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A NEW TYPE OF DRUG ENHANCEMENT: INCREASED MAXIMUM RESPONSE TO CUMULATIVE NORADRENALINE IN THE ISOLATED RAT VAS DEFERENS

BY

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Drugs such as the tricyclic antidepressants, some antihistamines and cocaine have been claimed to potentiate the actions of noradrenaline by interfering with its uptake by the adrenergic neurone (Trendelenburg, 1959; Isaac & Goth, 1965; Iversen, 1965a). Generally, this enhancement is shown by a parallel shift to the left of the noradrenaline dose-response curve. In the present study we observed that cocaine, in concentrations which shifted the noradrenaline dose-response curve to the left, increased the maximum contractile response of the isolated rat vas deferens to cumulative doses of noradrenaline. This finding led to an investigation of the effects of other inhibitors of catecholamine uptake on maximum responses to noradrenaline.

METHODS

Male Sprague-Dawley rats, weighing 200–250 g, were killed by a blow on the head. The right vas deferens was excised, placed in a 10 ml. organ bath containing Tyrode solution maintained at 37° C and aerated with a mixture of 5% carbon dioxide and 95% oxygen. Isometric contractions were recorded using a Grass FT10 transducer and an Offner Type R Dynograph. Cumulative dose-response curves with noradrenaline were obtained by starting with a concentration of 1×10^{-7}M and increasing the concentration by 0.5 log unit increments (after the force of contraction reached a constant level for each concentration) until addition of noradrenaline produced no further increase (less than 5%) in the force of contraction. The sequence of noradrenaline administrations on each experimental day was as follows: a single dose of 10^{-5}M was followed by two cumulative curves, and then one cumulative curve in the presence of test drug; finally, one cumulative noradrenaline curve was obtained post-drug. Ten minutes was allowed between each part of the sequence, with the exception that an incubation time of 20 min was allowed with each test drug. Noradrenaline at 10^{-5}M usually produced an increase in contractile force which was 90–100% of maximum.

The Tyrode solution had the following composition: NaCl 0.8%; KCl 0.02%; $MgCl_2$ 0.02%; $CaCl_2$ 0.02%; NaH_2PO_4 0.005%; $NaHCO_3$ 0.1%; and glucose 0.1%. The following drugs were used: l-noradrenaline bitartrate, desmethylimipramine, cocaine hydrochloride, dexchlorpheniramine maleate, imipramine hydrochloride, amitriptyline hydrochloride and phentolamine methanesulphonate.

RESULTS

The local anaesthetic, cocaine; the antidepressants amitriptyline, imipramine or desmethylimipramine; the antihistamine, dexchlorpheniramine; and the adrenergic blocking agent, phentolamine, produced dose-related increases in the maximum response to cumulative doses of noradrenaline (Table 1). Higher concentrations (not shown in

Table 1) of these drugs produced no greater increases in the noradrenaline maximum response. With amitriptyline, a higher concentration (10^{-6}M) produced less enhancement than did one-tenth that dose (10^{-7}M).

TABLE 1

INCREASE IN MAXIMUM RESPONSE TO CUMULATIVE DOSES OF NORADRENALINE (NA)

*Mean of two responses for each concentration of drug and mean of three responses for saline.

†Percent increase $= \frac{\text{NA max. with drug} - \text{Mean NA control max.}}{\text{Mean NA control max.}} \times 100.$

Drug	Molar concentration	Percent increase*,† in maximum response
Desmethylimipramine	10^{-8}	2·0
	10^{-7}	15·6
	10^{-6}	28·2
Imipramine	10^{-7}	13·4
	10^{-6}	17·4
Amitriptyline	10^{-8}	8·5
	10^{-7}	15·2
	10^{-6}	1·1
Cocaine	10^{-6}	0
	10^{-5}	31·6
Dexchlorpheniramine	10^{-6}	15·4
	10^{-5}	36·8
Phentolamine	10^{-8}	0
	10^{-7}	9·5
	10^{-6}	27·6
Saline	—	0

The increases in maximum response were independent of other effects of these agents on the noradrenaline dose-response curve (Fig. 1). At concentrations producing the greatest increases in maximum response, cocaine (10^{-5}M) potentiated noradrenaline and shifted the dose-response curve to the left by about 1 log unit. Amitryptyline (10^{-7}M) had little or no effect, whereas phentolamine (10^{-6}M) antagonized noradrenaline, shifting the dose-response curve to the right by approximately 1.5 log units.

Further evidence that this increased maximum response can be dissociated from other effects of these drugs on noradrenaline responses was obtained from wash-out experiments. In each of the three examples the increased maximum response to cumulative doses of noradrenaline returned to control after washout and a 10 min recovery time. In contrast, the antagonistic effect of phentolamine and the potentiating effect of cocaine were, for the most part, still evident (Fig. 2). Amitriptyline failed to shift the noradrenaline curve so that this effect did not change after washout, whereas the increase in maximum response returned to normal. Another tricyclic antidepressant, desmethylimipramine (10^{-7}M and 10^{-6}M) did potentiate noradrenaline, shifting its dose-response curve to the left and increasing the maximum response. As with cocaine, only the leftward shift of the dose-response curve was still evident after washout.

A possible explanation for the ability of these drugs to increase the maximal noradrenaline response is that they may prevent noradrenaline auto-inhibiton. The response to single and cumulative doses of noradrenaline were compared in a total of twenty-one experiments and the response to a cumulative concentration of 10^{-5}M noradrenaline was 26.1% less than the responses of the same tissue to an initial single administration of the same concentration (Fig. 3). If the drug-induced increases in maximal response are

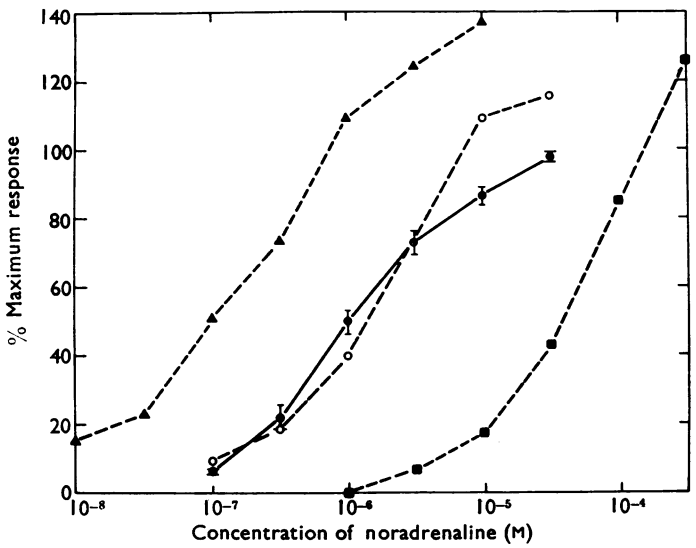

Fig. 1. Effects of cocaine 10^{-5}M (▲), phentolamine 10^{-6}M (■) and amitriptyline 10^{-7}M (○) on the contractile response of the rat vas deferens to noradrenaline, expressed as percentage of the maximum control response to noradrenaline (●). Each point represents the mean of three experiments except for the control curves for noradrenaline which represents the mean (±standard error) of the nine experiments in which these drugs were used.

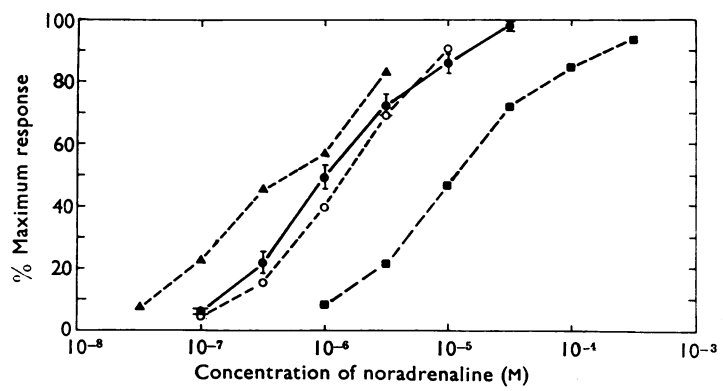

Fig. 2. Residual effects of cocaine 10^{-5}M (▲), phentolamine 10^{-6}M (■) and amitriptyline 10^{-7}M (○) (Fig. 1), after washout, on the noradrenaline dose-response curve, expressed as percentage of the maximum control response to noradrenaline (●). Each point represents the mean of three experiments, except for the control curve for noradrenaline which represents the mean (±standard error) of the same nine experiments in which these drugs were used.

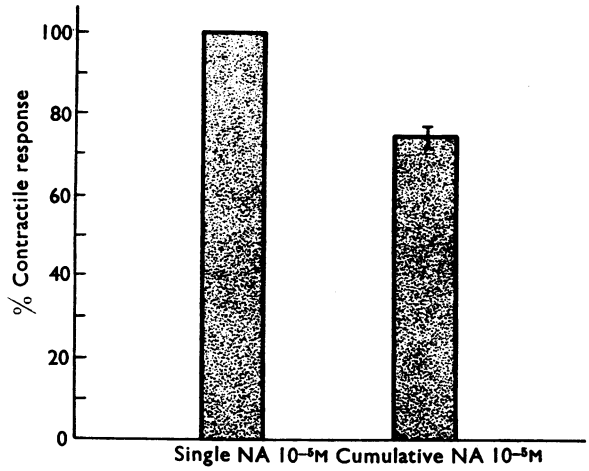

Fig. 3. The mean (±standard error) contractile response of the rat vas deferens to a cumulative concentration of noradrenaline (NA) 10^{-5}M, expressed as percentage of the contractile response to an initial single administration of noradrenaline 10^{-5}M (twenty-one experiments).

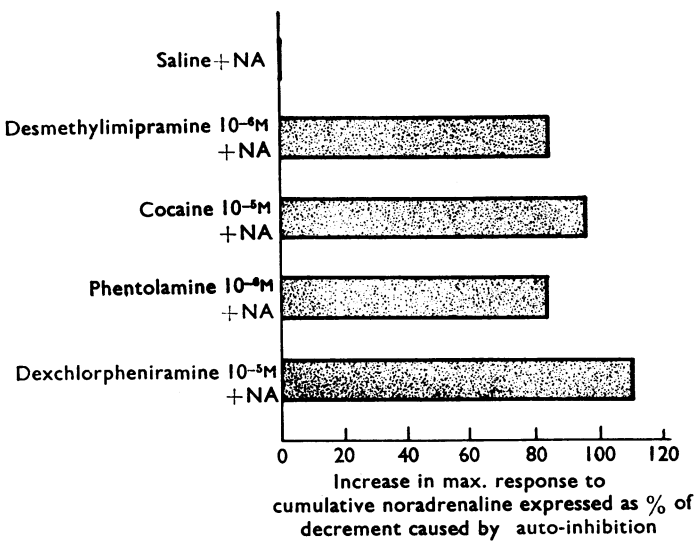

Fig. 4. Percent increase in maximum response to cumulative noradrenaline (NA) produced by optimal concentrations of representative drugs, expressed as a percentage of the decrement in response caused by auto-inhibition. Each response represents the mean of two experiments except for saline (three experiments).

calculated as a percentage of this difference between single and cumulative dose responses (Fig. 4), it can be seen that the increase in maximal response to cumulative doses of noradrenaline closely approximates (within 17%) the decrement caused by desensitization.

DISCUSSION

Drug potentiation is usually represented by a parallel shift to the left in the dose-response curve of the agonist (Trendelenburg, 1963). This study has demonstrated a new type of drug enhancement in which the maximum response to cumulative doses of noradrenaline has been increased regardless of the direction in which the noradrenaline dose-response curve was shifted.

The drugs which increased the maximum response to cumulative noradrenaline all inhibit noradrenaline uptake (Hertting, Axelrod & Whitby, 1961; Isaac & Goth, 1965; Iversen, 1965a; Iversen, 1965b). Their action of increasing the maximum noradrenaline response, however, does not correlate well with their relative ability to inhibit the uptake of noradrenaline. Phentolamine is a weak inhibitor of noradrenaline uptake in isolated rat heart (Iversen, 1965b) and in the intact cat (Hertting *et al.*, 1961) when compared with cocaine or imipramine, whereas phentolamine is at least as potent as any of the compounds tested in increasing the maximum noradrenaline response.

The noradrenaline potentiation produced by drugs such as cocaine or desmethylimipramine is thought to be caused by inhibition of noradrenaline uptake (Trendelenburg, 1959; Iversen, 1965a). The present studies on the rat vas deferens have shown that potentiation and increased maximum response to noradrenaline can be dissociated after washout. These results serve as additional evidence that inhibition of uptake cannot account for the ability of these drugs to increase maximal response to noradrenaline. Furthermore, it is difficult to visualize how inhibition of uptake could account for an increase in maximum response, assuming that the magnitude of the noradrenaline response is proportional to the number of receptors occupied by drug. The maximal cumulative response is determined by raising the concentration of noradrenaline until no further increase in contractile force results. At this point uptake inhibition would only increase the amount of noradrenaline present in an already saturated receptor area.

One possible explanation for our results is that drugs such as cocaine, imipramine, dexchlorpheniramine or phentolamine prevent the desensitization process of auto-inhibition. Auto-inhibition has been demonstrated in anaesthetized dogs with noradrenaline (Rosenthale & DiPalma, 1962) and in rabbit aortic strips with adrenaline (Furchgott & Bhadrakom, 1953; Pardo, Hong & LeLorier, 1967).

In the present experiments the magnitude of the largest increase in maximum response to cumulative noradrenaline produced by drugs such as cocaine was approximately equal to the decrement in response caused by auto-inhibition and this was found with all the drugs tested. If these drugs prevent auto-inhibition, their ability to increase maximum responses to noradrenaline should be independent of where these occur on the noradrenaline potency scale, as opposed to conventional potentiation which can be masked by concurrent adrenergic blockade. As indicated previously (Fig. 1), the increase in maximum response to noradrenaline is independent of the direction in which the noradrenaline curve is shifted.

SUMMARY

1. Cocaine, imipramine, amitriptyline, desmethylimipramine, dexchlorpheniramine and phentolamine increased the maximum response of the isolated rat vas deferens to cumulative doses of noradrenaline.

2. These increases in maximum response were independent of other effects on the noradrenaline dose-response curve for both cocaine, at concentrations which shifted the noradrenaline dose-response curves to the left, and phentolamine, at concentrations which shifted the noradrenaline curve to the right, increased the maximum response to cumulative noradrenaline.

3. The increases in maximum response to cumulative doses of noradrenaline were reversed by washing out, whereas dose-response curve shifts produced by drugs such as cocaine and phentolamine were still evident after washout.

4. Auto-inhibition with noradrenaline was found in these same experiments by comparing the decreased response to a cumulative concentration of 10^{-5}M with that obtained by a single administration of noradrenaline 10^{-5}M.

5. The magnitude of the peak increase in maximum response to cumulative doses of noradrenaline produced by each of the drugs in this study closely approximated the decrement in response caused by auto-inhibition.

6. A possible explanation for the ability of drugs such as cocaine to increase the maximum response to cumulative noradrenaline is that they prevent the desensitization process of auto-inhibition.

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