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DESENSITIZATION BY NORADRENALINE OF RESPONSES TO STIMULATION OF PRE- AND POSTSYNAPTIC ADRENOCEPTORS

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1 The effect of exposing isolated preparations of rat aortic strip, rat atria and mouse vas deferens to perfusions of Krebs solution containing various concentrations of noradrenaline on their sensitivity to the drug has been determined.

2 The responses evoked by stimulation of postsynaptic adrenoceptors in all the tissues and presynaptic α-adrenoceptors in the mouse vas deferens were diminished by the perfusion of noradrenaline through the organ bath for 30 min.

3 The concentration of noradrenaline required to produce desensitization was higher in the mouse vas deferens than in the other tissues and more was required to desensitize the chronotropic responses than the inotropic responses in rat isolated atria.

4 The inclusion of cocaine (10^{-5} M) in the bathing solution to block uptake$_1$ increased the sensitivity of most tissues to noradrenaline. With the possible exception of the response to stimulation of presynaptic receptors in the mouse vas deferens, desensitization was somewhat increased in its presence.

5 When uptake$_2$ was blocked by oestradiol (10^{-5} M), it was not possible to desensitize the contractor responses of the aortic strip and vas deferens to exogenous noradrenaline, nor the inotropic response of the atria to the drug. However, oestradiol failed to block the desensitization of chronotropic responses and responses to stimulation of presynaptic receptors in the vas deferens.

6 Blockade of monoamine oxidase (MAO) with iproniazid (7.2×10^{-4} M) or with pargyline (5×10^{-4} M) did not affect the desensitization process in the aortic strip.

7 Blockade of catechol-*O*-methyltransferase (COMT) with U-0521 (5.3×10^{-5} M) greatly increased desensitization in the aortic strip and desensitization of inotropic responses in the atria. It had no effect on desensitization of chronotropic responses. Its effect on responses in the mouse vas deferens was not determined.

8 The perfusion of methoxamine at concentrations about 1000 times higher than those of noradrenaline also produced desensitization in the aortic strip.

9 The desensitization of presynaptic receptors in the mouse vas deferens was shown to be specific and that of the responses to postsynaptic receptor stimulation to be non-specific.

10 It is concluded that responses to adrenoceptor stimulation may be desensitized by accumulation of noradrenaline inside the cells bearing the receptors and that the desensitization is caused by noradrenaline itself not by a metabolite. Desensitization may also be caused without accumulation of noradrenaline in uptake$_2$ and for some receptors these may not be alternative mechanisms.

Introduction

Desensitization is a well known phenomenon which may be defined as the reduced ability of a muscle to respond to a given dose of an agonist after prolonged exposure to it. Most investigations of the phenomenon have been concerned with responses of tissues to acetylcholine and related drugs and various models have been proposed to explain the phenomenon. The most successful of these seems to have been that of Katz & Thesleff (1957). However, their hypothesis would only be applicable to specific desensitization such as that observed by Barsoum & Gaddum (1935), where soaking the fowl rectal caecum in histamine abolished responses to histamine but not those to barium, acetylcholine or adrenaline. The model proposed by Katz & Thesleff would not be applicable to the kind of desensitization observed by Cantoni & Eastman (1946) in which a large concentration of acetylcholine applied to the guinea-pig ileum rendered it insensitive to both acetylcholine and histamine. If the system in which the phenomenon of desensitization is being examined can only be activated by one type of agent acting on one kind of

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receptor, then it is not even possible to say whether the desensitization is specific or non-specific. It seems important therefore to characterize the desensitization as far as possible and this work has been confined to that aim.

Methods

Rat aortic strip

Male Wistar rats (200–400 g) were killed by a blow on the head and exsanguinated. The aorta was removed from the aortic arch to the level of the renal arteries and spiral strips approximately 4 cm in length were cut from it. The strips were mounted in an overflow organ bath (5 ml capacity) under a tension of 1.0 g in Krebs solution at 37°C and of the following composition (mM): NaCl119, KCl4.7, $CaCl_2$2.5, $MgSO_4.7H_2O$ 1.2, $NaH_2PO_4.2H_2O$ 1.2, $NaHCO_3$ 25.0 and glucose 11. The solution was oxygenated via a sintered glass filter with 95% O_2 and 5% CO_2. Contractions were recorded isometrically via a Grass FTO3 force-displacement transducer on a Grass model 7C polygraph.

After an equilibration period of 90 min, dose-response curves were constructed by repeating each dose of agonist four times in random order with a contact time of 90 s and an interval of 10–15 min between doses.

Desensitization

After establishing a control dose-response curve, Krebs solution was perfused through the organ bath at a rate of 10 ml min^{-1} and noradrenaline was injected into it via a hypodermic needle in the supply tubing about 20 cm from its opening in the organ bath. The injection was sustained at a constant rate for 30 min by means of a Palmer Slow Injection apparatus. When the injection of noradrenaline was stopped, perfusion of Krebs solution continued until the issue had relaxed and returned to its original tension. The dose-response curve was then re-established.

Rat isolated atria

Adult male Wistar rats (175–200 g) were killed by a blow on the head and exsanguinated. The rib cage was removed and the heart was dissected out and placed in cold Krebs solution of the same composition as that used for aortic strips. The ventricles were cut away from the auricles taking care not to pinch or damage the latter. The auricles were mounted in a 40 ml overflow organ bath containing Krebs solution maintained at 37°C and oxygenated via a glass sinter with 95% O_2 and 5% CO_2. The force of contraction (inotropism) of the isolated spontaneously beating atria was recorded on a Grass polygraph via an FTO3 force-displacement transducer and the rate of beating (chronotropism) was recorded on a tachograph on the same instrument.

After a 45 min equilibration period a 3 or 4 point dose-response curve was constructed, each dose being left in contact with the tissue for 3 min and randomized at intervals of 10 min. The resting tension and the resting rate varied during the experiments, but the tension or rate developed in response to a given dose always approximated the same value independently of the resting value. Accordingly, the results are expressed as a percentage of the maximum response, the latter being established after completion of the control dose-response curve.

Desensitization

This was effected as described for the aortic strip except that the infusion of Krebs and noradrenaline were stopped together, the bath was washed with Krebs solution at a rate of 50 ml min^{-1} for 2 min and the dose-response curve to noradrenaline was re-established 10 min later.

Mouse isolated vas deferens

Male mice (30–50 g) were killed by a blow on the head and exsanguinated. The abdomen was opened and the vasa deferentia were dissected out and placed in a Petri dish containing Mg^{2+}-free Krebs solution whose composition was otherwise identical to that described for the aortic strip. Magnesium sulphate was omitted from the solution because the twitch response to electrical stimulation is much enhanced by its absence (Hughes, Kosterlitz & Leslie, 1975). The vasa were desheathed by careful removal of closely adjoining blood vessels, mesenteric tags and connective tissue.

The vasa were finally mounted under a resting tension of 0.5 g in a 2.0 ml organ bath containing Mg^{2+}-free Krebs maintained at 37°C and oxygenated with 95% O_2 and 5% CO_2. Twitch responses were elicited by field stimulation (68 V, 2 ms, 0.2 Hz) by a Grass S44 stimulator via gutter electrodes (Birmingham & Wilson, 1963) and recorded isometrically with a Grass FTO3 force-displacement transducer on a Grass polygraph.

After allowing 60 min with periodic washing for the tissue to equilibrate, the effect of noradrenaline on presynaptic α-adrenoceptors was measured by determining the reduction in twitch height produced by various doses of noradrenaline and expressing the reduction as a percentage of the twitch height before adding the drug. One and a half minutes after starting

stimulation, doses of noradrenaline were added to the bath via a micrometer syringe in volumes not exceeding 50 μl and allowed to act for 1 min. The tissue was then washed twice and allowed to rest unstimulated for 3.5 min before repeating the procedure. Dose-response curves were constructed by determining the inhibition of the twitch produced by 4 or 5 different concentrations of noradrenaline applied 4 times each in random order.

Contractor (postsynaptic) responses were produced by the higher concentrations used to determine the effects on presynaptic receptors, but it was necessary to increase the concentration of noradrenaline applied to obtain the upper part of the dose-response curve for contractor responses in some experiments.

The inhibition of twitch responses produced by morphine was less easily reversed than was that produced by noradrenaline, so cumulative dose-responses were determined during continued stimulation for 7.5 min. An interval of 20 min was left between each 7.5 min period of stimulation, during which stimulation was stopped and the tissue was washed 12 times (3 washes at a time at 2 min intervals) followed by single washes 10 min and 5 min before stimulation was due to start again. Four different concentrations of morphine were used and each cumulative response was repeated 4 times to construct the dose-response curve. Inhibitory responses were expressed as a percentage decrease in initial twitch height.

Desensitization

The tissue was not mounted in an overflow bath in this case, so the drain tube clamp was adjusted to take 30 s to empty the bath and fresh Krebs solution containing the appropriate concentration of noradrenaline was delivered to the top of the bath at the rate of 4 ml min^{-1}. The perfusion of Krebs solution containing noradrenaline was maintained for 30 min. The tissue was then washed twice, four times at 2 min intervals before re-establishing the dose-response curve.

Enzyme inhibition

In experiments on the aortic strip, monoamine oxidase (MAO) was inhibited by including iproniazid (7.2×10^{-4} M) in the Krebs solution bathing the tissue for the last 30 min of the equilibration period, or by including pargyline (5×10^{-4} M) in the Krebs solution throughout the experiment.

Catechol-*O*-methyl transferase (COMT) was inhibited in experiments on the aortic strip and the isolated auricles by including 3,4-dihydroxy-2 methyl-1-propriophenone (U-0521) (5.3×10^{-5} M) in the solution bathing the tissues throughout the experiment.

In one experiment on isolated auricles, tropolone (1.0 mM) was used to inhibit COMT.

Uptake inhibition

Cocaine (10^{-5} M) was used to block $uptake_1$ in experiments on isolated auricles and on the vas deferens.

Concentrations of oestradiol ranging from $1-3 \times 10^{-5}$ M were included in the Krebs bathing the tissues to block $uptake_2$.

Drugs

The following drugs were used: (−)-noradrenaline bitartrate (Levophed, Winthrop); cocaine hydrochloride (10^{-5} M) (St Mary's Hospital Pharmacy); 1,3,5(10)-oestratien-3,17,-diol (oestradiol, Koch-Light Laboratories Ltd, batch No. 84170); pargyline hydrochloride (Abbot Laboratories); theophylline sodium acetate; iproniazid phosphate (Sigma); 3,4 dihydroxy-2-methyl propriophenone (U-0521) Upjohn); tropolone 98% (Aldrich); morphine sulphate 15 mg ml^{-1}, containing sodium metabisulphite 0.1% w/v (St Mary's Hospital Pharmacy); acetylcholine (Sigma); hexamethonium bromide (Vegolysen, May & Baker); L-ascorbic acid (B.D.H.).

Ascorbic acid (20 μg ml^{-1}) was added to all dilutions of noradrenaline unless otherwise stated. Oestradiol was dissolved in alcohol to give a stock solution containing 5 mg ml^{-1}. Dilutions of this stock solution to give the concentrations of oestradiol used in the experiments were made with Krebs solution.

Results

Rat aortic strip

In 3 experiments in which Krebs solution alone was perfused through the bath for 30 min, it was without effect on the dose-response curve to noradrenaline. When noradrenaline (800 ng kg^{-1} min^{-1}) was injected into the perfusing Krebs solution (the kg weight referring to the weight of the animal from which the tissue had been removed) the dose-response curve to noradrenaline was shifted to the right by one third of a log unit at the ED_{50} level. The curves, derived from 3 experiments, exhibited some convergence at the higher dose levels, so that at the ED_{70} the shift was insignificant. The inclusion of iproniazid (7.2×10^{-4} M) in the solution bathing the tissue for the last 30 min of the equilibration period insignificantly increased the sensitivity of the aortic strip to noradrenaline and did not change the degree of desensitization produced by infusing norad-

renaline (800 ng kg^{-1} min^{-1}). However, it did prevent the convergence in the dose-response curves so that they were displaced in a parallel fashion and the desensitization was significant at all points. Pargyline (5×10^{-4} M) included in the Krebs throughout the experiment did not change the nature or the degree of desensitization produced by infusing noradrenaline (800 ng kg^{-1} min^{-1}).

By contrast, when COMT was blocked with U-0521 (5.3×10^{-5} M), it was only possible to construct dose-response curves to noradrenaline in 3 out of 5 experiments in which the curve to noradrenaline was shifted to the right by half a log unit after the infusion of noradrenaline (800 ng kg^{-1} min^{-1}). In the other two experiments, responses to all four doses of noradrenaline used were progressively reduced almost to the point of extinction during the construction of the curves (Figure 1). The reduction in the response was not due to an effect of U-0521 on the ability of the muscle to contract since responses to barium chloride were unaffected (Figure 1).

In four experiments in which extraneuronal uptake was blocked by oestradiol (1.2×10^{-5} M), infusions of noradrenaline failed to produce desensitization. In view of this result, the ability of methoxamine (which is not subject to uptake$_1$ or uptake$_2$, nor is it attacked by MAO or COMT) to produce desensitization was determined.

Methoxamine was approximately 1000 times less potent than noradrenaline on the rat aortic strip, but

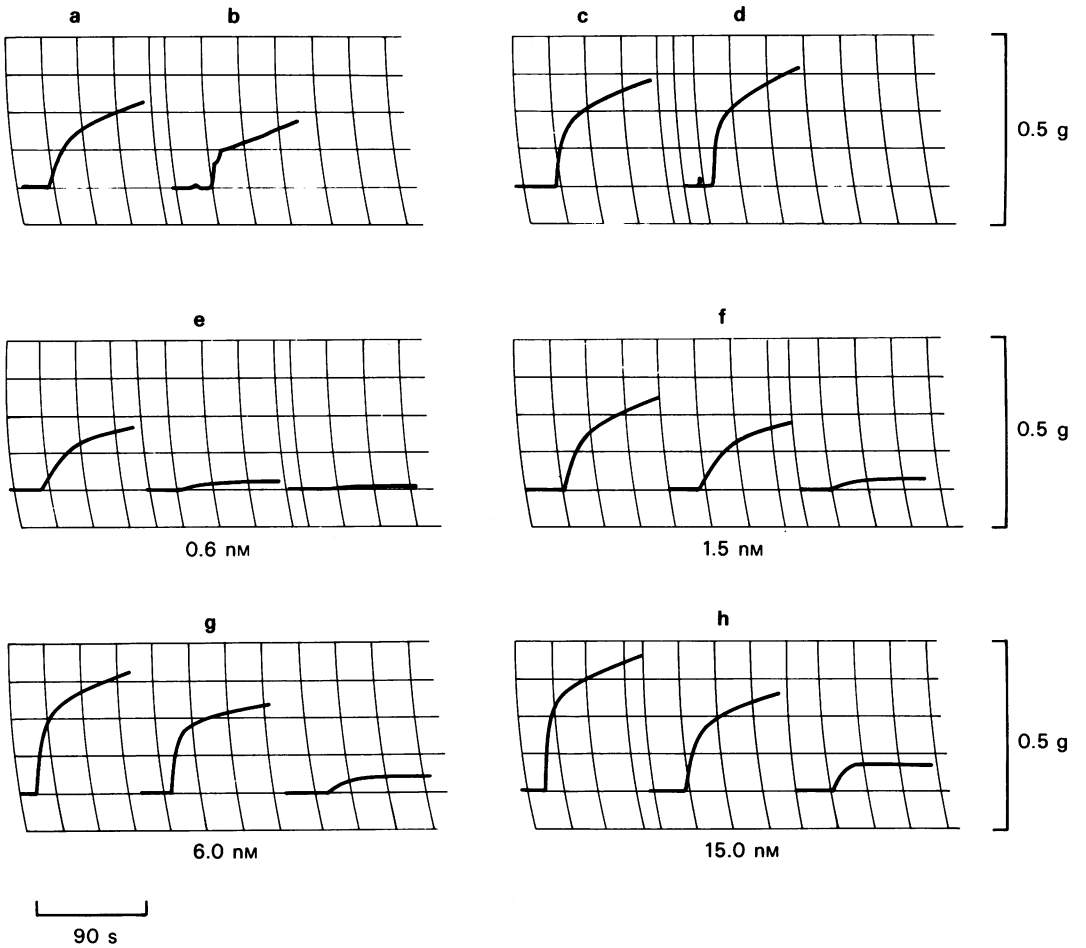

Figure 1 The influence of the catechol-*O*-methyltransferase blocking agent, U-0521, on responses to $BaCl_2$ and to noradrenaline. Panels (a) and (c) show the responses to $BaCl_2$ (1 mg and 2 mg respectively) in the absence of U-0521 and (b) and (d) in the presence of U-0521. Panels (e), (f), (g) and (h) show successive responses to the concentration of noradrenaline indicated during an attempt to prepare a dose-response curve in the presence of U-0521.

the relative potencies were first determined in each preparation used and the amount of methoxamine to be perfused through the bath was then calculated as 800 ng kg^{-1} min^{-1} × the value obtained. This ensured that infusions of methoxamine used were equipotent with those of noradrenaline and in 3 experiments it produced a rightward shift in the dose-response curve to noradrenaline similar to that produced by noradrenaline itself.

Rat isolated atria

Table 1 shows the effects of perfusing Krebs solution alone through the organ bath for 30 min, or Krebs solution into which an infusion of 800 ng kg^{-1} min^{-1} of noradrenaline was injected in the presence of different drugs calculated to affect either its uptake or metabolism on the dose-response curve for the inotropic effects of noradrenaline.

It is clear that Krebs solution alone did not significantly alter the dose-response curve for the inotropic effect of noradrenaline but the infusion of 800 ng kg^{-1} min^{-1} did produce a significant desensitization which was greater in the presence of cocaine (10^{-5} M). When uptake$_2$ was blocked with oestradiol (10^{-5} M) desensitization was prevented as it had been in the aortic strip.

The blockade of COMT by U-0521 increased the desensitization produced by noradrenaline though Table 1 shows two values that appear to be insignificantly different. This was because during the construction of the dose-response curve to noradrenaline in the presence of U-0521 before an infusion was given, there was a progressive desensitization which resulted in the large standard errors shown. In another experiment the process was completed and in

Table 1 The effect of infusing Krebs solution (10 ml min^{-1}) or Krebs solution into which noradrenaline (NA, 800 ng kg^{-1} min^{-1}) was injected (kg^{-1} referring to the weight of the animal from which the preparation was made) on inotropic dose-response curves to noradrenaline in isolated spontaneously beating rat atria in the presence and absence of drugs calculated to affect its uptake or metabolism

Dose NA (ng ml^{-1})	*% max response pre-infusion*	*% max response post-infusion*	*Difference between means (%)*	n	*Significance of difference*
Krebs solution alone					
2.50	38.0± 2.3	35.0±2.5	−3.0	6	NS
3.75	51.0± 1.9	52.0±3.3	+1.0	6	NS
5.00	61.0± 2.6	60.0±2.2	−1.0	6	NS
6.25	74.0± 3.0	75.0±2.7	+1.0	6	NS
NA (800 ng kg^{-1} min^{-1})					
1.25	35.0± 2.0	25.0±2.0	−10.0	22	$P<0.01$
2.50	47.5± 1.8	36.0±1.6	−11.5	22	$P<0.01$
3.75	63.0± 1.6	50.5±2.0	−12.5	22	$P<0.01$
5.00	72.5± 3.4	62.0±2.9	−10.5	22	$P<0.02$
NA (800 ng kg^{-1} min^{-1}) in presence of cocaine (10^{-5} M)					
0.25	33.3± 0.9	19.0±1.4	−14.3	6	$P<0.01$
0.50	41.0± 1.2	26.0±2.8	−15.0	22	$P<0.01$
1.00	54.0± 1.3	35.0±2.1	−19.0	22	$P<0.01$
1.50	66.0± 3.5	44.0±1.9	−22.0	22	$P<0.01$
2.00	73.0± 2.2	61.0±3.5	−12.0	14	$P<0.01$
NA (800 ng kg^{-1} min^{-1}) in presence of oestradiol (10^{-5} M)					
1.25	50.0± 1.9	51.0±1.4	+1.0	14	NS
2.50	59.0± 2.0	57.0±2.4	−2.0	22	NS
3.75	62.5± 1.7	63.3±1.4	+0.8	22	NS
5.00	70.5± 2.5	71.0±2.8	+0.5	22	NS
7.50	74.0± 2.0	76.0±2.2	+2.0	6	NS
NA (800 ng kg^{-1} min^{-1}) in presence of U-0521 (5.3×10^{-5} M)					
1.25	44.0± 5.9	28.0±3.5	−16.0	6	$P<0.1$
2.50	52.0± 5.0	30.0±3.8	−22.0	6	$P<0.02$
3.75	56.0± 9.7	34.0±2.9	−22.0	6	$P<0.1$
5.00	65.0±11.0	37.0±3.3	−28.0	6	$P<0.02$

Where $n = 6$ the observations recorded were from a single preparation, but the experiment was repeated at least 3 times in other preparations with the same result unless otherwise stated in the text. In such experiments the doses of noradrenaline required to construct the curve were widely different and could not, therefore, be amalgamated. Where n is greater than 6 the figures are the means from all the experiments (usually 4); values are given ± s.e.mean.

another it became total after the infusion of noradrenaline. One other experiment was similar to that shown in Table 1. The results obtained with U-0521 therefore closely resembled those in the aortic strip. In one experiment only, no inotropic response to noradrenaline could be obtained in the presence of tropolone (1.2×10^{-4} g ml^{-1}) to block COMT, but inotropic responses could be obtained with theophylline suggesting that the tropolone had not produced any particularly profound non-specific inhibition.

Table 2 shows the dose-response curves for chronotropism in response to noradrenaline. It may be remarked that the curves usually begin at 60–70% of the maximum response. This is because the spontaneous rate is usually at least 50% of the maximum.

Neither the perfusion of Krebs alone nor Krebs into which was injected 800 ng kg^{-1} min^{-1} of noradrenaline had any significant effect on the dose-response curve to noradrenaline. This contrasted with the inotropic responses which were desensitized by this amount of noradrenaline. It was not until 10

Table 2 The effect of perfusing Krebs solution (10 ml min^{-1}) or Krebs solution into which noradrenaline (NA, 800 ng or 8 μg kg^{-1} min^{-1}) was injected (kg^{-1} referring to the weight of the animal from which the preparation was made) on chronotropic dose-response curves to noradrenaline in isolated, spontaneously beating rat atria in the presence and absence of drugs calculated to affect its uptake or metabolism

Dose of NA (ng ml^{-1})	*% max response pre-fusion*	*% max response post-fusion*	*Difference between means* (%)	n	*Significance of difference*
Krebs solution alone					
0.25	69.5 ± 0.8	69.0 ± 0.5	−0.5	22	NS
0.50	73.0 ± 0.7	72.0 ± 0.8	−1.0	22	NS
0.75	79.5 ± 0.6	80.5 ± 0.8	+1.0	22	NS
1.00	86.5 ± 0.8	88.0 ± 1.5	+1.5	22	NS
NA (800 ng kg^{-1} min^{-1})					
0.25	75.0 ± 1.5	74.0 ± 2.0	−1.0	16	NS
0.40	77.0 ± 1.0	76.0 ± 1.0	−1.0	10	NS
0.50	77.5 ± 2.5	78.0 ± 2.0	+0.5	10	NS
0.75	81.0 ± 4.0	80.0 ± 3.5	−1.0	16	NS
1.00	85.5 ± 5.0	83.0 ± 3.0	−2.5	10	NS
NA (8 μg kg^{-1} min^{-1})					
0.25	74.0 ± 1.5	67.0 ± 1.0	−7.0	4	*P* 0.02
0.50	75.0 ± 1.0	69.0 ± 0.5	−6.0	12	*P* 0.01
0.75	77.5 ± 1.5	70.0 ± 1.3	−7.5	18	*P* 0.01
1.00	82.0 ± 1.0	74.5 ± 1.0	−7.5	18	*P* 0.01
1.25	87.0 ± 1.8	80.0 ± 2.0	−7.0	4	*P* 0.05
1.50	88.0 ± 2.0	86.0 ± 0.8	−2.0	12	NS
NA (8 μg kg^{-1} min^{-1}) in presence of cocaine (10^{-5} M)					
0.03	59.5 ± 1.3	30.5 ± 2.3	−29.0	6	*P* 0.01
0.06	66.0 ± 2.1	56.8 ± 2.9	−9.2	22	*P* 0.02
0.13	71.5 ± 2.4	62.5 ± 2.1	−9.0	22	*P* 0.01
0.19	77.0 ± 1.7	69.5 ± 3.0	−7.5	22	*P* 0.02
0.25	84.0 ± 1.8	74.5 ± 1.2	−9.5	14	*P* 0.01
NA (8 μg kg^{-1} min^{-1}) in presence of oestradiol (10^{-5} M)					
0.25	68.0 ± 2.3	60.0 ± 1.6	−8.0	6	*P* 0.05
0.50	76.0 ± 2.2	68.0 ± 1.2	−8.0	6	*P* 0.02
0.75	81.5 ± 1.0	73.0 ± 1.6	−8.5	6	*P* 0.01
1.00	87.0 ± 0.7	80.0 ± 1.4	−7.0	6	*P* 0.01
NA (8 μg kg^{-1} min^{-1}) in presence of U-0521 (5.3×10^{-5} M)					
0.06	75.0 ± 1.1	69.0 ± 0.5	−6.0	6	*P* 0.01
0.13	80.0 ± 1.4	72.5 ± 0.2	−7.5	6	*P* 0.01
0.19	82.0 ± 1.6	78.0 ± 0.4	−4.0	6	*P* 0.05
0.25	90.0 ± 1.5	84.0 ± 1.1	−6.0	6	*P* 0.02

Where $n = 6$ the observations were from a single preparation, but the experiment was repeated at least 3 times in other preparations. In such experiments the dose of noradrenaline required to construct the curves were widely different and could not, therefore, be amalgamated. Where n is greater than 6 the figures are the means from all the experiments (usually 4); values are given ± s.e.mean.

times this amount of noradrenaline was injected into the perfusing Krebs that desensitization was obtained.

When cocaine (10^{-5} M) was included in the bathing and perfusing solutions, the sensitivity of the tissue to the chronotropic effect of noradrenaline was greatly increased and the desensitization produced by noradrenaline ($8\,\mu g\,kg^{-1}\,min^{-1}$) was a little greater.

The inclusion of oestradiol (10^{-5} M) in the bathing and perfusing solution to block uptake$_2$ did not sensitize the tissue to the effect of noradrenaline as cocaine had done and did not significantly affect the desensitization of the responses when noradrenaline ($8\,\mu g\,kg^{-1}\,min^{-1}$) was perfused through the organ bath for 30 min. This was in contradistinction to the effect on inotropic responses which could not be desensitized in its presence.

Likewise blockade of the metabolizing enzyme COMT by the inclusion of U-0521 (5.3×10^{-5} M) in the bathing and perfusing solutions failed to increase the desensitization as it had done in the case of inotropic responses.

Mouse isolated vas deferens

In preliminary experiments it was shown that when Krebs solution containing ascorbic acid ($20\,\mu g\,ml^{-1}$) was perfused through the organ bath at the rate of $4\,ml\,min^{-1}$ for 30 min the response of the postsynaptic receptors to noradrenaline was subsequently increased resulting in displacement of the dose-response curve to the left. When ascorbic acid was omitted and Krebs solution alone was perfused through the bath for 30 min, the responses of the postsynaptic receptors to noradrenaline were unaffected. For this reason, ascorbic acid was omitted from the Krebs solution used in the desensitization procedures, but was included in the noradrenaline solutions used to construct dose-response curves. Because the Krebs solution used in the desensitization procedures did not contain ascorbic acid the noradrenaline added to it was always prepared and added immediately before use.

Table 3 shows that as the concentration of noradrenaline in the desensitizating solution was increased from 3×10^{-6} M to 2×10^{-5} M desensitization of the postsynaptic receptor was increased from significance at only one point on a 5 point dose-response curve to significance at all points except one. When the uptake of noradrenaline into the neurones was prevented with cocaine (10^{-5} M) significant desensitization of all points on the dose-response curve was produced by the perfusion of noradrenaline (2×10^{-5} M) and the rightward displacement of the curve was greater than that produced by the same concentration of noradrenaline in the absence of cocaine. The inclusion of oestradiol (3×10^{-5} M) in the bathing and desensitizing solutions to prevent the uptake of noradrenaline into the muscle fibres of the vas deferens prevented desensitization from occurring as it had done in the aortic strip and in the auricles.

Table 3 shows that contractor responses to acetylcholine were also desensitized by the perfusion of noradrenaline (2×10^{-5} M) through the organ bath for 30 min. It was further demonstrated that the addition of hexamethonium (10^{-4} M) to the bath did not shift the dose-response curve to acetylcholine, showing that the contractions it produced were not mediated by noradrenaline released as a result of ganglionic stimulation.

Table 4 shows the effect of the same concentrations of noradrenaline (3×10^{-6} M–2×10^{-5} M) perfused through the bath for 30 min on the inhibition of the electrically stimulated twitch response produced by the action of noradrenaline on presynaptic receptors. Again desensitization was produced by the perfusion of noradrenaline that increased as its concentration in the perfusing solution was increased. Consistent with this desensitizing effect was the fact that the inhibition of the response to electrical stimulation produced by the desensitizing perfusion decreased as the perfusion progressed.

In the presence of cocaine (10^{-5} M) the sensitivity of the presynaptic receptor to noradrenaline was increased 20 to 30 times but the ability of the drug to cause autodesensitization was little affected. During the perfusion of noradrenaline the twitch response was totally inhibited and did not re-appear until 25 to 35 min had elapsed after the washing period following the infusion.

Like cocaine, oestradiol (10^{-5} M) sensitized the presynaptic receptors to noradrenaline but only about 10 times. The inhibition of noradrenaline uptake into the muscle fibres with oestradiol had little or no effect on the desensitization produced by perfusing noradrenaline through the organ bath.

Table 4 also shows that where the perfusion of noradrenaline (2×10^{-5} M) had produced desensitization of the presynaptic α-adrenoceptor response, it failed to affect the inhibitory responses to morphine (3×10^{-8}–10^{-6} M).

Discussion

That the long exposure to catecholamines of tissues receiving a sympathetic innervation results in a reduced response of those tissues to catecholamines is well established (Blacket, Pickering & Wilson, 1950; Duner & von Euler, 1957; Trendelenburg, 1971; Eden & Nasmyth 1974; Langer & Dubocovich, 1977). The latter authors showed for the first time that presynaptic as well as postsynaptic responses are

Table 3 The effect of noradrenaline (NA) and acetylcholine on the tone of the mouse isolated vas deferens before and after perfusing magnesium-free Krebs solution containing noradrenaline (3×10^{-6} M–2×10^{-5} M) through the organ-bath for 30 min; the effects of the addition of cocaine (10^{-5} M) and of oestradiol (3×10^{-5} M) are also shown

Conc. of NA (M)	*Increase in resting tone* (mg) ± *s.e.* *pre-infusion*	*post-infusion*	n	*Significance of difference*
NA (3×10^{-6} M)				
2×10^{-7}	52 ± 15.0	47 ± 3.3	6	NS
5×10^{-7}	88 ± 19.8	70 ± 11.5	6	NS
10^{-6}	144 ± 23.4	107 ± 3.3	6	NS
1.5×10^{-6}	178 ± 28.9	130 ± 10.0	6	NS
3×10^{-6}	293 ± 12.0	217 ± 18.6	6	*P* 0.05
NA (10^{-5} M)				
2×10^{-7}	50 ± 10.0	38 ± 10.7	6	NS
5×10^{-7}	92 ± 10.7	70 ± 9.5	6	NS
10^{-6}	158 ± 9.2	122 ± 10.4	6	*P* 0.05
1.5×10^{-6}	208 ± 9.7	154 ± 13.3	6	*P* 0.02
3×10^{-6}	320 ± 24.5	204 ± 22.0	6	*P* 0.02
NA (2×10^{-5} M)				
2×10^{-7}	70 ± 7.1	64 ± 6.8	6	NS
5×10^{-7}	168 ± 21.3	106 ± 9.8	6	*P* 0.05
10^{-6}	256 ± 12.9	184 ± 14.7	6	*P* 0.02
1.5×10^{-6}	310 ± 17.4	228 ± 23.7	6	*P* 0.05
3×10^{-6}	456 ± 23.4	274 ± 31.4	6	*P* 0.01
NA (2×10^{-5} M) in presence of cocaine (10^{-5} M)				
2×10^{-7}	63 ± 7.2	14 ± 5.9	6	*P* 0.01
5×10^{-7}	154 ± 18.3	82 ± 8.0	6	*P* 0.02
10^{-6}	325 ± 25.0	160 ± 10.7	6	*P* 0.01
1.5×10^{-6}	396 ± 39.9	206 ± 18.8	6	*P* 0.01
3×10^{-6}	633 ± 41.7	300 ± 27.0	6	*P* 0.01
NA (2×10^{-5} M) in presence of oestradiol (10^{-5} M)				
2×10^{-7}	20 ± 3.2	28 ± 3.7	6	NS
5×10^{-7}	56 ± 4.0	64 ± 9.8	6	NS
10^{-6}	116 ± 15.2	112 ± 13.9	6	NS
1.5×10^{-6}	174 ± 14.4	172 ± 22.5	6	NS
3.0×10^{-6}	263 ± 17.4	260 ± 15.8	6	NS
Conc. of Acetylcholine (M)				
NA (2×10^{-5} M)				
5×10^{-7}	44 ± 5.9	28 ± 6.6	6	NS
10^{-6}	76 ± 13.8	34 ± 2.4	6	*P* 0.05
2×10^{-6}	138 ± 20.9	55 ± 3.5	6	*P* 0.01
5×10^{-6}	229 ± 23.4	84 ± 12.0	6	*P* 0.01
10^{-5}	356 ± 30.3	155 ± 25.3	6	*P* 0.01
	Before adding C_6 (10^{-4} M)	*After adding* C_6 (10^{-4} M)		
5×10^{-7}	14 ± 1.3	16 ± 2.4	6	NS
2×10^{-6}	47 ± 6.0	45 ± 4.6	6	NS
10^{-5}	144 ± 24.3	98 ± 10.5	6	NS

affected. The present work has confirmed that autodesensitization occurs in responses to both α- and β-postsynaptic adrenoceptor stimulation and to stimulation of presynaptic α-adrenoceptors. Examination of the influences of the uptake of catecholamines into the nerve fibres or the tissues that they innervate and blockade of metabolizing enzymes has, however, revealed that the site and mechanism of autodesensitization is not always the same.

In all these experiments the tissues were exposed to continually renewed concentrations of noradrenaline for 30 min. Whilst concentrations of approximately 10^{-7} M desensitized responses to postsynaptic α-adrenoceptor stimulation in the rat aortic strip and to β-adrenoceptors mediating inotropism in the rat atrium, it required 10 times as much to desensitize the β-adrenoceptors mediating chronotropism. Even higher concentrations of noradrenaline, namely 2×10^{-5} M, were required to desensitize pre- and

Table 4 The effects of noradrenaline (NA) and of morphine on the twitch response of the mouse isolated vas deferens to field stimulation before and after perfusing magnesium free Krebs containing noradrenaline (3×10^{-6} M–2×10^{-5} M) through the organ bath for 30 min; the effects of the addition of cocaine (10^{-5} M) and oestradiol (10^{-5} M) are also shown

Conc. of NA (M)	*% inhibition of twitch ± s.e.* *pre-infusion*	*post-infusion*	n	*Significance of difference*
NA (3×10^{-6} M)				
2×10^{-7}	10.1 ± 3.5	3.8 ± 2.3	6	NS
5×10^{-7}	20.3 ± 2.2	13.4 ± 0.8	6	*P* 0.05
10^{-6}	38.1 ± 0.7	26.0 ± 5.8	6	NS
1.5×10^{-6}	46.6 ± 1.2	26.6 ± 3.0	6	*P* 0.01
3×10^{-6}	68.3 ± 1.5	50.9 ± 4.2	6	*P* 0.02
NA (10^{-5} M)				
2×10^{-7}	8.8 ± 3.4	5.6 ± 3.7	6	NS
5×10^{-7}	26.9 ± 2.4	12.1 ± 2.7	6	*P* 0.01
10^{-6}	43.0 ± 2.0	20.3 ± 4.7	6	*P* 0.01
1.5×10^{-6}	47.1 ± 3.4	28.3 ± 3.1	6	*P* 0.01
3×10^{-6}	69.3 ± 2.7	49.5 ± 3.4	6	*P* 0.01
NA (2×10^{-5} M)				
2×10^{-7}	14.3 ± 2.0	5.0 ± 1.5	6	*P* 0.01
5×10^{-7}	29.3 ± 3.5	9.8 ± 2.6	6	*P* 0.01
10^{-6}	43.2 ± 1.3	29.3 ± 3.8	6	*P* 0.02
1.5×10^{-6}	54.8 ± 2.4	35.0 ± 3.1	6	*P* 0.01
3×10^{-6}	67.1 ± 2.0	53.1 ± 1.9	6	*P* 0.01
NA (2×10^{-5} M) in presence of cocaine (10^{-5} M)				
10^{-8}	14.4 ± 3.0	5.6 ± 2.3	6	*P* 0.05
2×10^{-8}	30.8 ± 2.1	22.1 ± 2.3	6	*P* 0.05
5×10^{-8}	55.8 ± 2.4	36.0 ± 2.4	6	*P* 0.01
10^{-7}	69.1 ± 1.2	51.9 ± 0.8	6	*P* 0.01
NA (2×10^{-5} M) in presence of oestradiol (10^{-5} M)				
10^{-8}	10.4 ± 1.7	4.0 ± 1.0	6	*P* 0.02
2×10^{-8}	18.8 ± 1.8	6.4 ± 1.3	6	*P* 0.01
5×10^{-8}	30.4 ± 0.8	11.5 ± 3.7	6	*P* 0.01
10^{-7}	41.7 ± 1.3	13.8 ± 2.8	6	*P* 0.01
Conc. of morphine (M)				
NA (2×10^{-5} M)				
3×10^{-8}	13.7 ± 3.2	12.0 ± 1.9	6	NS
10^{-7}	31.4 ± 2.4	28.3 ± 2.8	6	NS
3×10^{-7}	56.6 ± 1.8	50.6 ± 2.5	6	NS
10^{-6}	79.1 ± 1.4	73.0 ± 2.7	6	NS

postsynaptic receptor responses in the mouse vas deferens. It is tempting to explain these differences in terms of the density of sympathetic innervation in the various tissues. Thus the vas would require higher concentrations because its dense sympathetic innervation would remove the noradrenaline from the extracellular spaces more efficiently than would the less dense innervation in the other tissues. If this were so, then one would expect that in the presence of cocaine, desensitization in the vas would equate with that in other tissues. However, cocaine appeared to have less effect on the desensitization process in the vas than it did in the atria. It is conceivable that this is due to the avid uptake$_2$ process in the vas deferens (Burnstock, McCulloch, Story & Wright, 1972) especially as Hughes (1972) has shown that a balance exists between uptake$_1$ and uptake$_2$, so that when one process is blocked the other is able to compensate to a considerable extent.

Such an explanation would not supply the reason for the differences in concentration needed to desensitize the receptors mediating inotropism and chronotropism in the rat atrium, since here they exist side by side in the same tissue. More revealing was the effect of blocking uptake$_2$ with oestradiol. This prevented desensitization of the responses to stimulation of post-synaptic α-adrenoceptors in the aortic strip and the vas deferens and of β-adrenoceptors mediating inotropism in the rat atrium, but was without effect on desensitization of the chronotropic response or on inhibition of the twitch response in the vas deferens. This distinction extended to the effects of blocking COMPT with U-0521. This procedure massively potentiated the desensitization of those

responses in which the process was blocked by oestradiol and was without effect on those which were unaffected by the uptake$_2$ blocking agent. The implication of these results is that the mechanism by which the responses to postsynaptic α-adrenoceptors in the aortic strip, in the vas deferens and to postsynaptic β-adrenoceptors mediating inotropism in the rat atrium are desensitized is situated inside the cells bearing the receptors. Further, the desensitization is produced by the noradrenaline itself and not by a metabolite.

The mechanism by which chronotropic responses and those to stimulation of presynaptic α-adrenoceptors in the vas deferens are desensitized is clearly different from the others. Bearing in mind the relatively high concentrations of noradrenaline needed to affect these responses and the fact that a high concentration of methoxamine, which is not subject to either uptake mechanism or to metabolism by COMT, could desensitize the aortic strip, it seems likely that in these cases the phenomenon is mediated by receptor occupation along the lines suggested by Katz & Thesleff (1957). Such a mechanism would require that the phenomenon was specific and it proved to be so in the vas, where inhibition produced by morphine was unaffected when that produced by noradrenaline was desensitized. Waud's (1968) criterion for distinguishing specific from non-specific desensitization is that where two drugs produce the same response by two different receptors, desensitization of the response to one drug is not accompanied by desensitization of the response to the other. To this extent, the effects of noradrenaline and morphine in reducing the twitch response of the vas to field stimulation satisfy this criterion, since their effects are mediated by different receptors (Hughes *et al.*, 1975; Jenkins, Marshall & Nasmyth, 1975). By contrast, desensitization of the response to stimulation of the postsynaptic α-adrenoceptor in the vas has been shown to be non-specific, as the responses to both noradrenaline and to acetylcholine were both desensitized by prolonged contact of the tissue with noradrenaline. To demonstrate specificity or otherwise in the aortic strip or in the rat atrium is harder, since suitable agonists are not available.

It is concluded that some but not all postsynaptic adrenoceptors can be desensitized by accumulation of noradrenaline in the cells on which they are situated. This kind of desensitization is non-specific. Presynaptic α-adrenoceptors in the mouse vas deferens and postsynaptic β-adrenoceptors mediating chronotropism in the rat atrium are probably desensitized by an effect of noradrenaline on the receptors themselves, since the phenomenon is specific and does not depend upon the accumulation of noradrenaline in the tissue.

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