Br. J. Pharmac. (1969), 35, 440–455.

Effects of a haloalkylamine on responses to and disposition of sympathomimetic amines

S. KALSNER* AND M. NICKERSON†

Department of Pharmacology and Therapeutics, University of Manitoba Faculty of Medicine, Winnipeg, Canada

1. The mechanisms by which a haloalkylamine (GD-131) alters the inactivation of and potentiates responses to certain sympathomimetic amines, and the relationship of these actions to the similar effects of cocaine were investigated in rabbit aortic strips. The technique of oil immersion was used to assess rates of amine inactivation.

2. Exposures to GD-131, which produced no detectable α-adrenergic blockade, markedly slowed the inactivation of noradrenaline. It was concluded that it is unnecessary to postulate a role of adrenergic receptors in the inactivation of catecholamines to account for the reported effects of haloalkylamines on amine output during adrenergic nerve stimulation.

3. The reduction in the rate of noradrenaline inactivation produced by moderate exposure to GD-131 was approximately equivalent to that due to inhibition of both monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). Addition of GD-131 did not further slow inactivation in preparations in which MAO and COMT had been inhibited, but the effects of both GD-131 and of enzyme inhibition on noradrenaline disposition were additive with that of cocaine.

4. Cocaine consistently inhibited and GD-131 markedly potentiated responses to tyramine. The augmentation of responses by GD-131 was much greater than could be accounted for by the slight release of endogenous catecholamine by this agent. Thus the principal effect of the haloalkylamine appears not to involve inhibition of nerve cell membrane transport of amine.

5. Maximal exposure to GD-131 short of that which produced α-adrenergic blockade sometimes slowed the inactivation of noradrenaline as much as did inhibition of both MAO and COMT plus the maximal effect of cocaine.

6. These results seem best explained by postulating that GD-131 and other haloalkylamines inhibit the passage of sympathomimetic amines through biological membranes. Passage to sites of enzymatic inactivation, predominantly in non-neuronal tissue, is most readily inhibited. The "cocaine-sensitive mechanism," transport to sites of binding and storage, can also be inhibited, but is considerably less sensitive.

7. GD-131 potentiated responses to noradrenaline more than did the maximally effective concentration of cocaine. Cocaine produced very little addi-

* Present address: Department of Pharmacology, University of Ottawa, Ottawa, Canada.
† Present address: Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada.

tional potentiation when added in the presence of GD-131, whereas the latter had a reduced, but still significant effect in the presence of cocaine. Most of the effect of cocaine and at least half of that of GD-131 was due to a common action on effector cells, which is unrelated to any alteration of amine disposition. The balance of the potentiation by GD-131 may be due to inhibition of access of amine to sites of enzymatic inactivation, perhaps involving a reduction in the volume of distribution in intracellular water, and a very small part of the potentiation by cocaine may be secondary to inhibition of transport of amine to sites of binding and storage.

8. On the basis of the present observations, it is postulated that a major part of the noradrenaline released by adrenergic nerve activity is involved in the activation of tissue receptors and has its action terminated by movement away from the region of the receptors. A small portion of the mediator is removed by the circulation, some is taken up by adrenergic nerves, but the major part enters non-nervous cells and is distributed in intracellular water. The capacity of this intracellular compartment appears to be limited and enzymatic inactivation is essential to maintain its function. O-methylation is the dominant primary enzymatic process in the inactivation of physiological amounts of noradrenaline, but MAO appears to function "in series" as an effective alternate pathway of disposition.

The work of Brown & Gillespie (1957), Brown (1960), Bacq, Brown & Ferry (1960), Kirpekar & Cervoni (1963), Gillespie & Kirpekar (1965), and others has implicated α-receptors *per se* in termination of the action of noradrenaline, largely on the basis of effects of the haloalkylamine α-receptor blocking agents, dibenamine and phenoxybenzamine, on the output of noradrenaline from sympathetically innervated organs. A number of other workers (Paton, 1960; Hertting, Axelrod & Whitby, 1961; Thoenen, Huerlimann & Haefely, 1963, 1966; Farrant, Harvey & Pennefather, 1964), however, have attributed this effect of the blocking agents to inhibition of the "cocaine-sensitive mechanism" (uptake by adrenergic nerves), although the haloalkylamines appear to elevate the venous output of noradrenaline during sympathetic nerve stimulation more markedly and consistently than does cocaine (Blakely, Brown & Ferry, 1963; Kirpekar & Cervoni, 1963; Boullin, Costa & Brodie, 1967).

The experiments reported here were undertaken in an attempt to evaluate the contribution of α-receptors to the inactivation of sympathomimetic amines in a vascular smooth muscle preparation, and to compare the actions of a haloalkylamine with those of cocaine. GD-131 (N-cyclohexylmethyl-N-ethyl-β-chloroethylamine) was selected for study because its chemical reactivity is comparable with that of dibenamine and phenoxybenzamine (Harvey & Nickerson, 1954), but it has very little α-receptor blocking activity. It was felt that these properties might make it possible to distinguish between effects on α-receptors and on other processes affecting sympathomimetic amines and thus allow a more precise assessment of the contribution of the receptors to termination of action. GD-131 has been reported to potentiate certain cardiac responses to catecholamines which are mediated by β-receptors (Furchgott, 1960). It has also been reported to release catecholamines

from the heart (Furchgott & Kirpekar, 1960). Consequently, except where otherwise indicated, all experiments were performed on aortic strips from rabbits pretreated with reserpine to minimize complications which might arise from the release of endogenous noradrenaline.

The oil immersion technique (Kalsner & Nickerson, 1968a) was used to measure rates of amine inactivation. After a steady state contraction had been produced in an aqueous medium, the bathing solution was replaced by mineral oil to eliminate loss by diffusion out of the tissue. The relaxation curve then provided a direct measure of termination of action by intrinsic mechanisms.

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 the 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 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 in terms of the free base, and histamine diphosphate, iproniazid phosphate, and tyramine, methoxamine, GD-131 and cocaine hydrochlorides in terms of the salts. Reserpine powder was dissolved in a 10% solution of ascorbic acid, and rabbits were injected intramuscularly with 5.0 mg/kg, 18 to 24 hr before death. Angiotensin amide (Hypertensin) was prepared from vials containing: angiotensin amide 2.5 mg, mannitol 47.4 mg and thimerosal 0.1 mg. GD-131 was usually prepared on the day of use in a stock concentration of 1.0 mg/ml. in distilled water containing 0.1 N HCl and was diluted with 0.9% NaCl containing 0.01 N HCl before addition to the muscle baths. 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. Details of drug preparation, enzyme inhibition and oil immersion were as described previously (Kalsner & Nickerson, 1968a).

Monoamine oxidase (MAO) was inhibited with iproniazid (2×10^{-4} g/ml. for 30 min) and catechol-O-methyl transferase (COMT) with tropolone (3×10^{-5} g/ml.). Evidence for the completeness and specificity of the procedures used to inhibit specific mechanisms of amine inactivation has been presented previously (Kalsner & Nickerson, 1968b, 1969a). 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 percent 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

Effects of GD-131 on aortic strips from rabbits not pretreated with reserpine

A limited study was carried out on aortic strips from animals not pretreated with reserpine. A 15 min exposure to GD-131 at a concentration of 1×10^{-5} g/ml. had little or no effect on the basal tone of the strips in the aqueous medium and considerably potentiated their responses to noradrenaline. Some of the strips exposed to GD-131 did contract somewhat after oil immersion, due to magnification of the effects of small amounts of catecholamine released from endogenous stores and trapped within the tissue by the oil barrier (Kalsner & Nickerson, 1968a). However, even strips which did not shorten in oil after pretreatment with GD-131 relaxed considerably more slowly after contractions produced by noradrenaline (1×10^{-8} g/ml.) than did control strips. This effect on amine disposition was studied more thoroughly on aortic strips from rabbits pretreated with reserpine.

Exposure of aortic strips for 10 min to a 1×10^{-4} g/ml. concentration of GD-131 depressed responses to noradrenaline, but did not affect the amplitude of responses to angiotensin or the rate of relaxation of angiotensin contracted strips in oil. This difference indicated that significant α-receptor blockade was produced by this higher concentration of the haloalkylamine. Exposures of up to 30 min to a concentration of 1×10^{-5} g/ml. did not appreciably block responses to noradrenaline.

Effects of GD-131 on aortic strips from rabbits pretreated with reserpine

In contrast to the effects of cocaine, those of GD-131 were persistent, a characteristic of the effects of the related haloalkylamines, dibenamine and phenoxybenzamine. For example, GD-131 (1×10^{-5} g/ml.) added 15 min before oil immersion

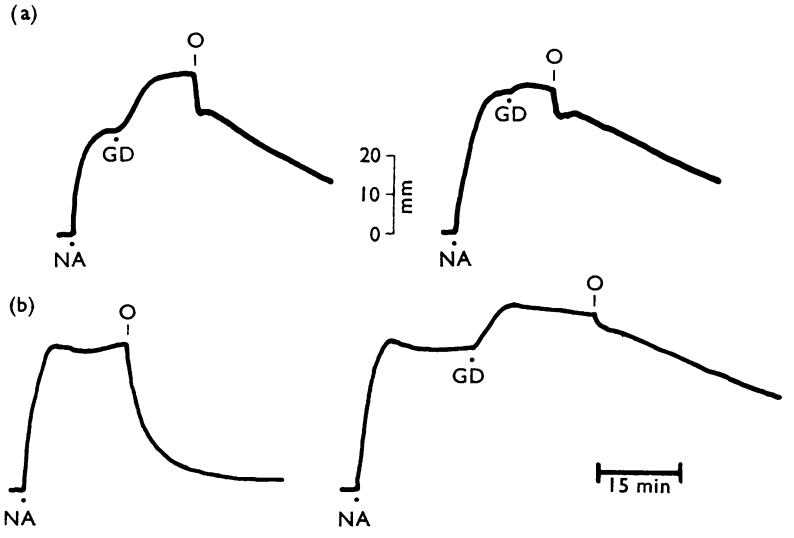

FIG. 1. Effects of GD-131 (GD) on aortic strips contracted by noradrenaline (NA). (a) Responses in Krebs solution and after oil immersion (O) to a first (left) and a second (right) exposure to GD-131 (1×10^{-5} g/ml.) after contractions produced by noradrenaline (1×10^{-8} g/ml.). (b) Left, response to noradrenaline (1×10^{-8} g/ml.) and subsequent relaxation in oil; right, same strip recontracted by noradrenaline and exposed to GD-131 (1×10^{-5} g/ml.) for 25 min before oil immersion.

considerably augmented the response to noradrenaline (1×10^{-8} g/ml.) and slowed subsequent relaxation in oil (Fig. 1a, left). After washout and recovery from the first test, the response to noradrenaline was almost equal to the previous potentiated response, and a second exposure to GD-131 produced a barely detectable increase in the amplitude of contraction, although it further decreased the rate of relaxation (Fig. 1a, right). These characteristics of a second exposure to GD-131 appear to be determined by the persistence of changes produced by the first.

The effects of a relatively long exposure of a strip contracted by noradrenaline (1×10^{-8} g/ml.) to GD-131 (1×10^{-5} g/ml. for 25 min) are shown in Fig. 1b. It appears that a large part of the action of GD-131 which potentiates responses to noradrenaline is exerted rapidly, like that of cocaine, but that the action which decreases the rate of relaxation develops more slowly.

The contractile response to GD-131 was considerably reduced but not completely eliminated by reserpine pretreatment. Strips exposed to GD-131 (1×10^{-5} g/ml.) did not contract in the aqueous medium, but some showed a gradual increase in tone during oil immersion. This occurred both in experiments in which the GD-131 was left in the muscle bath and those in which it was washed out before oil immersion, and was not altered by cocaine. The release of endogenous catecholamine was estimated in seven strips treated for 10 min with GD-131 (1×10^{-5} g/ml.), which was washed out of some of the baths before oil immersion. The strips contracted a mean of 1.8 mm in 15 min and of 5.2 mm in 30 min after oil immersion. The concentration of noradrenaline required to produce a 5.2 mm contraction of aortic strips from animals pretreated with reserpine was estimated from separately determined dose-response curves to be about 4×10^{-10} g/ml. This was negligible in terms of either the initial concentration of exogenous noradrenaline (1×10^{-8} g/ml.) or of that present in strips treated with GD-131 during the 30 min period of oil immersion.

Effects of GD-131 and of cocaine on the relaxation of aortic strips contracted by noradrenaline

Cocaine (1×10^{-5} g/ml.) significantly slowed the relaxation in oil of aortic strips contracted by noradrenaline (1×10^{-8} g/ml.), and a 10-fold higher concentration of cocaine had a slightly greater effect ($0.1 < P > 0.2$). GD-131 (1×10^{-5} g/ml.) added to the baths 10 to 15 min before oil immersion, after the contractions had reached a plateau, slowed the relaxation distinctly more than did either concentration of cocaine. Relaxation of strips contracted by noradrenaline was almost completely prevented by combined treatment with cocaine (1×10^{-4} g/ml.) and GD-131 (1×10^{-5} g/ml.), irrespective of the sequence in which the drugs were added (Fig. 2).

The effect of GD-131 on the rate of inactivation of noradrenaline and its relationship to that of cocaine were studied on four complete series of experiments in which GD-131 (1×10^{-5} g/ml.) was added to baths containing quiescent strips and washed out after 10 min. The strips were washed at frequent intervals for 30 min and then contracted by noradrenaline (1×10^{-8} g/ml.). Some of these strips were also exposed to cocaine (1×10^{-4} g/ml.) after the noradrenaline contractions had reached a plateau. Other preparations from the same aortas were contracted with noradrenaline without GD-131 pretreatment, and some of these were then exposed to cocaine (1×10^{-4} g/ml.). The relaxation of all strips in oil was recorded. The

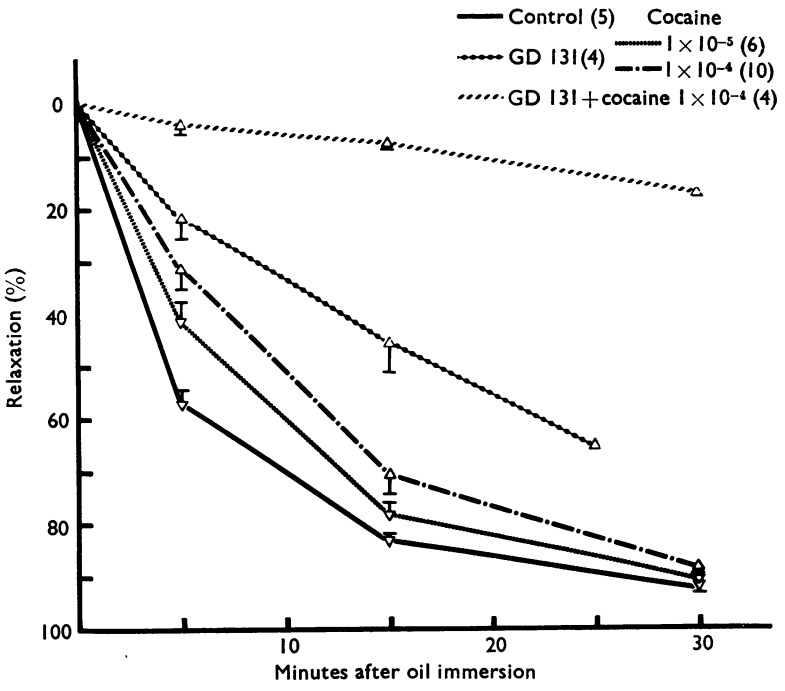

FIG. 2. Effects of GD-131 and of cocaine on the relaxation after oil immersion of aortic strips contracted by noradrenaline. Cocaine and GD-131 (1×10^{-5} g/ml.) were added after responses to noradrenaline (1×10^{-8} g/ml.) had reached a plateau, 10 to 15 min before oil immersion. In the combined treatment, GD-131 and cocaine were added sequentially. Figures in parenthesis indicate number of preparations represented by each curve, and vertical bars the standard errors of means.

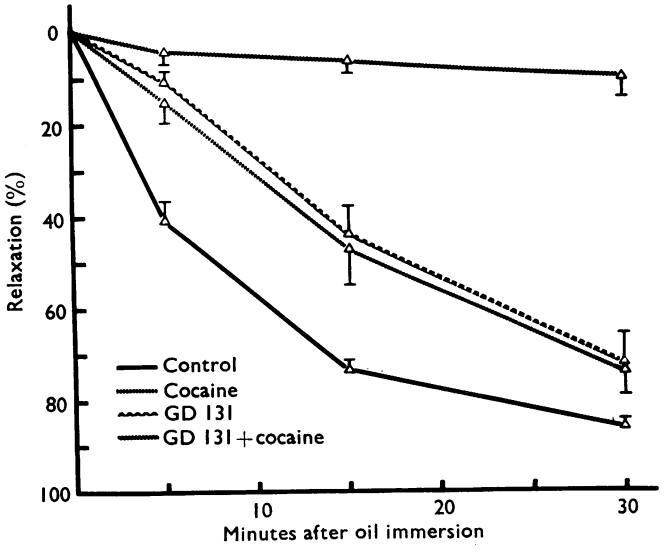

FIG. 3 Effects of GD-131 and of cocaine on the relaxation after oil immersion of aortic strips contracted by noradrenaline. All strips were contracted by noradrenaline (1×10^{-8} g/ml.). Treatment with GD-131 (1×10^{-5} g/ml. for 10 min) was 30 min before noradrenaline contraction ; cocaine (1×10^{-4} g/ml.) was added after noradrenaline contraction had reached a plateau, and 10 min before oil immersion. Curves depict the results of four complete experiments and vertical bars the standard errors of means.

results from this series of experiments are shown in Fig. 3, and the changes in time for 50% relaxation are summarized in Table 1.

The 10 min exposure of quiescent strips to GD-131 followed by washout inhibited relaxation less than did the same concentration of GD-131 added to the chambers 10 min before oil immersion. This difference was probably due to the continued action of GD-131 trapped in the tissue during the period of oil immersion. Strips treated with cocaine (1×10^{-4} g/ml.) relaxed in oil at almost the same rate as those pretreated with GD-131. Relaxation was almost completely prevented by the combined treatment.

Pretreatment with GD-131 (1×10^{-5} g/ml.) for 30 min inhibited the relaxation of noradrenaline contracted strips considerably more than did a 10 min exposure. Three strips treated for the longer period relaxed a mean of only 33.2% in 30 min after oil immersion, and one relaxed only 18.9%. This rate of relaxation was comparable with that of similar strips treated with the combination of iproniazid, tropolone and cocaine (1×10^{-4} g/ml.) (Kalsner & Nickerson, 1969a). This very slow rate of relaxation could not be obtained consistently with GD-131 alone, probably because the development of α-receptor blockade prevented exposure of aortic strips to GD-131 (1×10^{-5} g/ml.) for periods of longer than 30 min. Prolonging the exposure to cocaine does not increase the inhibition of relaxation in oil (Kalsner & Nickerson, 1969a).

Effects of GD-131 on the relaxation of noradrenaline contracted aortic strips after enzyme inhibition

The effect of cocaine on the rate of relaxation of aortic strips contracted by a low concentration of noradrenaline was previously shown to be approximately additive with the effects of inhibiting the enzymes involved in catecholamine inactivation (Kalsner & Nickerson, 1969a). The interaction of GD-131 with enzyme inhibition was studied in a similar way. Five strips pretreated with iproniazid were contracted by noradrenaline (1×10^{-8} g/ml.) in the presence of tropolone, and their relaxation during 30 min of oil immersion was recorded. After return to Krebs solution and recovery of basal tone, they were again contracted by noradrenaline in the presence of tropolone. Two of the strips were exposed to cocaine (1×10^{-4} g/ml.) and the remainder to GD-131 (1×10^{-5} g/ml.) for 10 min before oil immersion. The relaxation curves obtained in these experiments are plotted in Fig. 4. As reported previously, cocaine significantly slowed the rate of relaxation after inhibition of both COMT and MAO. In contrast, GD-131 did not alter the rate from that produced by the enzyme inhibition alone.

TABLE 1. *Effects of GD-131 and of cocaine on aortic strips contracted by noradrenaline*

Treatment	No. strips	Increment in amplitude		Relaxation in oil (multiple of control time)
		mm	%	
Cocaine	4	8·5	17·9±2·4	2·1
GD-131	4			2·4
Cocaine after GD-131	4	4·0	7·9±3·3	24·0

All strips were contracted by noradrenaline (1×10^{-8} g/ml.). Exposure to GD-131 (1×10^{-5} g/ml.) was for 10 min, and strips were then washed for 30 min before addition of noradrenaline. Cocaine (1×10^{-4} g/ml.) was added after noradrenaline contractions had reached a plateau.

Effects of GD-131 and of cocaine on the amplitude of responses to noradrenaline

As noted above, the addition of GD-131 to baths containing aortic strips contracted by noradrenaline considerably increased the amplitude of contraction. This effect was qualitatively similar to that of cocaine, but of somewhat greater magnitude than that produced by cocaine in a concentration of either 1×10^{-5} or 1×10^{-4} g/ml. When aortic strips contracted by noradrenaline (1×10^{-8} g/ml.) were exposed to cocaine and to GD-131 in sequence, the maximal total potentiation was achieved with a concentration of 1×10^{-5} g/ml. of each drug. Higher concentrations produced less effect, perhaps due to the same processes which often lead to a reduction in the height of contraction when the concentration of cocaine is increased after the response to 1×10^{-5} g/ml. is fully developed.

The results of experiments in which cocaine and GD-131 were added sequentially in a concentration of 1×10^{-5} g/ml. are summarized in Table 2. Cocaine added after GD-131 produced a barely detectable augmentation, whereas GD-131 had a

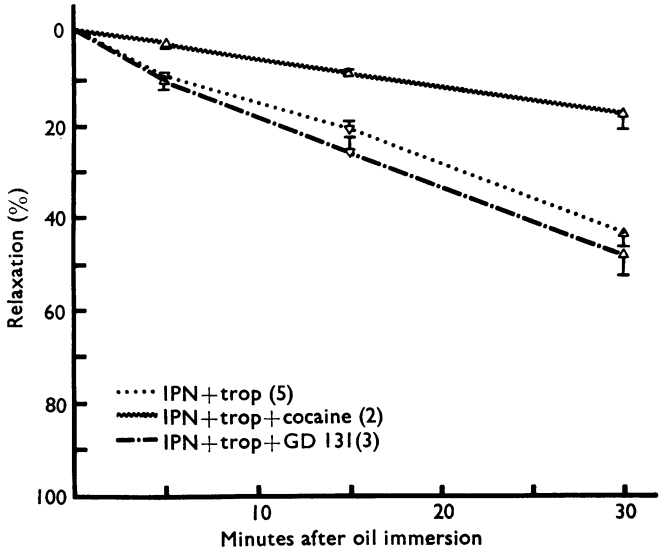

FIG. 4. Effects of GD-131 and of cocaine on the relaxation in oil of aortic strips contracted by noradrenaline after enzyme inhibition. All strips were treated with iproniazid (IPN) and tropolone (trop) and contracted by noradrenaline (1×10^{-8} g/ml.). Cocaine (1×10^{-4} g/ml.) or GD-131 (1×10^{-5} g/ml.) was added 10 min before oil immersion. Figures in parentheses indicate the number of preparations represented by each curve, and vertical bars the standard errors of means.

TABLE 2. *Effects of GD-131 and of cocaine on the amplitude of contractions produced by noradrenaline (1×10^{-8} g/ml.)*

Treatment	No. strips	Increment in amplitude mm	Increment in amplitude %	Final equiv. conc. of noradrenaline ($\times 10^{-8}$ g/ml.)
Cocaine (1×10^{-5} g/ml.)	10	7·0	19·7±2·3	2·4
GD-131 (1×10^{-5} g/ml.)	17	8·9	27·5±2·5	3·7
Cocaine after GD-131	4	0·6	2·0±0·3	4·1
GD-131 after cocaine	4	4·1	14·0±0·5	5·8

Potentiating drugs were added after previous contractions had reached a plateau value.

reduced, but much more definite, effect after cocaine. Similar results were obtained when cocaine (1×10^{-4} g/ml.) was combined with GD-131 on some strips which appeared to be relatively resistant to the depressant effect of the higher concentration. In other experiments, pretreatment with GD-131 (1×10^{-5} g/ml.) for 10 min, followed by washout and repeated changes of the bath fluid for 30 min, greatly reduced the potentiation of subsequent noradrenaline contractions by cocaine (1×10^{-4} g/ml.) (Table 1), although this exposure to the haloalkylamine produces a submaximal effect.

Effects of GD-131 and of cocaine on aortic strips contracted by other agonists

The observations on the effects of GD-131 and of cocaine on aortic strips contracted by noradrenaline indicated both similarities and differences in their effects. In an attempt to define these properties more precisely, the effects of the two agents on responses to other amines were determined.

Tyramine responses were studied on aortic strips from rabbits not pretreated with reserpine because reserpine greatly decreases responses to this agonist. All strips were treated with iproniazid to slow the destruction of the tyramine, which is rapidly deaminated by MAO. In agreement with previous observations, increasing concentrations of cocaine progressively depressed contractions induced by tyramine. In contrast, GD-131 considerably increased the response (Fig. 5a). Depression of the response by GD-131 occurred only at a concentration of 1×10^{-4} g/ml., which produces a considerable α-receptor blockade. After exposure to both iproniazid and tropolone, potentiation of responses to tyramine by GD-131 was still apparent, but it was considerably less than in strips which had not been exposed to the COMT inhibitor (Fig. 5b).

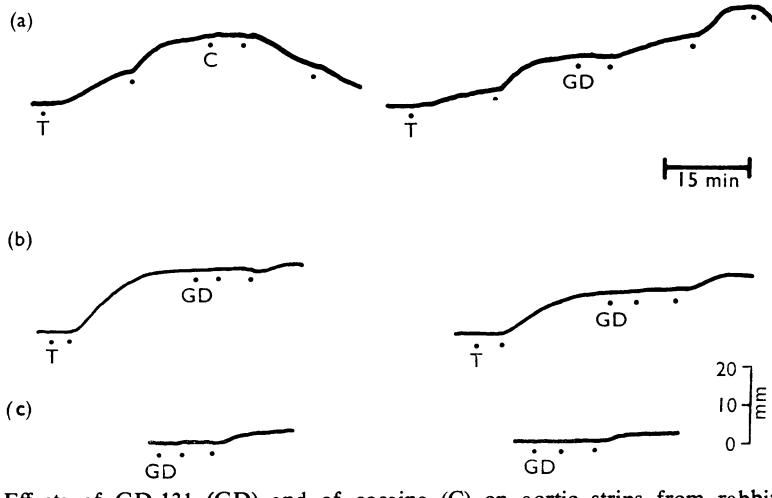

FIG. 5. Effects of GD-131 (GD) and of cocaine (C) on aortic strips from rabbits not pretreated with reserpine and contracted by tyramine (T). (a) Responses of iproniazid-treated strips from the same aorta to tyramine (1 and 3×10^{-7} g/ml.), followed by cocaine (1×10^{-7}, 1×10^{-6}, 1×10^{-5} g/ml.) or GD-131 (1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4} g/ml.). (b) Responses of strips treated with both iproniazid and tropolone to tyramine (1 and 3×10^{-7} g/ml.), followed by GD-131 (1×10^{-7}, 1×10^{-6}, 1×10^{-5} g/ml.). (c) Responses of iproniazid treated strips to GD-131 (1×10^{-7}, 1×10^{-6}, 1×10^{-5} g/ml.) alone.

The contribution of the known catecholamine releasing action of GD-131 to its potentiation of responses to tyramine was assessed on strips exposed only to the haloalkylamine. The contractile response to the GD-131 was obviously inadequate to account for the potentiation of responses to tyramine (Fig. 5c). This amount of noradrenaline would make a negligible contribution to the amplitude of the responses of strips already contracted by tyramine.

Methoxamine is not a substrate for either COMT or MAO and it appears not to be inactivated by any mechanism in aortic strips (Kalsner & Nickerson, 1968a, 1969c). When added cumulatively to the muscle baths after contractions produced by methoxamine had reached a plateau, GD-131 (1×10^{-7}, 1×10^{-6} and 1×10^{-5} g/ml.) caused a progressive potentiation similar in both form and amplitude to that produced by cocaine. Strips contracted by methoxamine and then treated with GD-131 also responded to oil immersion in the same way as did those exposed to cocaine. There was an initial sharp decrease in contraction amplitude to a new plateau, which remained relatively constant for long periods of time.

Histamine induced contractions were considerably decreased in aortic strips pretreated with GD-131 (1×10^{-5} g/ml.) and when this concentration was added to the muscle baths after contractions produced by histamine (3×10^{-6} g/ml.) had reached a plateau value, it caused a small reduction or no change in the contraction amplitude. Following oil immersion, two control strips relaxed 32.8% and 76.6% and three GD-131 pretreated strips 46.7% and 79.8% in 5 and 15 min. Thus, neither GD-131 nor cocaine (Kalsner & Nickerson, 1968a) slowed the relaxation of histamine contracted strips in oil. GD-131 clearly has a significant antihistamine effect,

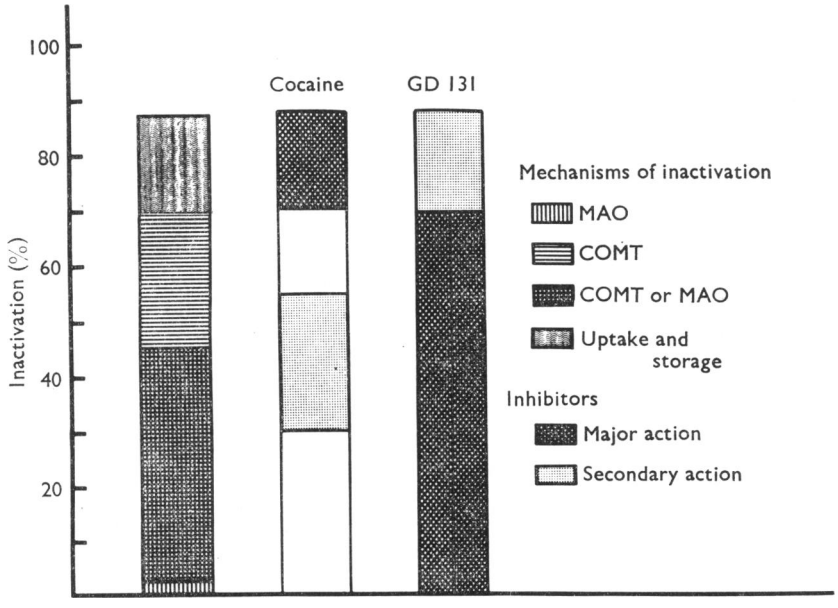

FIG. 6. Intrinsic mechanisms for the inactivation of noradrenaline in rabbit aortic strips and their relationship to the effects of GD-131 and cocaine. Column on the left depicts the relative contributions of various mechanisms to the inactivation of a low concentration (1×10^{-8} g/ml.) of noradrenaline (Kalsner & Nickerson, 1969a, b). Other columns illustrate the extent to which these are affected by the major and secondary actions of GD-131 and cocaine.

and this may have been involved in the tendency for strips pretreated with this agent to relax somewhat more rapidly than the controls.

Discussion

Dibenamine and phenoxybenzamine have been reported by a number of investigators to inhibit the inactivation of noradrenaline, presumably by blocking α-receptors. In the experiments reported above, a closely related haloalkylamine, GD-131, was found both to potentiate contractions of aortic strips induced by noradrenaline and to impair the inactivation of this amine in concentrations which produced no detectable α-receptor blockade. These findings make it unnecessary to implicate these receptors in the termination of action of noradrenaline or in the alteration of amine disposal caused by dibenamine or phenoxybenzamine.

Interpretation of the action of this haloalkylamine requires a careful comparison of its effects with those of cocaine. Both GD-131 and cocaine impaired the inactivation of noradrenaline in aortic strips. However, their major effects were approximately additive and, in addition, the effect of cocaine was additive with that of enzyme inhibition, but the major effect of GD-131 was not. Thus, moderate exposure to GD-131 (1×10^{-5} g/ml. for 10 to 15 min) appears not to affect the major mechanism by which cocaine inhibits the inactivation of noradrenaline, presumably blockade of transport of amine to sites of binding and storage (Whitby, Hertting & Axelrod, 1960; Muscholl, 1961; Dengler, Spiegel & Titus, 1961; Furchgott, Kirpekar, Rieker & Schwab, 1963; Van Zwieten, Widhalm & Hertting, 1965; Kalsner & Nickerson, 1968b, 1969a). This was confirmed in experiments in which GD-131 potentiated responses to tyramine. In contrast to the action of cocaine, moderate exposure to GD-131 clearly did not block the transport of tyramine to sites of endogenous catecholamine storage. These observations indicate that the major actions of GD-131 and of cocaine are distinct.

Although the major actions of GD-131 and of cocaine which affect amine inactivation are different, treatment with GD-131 to a point just short of producing α-receptor blockade appears also to inhibit the " cocaine sensitive mechanism." The ability of aortic strips to inactivate noradrenaline was almost eliminated by exposure to GD-131 (1×10^{-5} g/ml.) for 30 min. In some strips this single treatment reduced the rate of inactivation to essentially the same degree as did treatment with the combination of iproniazid, tropolone and cocaine. It is felt that this provides important information on the action of GD-131, although the maximal inhibition of noradrenaline inactivation could not be consistently reproduced, probably because the concentration and exposure time required were very close to those which produced significant α-receptor blockade.

The data suggest that GD-131 may inhibit all routes of noradrenaline inactivation in our preparation by a single basic action, to which the " cocaine sensitive pathway " is quantitatively the most resistant. Whether this relationship of effectiveness on the different pathways is constant within the series of haloalkylamines or varies widely, as do α-receptor blocking and antihistamine activities, cannot be accurately assessed from the information now available. However, the fluorescence microscopic observations of Malmfors (1965) showed no important effect of phenoxy-

benzamine on the uptake of amines into nerve cells. It appears very unlikely that interference with uptake and storage by nerve endings plays an important role in the augmentation by haloalkylamines of the noradrenaline output from organs during stimulation of their adrenergic nerves.

The haloalkylamines have been reported not to inhibit COMT (Axelrod, 1960; Eisenfeld, Krakoff, Iversen & Axelrod, 1967), or MAO (Eisenfeld *et al.*, 1967). Moderate exposure to GD-131 (1×10^{-5} g/ml. for 10 to 15 min), however, produced essentially the same effect on noradrenaline inactivation as inhibition of both MAO and COMT; the magnitudes of the effects were similar, and GD-131 did not further slow the relaxation of strips in which the enzymes had been inhibited. In addition, the effects of both GD-131 and of enzyme inhibition were additive with the major effect of cocaine. Thus the drug profoundly alters the effects of MAO and COMT without acting on the enzymes *per se*.

We wish to propose that the effects of GD-131 and of other haloalkylamines on amine inactivation are due to a primary action which specifically inhibits the movement of noradrenaline and other sympathomimetic amines across biological membranes. The major effect of GD-131 appears to be blockade of access of amine to sites of enzymatic degradation. These are predominantly extraneuronal, in " effector cells " (Kalsner & Nickerson, 1969a). Longer incubation with GD-131 also affects the transport of amine to sites of binding and storage, the major " cocaine sensitive mechanism." Conversely, cocaine can reduce the access of amine to sites of enzymatic degradation, the primary haloalkylamine mechanism, as indicated by some overlap of the effects of cocaine and of enzyme inhibition. This is most readily demonstrable under conditions where enzymic processes fully dominate the overall inactivation of a sympathomimetic amine (Kalsner & Nickerson, 1968b, 1969a), and appears to have been of limited magnitude under the conditions of the present experiments. The proposed involvement of the two effects of GD-131 and of cocaine on noradrenaline inactivation is illustrated diagramatically in Fig. 6.

It has been reported that dibenamine potentiates responses of rabbit intestine to isoproterenol (Furchgott, 1960), and that cocaine does not potentiate responses of this organ to catecholamines (Stafford, 1963). The present results indicate a sufficient difference in the potentiation produced by GD-131 and by cocaine to provide a basis for the above reports, but they also demonstrate a considerable overlap of the mechanisms of potentiation. Unfortunately, depression of responses by large doses of these agents and the fact that the primary action of each on amine disposition appears to be a secondary action of the other make it difficult to assess quantitatively the extent to which the potentiation produced is due to unshared mechanisms. However, it appears that most of the effect of cocaine is duplicated by GD-131, which has, in addition, a significant component of action not shared by cocaine. GD-131 potentiated responses to noradrenaline more than did cocaine. In addition, cocaine had only a slight effect when added after treatment with GD-131, whereas the latter still potentiated responses to noradrenaline considerably when added in the presence of cocaine.

The important common action of cocaine and GD-131 appears to be directly on effector cells because the two drugs produced essentially equal potentiation of responses to methoxamine, which is not inactivated in the aortic strips. Thus the potentiation of responses to sympathomimetic amines by cocaine can be attributed

almost entirely to an action on effector cells which makes them hyperresponsive, and which has been dissociated from effects of this agent on amine disposition in a wide variety of experiments (Kalsner & Nickerson, 1969c). In the present study, the independence of potentiation and inhibition of amine inactivation was also demonstrated by the fact that when administered in the presence of GD-131, cocaine exerted its typical effect on the rate of noradrenaline inactivation, but produced only a very slight additional augmentation of the amplitude of contraction. It is possible, however, that in some conditions a significant component of the potentiation by cocaine may be secondary to decreased inactivation of amines.

The consistent augmentation of responses to tyramine by GD-131 indicates that potentiation by this drug does not involve blockade of transport to sites of storage and binding, except possibly after exposure approaching that which produces α-receptor blockade. However, the action of GD-131 on amine disposition which simulates enzyme inhibition may contribute to potentiation. This is indicated by an effect greater than that which can be attributed to the action on effector cells shared with cocaine, and by the reduced potentiation of responses to tyramine after inhibition of both MAO and COMT. Potentiation by cocaine is only slightly reduced by prior enzyme inhibition (Kalsner & Nickerson, 1969c).

The major effect of GD-131 on noradrenaline inactivation is almost exactly equivalent to that produced by inhibiting both MAO and COMT, but the component of potentiation attributable to slowed inactivation of amines appears to be appreciably greater than the potentiation caused by enzyme inhibition. The degree of potentiation produced by inhibiting a pathway of inactivation is related to both the contribution of the mechanism to overall inactivation rate and its position relative to other mechanisms, including diffusion out of the tissue, in competition for "substrate." It may be postulated that by limiting passage through membranes GD-131 acts to decrease the volume of distribution of noradrenaline and certain other amines within cells, and that dissipation by passage into intracellular water is the first step in terminating the action of these amines. This concept would reconcile the relative effects of GD-131 and of enzyme inhibition on the inactivation of and on responses to sympathomimetic amines, but it is difficult to substantiate further until the location and nature of the pertinent barriers are established.

Uptake and storage by adrenergic neurones is widely accepted as the predominant mechanism terminating the action of both endogenous and exogenous noradrenaline (Whitby *et al.*, 1960; Hertting & Axelrod, 1961; Hertting, Axelrod, Kopin & Whitby, 1961; Hertting, Axelrod & Whitby, 1961; Muscholl, 1961; Whitby, Axelrod & Weil-Malherbe, 1961; Wolfe, Potter, Richardson & Axelrod, 1962; Rosell, Kopin & Axelrod, 1963; Kopin, 1964; Malmfors, 1965; Van Zwieten *et al.*, 1965). It is clear that such uptake and storage does occur, but its importance in terminating the action of amine actually involved in any given response is less clear. In the past, support for a major role of nerve uptake has largely come from the potentiation produced by agents such as cocaine which block the uptake mechanism. The oil immersion technique, however, provides data on the termination of action of amines which is independent of any potentiation. Observations made with this technique on vascular tissue (Kalsner & Nickerson, 1968a, b, 1969a, b, c and the present report) now provide a basis on which termination of the action of noradrenaline in this important effector complex can be re-evaluated. Observations particularly pertinent to this assessment include the following:

1. The major part of a physiological concentration of either noradrenaline or adrenaline penetrates " effector cells " to sites of enzymatic inactivation (Kalsner & Nickerson, 1969a).

2. Metabolic inactivation competes effectively with nerve uptake and storage as a mechanism terminating the action of catecholamines. COMT appears to be responsible for most of the primary inactivation of physiological concentrations of noradrenaline or adrenaline, but MAO functions as an effective alternate pathway (Kalsner & Nickerson, 1969a).

3. The increased sensitivity of vascular tissues from animals pretreated with reserpine is not due to a decreased ability to inactivate catecholamines (Kalsner & Nickerson, 1969b).

4. Potentiation of responses to sympathomimetic amines by cocaine is predominantly due to an increased responsiveness of effector cells, unrelated to any effect of this drug on mechanisms of amine disposition (Kalsner & Nickerson, 1969c).

5. Inhibition of amine inactivation by a haloalkylamine appears to be due to blockade of the access of amines to sites of enzymic degradation in effector cells, and may be associated with a decreased intracellular volume of distribution of these amines. This appears to be adequate to explain the reported augmentation by haloalkylamines of the venous effluent output of noradrenaline during sympathetic nerve stimulation, which is probably greater than that produced by cocaine.

6. Potentiation of responses to sympathomimetic amines by cocaine and other agents is an unreliable index of inhibition of amine inactivation.

These findings and the published observations of others appear to be most adequately accommodated within the following concept of mechanisms terminating the action of the adrenergic mediator.

A major part of the mediator released by sympathetic nerve activity participates in the activation of tissue receptors and its actions are terminated by movement away from the region of the receptors. A portion of the mediator diffuses through the interstitial fluid and is removed by the circulation. This pathway probably accounts for only a small part of the amine released by physiological frequencies of nerve activity (Brown & Gillespie, 1957). Of the remainder, a portion is taken up by adrenergic nerves, but the largest part of the released mediator is distributed in the intracellular water of nonnervous elements of the tissues. It is this movement which terminates receptor activation and which is inhibited by haloalkylamines. Although it is assumed that distribution in cell water is the immediate event which terminates the action of noradrenaline, the capacity of this process appears to be limited, and its contribution to the body economy is dependent on the associated enzymic processes for definitive inactivation of the mediator. O-methylation is the dominant primary enzymic step in the inactivation of physiological amounts of mediator, but MAO appears to function " in series " with COMT as an effective alternate pathway of disposition.

This work was supported by grants-in-aid from the Medical Research Council of Canada and the Manitoba Heart Foundation. The authors are indebted to Mr. Gordon N. Boyd for valuable technical assistance. Data from a thesis presented to the University of Manitoba by S. K. in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June 1966. Work done during support of S. K. by training grant 5 T01 GM 8154 from the National Institute of General Medical Sciences, U.S. Public Health Service.

REFERENCES

AXELROD, J. (1960). Discussion. In *Ciba Foundation Symposium on Adrenergic Mechanisms*, ed. Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M., pp. 56–60. London: Churchill.

BACQ, Z. M., BROWN, G. L. & FERRY, C. B. (1960). Action du pyrogallol sur la quantité de noradrénalin libérée par la stimulation des nerfs spléniques. *C.r. hebd. Séanc. Acad. Sci., Paris*, **250**, 194–195.

BLAKELEY, A. G. H., BROWN, G. L. & FERRY, C. B. (1963). Pharmacological experiments on the release of the sympathetic transmitter. *J. Physiol., Lond.*, **167**, 505–514.

BROWN, G. L. (1960). Release of sympathetic transmitter by nerve stimulation. In *Ciba Foundation Symposium on Adrenergic Mechanisms*, ed. Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M., pp. 116–130. London: Churchill.

BROWN, G. L. & GILLESPIE, J. S. (1957). The output of sympathetic transmitter from the spleen of the cat. *J. Physiol., Lond.*, **138**, 81–102.

BOULLIN, D. J., COSTA, E. & BRODIE, B. B. (1967). Evidence that blockade of adrenergic receptors causes overflow of norepinephrine in cats colon after nerve stimulation. *J. Pharmac. exp. Ther.*, **157**, 125–134.

DENGLER, H. J., SPIEGEL, H. E. & TITUS, E. O. (1961). Effects of drugs on uptake of isotopic norepinephrine by cat tissues. *Nature, Lond.*, **191**, 816–817.

EISENFELD, A. J., KRAKOFF, L., IVERSEN, L. L. & AXELROD, J. (1967). Inhibition of the extraneuronal metabolism of noradrenaline in the isolated heart by adrenergic blocking agents. *Nature, Lond.*, **213**, 297–298.

FARRANT, J., HARVEY, J. A. & PENNEFATHER, J. N. (1964). The influence of phenoxybenzamine on the storage of noradrenaline in rat and cat tissues. *Br. J. Pharmac. Chemother.*, **22**, 104–112.

FURCHGOTT, R. F. (1960). Receptors for sympathomimetic amines. In *Ciba Foundation Symposium on Adrenergic Mechanisms*, ed. Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M., pp. 246–252. London: Churchill.

FURCHGOTT, R. F. & KIRPEKAR, S. M. (1960). Release of catecholamines in heart by β-haloalkylamines and by bretylium. *Pharmacologist*, **2**, 93.

FURCHGOTT, R. F., KIRPEKAR, S. M., RIEKER, M. & SCHWAB, A. (1963). Actions and interaction of norepinephrine, tyramine and cocaine on aortic strips of rabbit and left atria of guinea pig and cat. *J. Pharmac. exp. Ther.*, **142**, 39–58.

GILLESPIE, J. S. & KIRPEKAR, S. M. (1965). The inactivation of infused noradrenaline by the cat spleen. *J. Physiol., Lond.*, **176**, 205–227.

HARVEY, S. C. & NICKERSON, M. (1954). Reactions of Dibenamine and some congeners with substances of biological interest in relation to the mechanism of adrenergic blockade. *J. Pharmac. exp. Ther.*, **112**, 274–290.

HERTTING, G. & AXELROD, J. (1961). Fate of tritiated noradrenaline at the sympathetic nerve-endings. *Nature, Lond.*, **192**, 172–173.

HERTTING, G., AXELROD, J., KOPIN, I. J. & WHITBY, L. G. (1961). Lack of uptake of catecholamines after chronic denervation of sympathetic nerves. *Nature, Lond.*, **189**, 66.

HERTTING, G., AXELROD, J. & WHITBY, L. G. (1961). Effect of drugs on the uptake and metabolism of H^3-norepinephrine. *J. Pharmac. exp. Ther.*, **134**, 146–153.

KALSNER, S. & NICKERSON, M. (1968a). A method for the study of mechanisms of drug disposition in smooth muscle. *Can. J. Physiol. Pharmac.*, **46**, 719–730.

KALSNER, S. & NICKERSON, M. (1968b). Disposition of phenylephrine in vascular tissue, determined by the oil immersion technique. *J. Pharmac. exp. Ther.*, **163**, 1–10.

KALSNER, S. & NICKERSON, M. (1969a). Disposition of norepinephrine and epinephrine in vascular tissue, determined by the technique of oil immersion. *J. Pharmac. exp. Ther.*, in the Press.

KALSNER, S. & NICKERSON, M. (1969b). Effects of reserpine on the disposition of sympathomimetic amines in vascular tissue. *Br. J. Pharmac.*, **35**, 394–405.

KALSNER, S. & NICKERSON, M. (1969c). Mechanism of cocaine potentiation of responses to amines. *Br. J. Pharmac.*, **35**, 428–439.

KIRPEKAR, S. M. & CERVONI, P. (1963). Effect of cocaine, phenoxybenzamine and phentolamine on the catecholamine output from spleen and adrenal medulla. *J. Pharmac. exp. Ther.*, **142**, 59–70.

KOPIN, I. J. (1964). Storage and metabolism of catecholamines: the role of monoamine oxidase. *Pharmac. Rev.*, **16**, 179–191.

MALMFORS, T. (1965). Studies on adrenergic nerves. The use of rat and mouse iris for direct observations on their physiology and pharmacology at cellular and subcellular levels. *Acta physiol. scand.*, **64**, *Suppl.* **248**, 1–95.

MUSCHOLL, E. (1961). Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. *Br. J. Pharmac. Chemother.*, **16**, 352–359.

PATON, W. D. (1960). Discussion. In *Ciba Foundation Symposium on Adrenergic Mechanisms*, ed. Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M., pp. 124–130. London: Churchill.

ROSELL, S., KOPIN, I. J. & AXELROD, J. (1963). Fate of H^3-noradrenaline in skeletal muscle before and following sympathetic stimulation. *Am. J. Physiol.*, **205**, 317–321.

STAFFORD, A. (1963). Potentiation of some catecholamines by phenoxybenzamine, guanethidine and cocaine. *Br. J. Pharmac. Chemother.*, **21**, 361–367.

THOENEN, H., HÜRLIMANN, A. & HAEFELY, W. (1963). Removal of infused norepinephrine by the cat's spleen; mechanism of its inhibition by phenoxybenzamine. *Experientia*, **19**, 601–602.

THOENEN, H., HUERLIMANN, A. & HAEFELY, W. (1966). Interaction of phenoxybenzamine with guanethidine and bretylium at the sympathetic nerve endings of the isolated perfused spleen of the cat. *J. Pharmac. exp. Ther.*, **151**, 189–195.

VAN ZWIETEN, P. A., WIDHALM, S. & HERTTING, G. (1965). Influence of cocaine and of pretreatment with reserpine on the pressor effect and the tissue uptake of injected dl-catecholamines-2-H^3. *J. Pharmac. exp. Ther.*, **149**, 50–56.

WHITBY, L. G., HERTTING, G. & AXELROD, J. (1960). Effect of cocaine on the disposition of noradrenaline labelled with tritium. *Nature, Lond.*, **187**, 604–605.

WHITBY, L. G., AXELROD, J. & WEIL-MALHERBE, H. (1961). The fate of H^3-norepinephrine in animals. *J. Pharmac. exp. Ther.*, **132**, 193–201.

WOLFE, D. E., POTTER, L. T., RICHARDSON, K. C. & AXELROD, J. (1962). Localizing tritiated norepinephrine in sympathetic axons by electron microscopic autoradiography. *Science, N.Y.* **138**, 440–442.

(*Received August* 12, 1968)