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Noradrenaline and tyramine action on isolated atrial muscle of endotoxin-treated guinea-pigs

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Summary

1. Isolated left-atrial strips of guinea-pigs were driven electrically at a constant rate and log-concentration curves were determined for the positive inotropic effect of noradrenaline and tyramine.

2. The atrial tissue from endotoxin-treated animals had a reduced sensitivity to noradrenaline and tyramine.

3. After endotoxin, the sensitizing effect of cocaine on the response to noradrenaline was normal but accumulation of ^{3}H-noradrenaline in nerve terminals was markedly reduced.

4. Atrial tissue of endotoxin-treated guinea-pigs responded normally to stretch.

Introduction

It has been shown that the intravenous administration of *E. coli* endotoxin to animals produced a progressive fall in aortic blood pressure, a drastic reduction in total blood volume, an increase in total peripheral resistance, a decrease in venous return and a diminished cardiac output (Weil, MacLean, Spink & Visscher, 1956; Heiffer, Mundy & Mehlman, 1960; Cavanagh & Rao, 1969). Changes in cardiac activity are assumed to be secondary to this circulatory response.

The function of the heart in endotoxin shock and its response to sympathetic stimulation remain to be clarified. The present study was undertaken to gain information on this point. Since the cardiac responses in the intact animal may be modified by reflex responses, an isolated atrial preparation has been used, thus eliminating these indirect actions. Responses to sympathetic stimuli in cardiac tissue, obtained from guinea-pigs pretreated with endotoxin, were examined. The effect of stretching on tension was also studied in an attempt to evaluate the effect of endotoxin on the contractility of the tissue.

Methods

Male guinea-pigs of 300 to 600 g body weight were stunned with a blow on the head and their hearts were rapidly removed. The left atrium was dissected from the heart and suspended in a modified Tyrode solution maintained at 34° C. The solution used in all experiments had the following composition: NaCl 0·09%; KCl 0·042%; $CaCl_2$ 0·024%; $NaHCO_3$ 0·05%, glucose 0·2%. It was aerated with

95% oxygen and 5% carbon dioxide and the bicarbonate concentration used maintained the pH at approximately 7·4.

The lower end of the atrium was tied to a plastic holder which contained punctate electrodes. The upper end was tied to a force transducer (Grass FT-303) and contractions were recorded on a Grass ink writing oscillograph. Two atria (control and experimental) were mounted in an organ bath of 70 ml capacity. The atrium was electrically driven via platinum electrodes, placed parallel to but not touching the tissue, with square-wave pulses of 5 ms duration, delivered at a frequency of 1 Hz and with an above threshold voltage. Unless otherwise indicated, the resting tension on the atria was 1 g. The atria were allowed to equilibrate for at least 1 h after being placed in the bath and were washed repeatedly after each addition of a drug. In the experiments in which noradrenaline was used, ethylenediaminetetraacetic acid (EDTA) was always present in a concentration of 10 μg/ml ($2{\cdot}7 \times 10^{-5}$M) in the bath during the periods of exposure to this amine, to retard oxidation (Iversen, 1967). This concentration of EDTA had no effect on the force of contraction, the uptake, release, metabolism, or effectiveness of noradrenaline.

It took approximately 10 min from the time of killing an animal to suspension of the atria in the organ bath.

Log-concentration curve to sympathomimetic amines

Cumulative log-concentration curves to sympathomimetic amines were determined by stepwise increases of the total concentration whenever the response to a given concentration was maximal (at intervals of 1 to 4 min).

All responses were expressed as a percentage of the maximal tension obtained for that dose-response curve. The log concentration of the amine was plotted against per cent of the maximum response and from each individual log-concentration curve, the concentration which caused 50% of the maximum response was calculated. This concentration is referred to as ED50 in the text. The ratio ED50 of atria from guinea-pigs pretreated with (coliform) endotoxin over ED50 of control atria was used as a measure of sensitivity changes.

In additional experiments the accumulation of (±)-^{3}H-noradrenaline (5·0 Ci/mmol, purchased from New England Nuclear Corp.) in isolated atria from guinea-pigs pretreated with endotoxin was determined. After incubation with 0·2 μCi/ml ^{3}H-noradrenaline for 30 min at 34° C, the atria were washed 6 times with the modified Tyrode solution at 2 min intervals at room temperature, blotted with filter paper, and weighed. They were then homogenized in 10 ml ice-cold 0·4 N perchloric acid. The protein-free supernatant obtained by centrifugation was passed over alumina at pH 8·6 and eluted with 0·04 N perchloric acid and assayed for tritiated noradrenaline (Whitby, Axelrod & Weil-Malherbe, 1961). EDTA (10 μg/ml, i.e. $2{\cdot}7 \times 10^{-5}$M) was always present in the bath during incubation with noradrenaline to retard the oxidation of the amine (Iversen, 1967).

Length tension studies

Atria to which a starting tension of 0·5 g had been applied were extended by 1 mm increments, allowing 1 h for equilibrium to be established in each case.

Drugs used

The following substances were used: (−)-noradrenaline bitartrate monohydrate, cocaine hydrochloride, tyramine hydrochloride and *E. coli* endotoxin (Difco Labs, Detroit, Michigan, U.S.A.).

Tests of significance were performed according to Student's *t* test (Snedecor, 1956). $P<0.05$ was regarded as significant.

Results

Response to noradrenaline of isolated atria from guinea-pigs pretreated with endotoxin

Guinea-pigs were given *E. coli* endotoxin (2 to 3 mg/kg), intraperitoneally. Left atrial strips were prepared at various times thereafter, and log-concentration curves for the positive inotropic effect of (−)-noradrenaline were determined. For each dose-response curve the ED50 was calculated. Mean values are presented in Table 1. The results show that endotoxin pretreatment reduced the sensitivity to (−)-noradrenaline and shifted the log-concentration curve to the right (Fig. 1). The reduction in sensitivity to noradrenaline increased with time and reached its maximum 6 to 18 h after endotoxin pretreatment. The response to noradrenaline began to return to normal at 23 h and was normal 42 h after endotoxin pretreatment. A similar reduction in sensitivity to noradrenaline was observed on left atrial strips taken from a baboon pretreated with endotoxin.

In another series of experiments, the response to tyramine, which acts by the release of noradrenaline from its stores in adrenergic nerve endings, was determined in isolated atrial preparations from guinea-pigs pretreated with endotoxin, 1 h before the experiment. The results (Fig. 2) show that endotoxin pretreatment shifted the log-concentration curve of tyramine to the right. The ratio ED50 for the atria from endotoxin-pretreated guinea-pigs over ED50 for the atria from control guinea-pigs was 1.8 ± 0.2 (six experiments).

In the third series of experiments the sensitizing effect of cocaine on the response of atrial strips to (−)-noradrenaline was determined. Log-concentration curves for (−)-noradrenaline were obtained in the absence and in the presence of 10 μg/ml of cocaine hydrochloride. There was an interval of about 60 min between the two

TABLE 1. *Sensitivity to noradrenaline after endotoxin treatment*

Time of killing after endotoxin (h)	ED50				
	Normal M$\times 10^{-7}$	No. of exp.	Endotoxin-treated M$\times 10^{-7}$	No. of exp.	Sensitivity ratio
1	6·93±0·7	7	13·25±1·2	7	1·91±0·20
6	5·2±0·6	4	19·55±1·2	4	3·75±0·12
18	5·63±0·3	4	22·50±1·2	4	4·00±0·06
23	6·16±0·8	5	9·22±0·6	5	1·50±0·20
42	6·22 (6·50; 5·99)	2	6·50 (6·22; 6·78)	2	1·05 (1·15; 0·95)

Log-concentration curves for (±)-noradrenaline were made on atria from normal guinea-pigs and guinea-pigs pretreated with endotoxin. From each individual log-concentration curve, the concentration (ED50) which caused 50% of the maximum response was calculated. The ratio of the ED50 of the preparation made from guinea-pigs pretreated with endotoxin over ED50 of control is a measure of sensitivity (sensitivity ratio). Results are given as means±S.E.

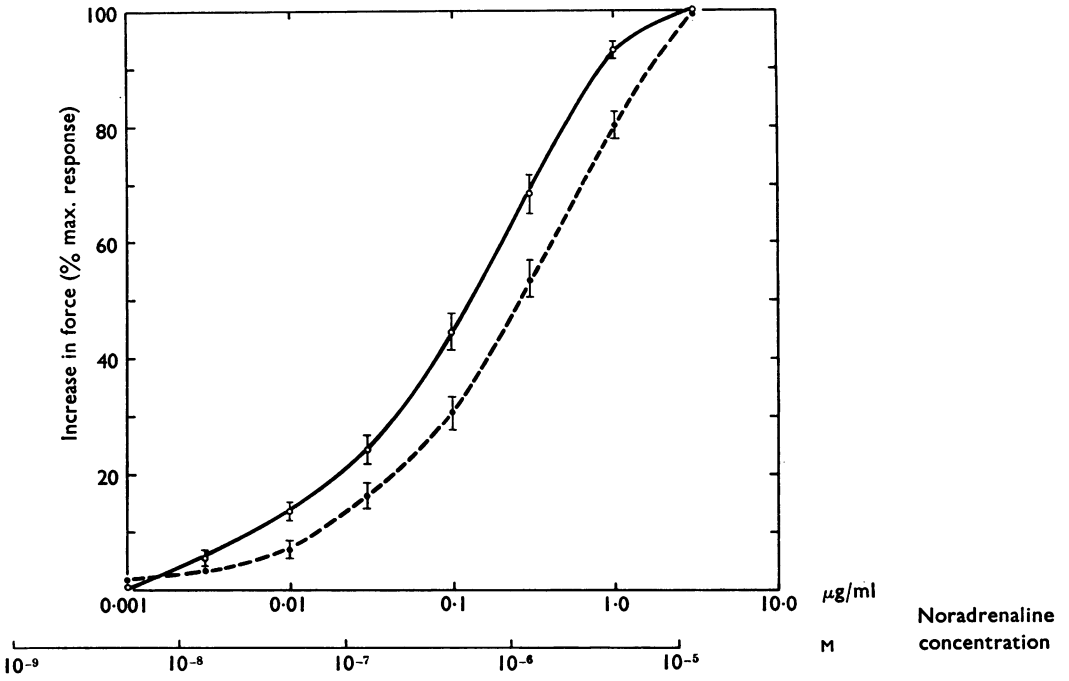

FIG. 1. Responses to noradrenaline. Isolated atrial strips from guinea-pigs (controls (——) and animals pretreated with endotoxin 1 h before the experiment (- - -)) driven electrically at 1 Hz in Tyrode solution at 34° C. Ordinate, Increase in force of contraction in % of maximal developed tension at the end of log-concentration curve. Abscissa, Log concentration of (−)-noradrenaline. Mean values ± S.E. for seven observations per point.

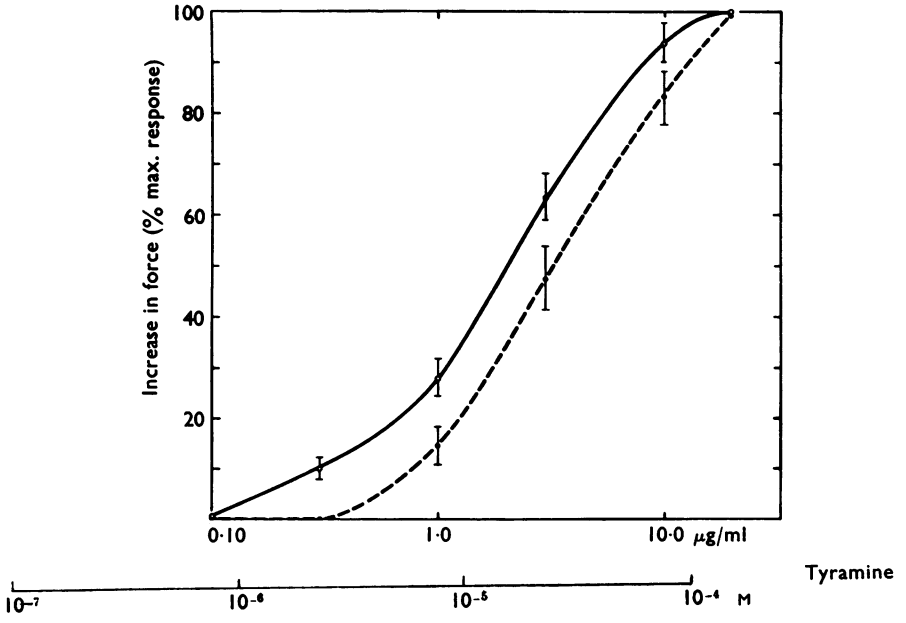

FIG. 2. Responses to tyramine. Isolated atrial strips from guinea-pigs (controls (——) and animals pretreated with endotoxin 3 mg/kg. 1 h before the experiment (- - -)) driven electrically at 1 Hz in Tyrode solution at 34° C. Ordinate, Increase in force of contraction in % of maximal developed tension at the end of log-concentration curve. Abscissa, Log concentration of tyramine. Mean values ± S.E. for six observations per point.

experiments and cocaine was added to the organ bath 20 min before the second. Cocaine increased the sensitivity of the normal atrial strips to (−)-noradrenaline about 18-fold. On the other hand, it increased the sensitivity 15 times in atria from endotoxin-treated guinea-pigs (injected 1 h before the experiment) (Fig. 3). The mean ED50s of (−)-noradrenaline obtained on atria from controls and atria from endotoxin-treated guinea-pigs (five experiments each) were 0·126 (S.E. ±0·01) and 0·270 (S.E. ±0·02) μg/ml (or 7·45 ± 0·7 and 15·9 ± 1·2 M × 10^{-7}), respectively, and in the presence of 10 μg/ml (3×10^{-5}M) of cocaine hydrochloride 0·007 (S.E. ±0·001) and 0·018 (S.E. ±0·002) μg/ml (or 4·14 ± 0·6 and 10·6 ± 1·2 M × 10^{-8}), respectively.

Length tension studies

Atrial strips from guinea-pigs pretreated with endotoxin had a reduced sensitivity to noradrenaline. Length tension studies, therefore, were made in order to determine whether or not any changes in extensibility were associated with it. Animals were injected with 2 mg/kg of endotoxin 12 h before the experiment. Atria were subjected to a starting tension (0·5 g) and then extended by 1 mm increments, allowing 1 h to reach equilibrium in each case. Figure 4 shows that atrial strips from endotoxin-treated guinea-pigs and control animals had the same extensibility with each stretch. Thus, endotoxin did not alter the diastolic compliance.

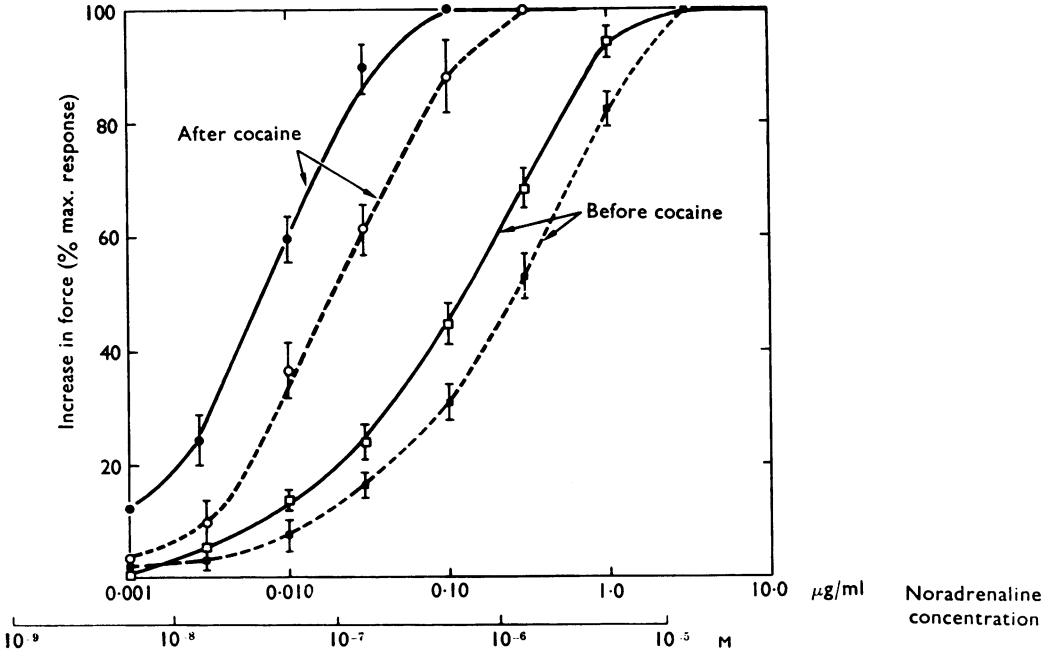

FIG. 3. Effect of cocaine on the positive inotropic response to (−)-noradrenaline. Isolated atrial strips from guinea-pigs (controls (——) and animals pretreated with endotoxin 1 h before the experiment (- - -)) driven electrically at 1 Hz in Tyrode solution at 34° C. Ordinate, Increase in force of contraction in % of maximal developed tension at end of log-concentration curve. Abscissa, Log concentration for (−)-noradrenaline. Log-concentration curves were determined for (−)-noradrenaline in the absence (right) and presence (left) of 10 μg/ml (3×10^{-5}M) of cocaine hydrochloride added 20 min before log-concentration curve. Mean values ± S.E. for five observations per point.

Effect of endotoxin pretreatment on accumulation of ^{3}H-noradrenaline in atrial strips

Reduced sensitivity to noradrenaline could be explained if endotoxin pretreatment caused an increase in the uptake of noradrenaline. A direct consequence of this would be that noradrenaline reaches the receptor sites of the effector organ in low concentrations, resulting in a reduced sensitivity to this amine. This hypothesis was tested in the following experiments. Animals were injected with 2 mg/kg of endotoxin 12 h before the experiment. Atrial preparations, made from untreated and endotoxin-treated guinea-pigs, were exposed to 0·2 μCi/ml of (±)-^{3}H-noradrenaline. Thirty minutes later they were washed and analysed for ^{3}H-noradrenaline. The mean value (±S.E.) for eight atrial strips obtained from endotoxin-treated guinea-pigs was 760 (±25) d.p.m./mg. The comparable value obtained for eight control atrial strips was 1,150 (±43). This difference (34%) is significant (P=0·05).

Discussion

The results of this study clearly indicate that in guinea-pigs which have received coliform endotoxin, the sensitivity of cardiac tissue to noradrenaline is reduced. Similarly dose-response curves to tyramine, an amine which stimulates the cardiac tissue indirectly (Burn & Rand, 1958; Bhagat, 1963; Bhagat & Gilliam, 1965, 1966) through the release of noradrenaline, also showed a shift to the right.

The response of an organ to noradrenaline depends on at least four factors: (1) extensibility of the tissue, (2) the amount of noradrenaline reaching the receptor sites, (3) the sensitivity of the receptors to noradrenaline, and (4) the sequence of the events between the occupation of the receptors by the agonist and the contraction of the myofibrils.

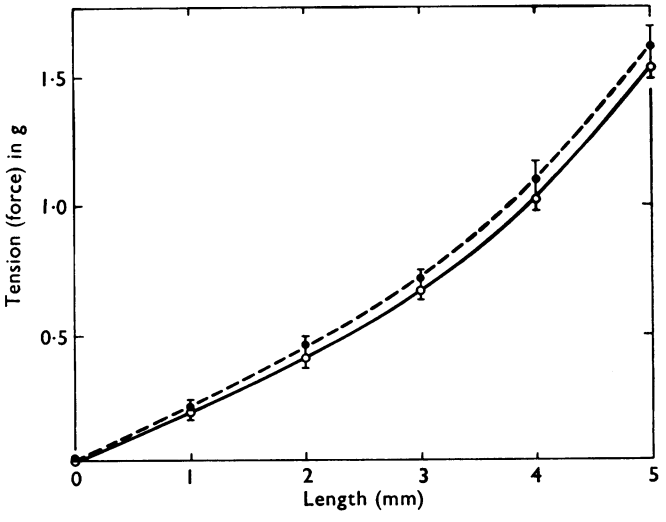

FIG. 4. Length-tension relationship in atria of controls and endotoxin-treated guinea-pigs. Isolated atrial strips from guinea-pigs (controls (——) and animals pretreated with endotoxin 12 h before the experiment (- - -)) driven electrically at 1 Hz in Tyrode solution at 34° C. Ordinate, Increase in tension (force). Abscissa, Increase in length. Mean values±S.E. for five observations per point.

Comparison of the length-tension curves showed that extensibility of the atria, from control guinea-pigs and guinea-pigs pretreated with endotoxin, was the same.

It is well known that cardiac tissue takes up catecholamine from the surrounding fluid and retains it in post-ganglionic sympathetic nerve endings and that this is more important in limiting the action of noradrenaline than metabolic degradation. If endotoxin pretreatment could increase the capacity of an organ to take up the neurohormones, this would result in a diminution in the response to this amine. In the present study, however, accumulation of ^{3}H-noradrenaline was reduced in the atria from endotoxin-treated guinea-pigs.

The reduced responsiveness of atrial tissue from endotoxin-treated guinea-pigs to noradrenaline could not be explained on the basis of changes in extensibility of the tissue either. Thus, reduced sensitivity after endotoxin may be due either to its effect on the sensitivity of the adrenoceptors or on the sequential steps subsequent to the receptor activation which leads to the response of the cell or on both. However, at the present time there is no way of proving or disproving this last possibility.

Although endotoxin pretreatment reduced the sensitivity to noradrenaline, the effect was not permanent. The sensitivity of atrial tissue from endotoxin-treated guinea-pigs began to increase again at 23 h and was normal 42 h after the administration of endotoxin.

The retention of exogenous noradrenaline must be regarded as a combination of two steps: (1) actual uptake into the nerve terminal, and (2) subsequent storage (presumably in vesicles). Compounds like cocaine, which interfere with the uptake of noradrenaline across the neuronal membrane, cause supersensitivity to noradrenaline. On the other hand, pretreatment with reserpine leaves the first step intact, but prevents the second (Lindmar & Muscholl, 1964). Thus, in the reserpine-pretreated tissue, noradrenaline is transported at a normal rate, but instead of being stored in vesicles, it is exposed to monoamine oxidase and is inactivated enzymically. This explains why there is no supersensitivity to noradrenaline after pretreatment with reserpine. In the present study, there was a marked reduction in the accumulation of ^{3}H-noradrenaline in the atria from guinea-pigs pretreated with endotoxin. If this reduction in accumulation of noradrenaline was due to a decrease in its uptake into the nerve terminal, this would have resulted in a supersensitivity to noradrenaline and a reduced effect of cocaine on sensitivity. Since after endotoxin, atrial responses to noradrenaline were reduced, and the sensitizing effect of cocaine was normal, it is suggested that endotoxin pretreatment did not affect the transport of noradrenaline into the nerve ending, but rather affected the storage and binding. At this time, however, the reduced uptake of ^{3}H-noradrenaline by tissue from endotoxin-treated animals cannot be unequivocally attributed to an alteration in intraneuronal binding. The results with tyramine clearly indicate that at least the tyramine-releasable fraction (store) of noradrenaline is unaffected by endotoxin.

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