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Effects of reserpine on the disposition of sympathomimetic amines in vascular tissue

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1. The effects of reserpine pretreatment on the intrinsic inactivation of low concentrations of phenylephrine and noradrenaline in strips of rabbit thoracic aorta were assessed by measuring the rates of relaxation, after oil immersion to prevent loss of active amine by diffusion into the surrounding medium.

2. Reserpine pretreatment considerably augmented the amplitude of responses to low concentrations of phenylephrine, noradrenaline and nordefrine (Cobefrine).

3. Reserpine pretreatment did not reduce the overall rate of inactivation of either phenylephrine or noradrenaline, but it did appear to decrease the contribution of uptake and storage, measured as an increased effect of enzyme inhibition and a decreased effect of cocaine on the rate of inactivation.

4. The role of catechol-O-methyl transferase (COMT), but not that of monoamine oxidase (MAO), in terminating the action of noradrenaline was increased in strips from animals pretreated with reserpine. Thus it appears that interference with intraneuronal storage diverts active amine to inactivation by COMT in vascular tissue, rather than by MAO as has been previously suggested.

5. As in preparations not treated with reserpine, inhibition of MAO alone had little effect on the rate of inactivation of noradrenaline, and this enzyme appears to function predominantly as an alternate pathway of little importance as long as COMT activity is unimpaired. Enzymatic processes accounted for about 85 and 70% of the inactivation of a low concentration of noradrenaline in reserpine pretreated and untreated preparations, respectively.

6. Cocaine potentiated responses to noradrenaline and phenylephrine as effectively in reserpine pretreated as in untreated preparations, and inhibition of the pathways of enzymatic inactivation did not appreciably decrease the potentiation produced by this agent.

7. The present results cannot be explained by the hypothesis that interference with amine inactivation by nerve uptake and storage is responsible for the potentiation of responses to noradrenaline or phenylephrine by either reserpine

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or cocaine, and emphasize the unrealiability of potentiation as an index of interference with mechanisms involved in terminating the action of sympathomimetic amines.

The " uptake " and storage of noradrenaline and other sympathomimetic amines in tissues is currently considered to have two components. The first is transport of amine across the nerve cell membrane, and the second, the incorporation of intraneuronal amine into storage granules or vesicles (Furchgott, Kirpekar, Rieker & Schwab, 1963 ; Kopin, 1964). Cocaine is believed to block transport at the nerve membrane and reserpine to inhibit incorporation into storage granules (Furchgott *et al.*, 1963 ; Kopin, 1964 ; Malmfors, 1965 ; Carlsson & Waldeck, 1965 ; Dahlström, Fuxe & Hillarp, 1965). The most prominent effect of reserpine is depletion of tissue stores of catecholamine (Carlsson, Rosengren, Bertler & Nilsson, 1957 ; Burn & Rand, 1958 ; Iversen, Glowinski & Axelrod, 1965), but it is still not clear whether this is due entirely to block of incorporation into storage units. Reserpine has also been reported to alter catecholamine metabolism in such a way that amine which would normally be stored in granules is deaminated intraneuronally (Kopin & Gordon, 1962, 1963 ; Stjärne, 1964 ; Iversen *et al.*, 1965).

It is difficult to assess the significance of the above observations in terms of the overall effects of reserpine on catecholamine disposition, or to relate changes in disposition to the potentiation by reserpine of responses to catecholamines and certain other sympathomimetics (Burn & Rand, 1958 ; Trendelenburg, 1963). Obstacles to a full evaluation of the data currently available include the difficulty of accounting quantitatively for the fate of all of the free catecholamine involved in a given response, uncertainties in relating ultimate biochemical fate to termination of action, the pharmacologically important parameter, and the questionable reliability of potentiation as a measure of altered amine inactivation.

The recently described technique of oil immersion (Kalsner & Nickerson, 1968a) permits the rate of relaxation of aortic strips in mineral oil after contractions produced in an aqueous medium to be directly equated with the rate of intrinsic inactivation of agonist involved in the response. It also enables alterations in response (potentiation) and in rates of inactivation to be studied in the same test preparation. In the experiments reported here, this technique was used in an attempt to determine: (1) if the supersensitivity of aortic strips to sympathomimetic amines produced by reserpine is due to a decreased rate of inactivation of the agonist with a consequent increase in its concentration at the tissue receptors, and (2) if the contributions of the cocaine sensitive mechanism(s) and of enzymatic processes to the termination of action (inactivation) of sympathomimetic amines are altered in tissues from animals pretreated with reserpine.

Methods

Helically cut strips of rabbit thoracic aorta about 2.5×23 mm were prepared for isotonic recording as described previously (Kalsner & Nickerson, 1968a). All experiments were done at 37° C and the strips were kept under a tension of 2 g. Responses were recorded on a kymograph with 6.8-fold amplification. 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 oxygen-carbon dioxide mixture through the muscle baths was maintained both when they were filled with the Krebs solution and when the tissues were immersed in oil. Oil immersion was accomplished, after a given response had reached a 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 bitartrate, nordefrine (Cobefrine) and (−)-phenylephrine hydrochlorides in terms of the free base, iproniazid phosphate and cocaine hydrochloride in terms of the salts. Reserpine powder was dissolved in 10% ascorbic acid, 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, 18-24 hr before death. No differences between the responses of aortic strips from animals on the two reserpine dosage schedules were detected, 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. Other details of the procedures used have been described previously (Kalsner & Nickerson, 1968a).

Four and eight treatment conditions were used to evaluate the inactivation of phenylephrine and noradrenaline, respectively. In the majority of experiments, strips cut from the same aorta were used for all groups to reduce variability. Monoamine oxidase (MAO) was inhibited with iproniazid and catechol-O-methyltransferase (COMT) with tropolone. Evidence for the completeness and specificity of the procedures used to inhibit specific mechanisms of amine inactivation has been presented previously (Kalsner & Nickerson, 1968b, c). To compare the rates of relaxation in oil, the time required for each strip to relax 50% was measured and the mean time calculated for each treatment. Under experimental conditions where 50% relaxation was usually not achieved within 30 min after oil immersion, comparisons were made at some lesser percentage relaxation. Mean values were compared by Student's two-tailed t test, and differences with P values of 0.05 or less were considered significant.

Results

Responses to sympathomimetic amines

Aortic strips from rabbits treated with reserpine responded to standard concentrations of a number of sympathomimetic amines with greater contractions than did strips from untreated animals. For example, six control strips and eight from reserpine-treated animals responded to nordefrine (Cobefrine) (1×10^{-8} g/ml.) with mean recorded contraction amplitudes of 2.2 and 10.1 mm, respectively. Responses to noradrenaline and phenylephrine were similarly potentiated (Table 1 and Fig. 2).

Relaxation of strips contracted by phenylephrine

Sixteen aortic strips from four rabbits treated with reserpine were studied by the same procedures used previously (Kalsner & Nickerson, 1968b), and the rates of

relaxation in oil after contractions produced by phenylephrine (3×10^{-8} g/ml.) compared with those of twenty-four strips from six untreated animals (Fig. 1). Reserpine treatment did not alter the rate of relaxation; the process was 36.8 and 38.8% complete for treated and untreated preparations, respectively, in 5 min. Reserpine also had no apparent effect on the ability of either cocaine or iproniazid to delay the relaxation of strips contracted by phenylephrine. Cocaine (1×10^{-5} g/ml.) increased the intervals required for half relaxation to 1.94 and 1.76 times those of the controls in strips from treated and untreated animals and, similarly, iproniazid increased the periods required for strips to relax 20% (50% relaxation was not achieved during the 30 min period of observation) to 10.9 and 9.3 times those of the controls.

TABLE 1. *Responses of aortic strips from reserpine-treated and untreated rabbits to several sympathomimetic amines*

Agonist (g/ml.)	Contraction amplitude (mm)		
	Untreated	Reserpine-treated	Significance of difference
Nordefrine			
1×10^{-8}	2·2±0·4 (6, 2)	10·1±1·1 (8, 3)	$P<0·001$
Phenylephrine			
3×10^{-9}	3·3±1·1 (16, 5)	18·1±1·4 (14, 5)	$P<0·001$
1×10^{-8}	12·5±0·7 (24, 7)	28·0±1·2 (4, 3)	$P<0·001$
3×10^{-8}	27·1±1·4 (16, 11)	35·6±2·9 (10, 6)	$P<0·01$
Noradrenaline			
1×10^{-8}	28·3±2·8 (10, 10)	38·9±3·5 (8, 8)	$P<0·05$

The number of strips and number of animals from which they were obtained are given in parentheses.

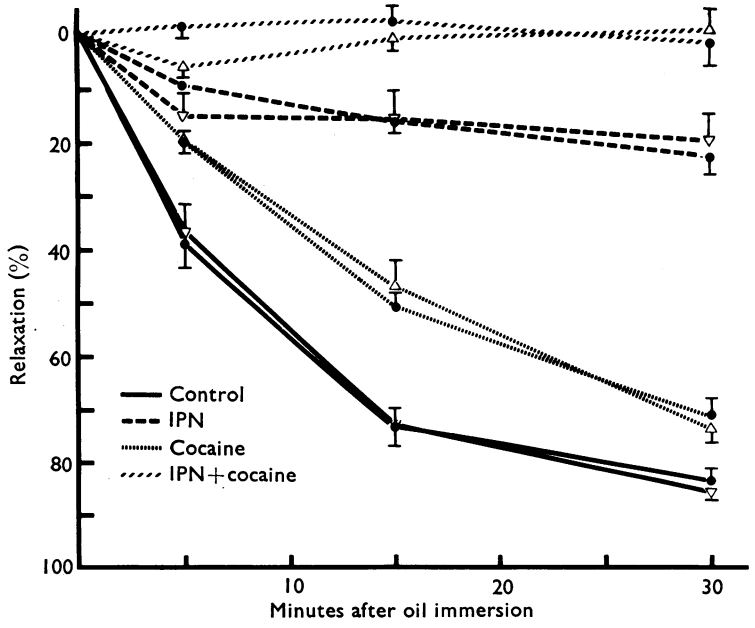

FIG. 1. Effects of reserpine, iproniazid (IPN) and cocaine on the relaxation of phenylephrine contracted aortic strips after oil immersion. All strips were contracted by phenylephrine (3×10^{-8} g/ml.); cocaine concentration was 1×10^{-5} g/ml. Results from four complete experiments with strips from reserpine-treated rabbits (△) and from six with strips from untreated animals (●). Vertical bars indicate standard errors of means.

A comparison of complete phenylephrine concentration-response curves determined on aortic strips from reserpine-treated and untreated animals showed the maximal response of the former to be greater (Fig. 2). Because of this difference and the general shift of the phenylephrine concentration-response curve by reserpine, any given percent relaxation corresponded to different residual amine concentrations in the reserpine treated and untreated preparations. For example, the effective concentrations of the sympathomimetic, determined from the concentration-response curves, at 10 and 30% relaxation of untreated strips are equivalent to those present at 8.3 and 26.2% relaxation of strips from reserpine-treated animals. This disparity increased with further relaxation ; 50 and 39.3% relaxation represented equivalent concentrations of phenylephrine. The untreated strips relaxed 50% in 8.2 ± 1.2 and those from reserpine-treated animals 39.3% in 5.4 ± 0.9 min. This difference in time was not statistically significant ($0.2 < P > 0.1$), but it suggests that phenylephrine may have been inactivated slightly more rapidly in the reserpine treated strips. Correction for differences in effective concentration resulting from potentiation by reserpine would not appreciably alter interpretation of the results. All data on which analysis of the contributions of individual intrinsic mechanisms of amine inactivation are based, however, are presented in terms of changes in the time required for a given percentage relaxation relative to controls from the same animals (Table 2 and Fig. 1). This is a " null " comparison which does not require assumptions regarding the shape or position of the concentration-response curves.

Effects of iproniazid and of cocaine on contraction amplitude in response to phenylephrine

Iproniazid pretreatment did not significantly alter the magnitude of responses of strips from reserpine-treated animals to phenylephrine (3×10^{-8} g/ml.). The peak contractions of ten control and eight iproniazid treated strips were 35.6 ± 2.9 and

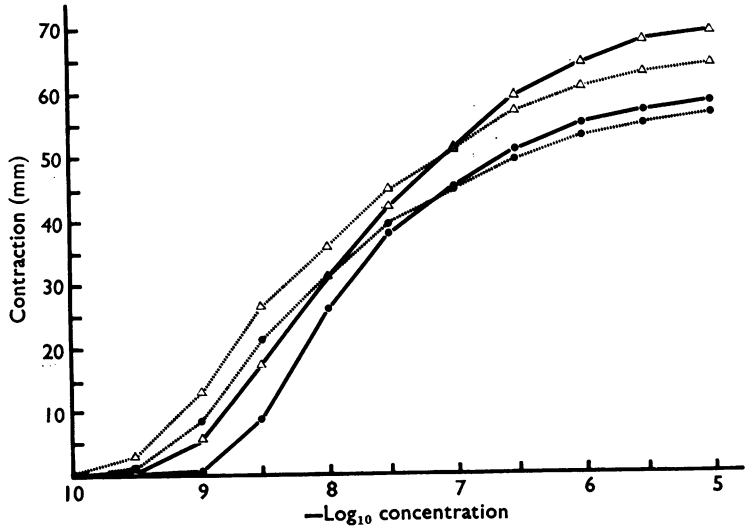

FIG. 2. Cumulative log concentration-response curves for contractions of rabbit aortic strips produced by noradrenaline (· · · ·) or phenylephrine (——). Each curve is the mean of results from three complete experiments on strips from reserpine-treated (△) or untreated (●) animals.

38.6 ± 4.0 mm, respectively. This finding is similar to that previously reported for strips from animals not pretreated with reserpine (Kalsner & Nickerson, 1968b).

Cocaine (1×10^{-5} g/ml.) added to the muscle baths after contractions produced by phenylephrine (3×10^{-8} g/ml) had reached a plateau value increased the response of six strips from reserpine-treated animals by a mean of 4.2 mm (13.3%). This potentiated response was equivalent to the contraction which would have been produced by a concentration of 6.4×10^{-8} g/ml. in the absence of cocaine. Similarly, five strips treated with iproniazid and then contracted by phenylephrine responded to cocaine with an increase of 3.8 mm (13.4%). These results are similar to those obtained with strips from animals not pretreated with reserpine (Kalsner & Nickerson, 1968b). Neither reserpine treatment *per se* nor the additional inhibition of MAO by iproniazid altered the action of cocaine which potentiates responses to phenylephrine.

Relaxation of strips contracted by noradrenaline

The procedures used to assess tissue mechanisms for the inactivation of noradrenaline in aortic strips from rabbits treated with reserpine were identical to those used in studies on strips from untreated animals (Kalsner & Nickerson, 1968c). The results of experiments on fifty nine strips from nine reserpine-pretreated animals are summarized in Table 2 and Fig. 3. As in the case of phenylephrine, each point on the relaxation curves of noradrenaline contracted strips corresponded to a different effective amine concentration in tissues from treated and untreated animals. For example, the residual concentrations of noradrenaline, determined from concentration-response curves (Fig. 2), at 10, 30 and 50% relaxation of untreated strips were equivalent to those present at 9.0, 25.8 and 44% relaxation of strips from reserpine-treated animals. The former relaxed 50% in 5.3 ± 0.5 min and the latter 44.0% in 4.0 ± 0.5 min. This difference in time was not statistically significant at the 5% level of probability ($0.1 < P > 0.05$), but suggests a somewhat greater overall rate of inactivation of noradrenaline in the tissues of animals treated with reserpine.

TABLE 2. *Relaxation in oil of aortic strips from reserpine-treated and untreated rabbits after contractions produced by noradrenaline* (1×10^{-8} *g/ml.*)

Treatment of strips *in vitro*	Untreated			Reserpine-treated		
	No. of expts.	Time to relax 50% (min)	Multiple of control time	No. of expts.	Time to relax 50% (min)	Multiple of control time
Control	10	5·3±0·5 1·8±0·1*		8	4·7±0·5 1·7±0·2*	
Iproniazid	10	5·1±0·3		6	5·4±0·7	1·13
Tropolone	6	7·0±0·7	1·33	7	9·9±1·2	2·09
Cocaine	9	7·9±1·3	1·49	7	6·4±0·7	1·36
Cocaine (1×10^{-4})	2	8·2±1·1	1·55	8	8·7±1·1	1·84
Iproniazid+cocaine	8	8·1±1·2	1·54	5	6·0±0·7	1·27
Tropolone+cocaine	5	13·9±0·7	2·62	5	14·0±1·0	2·95
Iproniazid+tropolone	7	17·0±3·1	3·21	5	32·4±3·8	6·84
Iproniazid+tropolone +cocaine	9	15·2±2·3*	8·66*	6	26·2±5·0*	15·6*
Iproniazid+tropolone +cocaine (1×10^{-4})	6	34·3±10·2*	19·58*	2	37·9±1·5*	22·5*

Cocaine concentration was 1×10^{-5} g/ml. except where otherwise indicated. Asterisks indicate determinations at 20 rather than 50% relaxation.

Comparisons made either with or without correction for the slight differences in residual amine concentration showed clearly that reserpine treatment did not decrease the ability of the strips to terminate the action of noradrenaline.

Iproniazid did not appreciably alter the rate of relaxation of noradrenaline contracted strips from reserpine-treated animals. Inhibition of COMT by tropolone slowed the relaxation of strips from reserpine-treated animals more than that of untreated strips; the periods required for 50% relaxation were increased to 2.09 and 1.33 times those of their controls, respectively. Inhibition of both MAO and COMT slowed the relaxation of reserpine-treated strips much more than it did that of untreated strips, to 6.84 and 3.21 times their control values, respectively.

Cocaine (1×10^{-5} g/ml.) somewhat slowed relaxation; the difference from the behaviour of control strips was greatest 5 min after oil immersion, but this effect was significant only at the 10% level of probability. The periods required for 50% relaxation of reserpine-treated and untreated strips were increased by cocaine (1×10^{-5} g/ml.) to 1.36 and 1.49 times those of their controls, respectively. A higher concentration of cocaine (1×10^{-4} g/ml.) significantly slowed the relaxation of reserpine-treated strips. The high concentration increased the time required for 50% relaxation to 1.36 times that with the lower concentration of cocaine in these

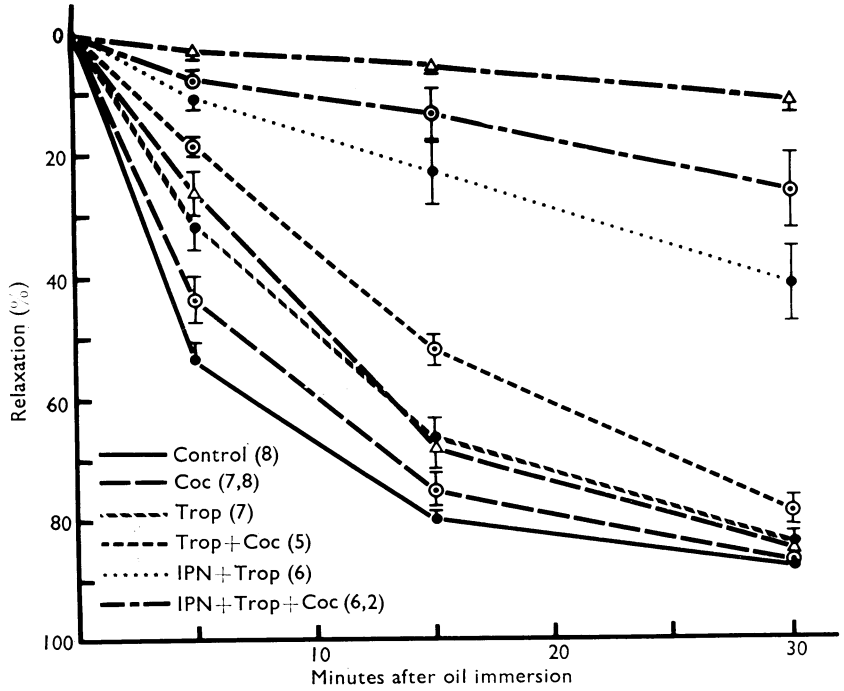

FIG. 3. Effects of iproniazid (IPN), tropolone (Trop) and cocaine (Coc) on the relaxation after oil immersion of noradrenaline-contracted aortic strips from reserpine-pretreated rabbits. All strips were contracted by noradrenaline (1×10^{-8} g/ml.); cocaine concentration was 1×10^{-5} g/ml. (⊙) or 1×10^{-4} g/ml. (△). Figures in parentheses indicate the number of experiments on which each curve was based, and vertical bars the standard errors of means. (Curves for strips exposed to iproniazid alone and iproniazid plus cocaine (1×10^{-5} g/ml.) did not differ appreciably from the control curve and are not shown.)

strips, but did not have an appreciably greater effect on those from animals not treated with reserpine.

Reserpine-treated strips exposed to iproniazid, tropolone and cocaine (1×10^{-5} g/ml.) relaxed in oil at a rate not significantly different from that of strips treated with iproniazid plus tropolone. However, a higher concentration of cocaine (1×10^{-4} g/ml.) did add significantly to the delay produced by the enzyme inhibitors. In all combinations, cocaine had a smaller effect on strips from animals pretreated with reserpine than on those from untreated animals. Addition of cocaine 1×10^{-5} g/ml. to treatment with iproniazid or tropolone and addition of cocaine 1×10^{-5} and 1×10^{-4} g/ml. to treatment with iproniazid plus tropolone increased the relaxation times to 1.59, 1.97, 2.70 and 6.10 times the values without cocaine in strips from control animals and to only 1.11, 1.41, 2.28 and 3.29 in those from reserpine-treated rabbits.

Effects of iproniazid, tropolone and cocaine on contraction amplitude in response to noradrenaline

The effects of iproniazid and tropolone on the magnitude of responses to noradrenaline were assessed on the basis of all peak contractions obtained after treatment with these inhibitors, including those of strips which subsequently were exposed to cocaine. The amplitude of contractions produced by noradrenaline was not altered by iproniazid, tropolone or the two enzyme inhibitors together in strips from either reserpine-treated or untreated animals. Strips from reserpine-treated rabbits exposed to both iproniazid and tropolone and their controls responded to noradrenaline (1×10^{-8} g/ml.) with almost identical peak contractions, 38.3 ± 4.3 and 37.3 ± 2.5 mm, respectively; the corresponding values for untreated strips were 30.4 ± 2.0 and 27.5 ± 2.0 mm.

Contractions of seven strips from reserpine-treated rabbits in response to noradrenaline (1×10^{-8} g/ml.) were increased a mean of 7.0 mm ($19.1 \pm 2.6\%$) by cocaine (1×10^{-5} g/ml.), and effect comparable with the mean potentiation of 4.6 mm ($18.3 \pm 1.6\%$) of the responses of ten similar strips from untreated animals. In both groups the potentiated responses were approximately equivalent to those which would have been produced by doubling the concentration of noradrenaline. Neither iproniazid nor tropolone treatment altered the potentiation produced by cocaine in either group. Exposure to both enzyme inhibitors only slightly decreased the effect of cocaine. The contractions of six and two reserpine-treated strips in which both MAO and COMT had been inhibited were increased 12.2 ± 2.2 and $14.5 \pm 2.5\%$ by cocaine in concentrations of 1×10^{-5} g/ml. and 1×10^{-4} g/ml., respectively. This potentiation differed from that due to cocaine in the absence of enzyme inhibition only at the 10% level of probability, a difference equivalent to that recorded in a similar comparison on strips from animals which had not received reserpine.

Discussion

It is now widely accepted that reserpine inhibits the accumulation of catecholamines in adrenergic nerves by blocking their incorporation into intraneuronal granules (Furchgott *et al.*, 1963; Kopin & Gordon, 1963; Kopin, 1964; Carlsson & Waldeck, 1965; Malmfors, 1965). It also potentiates responses of a variety of effectors to catecholamines and certain other sympathomimetics, and this potentia-

tion has been assumed by some workers to result from decreased amine inactivation by nerve uptake, which allows more of the available agonist to reach tissue receptors (MacMillan, 1959; Brodie & Beaven, 1963; Axelrod, 1965; Hertting, 1965). To reconcile this view with the fact that cocaine still effectively potentiates responses to sympathomimetic amines after treatment with reserpine, it has been suggested that after reserpine the net uptake into nervous structures is unaltered, but the amine is rapidly deaminated intraneuronally rather than stored in granules (Furchgott *et al.*, 1963; Iversen *et al.*, 1965).

The effects of inhibiting various pathways involved in the inactivation of a low concentration of noradrenaline in aortic strips from reserpine-pretreated and untreated animals are compared in Table 2. Cocaine alone and in combination with other agents slowed the relaxation in oil of strips from the pretreated animals less than it did that of preparations not exposed to reserpine. This is compatible with the concept that reserpine reduces the contribution of uptake and storage in adrenergic neurones to the inactivation of noradrenaline, and suggests that reserpine and cocaine act on overlapping components of some pathway of amine inactivation. These could be nerve membrane transport and intraneuronal incorporation into storage granules.

The results also indicate that a part of the effect of cocaine on amine inactivation persists after treatment with reserpine plus the enzyme inhibitors. The combination of reserpine, iproniazid and tropolone should have eliminated all known intraneuronal mechanisms for the inactivation of noradrenaline and, after a period of equilibration, inhibition of nerve membrane transport by cocaine should have had little effect on the rate of inactivation. The residual action of cocaine demonstrable in these conditions may be on processes involved in extraneuronal binding and storage of amine, and the fact that differences between the effects of the two concentrations of cocaine tended to be greater in the reserpine-treated preparations, where intraneuronal inactivation is less important, suggests that the extraneuronal processes are somewhat less sensitive than nerve membrane transport to inhibition by this agent. Other evidence for a significant extraneuronal action of cocaine has been previously presented (Kalsner & Nickerson, 1968b, c).

It is now generally assumed that inhibition of storage in intraneuronal granules by reserpine diverts catecholamine to metabolic inactivation by intraneuronal MAO, largely because much of the endogenous catecholamine released by reserpine is inactivated by deamination before leaving the nerves (Chessin, Kramer & Scott, 1957; Kopin & Gordon, 1962, 1963; Stjärne, 1964). Demonstration of an increase in deaminated metabolites of exogenous noradrenaline in perfused hearts from reserpine-treated animals (Iversen *et al.*, 1965) does not establish either that the deamination is intraneuronal or that it contributes to termination of action.

The present experiments provided no support for the concept that reserpine increases the contribution of MAO to termination of the action of noradrenaline. Iproniazid did not slow the relaxation of aortic strips from rabbits treated with reserpine and contracted by noradrenaline. When added to treatment with both tropolone and cocaine, it had no greater effect than it did on strips from rabbits not pretreated with reserpine. This effect of iproniazid probably reflects predominantly inhibition of extraneuronal MAO because the strips were exposed to a concentration of cocaine which should have markedly reduced transport of noradrenaline into the nerves (Iversen, 1963).

The results presented above indicate that inhibition of storage by reserpine diverts amine involved in the response of aortic tissue to metabolic inactivation by COMT rather than by MAO. Tropolone alone slowed relaxation more than it did in strips from untreated animals. Inhibition of MAO alone did not have an appreciable effect on either preparation, indicating that this enzyme functions predominantly as an alternate pathway of little importance as long as COMT is active (Kalsner & Nickerson, 1968c). Thus, the data indicate that in the absence of enzyme inhibition COMT is responsible for the inactivation of about 85 and 70% of a low concentration of noradrenaline in aortic tissues from reserpine-treated and untreated rabbits, respectively.

The data presented and discussed here reflect changes in the concentration of active amine available to act on tissue receptors. In view of the predominantly extraneuronal location of COMT (Potter, Cooper, Willman & Wolfe, 1965 ; Iversen, Glowinski & Axelrod, 1966), it seems that amine which cannot be stored intraneuronally because of the action of reserpine diffuses out of the nerves to sites of COMT activity without being first deaminated by MAO. Other possibilities which alone or in combination might explain the increased role of COMT in the inactivation of noradrenaline in aortic tissues from reserpine-treated animals include : (1) some of the storage or binding sites which are inactivated by reserpine and access to which is prevented by cocaine are extraneuronal ; (2) some COMT is intra-

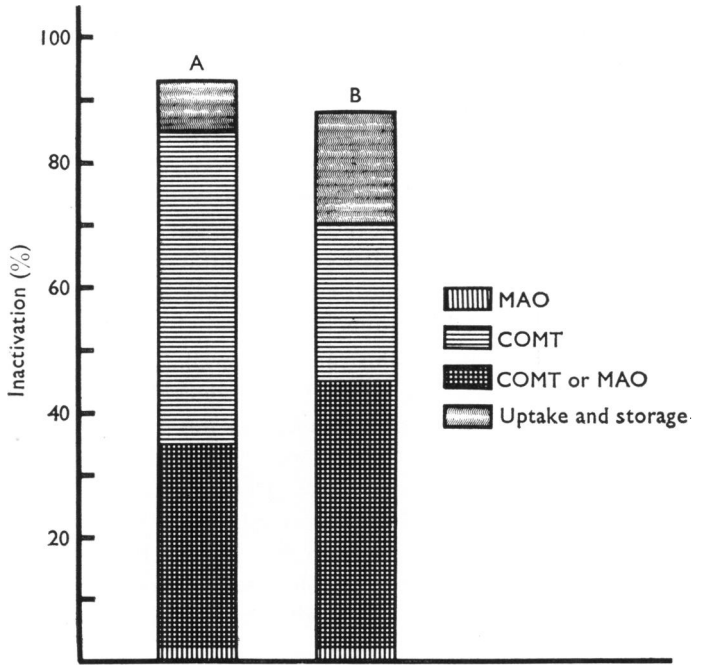

FIG. 4. Diagrammatic representation of the relative importance of various pathways in the inactivation of a low concentration of noradrenaline in aortic tissues from rabbits pretreated with reserpine (A) and from untreated animals (B). Monoamine oxidase (MAO) seems to contribute little to the inactivation as long as catechol-O-methyl transferase (COMT) activity is unimpaired, and the area indicated as COMT or MAO represents the extent to which the latter functions as an effective alternate pathway after COMT inhibition. "Uptake and storage" is believed to be predominantly a function of adrenergic neurones, but non-neuronal sites may also be involved.

neuronal, but its action is apparent only after blockade of granule storage ; and (3) reserpine treatment increases COMT activity.

On the basis of these data and of observations previously reported (Kalsner & Nickerson, 1968c), it is possible to formulate a general concept of the pathways by which the action of a low concentration of noradrenaline is terminated in aortic tissues from rabbits pretreated with reserpine and from untreated animals (Fig. 4). The mechanisms shown account for 93 and 88% of the inactivation of noradrenaline in the two preparations. The nature of the small residual inactivation is not clear. It could reflect slightly incomplete inhibition of one or more of the indicated pathways in the experiments on which the diagrams are based, or it could represent very slow inactivation by nonenzymatic oxidation.

A very marked decrease in the rate of amine inactivation, at least 80%, would be necessary to account for the observed potentiation by reserpine. No decrease in the rate of inactivation attributable to reserpine was, however, found in any experiment. Indeed, the results suggest that both noradrenaline and phenylephrine may be somewhat more rapidly inactivated in aortic strips from rabbits treated with reserpine than in those from untreated animals. Consequently, the considerable potentiation of responses to these amines by reserpine must be due to some action unrelated to their inactivation.

Cocaine also decreases the uptake and storage of certain sympathomimetic amines by adrenergic nerves and potentiates responses to these amines. It is generally assumed that the latter is entirely a consequence of the former (MacMillan, 1959 ; Muscholl, 1961 ; Furchgott *et al.*, 1963 ; Kopin, 1964 ; Trendelenburg, 1965). However, the present results seem to be incompatible with this interpretation. Cocaine potentiated responses to noradrenaline as effectively in aortic strips from rabbits treated with reserpine as in strips from untreated animals, in agreement with the observations of Furchgott *et al.* (1963), although the role of nerve uptake and storage in the inactivation of noradrenaline was considerably decreased in the former. In addition, inhibition of other possible intraneuronal mechanisms of amine inactivation by treatment with iproniazid, tropolone or a combination of both enzyme inhibitors altered the potentiation very little. These findings strongly suggest that cocaine decreases the rate of inactivation of amines and potentiates responses to them by independent actions.

Many of the present observations illustrate the unreliability of potentiation as an index of altered amine inactivation. The lack of correlation between decreased inactivation and potentiation is particularly clear in the responses to noradrenaline of strips in which both MAO and COMT were inhibited before the addition of cocaine. Inhibition of the two enzymes removed about 85 and 70% of the capacity of tissues from reserpine-treated and untreated rabbits to inactivate noradrenaline, but did not appreciably augment responses to this amine. In contrast, the relatively slight further reduction in inactivation due to cocaine, to totals of 93 and 88%, was associated with a clear potentiation which was essentially the same in the two types of preparation.

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