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Action of various sympathomimetic amines on the isolated stripped vas deferens of the guinea-pig

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1. The contractile potencies of some sympathomimetic amines and cholinergic drugs were studied in the isolated, stripped vas deferens of the guinea-pig.

2. α-Methylnoradrenaline, adrenaline and metaraminol produced the same relative maximal responses as noradrenaline, which served as reference drug; on the other hand, octopamine, dopamine and α-methyldopamine were much less active. Short term pretreatment of the animals with reserpine did not affect the maximal relative responses.

3. Tyramine, α-methyl-*m*-tyramine and hordenine methiodide had little effect on the untreated preparation. In the presence of just threshold concentrations of noradrenaline, the relative maximal responses to tyramine and hordenine methiodide were markedly increased.

4. The relative maximal response to carbachol was the same as that to noradrenaline, while that to DMPP was smaller. In contrast to carbachol, the action of DMPP was potentiated by pretreatment of the preparation with noradrenaline.

The humoral transmitter in sympathetic postganglionic nerve fibres, noradrenaline, can be replaced by various sympathomimetic amines, which act as false neurochemical transmitters when they are released (for review, see Holtz & Palm, 1966; Kopin, 1966). The ability of these amines to substitute for noradrenaline depends *inter alia* on their potency relative to noradrenaline. In this paper the contractile response of the muscle of the guinea-pig isolated vas deferens (Waddell, 1916) to several sympathomimetic amines, some of which can serve as false transmitters, was measured.

A preliminary account of part of this work was presented at the 6th Spring Meeting of the German Pharmacological Society in Mainz (Nedergaard & Westermann, 1965).

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Methods

Preparation

The isolated stripped vas deferens preparation (Waddell, 1916) from guinea-pigs weighing 300–500 g was used. The animals were decapitated, the abdominal cavity opened and both vasa deferentia removed and placed in an organ bath filled with oxygenated physiological salt solution maintained at 20° C. The mesenteric investment was carefully stripped off and a total length of vas deferens varying between 20 and 40 mm was obtained. The preparation was stretched slightly and then allowed to contract so as to assume a " resting " length. Two ligatures were placed 10 mm apart on the middle portion of the vas deferens. In some preliminary experiments the vas deferens with its mesenteric investment (" non-stripped ") was used.

Organ bath

The preparations were mounted vertically in a jacketed organ bath of 20 ml. capacity maintained at 30° C. The bath was equipped with a side arm through which the salt solution was circulated and preheated so as to keep the bath temperature constant when the preparation was washed.

Physiological salt solution

The ionic concentrations were (mM): Na^+ 143.9, K^+ 5.9, Ca^{2+} 1.9, Mg^{2+} 0.61, Cl^- 129.2, HCO_3^- 24.9, $H_2PO_4^-$ 0.59 and glucose 11.1. The solution was gassed with a mixture of 95% oxygen and 5% carbon dioxide for at least 30 min before the experiment began. Both the bath and supply reservoir were gassed throughout the experiment. The salt solution also contained 0.03 mmoles of disodium ethylendiamine tetra-acetic acid (Titriplex III, Merck) in order to decrease spontaneous oxidation of sympathomimetic amines during incubation experiments (Furchgott, 1960; Nedergaard, Vagne & Bevan, 1968). This concentration of the chelating agent did not influence the concentration-response curve of noradrenaline.

Recording

A ligature from one end of the preparation was attached to a glass holder, and a ligature from the other end to an isotonic writing lever recording on smoked kymograph paper. The lever gave approximately 10-fold magnification and was counter-weighted to exert 4 g resting tension. As the preparation usually stretched slightly during an experiment, the base line had to be readjusted. The maximal contraction height elicited by high concentrations of noradrenaline in 124 preparations averaged 93 ± 2.2 mm (S.E. of the mean).

Reserpine treatment

Reserpine 500 μg/kg was injected intramuscularly 40, 24 and 16 hr before the commencement of an experiment.

Drugs

The following drugs were used: (−)-cocaine hydrochloride, tyramine hydrochloride, hexamethonium dibromide, carbachol (Doryl) (E. Merck A.G., Darmstadt);

dopamine (Hoffman La Roche A.G., Grenzach); (±)-metaraminol (Aramine), (−)-noradrenaline, (−)-α-methylnoradrenaline hydrochloride (Corbasil), (−)-adrenaline bitartrate (Suprarenin) (Farbwerke Hoechst A.G., Frankfurt-Höchst); (±)-α-methyldopamine (Farbenfabriken Bayer A.G., Leverkusen); (±)-α-methyl-*m*-tyramine (Merck, Sharp & Dohme, Rahway, N.J.); reserpine (Sedaraupin) (Boehringer und Soehne, Mannheim-Waldhof); (−)-isoprenaline hydrochloride, 1-(3-methylphenoxy)-3-isopropyl-amino-propanol-2-hydrochloride (Kö 592) (C. H. Boehringer u. Sohn, Ingelheim); (−)-octopamine hydrochloride (Sterling Winthrop, Rensselaer); 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (Fluka, Buchs); hordenine methiodide (May & Baker, Dagenham).

Sympathomimetic amine bases were dissolved in equivalent amounts of HCl and diluted with 0.01 N-HCl. Stock solutions were prepared and refrigerated when not in use. They were diluted appropriately on the day of experiment with 0.01 N-HCl. The concentrations of the working solutions were so chosen that the volume added to the bath did not exceed 0.6 ml. and usually was about 0.2 ml.

The effect of varying the time interval between the additions of noradrenaline was examined for various concentrations of this amine. An interval of 10 min was found to be sufficient if the preparation was washed at least twice between each addition of noradrenaline. The contact time was 60–90 sec.

Results

Sensitivity changes

The vas deferens showed a marked increase in sensitivity to noradrenaline with time, the course of which was affected by the tearing of the mesenteric investment. When the non-stripped muscle was mounted in the bath, the response to a standard dose of noradrenaline (3×10^{-5}M) increased slowly during the initial 2–2.5 hr and then remained almost constant for several hours. The increase occurred more rapidly, usually within 1 hr, when the muscle was stripped of its mesenteric investment. After a suitable incubation period the sensitivity of either the stripped or non-stripped preparation to a wide range of noradrenaline concentrations apparently did not differ markedly, as was previously stated by others (Bentley & Sabine, 1963; Della Bella, Benelli & Gandini, 1964). Similar results were obtained also with preparations from guinea-pigs pretreated with reserpine. A constant response to a test dose of noradrenaline (3×10^{-5}M) was obtained more rapidly when the preparation was exposed a few times to higher concentrations of noradrenaline (0.3–3×10^{-3}M).

Contraction and "fade"

Figure 1 shows the characteristic response of the vas deferens to two concentrations of noradrenaline. After addition of the drug to the bath the vas deferens began to shorten after a latent period of 2–5 sec. Depending on the noradrenaline concentration, the contraction usually reached a maximum between 10 and 60 sec after addition of the drug. The muscle then began to relax spontaneously in the continued presence of the drug in the bath; this phenomenon is referred to as "fade" (Paton, 1961). With a noradrenaline concentration of 3×10^{-5}M, the tension returned almost to the initial resting level. With higher concentrations the fade

was usually followed by a slow secondary rise in tension, which began to level off 30–60 min later. The fade was interrupted by rhythmic contractions which often showed a very regular pattern. The rhythmic activity persisted for several hours if noradrenaline was present in the bath.

" Wash-out effect "

Removal of noradrenaline from the bath caused a contraction of the muscle, whereas the change of the salt solution itself had no effect. A similar " wash-out " effect was observed by Ohlin & Strömblad (1963) after removal of acetylcholine from the bath. When we tested a series of increasing concentrations of noradrenaline,

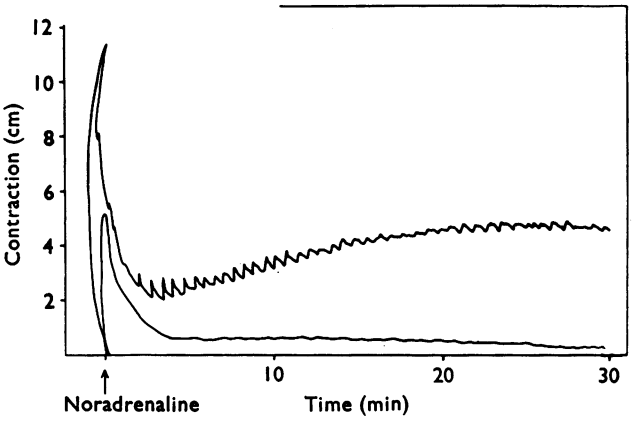

FIG. 1. Effect of the continued presence of 3×10^{-5}M (lower curve) and 3×10^{-4}M (upper curve) of noradrenaline on the isolated stripped vas deferens of the guinea-pig. The two curves have been superimposed for the sake of comparison.

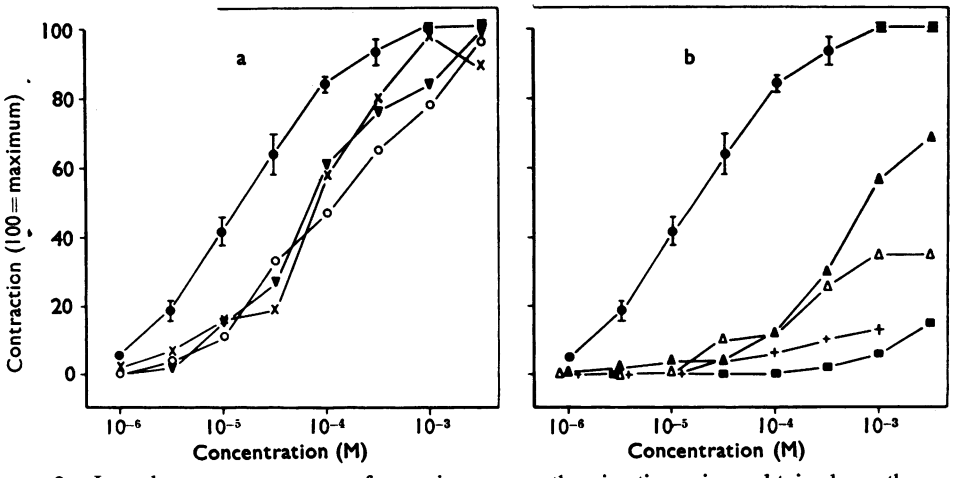

FIG. 2. Log dose-response curves for various sympathomimetic amines obtained on the vas deferens. Each curve is the mean from three to seven different preparations. The preparations were washed at least twice during the 10 min interval between each addition of a drug. ●, Noradrenaline (seven); ×, metaraminol (five); ○, adrenaline (four); ▼, α-methylnoradrenaline (five); ▲, octopamine (five); △, dopamine (four); +, α-methyldopamine (three); ■, isoprenaline (five). The numbers in parentheses are the numbers of preparations used and the vertical bars the S.E. of the means.

the effect of washing out low concentrations was always a contraction. The threshold concentration for the " wash-out " response was usually one tenth of the threshold concentration required to obtain a contractile response on addition of the drug. As the contractile response to addition of higher concentrations of noradrenaline increased, the " wash-out " effect decreased.

Dose-response curves of various sympathomimetic amines

Increasing concentrations of noradrenaline, α-methylnoradrenaline, adrenaline and metaraminol produced dose-dependent increases in the contractions, which were maximal with concentrations of about 3×10^{-3}M (Fig. 2a). Octopamine and dopamine proved to be much less active, and α-methyldopamine and isoprenaline had only a small effect on the vas deferens, even in high concentrations (Fig 2b).

According to many investigators, tyramine acts indirectly by releasing noradrenaline from peripheral tissue stores. Although the guinea-pig vas deferens has a high content of noradrenaline, tyramine proved to be relatively inactive (Fig. 3). The response to tyramine, however, was potentiated markedly after exposure of the preparation to noradrenaline in concentrations higher than 10^{-5}M for 30–60 sec followed by several washings. In the presence of just threshold concentrations of noradrenaline, the tyramine response was increased markedly (Fig. 3), a finding which is reflected also in the five-fold increase in the maximal responses (Table 1).

The quaternary N-methyl analogue of tyramine, hordenine methiodide, was more potent than tyramine, but the potentiation seen in the presence of just threshold concentrations of noradrenaline was less marked than with tyramine (Fig. 3).

Maximal responses and ED50 values obtained with different amines

With noradrenaline as the reference drug, the relative maximal responses were obtained from dose-response curves; the negative logarithms of the ED50 values (pD_2) were also calculated (Table 1). Adrenaline, α-methylnoradrenaline and metaraminol produced relative maximal responses of 1, but for other amines lower relative maximal responses were obtained in the following decreased order: octopamine > dopamine > hordenine methiodide > isoprenaline > α-methyl-*m*-tyramine > α-methyldopamine > tyramine. In the presence of just threshold concentrations of noradrenaline, however, the relative maximal responses to tyramine and hordenine methiodide were markedly increased (see also Fig. 3). Cocaine, hexamethonium, and the β-receptor blocking agent, Kö 592, were devoid of any stimulating action on the vas deferens even in concentrations as high as 10^{-3}M (Table 1).

The dose-response curve of carbachol showed an inflection point at a concentration of 3×10^{-4}M (Fig. 4). The resultant shape of the curve may possibly reflect the activation by carbachol of two different sets of receptors in the vas deferens. Pretreatment of the preparation with noradrenaline (up to 3×10^{-3}M) did not influence the response to carbachol (10^{-5}M). Tachyphylaxis was not seen with carbachol (10^{-5}M or 3×10^{-4}M) for up to 2.5 hr. It appears, therefore, that the response to carbachol does not involve the release of either endogenous or exogenous noradrenaline.

In contrast, the response to DMPP showed tachyphylaxis with concentrations higher than 10^{-4}M (Fig. 4). Furthermore, the DMPP-induced contraction was markedly potentiated by pretreatment of the preparation with noradrenaline. Thus

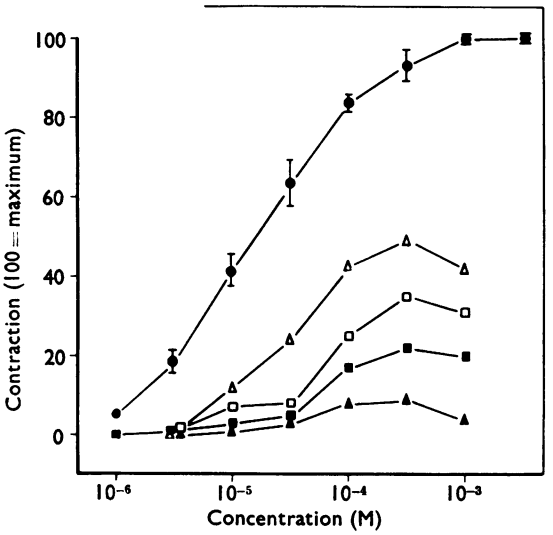

FIG. 3. Log dose-response curves obtained on the vas deferens for tyramine and hordenine methiodide alone and in the presence of noradrenaline. ▲, Tyramine alone (seven); ■, hordenine methiodide alone (four); ●, noradrenaline alone (seven). In the presence of noradrenaline (3×10^{-6}M) added to the bath 10 min before the experiment and maintained constant throughout: △, tyramine (two); □, hordenine methiodide (two). The numbers in parentheses are the numbers of different preparations used and the vertical bars the S.E. of the means.

TABLE 1. *Relative potencies of various drugs on the guinea-pig vas deferens*

Drug	Number of experiments	Relative maximal response (noradrenaline=1) Mean	Range	Mean pD_2 + S.E.	Min. effective conc. ($\times 10^{-6}$M)	Min. conc. causing max. contraction ($\times 10^{-3}$M)
Normal preparation:						
Noradrenaline	7	1·00	—	4·48±0·15	0·1	1
Adrenaline	4	1·00	0·92–1·00	3·96±0·22	3	3
α-Methylnoradrenaline	4	1·00	0·96–1·00	4·14±0·05	3	3
Metaraminol	5	1·00	1·00	4·09±0·09	1	1
Octopamine	5	0·69	0·52–0·77	3·39±0·06	1	3
Dopamine	4	0·35	0·32–0·45	3·67±0·05	10	3
α-Methyldopamine	3	0·09	0·08–0·10	3·92±0·06	30	1
α-Methyl-*m*-tyramine	3	0·12	0·10–0·14	4·33±0·04	3	0·1
Tyramine	7	0·09	0·03–0·13	4·16±0·12	10	0·1
Tyramine+noradrenaline	2	0·49	0·47–0·50	4·47	3	0·3
Hordenine methiodide	4	0·22	0·03–0·38	4·26±0·03	3	0·1
Hordenine methiodide +noradrenaline	2	0·34	0·30–0·38	4·77	3	0·3
Isoprenaline	5	0·15	0·05–0·26	3·20±0·23	300	3
Carbachol	6	1·00	—	4·56±0·21	0·3	3
DMPP	5	0·42	0·19–0·58	4·76±0·19	0·1	0·1
Cocaine	2	0	—	—	—	—
Kö 592	4	0	—	—	—	—
Hexamethonium	3	0	—	—	—	—
Preparation from reserpine-treated guinea-pigs:						
Noradrenaline	6	1·00	—	4·43±0·13	1	3
Adrenaline	3	1·00	1·00	4·45±0·08	1	3
α-Methylnoradrenaline	6	1·00	0·92–1·00	4·02±0·15	1	3
Metaraminol	3	1·00	1·00	4·12±0·10	3	1
Dopamine	2	0·31	0·29–0·33	3·85	1	0·3
DMPP	3	0·30	0·06–0·43	4·63±0·38	3	0·1
Tyramine	6	0	—	—	—	—

Noradrenaline was used as reference agonist. "Tyramine+noradrenaline" and "hordenine methiodide+noradrenaline": potencies of tyramine and hordenine methiodide in the presence of noradrenaline (3×10^{-6}M). $pD_2 = -\log ED50$.

in a typical experiment, after incubation for 5 min with noradrenaline (3×10^{-5}M), the response to the first addition of DMPP (10^{-4}M) was potentiated by 500%. Hence DMPP appears to require the presence of noradrenaline to elicit a response.

Effect of pretreatment of the guinea-pig with reserpine

The effect of short-term pretreatment with reserpine on the contractile response to DMPP varied. The minimum effective concentration was increased 30-fold and the relative maximal response decreased (Table 1). In several other experiments DMPP either had no effect or elicited only a small single response. It is possible that DMPP requires only a very minute amount of noradrenaline, which may still be present in the muscle despite pretreatment with reserpine, although no chemically identifiable noradrenaline was present. Reserpine did not affect the maximal responses obtained with noradrenaline, adrenaline, α-methylnoradrenaline, metaraminol and dopamine (Table 1); the dose-response curves of these amines obtained on preparations from reserpine-treated and untreated guinea-pigs were similar.

Discussion

It is suggested that the maximal sensitivity to noradrenaline was reached more rapidly with the stripped than with the non-stripped preparation, because the former stretched to longer resting length more rapidly than the latter. The increase in sensitivity cannot be related to a possible traumatic release of noradrenaline during the dissection (Furchgott & Bhadrakom, 1953), for it occurred also in preparations from reserpine-treated guinea-pigs.

A direct quantitative comparison between the values for the relative maximal responses and threshold concentrations of various sympathomimetic amines given in the paper and those of other authors is not possible because of differences

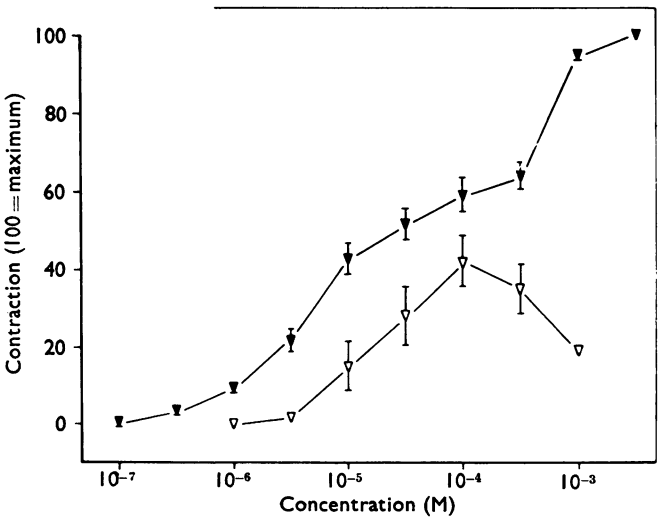

FIG. 4. Log dose-response curves for carbachol and DMPP obtained on the vas deferens. ▼, Carbachol (six); ▽, DMPP (five). The numbers in parentheses are the numbers of different preparations used. The bars represent the S.E. of the means.

in experimental conditions. These differences cannot, however, account for the small relative maximal responses obtained with α-methyldopamine and tyramine and the somewhat higher values found with dopamine and octopamine. Because these four compounds have relative maximal responses of 1 in the rat vas deferens (Van Rossum, 1965; Patil, Tye & Lapidus, 1965) this difference in potency presumably represents a true species difference which cannot be explained by the evidence available at present.

The almost complete absence of a response to tyramine is surprising, particularly because the guinea-pig vas deferens has a high content of noradrenaline (Sjöstrand, 1962, 1965) mainly localized in the adrenergic neurones (Falck, 1962; Falck, Owman & Sjöstrand, 1965; Sjöstrand, 1965). This finding is in disagreement with the view that tyramine acts indirectly by releasing noradrenaline from the presynaptic adrenergic nerve terminals (Fleckstein, 1953; Burn & Rand, 1958). A lack of correlation between the response to tyramine and the noradrenaline content has been shown for the heart (Muscholl, 1960; Muscholl & Lindmar, 1965), where the response to tyramine in hearts from rats treated with reserpine was restored by perfusion with noradrenaline without a chemically measurable refilling of the noradrenaline stores. Trendelenburg & Crout (1964), however, showed that replacement of less than 1% of the total noradrenaline content is sufficient to restore the action of tyramine in reserpine-treated animals.

The potentiation of the tyramine response after brief exposure to noradrenaline emphasizes the need to avoid the priming of the muscle with test doses of noradrenaline, even if the preparation is washed repeatedly between the additions of noradrenaline and tyramine. Kuschinsky, Lindmar, Lüllman & Muscholl (1960) emphasized the influence of the presence of small concentrations of extracellular noradrenaline. The marked potentiation of the response to tyramine seen in the presence of just threshold concentrations of exogenous noradrenaline is consistent with this view. This potentiation may be due to a release of exogenous noradrenaline bound to extraneuronal sites. The "wash-out" response could be explained similarly: removal of noradrenaline from the bath causes the unloading of this amine from silent receptors, thereby raising its concentration gradient at adrenoceptive receptors sufficiently to trigger a contractile response.

Burn & Rand (1959) suggested that the sensitivity of an organ to noradrenaline is inversely related to its noradrenaline content and that depletion causes supersensitivity. The failure of short term pretreatment with reserpine to induce supersensitivity of the vas deferens to noradrenaline, adrenaline, α-methylnoradrenaline and metaraminol may be due to a failure of reserpine to affect the neuronal uptake of noradrenaline and thus alter the concentration of this amine at the receptors, as suggested for other tissues (Trendelenburg, 1966).

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