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Mechanism of cocaine potentiation of responses to amines

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1. Effects of cocaine on the magnitude of responses to several biologically active amines and on their rates of inactivation were studied in strips of rabbit thoracic aorta *in vitro*.

2. Although cocaine both potentiated responses to noradrenaline, adrenaline and phenylephrine and slowed their inactivation, the correlation between these two parameters under various experimental conditions was poor, and in all cases the delay in intrinsic inactivation was inadequate to account for the observed potentiation.

3. Potentiation of responses to noradrenaline by cocaine was little decreased in strips stored at 6° C for up to 10 days, although the response to low doses of tyramine was abolished much earlier. Similarly, cocaine clearly potentiated responses to noradrenaline for at least 28 hr at 37° C, at which time responses to noradrenaline alone were markedly decreased.

4. Cocaine potentiated responses to phenylephrine as well after 60 as after 10 min exposure to the amine in strips in which all intra-neuronal disposition of this amine had been eliminated by treatment with reserpine and iproniazid.

5. Cocaine effectively potentiated responses to histamine, but had only a slight and variable effect on those to 5-hydroxytryptamine (5-HT). It did not alter the tissue inactivation of histamine, but did significantly slow the inactivation of 5-HT.

6. Procaine slowed amine inactivation in the same way and to the same extent as did cocaine, but did not potentiate responses or affect the potentiation produced by cocaine added in its presence.

7. Cocaine potentiated responses to methoxamine to approximately the same degree as it did those to noradrenaline, although studies by the oil immersion technique clearly demonstrated that the aortic strips were entirely incapable of inactivating methoxamine.

8. The observations reported and discussed are incompatible with the hypothesis that cocaine potentiates responses to sympathomimetic amines because it prevents their inactivation by nerve uptake and storage and thus diverts larger amounts of agonist to tissue receptors. It is concluded that potentiation and inhibition of amine inactivation reflect two largely independent actions of

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cocaine in this vascular smooth muscle preparation, and probably in other organs, and that potentiation is a generally unreliable criterion of the blockade of processes inactivating sympathomimetic amines or of the importance of these processes in terminating the action of the amines.

Uptake and storage by adrenergic nerves is currently believed to play a dominant part in the regulation of responses to catecholamines. For example, the " unitary " hypothesis of cocaine action (MacMillan, 1959 ; Muscholl, 1961 ; Furchgott, Kirpekar, Rieker & Schwab, 1963 ; Kopin, 1964 ; Trendelenburg, 1965) postulates that the key action of cocaine is to block a specialized nerve membrane transport system for sympathomimetic amines (Whitby, Hertting & Axelrod, 1960 ; Dengler, Spiegel & Titus, 1961 ; Muscholl, 1961 ; Iversen, 1963 ; Malmfors, 1965 ; Van Zwieten, Wildhalm & Hertting, 1965), and that the block of uptake diverts amine to the vicinity of appropriate tissue receptors, thus potentiating the response.

Evidence supporting the unitary hypothesis of cocaine action and the key role of uptake and storage in terminating the action of noradrenaline and certain other sympathomimetic amines is largely indirect. It is obtained predominantly from (1) observations on the degree of potentiation of responses after inhibition of various pathways which might inactivate the sympathomimetic under study (Griesemer, Barsky, Dragstedt, Wells & Zeller, 1953 ; Furchgott, 1955 ; Crout, 1961 ; Furchgott *et al.*, 1963 ; Haefely, Hürlimann & Thoenen, 1964), and (2) biochemical assessment of tissue and effluent concentrations of amines and their metabolites (Hertting & Axelrod, 1961 ; Whitby, Axelrod & Weil-Malherbe, 1961 ; Iversen, 1963 ; Rosell, Kopin & Axelrod, 1963 ; Thoenen, Hürlimann & Haefely, 1964 ; Iversen, Glowinski & Axelrod, 1965). However, interpretation of the reported observations is difficult. The ultimate biochemical fate of amine present in a tissue or effluent may not adequately reflect the primary events terminating the action of that portion of the amine specifically involved in the response, and the validity of the assumption that potentiation is a quantitative reflection of interference with amine inactivation appears to be questionable (Maxwell, 1965a, b ; Maxwell, Daniel, Sheppard & Zimmerman, 1962 ; Kalsner & Nickerson, 1968b, 1969a, b).

The recently described technique of oil immersion permits potentiation and rate of amine inactivation to be assessed separately in the same test preparation (Kalsner & Nickerson, 1968a). Amine inactivation is determined from the rate of relaxation of isolated strips of vascular smooth muscle in mineral oil after contractions produced in an aqueous medium. Thus it provides a direct measure of intrinsic termination of action and makes it possible to account quantitatively for all of the agonist involved in a given response. This technique was combined with other procedures in the present experiments in an attempt to assess the relationship, if any, between enhancement of responses to certain biologically active amines and inhibition of their inactivation by cocaine.

Methods

Helically cut strips of rabbit thoracic aorta about 2.5×23 mm were prepared for isotonic recording as previously described (Kalsner & Nickerson, 1968a). All experiments were done at 37° C and the strips were kept under a tension of 2 g.

The muscle baths were of approximately 10 ml. working volume and contained a modified Krebs-Henseleit solution with disodium EDTA added to give a final concentration of 0.01 g/l. Flasks containing mineral oil (liquid petrolatum, U.S.P., 180–190 centistokes) were kept at 37° C in a water bath and constantly bubbled with 95% oxygen and 5% carbon dioxide. A flow of the gas mixture through the muscle baths was maintained both when they were filled with the Krebs solution and during oil immersion. The tissues were immersed in oil, after a given response had reached a stable plateau value, by draining the aqueous medium from the bath and rapidly refilling with the warm mineral oil, without any intervening washing of the tissue.

All drug concentrations are expressed as w/v (g/ml.), (−)-noradrenaline and (−)-adrenaline bitartrates, (−)-phenylephrine hydrochloride and 5-hydroxytryptamine creatine sulphate in terms of the free base, methoxamine, tyramine, procaine and cocaine hydrochlorides, histamine diphosphate, and iproniazid phosphate in terms of the salts. Reserpine powder was dissolved in a 10% ascorbic acid solution, and rabbits were injected intramuscularly either with two doses of 0.5 mg/kg, 48 and 24 hr before death or with one dose of 5.0 mg/kg, 18 to 24 hr before death. No differences between the responses of aortic strips from animals on the two reserpine dosage schedules were detected in these studies, and the results obtained with all strips from animals treated with reserpine were combined. Fresh stock solutions of all drugs were made every few days and were stored at 8° C. All solutions of catecholamines contained 0.01 N HCl. Monoamine oxidase (MAO) was inhibited with iproniazid. Evidence for the completeness and specificity of procedures used to inhibit specific mechanisms of amine inactivation have been previously presented (Kalsner & Nickerson, 1968b, 1969a). Other details of the procedures used have also been previously described (Kalsner & Nickerson, 1968a).

Results

Correlation between potentiation of responses and impairment of amine inactivation by cocaine

Cocaine (1×10^{-5} g/ml.) potentiated responses of aortic strips to phenylephrine, adrenaline and noradrenaline so that the final amplitude was equivalent to that which would have been produced by twice the concentration of agonist in the absence of cocaine, and a concentration of 1×10^{-4} g/ml. cocaine had a somewhat greater effect. Under steady state conditions, a reduction of at least 50% in the rate of amine inactivation would be required to double the effective concentration in the tissue. This is an absolute minimum which requires the unlikely assumption that a tendency for tissue amine concentration to increase would not increase the amount handled by alternate pathways, including diffusion out of the tissue (Kalsner & Nickerson, 1968b, 1969a). Estimates of the minimal percent inhibition of amine inactivation required to account for cocaine potentiation under various conditions were compared with values obtained by direct measurements of the effects of cocaine on rates of inactivation after oil immersion (Table 1). In all cases the measured reduction in rate of amine inactivation produced by cocaine was less than that required to explain the potentiation, even when diffusion out of the tissue, an alternate mechanism of inactivation operative in the aqueous medium, was eliminated by oil immersion.

Effect of cocaine on responses of stored aortic strips to noradrenaline

The effect of cocaine was studied on aortic strips which had been stored to permit degeneration of the severed sympathetic nerves. Four strips from the same aorta were contracted by noradrenaline (1×10^{-8} g/ml.) and exposed to cocaine (1×10^{-4} g/ml.). After the drugs had been washed out and the strips had regained their basal tone, they were placed in individual vials containing oxygenated Krebs solution, sealed with Parafilm and stored at 6° C. Four days later the strips were resuspended in Krebs solution at room temperature and the baths slowly raised to 37° C. After a 2–3 hr equilibration period they were again contracted by noradrenaline and exposed to cocaine. After recovery from the test, the strips were again stored at 6° C. This procedure was repeated after 7, 10 and 15 days of storage, except that one strip was tested with tyramine after 4 and one after 10 days. MAO was inhibited with iproniazid before the tyramine tests.

The results of this series of experiments are presented in Table 2. The effect of cocaine, expressed either as absolute magnitude or as a percentage of the contraction height before its addition, was not clearly decreased until after 10 days of storage. In fact, the cocaine increment was somewhat greater after 4 days of storage than it was in the initial test. Tyramine did not affect the stored strips except for a very small contraction produced by the high concentration of 1×10^{-5} g/ml. in the strip stored for 4 days. The strip tested after 10 days of storage was also immersed in oil to magnify the effect of any small amount of catecholamine released by the tyramine (Kalsner & Nickerson, 1968a), but no response was detected.

Cocaine potentiation was also studied on four strips kept continuously in the muscle baths at 37° C. Control responses to noradrenaline (1×10^{-8} g/ml.) followed by cocaine were obtained about 4 hr after the death of the animal. The baths were then washed out, and the strips retested about 22.5 and again 28 hr after the death of the animal. Cocaine still potentiated the responses 28 hr after preparation of the strips (axotomy), although the diminished responses to noradrenaline indicated that considerable muscle cell damage had occurred by this time (Table 2).

TABLE 1. *Effects of cocaine on responses to and inactivation of sympathomimetic amines*

Agonist	Pretreatment	Cocaine conc. (g/ml.)	Cocaine potentiation		Multiple of control time to relax 50% in oil	Inhibition of inactivation (%)	
			%	Multiple of agonist conc.		Measured	Min. calc. from potentiation
Noradrenaline (1×10^{-8} g/ml.)	None	1×10^{-5}	18·3	2·2	1·49	33	55
		1×10^{-4}	25·3	3·0	1·55	35	67
	Reserpine	1×10^{-5}	19·1	2·3	1·36	26	57
		1×10^{-4}	27·8	3·6	1·84	46	72
Phenylephrine (3×10^{-8} g/ml.)	None	1×10^{-5}	11·7	2·0	1·76	43	50
	Reserpine	1×10^{-5}	13·3	2·1	1·94	48	52
Adrenaline (1×10^{-8} g/ml.)	None	1×10^{-5}	21·1	2·1	1·57	36	52

Effect of cocaine on responses to phenylephrine

Reserpine is believed to prevent the storage of amines in intraneuronal granules and thus to promote their intraneuronal deamination (Kopin & Gordon, 1962, 1963; Stjärne, 1964; Iversen *et al.*, 1965). Inhibition of MAO in strips from animals pretreated with reserpine should eliminate all intraneuronal mechanisms for the inactivation of phenylephrine.

Aortic strips from rabbits pretreated with reserpine were treated with iproniazid, and cocaine (1×10^{-5} g/ml.) was added to the baths as soon as responses to phenylephrine (1×10^{-8} g/ml.) had reached a plateau (10 min). After the augmented response was recorded, the muscle baths were washed out and the strips allowed to return to basal tone. In the second test, cocaine was added after exposure to the phenylephrine for 60 min. Records from a typical experiment of this series are shown in Fig. 1. Potentiation by cocaine was not decreased by the very long exposure to phenylephrine.

Effects of cocaine on responses to 5-hydroxytryptamine and histamine

Cocaine was found in preliminary tests to potentiate responses of many aortic strips from both reserpine-pretreated and untreated animals to histamine and occasionally to augment responses to 5-hydroxytryptamine (5-HT, serotonin). To assess these effects and their possible association with altered disposition of the amines more accurately, ten strips from reserpine-pretreated animals were contracted by histamine (3×10^{-6} g/ml.) and 10 by 5-HT (1×10^{-6} g/ml.), and their relaxation in oil recorded. After thorough washing, they were re-contracted by the same agonists, cocaine (1×10^{-5} g/ml.) was added and their relaxation in oil again recorded. The responses to histamine were augmented a mean of 28.2% ($P<0.02$), whereas those to 5-HT were only slightly and variably affected. In contrast, cocaine did not alter the inactivation of histamine, but considerably reduced the rate of inactivation of 5-HT (Fig. 2); the time for 50% relaxation in oil was increased to 1.5 times the control value ($P<0.01$). In two additional experiments, cocaine (1×10^{-4} g/ml.) also did not affect the rate of relaxation in oil of strips contracted by histamine.

TABLE 2. *Effect of interval after axotomy on cocaine potentiation of responses to noradrenaline* (1×10^{-8} *g/ml.*)

Interval	Contraction amplitude (mm)	Increase after cocaine (mm)	Increment (%)
Strips stored at 6° C			
Days			
0	27·0	6·8	25
4	28·8	8·3	29
7	30·7	6·0	20
10	32·0	4·5	14
15	17·0	−2·5	−15
Strips in muscle baths at 37° C			
Hours			
4	17·5	5	29
22·5	11·0	2·4	21
28	5·3	2·0	53

Cocaine concentration was 1×10^{-4} g/ml. in all experiments except for the 28 hr test on strips kept at 37° C, when it was 1×10^{-5} g/ml. The higher concentration of cocaine depressed these damaged strips.

Effects of procaine on strips contracted by phenylephrine and noradrenaline

The cumulative addition of procaine (1×10^{-7}, 1×10^{-6} and 1×10^{-5} g/ml.) to the muscle baths had no observable effect on strips contracted by phenylephrine (3×10^{-8} g/ml.) or noradrenaline (1×10^{-8} g/ml.), except to cause a slight decrease in the contraction amplitude at the highest concentration. The presence of procaine did not alter the potentiation produced by a subsequent addition of cocaine (Fig. 3A). Procaine alone very significantly slowed the relaxation after oil immersion of strips contracted by either phenylephrine or noradrenaline. The times for 50% relaxation were increased to 1.68 and 1.64 times the control values, respectively, an effect very similar to that of cocaine. Like cocaine, it almost completely eliminated relaxation in oil of strips contracted by phenylephrine which had been treated with iproniazid (Fig. 3B). These results show that procaine slows amine inactivation in almost exactly the same manner as does cocaine, but that it does not potentiate responses of aortic strips to sympathomimetic amines or interfere with the potentiation produced by cocaine.

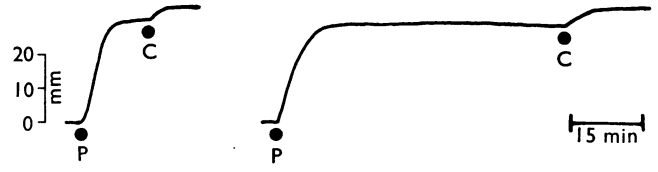

FIG. 1. Potentiation of responses by cocaine (C) after different periods of exposure to phenylephrine (P). Strips were contracted by phenylephrine (1×10^{-8} g/ml.); cocaine (1×10^{-5} g/ml.) was added after 10 min (left) or 60 min (right).

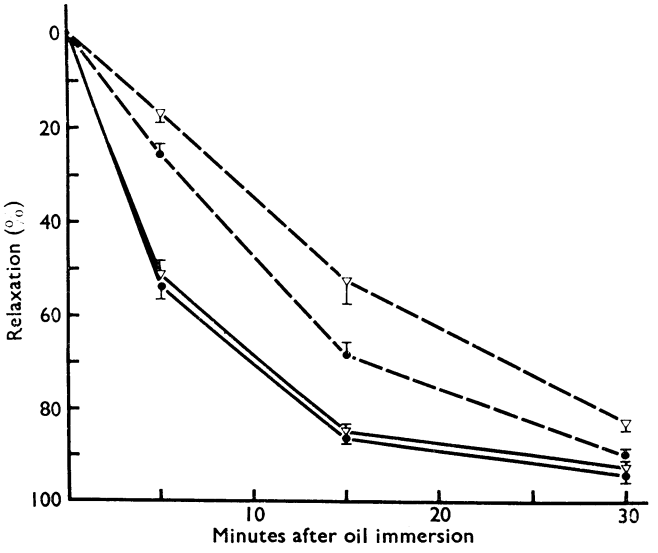

FIG. 2. Effect of cocaine (▽) on the relaxation after oil immersion of aortic strips contracted by (– – –) 5-HT (1×10^{-6} g/ml.) or (——) histamine (3×10^{-6} g/ml.). Curves depict the results of ten complete experiments with each agonist on strips from rabbits pretreated with reserpine. Vertical bars indicate standard errors of means.

Effects of cocaine on aortic strips contracted by methoxamine

All aortic strips used in this series of experiments were from rabbits pretreated with reserpine to minimize any possible complication from the release of endogenous noradrenaline. Strips contracted by methoxamine were followed for periods of up to 60 min after oil immersion and no appreciable progressive relaxation was observed in any experiment. This confirmed the inability of this preparation to inactivate methoxamine (Kalsner & Nickerson, 1968a). The strips relaxed somewhat immediately after oil immersion and then slowly regained their previous amplitude of contraction. A similar abrupt, transient relaxation at the time of oil immersion has been observed with other agonists when major mechanisms for their inactivation were blocked (Fig. 3B; Kalsner & Nickerson, 1968b), but no satisfactory explanation for this behaviour is available.

Cocaine added to the baths after the response to methoxamine had reached a plateau value caused a prompt increase in the amplitude of contraction, which in some instances had a considerably greater rate of rise that the initial response to the agonist. In all cases the increase was somewhat greater than that which would have been produced by doubling the concentration of methoxamine. Cocaine (1×10^{-5} g/ml.) augmented the responses of eight strips contracted by methoxamine (5×10^{-8} g/ml.) by $23.2 \pm 3.3\%$ and those of five from the same aortas contracted by noradrenaline (1×10^{-8} g/ml.) by $17.5 \pm 3.1\%$. A higher concentration of methoxamine (1×10^{-7} g/ml.) was potentiated less ($7.7 \pm 1.0\%$), but responses to this concentration are on the relatively flat part of the agonist concentration-response curve.

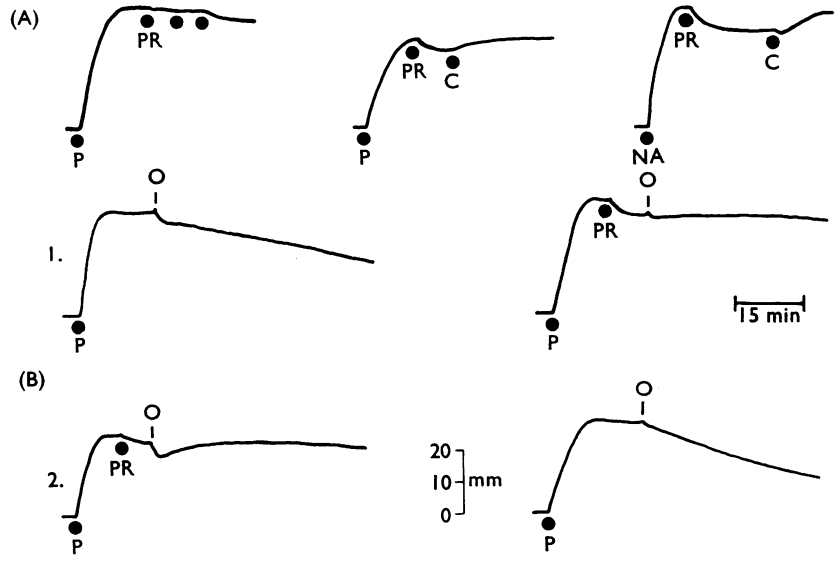

FIG. 3. Effects of procaine (PR) and cocaine (C) on aortic strips contracted by phenylephrine (P) or noradrenaline (NA). (A): Left, response of a strip contracted by phenylephrine (3×10^{-8} g/ml.) to the cumulative addition of procaine (1×10^{-7}, 1×10^{-6}, 1×10^{-5} g/ml.); centre, response of a strip contracted by phenylephrine to procaine (1×10^{-5} g/ml), followed by cocaine (1×10^{-5} g/ml.); right, response of a strip contracted by noradrenaline (1×10^{-8} g/ml.) to procaine (1×10^{-5} g/ml.), followed by cocaine (1×10^{-4} g/ml.). (B): Relaxation after oil immersion (O) of two iproniazid treated strips (1 and 2) contracted by phenylephrine with and without the addition of procaine (1×10^{-5} g/ml.).

After potentiation of methoxamine responses by cocaine, the sharp drop in amplitude associated with oil immersion was somewhat greater than in preparations not exposed to cocaine, 33.7 ± 3.4 and $23.2 \pm 1.4\%$ of the contraction height for strips contracted by methoxamine 5×10^{-8} and 1×10^{-7} g/ml., respectively, compared with 23.5 ± 1.7 and $19.4 \pm 2.2\%$ for the controls, and the tendency to return toward the level reached prior to oil immersion was somewhat less.

Discussion

In these experiments the technique of oil immersion was used to provide a measure, independent of potentiation, of the rates at which the actions of various amines were terminated in vascular tissue. A rough correlation between potentiation of responses to noradrenaline, adrenaline and phenylephrine by cocaine and impairment of their inactivation in aortic strips from both reserpine pretreated and untreated animals was observed. The inhibition of amine inactivation was, however, consistently less than the minimum required to explain the potentiation on this basis. A disparity in the opposite direction would be expected if potentiation were predominantly due to decreased amine inactivation because the inhibition of one pathway would increase the contribution of alternate mechanisms and because the oil immersion eliminated diffusion into the bathing medium as a mechanism of amine removal, which would magnify the contribution of intrinsic mechanisms of inactivation. The quantitative inadequacy of altered inactivation as a cause of potentiation was even more marked in other experiments in which the effects of cocaine were assessed after other pathways of inactivation had been blocked (Kalsner & Nickerson, 1968b, 1969a). In these conditions the percent inhibition of inactivation by cocaine was considerably decreased, but the degree of potentiation was little altered. A similar dissociation of inhibition of nerve uptake and potentiation of responses to noradrenaline by guanethidine, methylphenidate and cocaine was reported by Maxwell and co-workers (Maxwell, 1965a, b; Maxwell, Wastila & Eckhardt, 1966).

Cocaine effectively potentiated the responses of aortic strips that had been stored at 6° C for as long as 10 days, and of strips kept in the muscle chambers at 37° C for as long as 28 hr, at which time the response to noradrenaline alone was greatly reduced. No direct observations on the rate of degeneration of nerve terminals under the conditions of the present experiments are available, but it is probable that the nervous elements in the strips were nonfunctional long before the last demonstration of potentiation by cocaine in both series. Malmfors & Sachs (1965) found that a large portion of the adrenergic nerve terminals in the rat iris lost their noradrenaline between 12 and 16 hr after superior cervical ganglionectomy and that no accumulation of exogenous noradrenaline or other amines could be demonstrated after 24 hr. Far distal axotomy, as during removal of the aorta, should have caused even more rapid loss of nerve function (Emmelin & Malm, 1965; Dahlström, Fuxe & Hillarp, 1965).

The absence of a response to tyramine, which is believed to be predominantly due to noradrenaline released from adrenergic nerve terminals (Burn & Rand, 1958; Furchgott *et al.*, 1963), provided evidence of a functional loss of noradrenaline stores and/or the nerve membrane amine transport mechanism in our cold stored aortic strips. In fresh iproniazid pretreated aortic strips tyramine in a concentra-

tion of 1×10^{-6} g/ml. usually produces a nearly maximal response to this agent, and it is probable that the small response of the strip stored for 4 days to 1×10^{-5} g/ml. was due to the direct effect of tyramine on smooth muscle (Furchgott, 1955). The results of this series of experiments provide strong evidence that potentiation by cocaine is due to an action on effector cells rather than one on nervous elements.

The observation that cocaine potentiation of responses to phenylephrine was undiminished by treatment with reserpine and iproniazid provided additional evidence that the major action of cocaine in potentiating responses to sympathomimetic amines is unrelated to nerve uptake. The doses of reserpine used should have been more than sufficient to block any major storage of amine in intraneuronal granules (Stjärne, 1964 ; Malmfors, 1965), and the treatment with iproniazid should have prevented deamination. If the observed potentiation of responses of these strips to phenylephrine is to be attributed to block of nerve membrane transport, it must be assumed that net uptake was still undiminished by outward diffusion after a 60 min exposure to phenylephrine in conditions which prevented all intraneuronal inactivation of the amine, which seems to be very unlikely.

The effects of cocaine on the amplitude of responses to histamine and 5-HT and on the inactivation of these amines clearly dissociated the two. Responses to histamine were considerably potentiated, but inactivation of this amine was not slowed, even by a 10-fold higher concentration of cocaine than that required for potentiation. Conversely, responses to 5-HT were not significantly altered, although cocaine clearly slowed its inactivation. The observed effect of cocaine on 5-HT disposition may involve an action other than block of nerve membrane transport. Axelrod & Inscoe (1963) reported that 5-HT is not localized in the same subcellular fractions of heart as is noradrenaline, and tyramine is incapable of liberating this amine from tissues. In addition, fluorescence microscopic studies have shown adrenergic nerve endings to have negligible capacity to take up circulating 5-HT (Malmfors, 1965).

Comparison of the effects of cocaine and of procaine also demonstrated that potentiation can occur without delayed amine inactivation and vice versa. Procaine slowed the inactivation of phenylephrine, apparently by the same mechanism as did cocaine, because both eliminated the residual capacity of iproniazid treated aortic strips to inactivate this amine. However, procaine did not potentiate responses to phenylephrine in any experiment. Conversely, although cocaine had no detectable effect on inactivation except that shared with procaine, potentiation of responses to both phenylephrine and noradrenaline by this agent was undiminished in the presence of an effective concentration of procaine.

Inhibition of a significant pathway of amine inactivation by procaine might be expected to increase the effective tissue concentration of agonist. The absence of any detectable potentiation in the present experiments indicates that the procaine sensitive mechanism(s) of amine disposition was unimportant in the presence of alternate pathways, including diffusion out of the tissue. In other conditions the same inhibition of amine inactivation might result in some potentiation, and this effect of procaine could provide a basis for the conflicting reports that it does (Bacq & Lefebvre, 1934 ; Armin, Grant, Thompson, & Tickner, 1953) or does not (Tainter, 1930 ; Wirt & Tainter, 1932) potentiate in other smooth muscle preparations.

The most direct and unequivocal evidence that cocaine can potentiate responses by an action unrelated to any interference with inactivation of the agonist is from the experiments with methoxamine. This agent is not a substrate for either MAO or COMT, has no affinity for the amine transport mechanism of adrenergic nerves (Iversen, 1964), and the absence of any other pathway for its inactivation in our preparation was confirmed by the observation that there was no progressive decline in the amplitude of contractions produced by methoxamine during observation periods of up to 60 min after oil immersion. Despite the complete absence of tissue inactivation of the amine, cocaine potentiated responses to methoxamine as effectively as it did those to noradrenaline. It was also noted that in some strips the increment in contraction amplitude due to cocaine was much more rapid than the initial contraction produced by methoxamine. If the potentiation resulted from a build up of the tissue concentration of amine due to altered disposition, this increment would be expected to have a slope similar to that of the initial response to the agonist.

The results of the several series of experiments described in this report are incompatible with the " unitary " hypothesis of cocaine action which attributes the potentiation of responses to sympathomimetic amines to inhibition of their inactivation by transport into adrenergic neurones. The most acceptable interpretation of these data is that cocaine can act directly on effector cells to make them hyperresponsive. It is well known that the potentiation of responses of some effector organs by cocaine is considerably greater than that observed in the present experiments (Furchgott *et al.*, 1963 ; Haefely *et al.*, 1964 ; Trendelenburg, 1965), and it is conceivable that a greater effect on amine inactivation in other structures, such as the nictitating membrane and heart, could contribute to potentiation. Even if half the amine were extracted by the sympathetic plexus and there were no alternate mechanisms of inactivation, however, the maximal effect of inhibiting this process would be slight, equivalent to the effect of doubling the concentration of amine. In the presence of effective alternate pathways, including diffusion out of the tissue, it is difficult to conceive of circumstances in which block of nerve uptake would produce a 10 to 30 fold increase in amine concentration. Thus some other mechanism, probably a direct action on effector cells such as that demonstrated in the experiments described above, seems necessary to account for the magnitude of the potentiation produced by cocaine in other organs.

Cocaine does not potentiate responses of denervated organs to catecholamines, and this has been one of the important considerations supporting the hypothesis which attributes potentiation by this agent to block of transport of amines into nerve cells. However, an alternative interpretation of this observation is that the changes in effector cells following denervation are of the same type as those produced by cocaine and are maximal by the time the sensitization is fully developed. This alternative is attractive because it avoids the quantitative difficulty, mentioned above for cocaine, of explaining the magnitude of the effect on the basis of eliminating only one of several alternate mechanisms of amine inactivation.

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