thereafter was 35 min. When nor-

TABLE 1

EFFECT OF EXPOSING THE ATRIA TO NORADRENALINE ON THE RESPONSE OF THE ATRIAL RATE TO NICOTINE

Time in min	Substance added	Concentration of base μg/ml.	Time of action in min	Increase of atrial rate beats/min
0	Nicotine	2·2	6	17
48	Nicotine	2·2	6	0
94	Nicotine	2·2	6	0
100	Noradrenaline	1·0	25	
145	Nicotine	2·2		6
182	Nicotine	3·0		7
205	Nicotine	4·5		24
211	Noradrenaline	1·0	25	
240	Nicotine	4·5		94
246	Noradrenaline	1·0	25	
275	Nicotine	3·0	6	74
281	Noradrenaline	1·0	25	
308	Nicotine	2·2	6	70
335	Nicotine	3·0	6	10

adrenaline was added to the bath between applications of nicotine, it was always added for 25 min, and at the end of this time the bathing fluid was changed twice with an interval between the changes. There was then a period of 10 to 20 min for the direct effect of the noradrenaline to pass off.

In Table 1 it is seen that the first addition of a concentration of 2.2 μg/ml. nicotine increased the rate slightly by 17 beats per min, but that the second and third additions of this concentration had no effect. Noradrenaline (1 μg/ml.) was added to the bath for 25 min, and after it had been removed the concentration of 2.2 μg/ml. nicotine increased the rate by 6 beats. Thus noradrenaline in this trial had almost no effect. Higher concentrations of nicotine were then tested, 3 μg/ml. and 4.5 μg/ml., without further applications of noradrenaline ; they caused increases of rate of 7 and 24 beats respectively. The effect of noradrenaline was then determined on these higher concentrations. After application of noradrenaline (1 μg/ml.) the concentration of 4.5 μg/ml. nicotine caused an increase of 94 beats, the concentration of 3 μg/ml. an increase of 74 beats, and even the concentration of 2.2 μg/ml. caused an increase of 70 beats. This increased effect of nicotine was evident only for the application of nicotine made immediately after the exposure to noradrenaline, for the last application shown in Table 1 of 3 μg/ml. produced little more increase than it did when it was first tested.

A more lasting effect of noradrenaline, however, was observed in another experiment summarized in Table 2 as Expt. 2. Nicotine in a concentration of 2.7 μg/ml. caused the rate to increase in three successive applications by 62, 62, and 20 beats respectively. Noradrenaline (1 μg/ml.) was then added to the bath before each of the next 4 applications of nicotine and left in the bath for periods of 30, 60, 26, and 28 min respectively. The bath fluid was, as usual, changed twice and the rate returned to the initial value before nicotine was added. The increase in rate caused by nicotine (2.7 μg/ml.) was then 38, 90, 90, and 86 beats per min respectively. No further additions of noradrenaline were made, and at the next 4 applications of nicotine the rate increased by 90, 76, 72, and 72 beats. Thus the effect of the

noradrenaline in increasing the nicotine response persisted for more than 3 hr after the last application of noradrenaline.

Effect of noradrenaline on the response to tyramine. The addition of noradrenaline to the bath had a similar effect on the action of tyramine to that described on the action of nicotine. The results of three experiments (Nos. 4, 5 and 6) are summarized in Table 2. In Expt. 4 the first application of noradrenaline had little effect, for

TABLE 2

MEAN EFFECTS OF NICOTINE AND OF TYRAMINE ON ATRIAL RATE BEFORE AND AFTER EXPOSING THE ATRIA TO NORADRENALINE

Expt.	Substance	Concentration μg/ml.	Effect on atrial rate: Initial	Effect on atrial rate: After noradrenaline
2	Nicotine	2·7	48	90
3	Nicotine	2·2	25	82
4	Tyramine	0·5	26	90
5	Tyramine	3·0	10	72
6	Tyramine	1·0	6	54

the effect of tyramine (0.5 μg/ml.) was to cause an increase of rate of 27 beats per min (2 observations) before noradrenaline was added, and an increase of 32 beats per min after noradrenaline was added. The second and third applications of noradrenaline, however, were followed by a great increase in the action of tyramine. The rate rose by 82 and 90 beats per min. No further application of noradrenaline

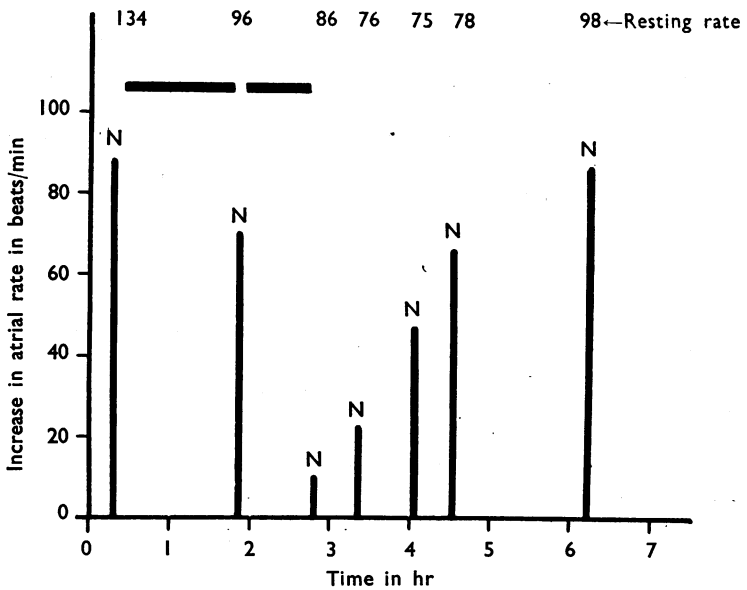

Fig. 1. The diagram shows the effect of adding reserpine to the bath on the action of nicotine. Initially nicotine (6 μg/ml.) caused the atrial rate to increase from 134 per min to 222 per min. After reserpine 4 μg/ml. had been added to the bath for the period shown by the horizontal bars, nicotine increased the rate from 86 per min to 95 per min. After changing the bath repeatedly the original effect of nicotine returned, more than 3 hr later. N=nicotine 6 μg/ml.

was made, but the next response to tyramine was still 82 beats per min. However, the next response after that was almost zero.

In two preparations the addition of tyramine caused a lasting increase in the atrial rate which persisted after tyramine was removed. In one of these the rate at the beginning was 139 per min. After the first addition of tyramine an increase of 50 beats per min was recorded. When the bath was changed to remove the tyramine, the rate remained at a higher level of 148 per min. Successive additions of tyramine caused progressively smaller increases in rate, but raised the atrial rate after the tyramine was removed from the bath. When noradrenaline was added to the bath for 43 min and then removed, the atrial rate was 192 per min. Although it was so high, the addition of tyramine raised the rate by 46 beats per min.

Effect of reserpine. We tested the effect of adding reserpine to the bath, and found that, as Fig. 1 shows, it blocked the action of nicotine in causing a large increase in the atrial rate. Reserpine was added to the bath in a concentration of 4 μg/ml. Fig. 1 shows that after reserpine was present in the bath for 82 min the effect of nicotine was slightly reduced, but that after it was added again in the same concentration for a further period of 47 min the effect of nicotine was then almost abolished. However, successive additions of nicotine (in the absence of added reserpine) were followed by a gradual return of the full nicotine effect seen at the beginning. Reserpine also reduced the atrial rate from an initial figure of 134 beats per min to 75 beats per min. This reduction lagged behind the reduction in the action of nicotine, since the rate was 86 when the nicotine effect was least, and was 75 when the nicotine effect was well on the way to recovery. However, the atrial rate rose again to 98 when the nicotine effect was fully recovered.

In view of the blocking action of reserpine, experiments were made to see if reserpine would reduce the action of noradrenaline. It was found that it did so. Thus, having observed that the maximum increase in atrial rate caused by a concentration of 0.5 μg/ml. noradrenaline was 97 and 114 beats per min in two successive trials, reserpine was added to the bath in a concentration of 4 μg/ml. for a period of 68 min and then for a further period of 58 min. After the bath fluid was changed the maximum increase caused by noradrenaline was 65 beats per min. We added no more reserpine, and successive tests of noradrenaline gave a maximum increase of 86, 90, and 105 beats per min.

In another experiment it was observed that not only did reserpine reduce the maximum effect following noradrenaline, but also the duration of the effect. This finding is illustrated in Fig. 2, where each effect of noradrenaline is shown during 25 min. Curve 1 shows the response after the atria had been exposed to reserpine (4 μg/ml.) for 80 min. Curves 2, 3 and 4, obtained at intervals of 30 min, show the response to successive additions of noradrenaline as the reserpine was removed by repeated washings. The difference between curve 1 and curve 4 is very large.

Finally, we tested the effect of reserpine on the action of tyramine. Reserpine blocked the action of tyramine, and the block appeared to persist longer than the block of the action of nicotine. Thus, in one experiment, tyramine (3 μg/ml.) increased the atrial rate by 74 and 88 beats in two trials. Reserpine (4 μg/ml.)

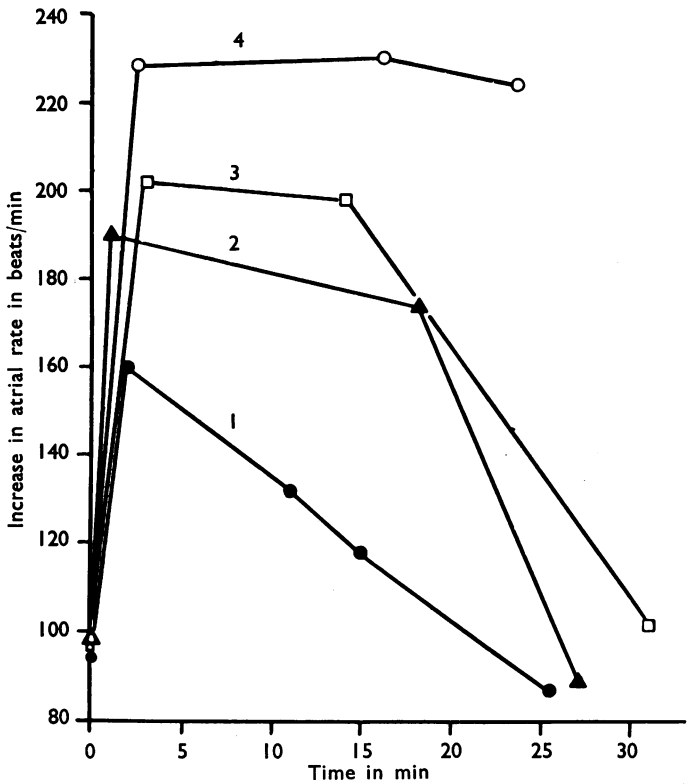

Fig. 2. After reserpine (4 μg/ml.) was added to the bath for 80 min, and then washed out, the effect of noradrenaline (0.5 μg/ml.) was small as shown in curve 1. The effect of this concentration was determined at intervals of 30 min, and curves 2, 3, and 4 were thus obtained.

was then added to the bath for 93 min and was washed out. Four successive trials of tyramine then caused no increase in rate, the fifth trial increased the rate by 14 beats, but the next four trials increased the rate only by 6, 10, 6 and 2 beats. Thus the reserpine blocked the action of tyramine for 4 hr at least.

Action of bretylium. Bretylium is a substance which blocks the effect of stimulating the postganglionic sympathetic fibres without affecting the action of noradrenaline. Its properties have been described by Boura & Green (1959). We tested the effect of bretylium on the actions of nicotine and tyramine, and observed that, while it blocked the action of nicotine, it increased the action of tyramine. In one experiment bretylium was added to the bath in a concentration of 3 μg/ml. Nicotine was tested in a concentration of 9 μg/ml., and, in two trials before the addition of bretylium, nicotine increased the atrial rate by 87 and 114 beats respectively. After bretylium had been present for 12 min nicotine increased the rate by 58 beats, and after it had been present for 56 min nicotine increased the rate by 4 beats. Tyramine, on the other hand, in a concentration of 20 μg/ml. increased the rate in the presence of bretylium by 116 beats, whereas before bretylium was added it increased the rate only by 68 beats. Bretylium thus had opposite effects on the actions of nicotine and tyramine.

DISCUSSION

From the evidence of different workers quoted in the introduction it appears that tissues such as the heart, spleen, kidney and uterus, which have a sympathetic innervation, can take up noradrenaline from the blood. In the second place, Schümann & Weigmann (1960) have shown that tyramine increases the rate of release of noradrenaline from chromaffin granules obtained from the splenic nerve by differential centrifugation.

In spite of this evidence, Kuschinsky, Lindmar, Lüllmann & Muscholl (1960) consider that tyramine does not act by liberating noradrenaline, but that, in order that tyramine may act, a certain quantity of noradrenaline must be *present*. Their evidence is based first on the finding of Muscholl (1960) that the heart of a rat treated with reserpine does not take up noradrenaline from the blood, and, second, on the ability of very low concentrations of noradrenaline to restore the action of tyramine on the isolated atria of a reserpine-treated rat. Moreover, they were able to show that the effect of the noradrenaline lasted only so long as it was present in the bath. A similar view has been expressed by Nasmyth (1960). He found that tyramine caused an increase in the amplitude of contraction of the isolated guinea-pig heart, but that this increase was no longer to be seen after several injections of tyramine had been given. At this point there was no decline in the concentration of noradrenaline in the heart. The effect of tyramine could be restored by an injection of noradrenaline. In addition Nasmyth found that perfusion of isolated hearts with Krebs solution containing tyramine for 30 min did not diminish the amount of noradrenaline present. Nasmyth concluded from this and other evidence that the potentiation of the effect of tyramine by noradrenaline occurred at a time when the noradrenaline was in the extracellular fluid.

The experiments described in this paper showed that, when atria were beating in a bath, the addition of noradrenaline to the bath increased the effect of both tyramine and nicotine on the atrial rate. This increased action was observed after the noradrenaline had been removed from the bath and the atrial rate had returned to the rate before noradrenaline was added. But the effect was not uniform. Often the first addition of noradrenaline had very little effect on the action of tyramine or of nicotine, while later additions had a great effect. Furthermore, to maintain this great effect it was usually necessary to add noradrenaline to the bath between each trial of tyramine or nicotine. But again this was not always so, and in some experiments the great effect was maintained, without further addition of noradrenaline, for as long as 3 hr. These observations are difficult to harmonize with the view that noradrenaline by its presence can so greatly potentiate the action of tyramine.

The idea that the potentiating action of noradrenaline depends on its presence in the extracellular fluid does not account for the potentiating action of noradrenaline on sympathetic stimulation which runs parallel with the effect on tyramine. Nor is it easy to conceive how the potentiating action of dopamine, of L-dopa, of *m*-tyrosine and of phenylalanine on tyramine can be explained as being due to an extracellular accumulation of noradrenaline (Burn & Rand, 1960b).

The experiments described in this paper also show that reserpine does more than displace noradrenaline from tissues where it is stored. After the atria had

been in contact with reserpine, the action of noradrenaline in increasing the atrial rate was greatly reduced, and many changes of the bath fluid were required before the reduction was no longer evident. From the experiments of Muscholl it appears that not only effector sites on which noradrenaline acts are blocked by reserpine, but storage sites also, for he found that noradrenaline was not taken up by the heart of an animal treated with reserpine (though this result was in conflict with those of Pennefather & Rand, 1960).

The block of the action of noradrenaline by reserpine is the likely explanation of various discrepancies between different observers on the effect of noradrenaline in tissues from reserpine-treated animals. For example, Macmillan (1959) observed that atria from reserpine-treated rabbits were more sensitive to the effect of noradrenaline on the rate than those from normal rabbits. Similarly, Leusen & Verbeke (1960) found that the sensitivity of the papillary muscle of the cat heart to the inotropic action of noradrenaline was regularly increased after reserpine pretreatment. On the other hand, Kuschinsky *et al.* (1960) failed to observe any increase in sensitivity to noradrenaline of the atria of the rat heart after reserpine pretreatment, no matter whether rate or force of contraction were observed. This failure may be explained by the persistence of the reserpine attachment to the receptors of the rat heart.

There are similar discrepancies for the nictitating membrane. Thus Fleming & Trendelenburg (1960) failed to observe that the nictitating membrane of the spinal cat previously treated with reserpine was supersensitive to the action of noradrenaline, but Schmitt & Schmitt (1960) recorded that supersensitivity was present in the cat under pentobarbitone anaesthesia.

There are now several examples of supersensitivity of organs to noradrenaline resulting from the administration of reserpine. It is certainly possible that, when this supersensitivity has not been observed, in some cases the supersensitivity has been masked by the blocking action of reserpine.

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