Br. J. Pharmac. (1968), **34**, 88–98.

Restoration of the chronotropic effect of tyramine on rat atria after reserpine

M. F. MURNAGHAN

Department of Physiology, University College, Dublin, Ireland

1. The restoration by various sympathomimetic amines of the chronotropic response to tyramine was studied on the isolated atria of rats pretreated with reserpine. The atria were exposed to the "restorative" sympathomimetic amine for 10 min, washed over a period of 1 hr and then tested with 10 μM tyramine. The effect of noradrenaline, dopamine and norphenylephrine before and after inhibition of monoamine oxidase by 0.5 mM iproniazid were compared with their α-methyl and N-alkyl analogues in their ability to restore the chronotropic response to tyramine.

2. Noradrenaline and adrenaline restored the chronotropic response to tyramine, the degree of restoration depending on the concentration of the restorative amine used. Noradrenaline after iproniazid and α-methylnoradrenaline were equipotent and were about 1,000 times more active than noradrenaline where monoamine oxidase was not inhibited. Dopamine, epinine, norphenylephrine, phenylephrine, octopamine, synephrine and isoprenaline in the absence of monoamine oxidase inhibition had no effect. Dopamine after iproniazid and α-methyldopamine were equipotent and were about 1/10 as active as α-methylnoradrenaline. Norphenylephrine after iproniazid and metaraminol were equipotent and were about 1/500 as active as α-methylnoradrenaline. Octopamine after iproniazid was even less active. The N-methylated analogues were about 1/10 as active as their nor-compounds but the N-isopropyl analogue, isoprenaline, was devoid of activity.

3. Dopamine after iproniazid and α-methyldopamine were inactive if a dopamine-β-hydroxylase inhibitor, disulphiram or sodium diethyldithiocarbamate, was present.

4. It is concluded that, in atria of reserpinized rats, (*a*) protection from monoamine oxidase increases ; (*b*) N-substitution decreases ; and (*c*) hydroxyl groups at the β-carbon and ring positions 3 and 4 increase the capabilities of a sympathomimetic amine to restore the chronotropic response to tyramine.

Tyramine is classified as an indirectly acting sympathomimetic amine because it is believed to exert its effect by liberating noradrenaline from its store in sympathetic nerve fibres. The liberated noradrenaline, a directly acting amine, can then

attach to the adrenergic receptor and produce the pharmacological response. When the noradrenaline store is depleted by reserpine, the effect of tyramine is reduced or abolished. The response to tyramine after reserpine in the whole animal or isolated tissue can be restored, respectively, by an infusion of, or by previous exposure to, noradrenaline (Burn & Rand, 1958; Crout, Muskus & Trendelenburg, 1962); it is believed that the noradrenaline enters the store and is then available for release by tyramine.

In an earlier study (Murnaghan, 1965) it had been found that the pressor response to tyramine in reserpinized cats was restored by an infusion of directly acting norsympathomimetic amines but not by their N-methylated analogues. Furthermore, α-methylnoradrenaline was the most active compound tested, which suggested that monoamine oxidase plays a part at storage sites of sympathomimetic amines. The study, however, possessed the disadvantages that the pressor response is due to the algebraic summation of a variety of different effects and in the whole cat catechol-*O*-methyltransferase apparently plays an important part in the metabolism of catecholamines (Whitby, Axelrod & Weil-Malherbe, 1961; Axelrod, Weil-Malherbe & Tomchick, 1959).

Rat isolated atria were therefore selected for study of the chronotropic response because in this tissue the metabolism of sympathomimetic amines occurs largely by way of monoamine oxidase, catechol-*O*-methyltransferase playing only an insignificant part (Goldberg & Shideman, 1962); moreover the heart of this species has been extensively used in studies of uptake, storage, turnover rates, release and metabolism of sympathomimetic amines (Iversen, 1963; Iversen, Glowinski & Axelrod, 1965; Montanari, Costa, Beaven & Brodie, 1963; Nash, Costa & Brodie, 1964; Kopin, Hertting & Gordon, 1962).

The procedure followed that described by Crout *et al.* (1962) for the isolated atria of the guinea-pig. The atria of the rat, however, possesses the advantage that their chronotropic response to sympathomimetic amines is greater than that of the guinea-pig. The atria of reserpinized rats were exposed to varying concentrations of sympathomimetic amines and then tested subsequently with tyramine to determine the degree of recovery of the chronotropic response to the latter. In order to determine the significance of the role of monoamine oxidase in these replenishment studies, either the enzyme was inhibited by iproniazid or the α-methyl derivatives of the sympathomimetic amines, which are resistant to the enzyme, were used. In addition, by selecting sympathomimetic amines of varying chemical structure an estimate of the relative importance of the various groupings on the molecule have been investigated, for example, beta carbon- and ring-hydroxyls, and N-alkylation.

Methods

The rats were injected intraperitoneally with reserpine (5 mg/kg). The rat was anaesthetized with ether 20 hr later, the throat cut and the atria removed and suspended in Locke solution of the following composition (g/l.): NaCl 9, KCl 0.42, $CaCl_2$ 0.24, dextrose 1 and $NaHCO_3$ 0.5. The solution was gassed with 1% carbon dioxide in oxygen and maintained at 30° C. The atria were attached to a hair-spring lever (Reiter, 1956), the excursions of which actuated a Thorp impulse counter to record the rate of beating. With the aid of a timing unit (Palmer) the counter was disengaged for 2 sec in each 10 sec period.

The atria were left for 1 hr to allow the rate to attain a steady level and then the chronotropic response to a bath concentration of 1 and 10 μM tyramine was tested. Each concentration was permitted to act for 5–7 min. The control chronotropic response in ninety reserpinized atria to 1 and 10 μM tyramine was 0.04 ± 0.4 and 3.7 ± 0.9 beats/min (mean $\pm$ S.E.M.) respectively; in twenty-two atria from non-reserpinized rats, the respective values were 39 ± 5.5 and 129 ± 7.7.

After removal of the tyramine, the lowest concentration of the "restorative" amine being tested was added to the bath; ethylenediamine tetraacetic acid in a concentration of 3 μM was included to protect the amine from oxidation. After exposure to the amine for 10 min, the preparation was washed 10 times over a period of 50 min. At the end of this time the atria had returned to the control rate and the chronotropic response to 1 and 10 μM tyramine was again tested. The effect of exposure to increasing concentrations of the "restorative" sympathomimetic amine on the subsequent tyramine response was then determined. Usually three increasing concentrations could be used during a single experiment; preliminary experiments indicated that the tyramine response after the second or third higher concentration did not differ significantly from the response after the same concentration when applied singly to the preparation.

In order to inhibit monoamine oxidase, the atria were exposed to 0.5 mM iproniazid for 30 min and then washed repeatedly. The tyramine response was invariably partially restored by such treatment, but this had usually disappeared on the second or third trial with tyramine. The atria were discarded if the tyramine response after iproniazid remained elevated.

Dose-response curves for noradrenaline, dopamine and metaraminol were obtained with atria from reserpinized rats after pretreatment with iproniazid. Tyramine, 1 μM concentration, was included to simulate the conditions present in the release experiments described above before the tyramine strength was increased to 10 μM.

Results

Table 1 and Figs. 1 and 2 summarize the results of the chronotropic response to tyramine after exposure of the rat atria for 10 min to the indicated concentration of the "restorative" sympathomimetic amine followed by repeated washing for 50 min. Only the response to a bath concentration of 10 μM tyramine is included in Fig. 2 and Table 1 because the response to 1 μM was often unaffected, particularly after exposure to the lower concentrations of the "restorative" amine.

In the last two columns of Table 1 and in the text the terms "potency" and "effectiveness" refer respectively to the relative concentrations of the sympathomimetic amines required to restore the tyramine chronotropic response and the relative magnitude of the response. They correspond to the respective terms "affinity" and "intrinsic activity" as described by Ariëns (1954), but the former terms are used in this paper in reference to the store while the latter had been introduced to apply to the receptor. To facilitate ready comparison of potency, the logarithm of the ratio of the concentration of noradrenaline without iproniazid (taken as standard) to that of the restorative amine being tested, producing equivalent effects, is listed in Table 1. Consequently a shift of potency of one unit indicates a ten-fold change. Effectiveness which indicates the expected maximum acceleration capable of being produced by 10 μM tyramine, compared with that in

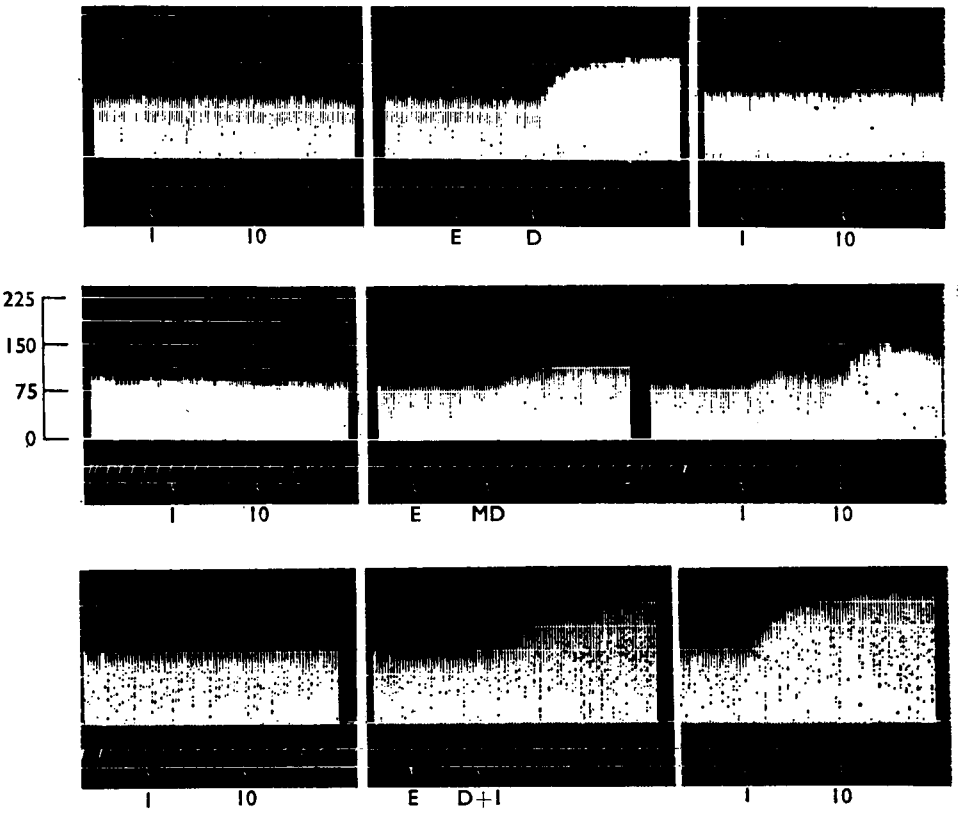

FIG. 1. Effect of dopamine and α-methyldopamine on the tyramine chronotropic effect of isolated atria of rats pretreated with reserpine. Upper record in each row, beats/min (scale on left); middle record, time in min; lower record, signal. The left column tracing in each row indicates the control responses to 1 and 10 μM tyramine, and the right column the tyramine responses after exposure of the atria to 3 μM dopamine (D) in the middle panel of the first row, 0.3 μM α-methyldopamine (MD) in the second row and 3 μM dopamine after 0.5 mM iproniazid (D+I) in the third row. E=3 μM disodium ethylenediamine tetraacetic acid.

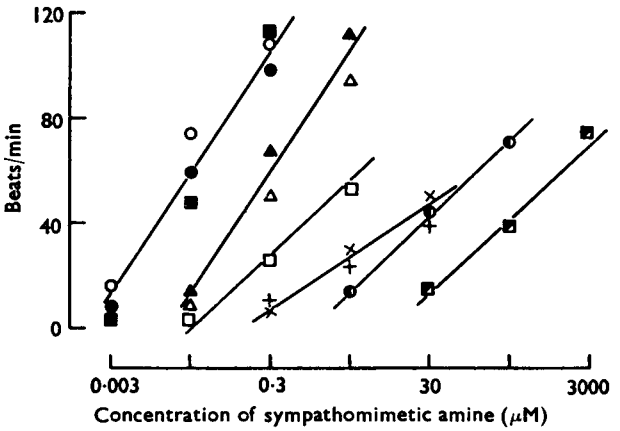

FIG. 2. Accelerator effect of 10 μM tyramine on isolated atria of reserpinized rats after previous exposure to various sympathomimetic amines. ◐, Noradrenaline; ○, noradrenaline + iproniazid (IPN); ●, α-methylnoradrenaline; ◪, adrenaline; □, adrenaline + IPN; ■, dihydroxyephedrine; △, dopamine + IPN; ▲, α-methyldopamine; ×, norphenylephrine + IPN; +, metaraminol.

the normal preparation (129 beats/min), is expressed in decimal fractions when less than unity. Concentrations of the restorative amine larger than the maximum listed in Table 1 could not be used because, although the effect was slightly larger, the atria, after repeated washing, would not resume their control rate of beating within the period of approximately 50 min before testing with tyramine. Consequently the expected maximum response has been estimated in the following manner. The line relating response to log dose is projected to cut the ordinate at 1,000 times the dose (highest) producing the lowest detectable response. The corresponding acceleration value is considered, and was confirmed in a few experiments, to be the expected maximum response and is expressed as a fraction of the response produced by tyramine on unreserpinized atria. In those cases where high concentrations resulted in decreasing responses, the maximum acceleration attained is the value recorded.

As the slope of the regression lines for noradrenaline and dopamine after iproniazid, α-methylnoradrenaline, α-methyldopamine and dihydroxyephedrine were similar, all lines were plotted with a common regression coefficient (*b*) of 46. The values for noradrenaline after iproniazid, α-methylnoradrenaline and dihydroxyephedrine apparently belong to the same population, while those for dopamine after iproniazid and α-methyldopamine belong to a second. Consequently a single regression line has been drawn for the first three and a second one for the other two compounds. Similarly the regression lines for noradrenaline and adrenaline alone, and adrenaline after iproniazid, have been drawn with the same slope ($b=27$). The data of norphenylephrine after iproniazid have been pooled with that of metaraminol to construct the common regression line, $b=20$.

TABLE 1. *Chronotropic response (increased beats/min) of atria from reserpinized rats to* 10 μM *tyramine after previous exposure to various sympathomimetic amines*

Group	Control (normal) 129±7·7 Control (reserpinized) 3·7±0·9	No. of trials	Micromolar concentration 0·003	0·03	0·3	3	30	300	3,000	Potency	Effectiveness
1	Noradrenaline	4				13	45*	70*		0	0·8
	Noradrenaline+iproniazid	3	16	75‡	108†					3	1
	α-Methylnoradrenaline	4	5	59	97					3	1
	Adrenaline	5					16*	40*	77	−1	0·8
	Adrenaline+iproniazid	7		3‡	27†‡	54				1·5	0·6
	Dihydroxyephedrine	3	4	48	113‡					3	1
	Isoprenaline	3			4	0	0			—	0
	Isoprenaline+iproniazid	3		7	4	6	0			—	0
2	Dopamine	4					3	3	7	—	0
	Dopamine+iproniazid	6		9	51	94				2	1
	α-Methyldopamine	5		11	73†	107†				2	1
	Epinine	2					0	0	0	—	0
	Epinine+iproniazid	4		5	9†	62†				1·5	1
3	Norphenylephrine	2					6	6		—	0
	Norphenylephrine+iproniazid	3			7	30*	51†			0·3	0·6
	Metaraminol	6			12	24	41			0·2	0·5
	Phenylephrine	2					7	0	0	—	0
	Phenylephrine+iproniazid	6			0	15*	13†	7		−1	0·1
4	Octopamine+iproniazid	3			5	23*	18	16		0	0·15
	Synephrine+iproniazid	3				6*	12	22	5	−2	0·15

Significant difference between amines within group: *$P<0.05$; †$P<0.01$; ‡$P<0.001$.

Effect of norsympathomimetic amines in the absence and presence of monoamine oxidase inhibition

Exposure of atria to noradrenaline in concentrations of 3–300 μM restored the chronotropic response to tyramine, the degree being proportional to the concentration of the noradrenaline used. When noradrenaline was protected from monoamine oxidase either by inhibition of the enzyme with iproniazid, or by using its alpha-carbon methyl analogue, α-methylnoradrenaline (cobefrine), the potency in restoring the tyramine response was increased approximately 1,000-fold. The maximal attainable accelerated rate (effectiveness) produced by tyramine was also increased.

The use of dopamine, norphenylephrine and their alpha-methylated analogues, and octopamine (norsynephrine) permitted a study of the influence of the beta carbon and ring-hydroxyl groups. In the absence of monoamine oxidase inhibition, dopamine, norphenylephrine and octopamine were ineffective. However, dopamine after iproniazid and alpha-methyldopamine restored the response to tyramine. They were equipotent and were about 1/10 as active as α-methylnoradrenaline; their effectiveness was similar to that of the latter. Norphenylephrine after iproniazid and its α-methyl derivative metaraminol were approximately equipotent and were about 500 times less active than α-methylnoradrenaline; their effectiveness was lower than that of the catecholamines. Octopamine seems to be less potent than norphenylephrine (see Table 1) but in view of its lower effectiveness a comparison of potency is probably unjustified.

In order to exclude the possibility that it was the tyramine which was destroyed by the monoamine oxidase, rather than the " stored " amine, paredrine was used in a few experiments as the releasing agent. This substance which is α-methyltyramine is resistant to destruction by the enzyme. While iproniazid pretreatment potentiated the effect of tyramine after dopamine about 100,000-fold, after dopamine without iproniazid the effect of paredrine was only 30 times greater than that of tyramine.

Effect of nitrogen alkylated compounds

Adrenaline in concentrations of 30, 300 and 3,000 μM also restored the response to tyramine. As the effects at the first two concentrations were statistically less than those for noradrenaline, the former compound seems to be approximately 10 times less potent than the latter. Inhibition of monoamine oxidase increased the potency of adrenaline about 300 times: after iproniazid, adrenaline was still significantly less active than noradrenaline. On the other hand, dihydroxyephedrine was not significantly different from α-methylnoradrenaline.

Isoprenaline before or after iproniazid was completely inactive as a restorative agent.

Epinine, phenylephrine and synephrine, like their nor-analogues dopamine, norphenylephrine and octopamine respectively, were inactive if monoamine oxidase was not inhibited. After pretreatment with iproniazid, however, they had a restorative effect but were significantly less potent than their nor-analogues. Concomitantly the effectiveness of the phenolic compounds was less than that of the catecholic.

Figure 1 shows that the chronotropic response to tyramine after exposure either to dopamine following iproniazid or to α-methyldopamine was greater than that produced directly by the restorative amine itself. This was particularly apparent

with the lower test concentrations of the restorative amine. This phenomenon was always seen with dopamine or epinine after iproniazid and α-methyldopamine but not with the other sympathomimetic amines which were tested, which suggests that perhaps these compounds were being converted, in part, into noradrenaline, adrenaline and α-methylnoradrenaline respectively before being released by tyramine. An attempt was made to study this phenomenon further in the following manner. The concentrations of noradrenaline, dopamine and metaraminol required to accelerate reserpinized atria by 40 and 80 beats/min, previously treated with iproniazid, were estimated from dose-response curves (direct effect) and are recorded in Table 2. In order to simulate the conditions during release by tyramine, this substance was added to the bath to give a concentration of 1 μM during the determination of the dose-response curve. This was done because the same concentration of tyramine would have been present in the bath during the "release" experiments before its concentration was increased to 10 μM. From Fig. 2 the concentrations of these three amines required to cause a similar acceleration after subsequent release by tyramine (release effect) are also recorded in Table 2. While the "direct" and "release" concentrations of noradrenaline to produce an equivalent acceleration were similar, the "release" concentration for dopamine was about 7 times less than the direct, suggesting a greater potency of the released dopamine. Conversely, metaraminol was less potent on release compared with its direct effect, suggesting a decreased quantitative release compared with that of noradrenaline. Several authors (Goldstein, Anagnoste, Lauber & McKereghan, 1964; Musacchio, Kopin & Snyder, 1964) have shown that disulphiram inhibits the β-hydroxylation of

TABLE 2. *Mean concentration (μM) of sympathomimetic amine required to produce in rat atria an acceleration in rate of 40 and 80 beats/min, directly (dose-response curve) or after release by tyramine*

	Beats/min			
	40		80	
Amine	Direct	Release	Direct	Release
Noradrenaline	0·013	0·013 ($P>0·9$)	0·075	0·053 ($P>0·4$)
Dopamine	1·2	0·16 ($P<0·02$)	6·1	0·84 ($P<0·05$)
Metaraminol	1·25	10·2 ($P<0·001$)	4·5	—

Significance value (P) between direct and release responses in parenthesis.

TABLE 3. *Chronotropic response (mean±S.E.M. beats/min) of atria from reserpinized rats to 10 μM tyramine pretreated with iproniazid and previously exposed to dopamine and a dopamine β-hydroxylase inhibitor*

Dopamine β-hydroxylase inhibitor	Dopamine μM	
	0·3	3
Control	51±11 (5)	91±14 (5)
33 μM Sodium diethyldithiocarbamate	9±3·2 (4) ($P=0·02$)	7±2·7 (4) ($P=0·001$)
33 μM Disulphiram	11±8·6 (4) ($P=0·05$)	27±4·6 (4) ($P<0·01$)

Number of experiments and significance levels (Student's t test) in parenthesis.

dopamine. The active inhibiting agent appears to be diethyldithiocarbamate, to which disulphiram is reduced (Goldstein, Lauber & McKereghan, 1965). Consequently the restorative effect of dopamine, after monoamine oxidase inhibition, on the tyramine chronotropic response was tested in the presence and absence of 33 μM disulphiram or sodium diethyldithiocarbamate. When either substance was present the restorative effect of dopamine was significantly decreased (see Table 3).

Discussion

The results of this and previous studies indicate that a variety of factors determine the optimal conditions for attachment of a directly acting sympathomimetic amine at the store so that it may be available in an active form for subsequent release by tyramine.

Kopin & Gordon (1963) have shown that noradrenaline is preferentially metabolized by monoamine oxidase in the reserpinized animal. As previous exposure of sympathetically innervated tissue to noradrenaline partially refills the stores depleted by reserpine, and the action of tyramine requires the release of such stored noradrenaline, then the protection of the stored noradrenaline from monoamine oxidase should increase the pharmacological effect of the tyramine. This study of the chronotropic effect of tyramine on isolated rat atria and the previous ones of the pressor effect of tyramine on the whole rat (Torchiana, Wenger, Stavorski, Ludden & Stone, 1966) and cat (Murnaghan, 1965) clearly indicate that protection of the stored amine from monoamine oxidase potentiates its power in restoring the response to tyramine as was originally demonstrated by Furchgott, Kirpekar, Rieker & Schwab (1963). Apparently the tissue of the reserpinized animal can take up the restorative amine but it can only be retained if it is protected from oxidative deamination by the monoamine oxidase located in close proximity to the storage granules (Potter & Axelrod, 1963). This study on the rat atria clearly indicates that the increased potency of α-methylated compounds is due to their built-in protection from monoamine oxidase because noradrenaline, dopamine and norphenylephrine after monoamine oxidase inhibition with iproniazid produced an identical effect to their α-methylated derivatives. Moreover the results with paredrine show that protection from monoamine oxidase of the " stored," rather than of the " releasing," amine is the significant mechanism in the restoration of the tyramine response.

In the whole cat where catechol-*O*-methyltransferase plays a significant role in the metabolism of catecholamines (Whitby *et al.*, 1961), protection of noradrenaline from monoamine oxidase caused less potentiation (3 times) than in the isolated rat atria (1,000 times), where the metabolism of sympathomimetic amines occurs almost exclusively by way of monoamine oxidase (Goldberg & Shideman, 1962). In the whole rat, where catechol-*O*-methyltransferase contributes significantly in the metabolism (Kopin & Gordon, 1963), methylation of the alpha-carbon increased the potency of noradrenaline 30 times in restoring the pressor response to tyramine (Torchiana *et al.*, 1966), so that after reserpinization monoamine oxidase in this species must play a greater part than in the cat. Apparently this enzyme is also of lesser importance in the dog because α-methylation did not potentiate the effect of noradrenaline or dopamine in restoring the tyramine pressor response (Torchiana *et al.*, 1966) or the chronotropic response to sympathetic nerve stimulation (Conradi, Gaffney, Fink & Vangrow, 1965). The inability of dopamine, in the absence of

monoamine oxidase inhibition, to restore the tyramine response on the rat atria is probably the result of the high affinity of the enzyme for this compound (Blaschko, Richter & Schlossmann, 1937 ; Zeller, 1959) ; in the cat and dog, where this enzyme plays a less important part, dopamine was moderately active. These findings suggest that the steps taken for the protection of the stored amine should therefore take cognizance of the catabolic pathways involved, which clearly vary with the tissue and species being investigated.

When the direct chronotropic response of the rat atria to dopamine or epinine after iproniazid, or to α-methyldopamine, is compared with the subsequent response due to release by tyramine, the latter is always greater than the former. This phenomenon was never seen with any of the other sympathomimetic amines tested. Furthermore, when the dose-response curves of dopamine and noradrenaline on the rat atria after iproniazid are compared, the former is found to possess about 1/80 the potency of the latter. When the concentrations of the two amines required to produce equivalent restoration of the response to tyramine are compared, however, the ratio is found to be about 1/12. These findings suggest that the dopamine when released is in a more active form than when applied directly. Musacchio, Goldstein, Anagnoste, Poch & Kopin (1966) have demonstrated the uptake by rat heart of tritiated noradrenaline after an injection of tritiated dopamine. Dopamine-β-hydroxylase, which converts dopamine to noradrenaline, being a copper-bearing protein, is inhibited by a variety of copper-chelating agents. Among these, disulphiram (Antabuse) was found to be effective *in vitro* and *in vivo* (Musacchio *et al.*, 1964 ; Goldstein *et al.*, 1964). Musacchio, Bhagat, Jackson & Kopin (1966) have shown that the restorative power of dopamine on the tyramine response in cats is reduced by disulphiram ; this has been confirmed in rat atria in this study. As the amine storage granules isolated from rat hearts have been shown to contain most of the dopamine-β-hydroxylase (Potter & Axelrod, 1963), the evidence is strongly in favour that dopamine is converted to noradrenaline at this site.

While hydroxylation at the β-carbon of dopamine probably occurs in rat atria, there is no evidence in these experiments to indicate that significant hydroxylation occurs at the 3- or 4- positions on the benzene ring to convert octopamine or norphenylephrine, respectively, to noradrenaline. It has been shown that tyramine is readily converted in tissues to octopamine (Carlsson & Waldeck, 1963) but only traces are converted slowly to noradrenaline (Creveling, Levitt & Udenfriend, 1962), which indicates that ring hydroxylation of sympathomimetic amines plays an insignificant part in biological synthesis. Metaraminol (α-methylnorphenylephrine) when applied directly to the atria was about 1/80 as potent as noradrenaline but only about 1/500 as potent in restoring the response to tyramine. This suggested that not only was this compound not converted to a more active one but also that less was released, or was available for release, when compared with noradrenaline.

In this and previous investigations (Trendelenburg & Crout, 1964 ; Murnaghan, 1965) the N-methylated derivatives have been found to be weaker than their nor-compounds in restoring the response to tyramine, and the isopropyl analogue was completely inactive. Direct studies on storage vesicles indicate that the tertiary nitrogen is essential for binding (dihydroxymandelic acid is not retained), and increasing the N-substitution decreases binding (Potter, 1966). It therefore seems plausible that one mechanism concerned with the binding of the sympathomimetic amine at the storage sites involves ionic pairing of the protonated ammonium head

of the amine with an anionic site, probably phosphatic, as was earlier suggested (Murnaghan, 1965); this hypothesis had previously been proposed by Belleau (1960) to account for attachment of catecholamines at the α-receptor. Consequently substitution on the nitrogen of the amine might be expected to interfere with ionic pairing caused by steric hindrance of the substituent group.

Belleau further suggested that attachment at the receptor might include a chelating action involving the ring hydroxyls. It has been demonstrated that only compounds with at least two hydroxyl groups—dopamine, norphenylephrine and octopamine, or their α-methylated derivatives—are retained in considerable amounts in the noradrenaline storage vesicles (Musacchio, Kopin & Weise, 1965): of these compounds the catecholic is bound more than the phenolic. While a chelating action could account for binding of catecholic compounds at the store it is highly improbable that such a mechanism could account for the attachment of di-hydroxyl compounds where one hydroxyl is on the β-carbon and the second on the ring, because of the interatomic distances involved. A single hydroxyl group apparently can contribute a weak attachment at the effector receptor because the laevo-rotatory form of phenylethanolamine (Burn & Rand, 1958) and metatyramine (Trendelenburg, Muskus, Fleming & Gomez de La Sierra, 1962) have been demonstrated to possess a weak direct effect at this site. Apparently a single hydroxyl group can also assist in binding at the storage site because phenylethanolamine and α-methyltyramine exhibit a small but measurable retention in microsomes (Potter, 1966). Octopamine and norphenylephrine with both a beta carbon and a ring-hydroxyl would attach more effectively at both sites and consequently are more active pharmacologically and are retained better by the storage vesicles than the mono-hydroxyl compounds. The closer the proximity of the two hydroxyls, the firmer is the attachment, because norphenylephrine is more active (Lands, 1949; Ariëns & Simonis, 1960) and is retained better by the vesicles than octopamine (Kopin, 1966).

This similarity in structure between the effector receptor site and the main site of inactivation (uptake into the storage vesicles) for noradrenaline, has its counterpart in the case of acetylcholine, where a structural resemblance between its effector receptor and acetylcholinesterase (the main inactivation site) has been suggested.

This work was supported by a grant from the Medical Research Council of Ireland. I thank Miss B. Dooley for her technical assistance, and Dr. S. Archer, of the Sterling-Winthrop Research Institute, Rensselaer, New York, for supplying me with some of the sympathomimetic amines used in this study.

REFERENCES

ARIËNS, E. J. (1954). Affinity and intrinsic activity in the theory of competitive inhibition. *Archs int. Pharmacodyn. Thér.*, **99**, 32–50.

ARIËNS, E. J. & SIMONIS, A. M. (1960). Autonomic drugs and their receptors. *Archs int. Pharmacodyn. Thér.*, **127**, 479–496.

AXELROD, J., WEIL-MALHERBE, H. & TOMCHICK, R. (1959). The physiological disposition of H^3-epinephrine and its metabolite metanephrine. *J. Pharmac. exp. Ther.*, **127**, 251–256.

BELLEAU, B. (1960). Relationships between agonists, antagonists and receptor sites. In *Adrenergic Mechanisms*, ed. Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M., pp. 223–244. London: J. A. Churchill Ltd.

BLASCHKO, H., RICHTER, D. & SCHLOSSMANN, H. (1937). The oxidation of adrenaline and other amines. *Biochem. J.*, **31**, 2187–2196.

BURN, J. H. & RAND, M. J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol., Lond.*, **144**, 314–336.

CARLSSON, A. & WALDECK, B. (1963). β-Hydroxylation of tyramine *in vivo*. *Acta pharmac. tox.*, **20**, 371–375.

CONRADI, E. C., GAFFNEY, T. E., FINK, D. A. & VANGROW, J. S. (1965). Reversal of sympathetic nerve blockade: A comparison of dopa, dopamine and norepinephrine with their α-methylated analogues. *J. Pharmac. exp. Ther.*, **150**, 26–33.

CREVELING, C. R., LEVITT, M. & UDENFRIEND, S. (1962). An alternative route for the biosynthesis of norepinephrine. *Life Sci.*, **1**, 523–526.

CROUT, J. R. & MUSKUS, A. J. & TRENDELENBURG, U. (1962). Effect of tyramine on isolated guinea-pig atria in relation to their noradrenaline stores. *Br. J. Pharmac. Chemother.*, **18**, 600–611.

FURCHGOTT, R. F., KIRPEKAR, S. M., RIEKER, M. & SCHWAB, A. (1963). Actions and interactions 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.

GOLDBERG, N. D. & SHIDEMAN, F. E. (1962). Species difference in the cardiac effects of a monoamine oxidase inhibitor. *J. Pharmac. exp. Ther.*, **136**, 142–151.

GOLDSTEIN, M., ANAGNOSTE, B., LAUBER, E. & MCKEREGHAN, M. R. (1964). Inhibition of dopamine β-hydroxylase by disulphiram. *Life Sci.*, **3**, 763–767.

GOLDSTEIN, M., LAUBER, E. & MCKEREGHAN, M. R. (1965). Studies in the purification and characterization of 3,4-dihydroxyphenylethylamine-β-hydroxylase. *J. biol. Chem.*, **240**, 2066–2072.

IVERSEN, L. L. (1963). The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmac. Chemother.*, **21**, 523–537.

IVERSEN, L. L., GLOWINSKI, J. & AXELROD, J. (1965). The uptake and storage of H^3-norepinephrine in the reserpine-pretreated rat heart. *J. Pharmac. exp. Ther.*, **150**, 173–183.

KOPIN, I. J. (1966). Biochemical aspects of release of norepinephrine and other amines from sympathetic nerve endings. In *Second Symposium on Catecholamines*, Milan, 1965, ed. Acheson, G. H. *J. Pharmac. exp. Ther.*, **18**, 513–523.

KOPIN, I. J. & GORDON, E. K. (1963). Metabolism of administered and drug-released norepinephrine-7-H^3 in the rat. *J. Pharmac. exp. Ther.*, **140**, 207–216.

KOPIN, I. J., HERTTING, G. & GORDON, E. K. (1962). Fate of norepinephrine-H^3 in the isolated rat heart. *J. Pharmac. exp. Ther.*, **138**, 34–40.

LANDS, A. M. (1949). The pharmacological activity of epinephrine and related dihydroxyphenylalkylamines. *Pharmac. Rev.*, **1**, 279–309.

MONTANARI, R., COSTA, E., BEAVEN, E. A. & BRODIE, B. B. (1963). Turn-over rates of norepinephrine in hearts of intact mice, rats and guinea pigs using tritiated norepinephrine. *Life Sci.*, **2**, 232–240.

MURNAGHAN, M. F. (1965). Potentiation of tyramine responses by nor-sympathomimetic amines in the reserpine-pretreated cat. *Archs int. Pharmacodyn. Thér.*, **155**, 332–355.

MUSACCHIO, J. M., BHAGAT, B., JACKSON, C. J. & KOPIN, I. J. (1966). The effect of disulphiram on the restoration of the response to tyramine by dopamine and α-methyldopa in the reserpine-treated cat. *J. Pharmac. exp. Ther.*, **152**, 293–297.

MUSACCHIO, J. M., GOLDSTEIN, M., ANAGNOSTE, B., POCH, G. & KOPIN, I. J. (1966). Inhibition of dopamine-β-hydroxylase by disulphiram *in vivo*. *J. Pharmac. exp. Ther.*, **152**, 56–61.

MUSACCHIO, J. M., KOPIN, I. J. & SNYDER, S. (1964). Effects of disulphiram on tissue norepinephrine content and subcellular distribution of dopamine, tyramine, and their β-hydroxylated metabolites. *Life Sci.*, **3**, 769–775.

MUSACCHIO, J. M., KOPIN, I. J. & WEISE, V. K. (1965). Subcellular distribution of some sympathomimetic amines and their β-hydroxylated derivatives in the rat heart. *J. Pharmac. exp. Ther.*, **148**, 22–26.

NASH, C. W., COSTA, E. & BRODIE, B. B. (1964). The actions of reserpine, guanethidine and metaraminol on cardiac catecholamine stores. *Life Sci.*, **3**, 441–449.

POTTER, L. T. (1966). Stores of norepinephrine in sympathetic nerves. In *Second Symposium on Catecholamines*, Milan, 1965, ed. Acheson, G. H. *J. Pharmac. exp. Ther.*, **18**, 439–451.

POTTER, L. T. & AXELROD, J. (1963). Properties of norepinephrine storage particles of the rat heart. *J. Pharmac. exp. Ther.*, **142**, 299–305.

REITER, M. (1956). Die Wirkung von Adrenalin, Veratramin und Chinidin auf die Reizbildung im Herzen. *Naunyn-Schmiedbergs Arch. exp. Path. Pharmak.*, **229**, 233–241.

TORCHIANA, M. L., WENGER, H. C., STAVORSKI, J., LUDDEN, C. T. & STONE, C. A. (1966). Effect of methyldopa and related agents on pressor responses to tyramine in reserpine-pretreated rats and dogs. *J. Pharmac. exp. Ther.*, **151**, 242–252.

TRENDELENBURG, U. & CROUT, J. R. (1964). The norepinephrine stores of isolated atria of guinea-pigs treated with reserpine. *J. Pharmac. exp. Ther.*, **145**, 151–161.

TRENDELENBURG, U., MUSKUS, A., FLEMING, W. W. & B. GOMEZ ALONSO DE LA SIERRA (1962). Modification by reserpine of the action of sympathomimetic amines in spinal cats; a classification of sympathomimetic amines. *J. Pharmac. exp. Ther.*, **138**, 170–180.

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

ZELLER, E. A. (1959). The role of amine oxidases in the destruction of catecholamines. In *Symposium on Catecholamines*, Baltimore, 1959, ed. Krayer, O. *Pharmac Rev.*, **11** (2), 387–393.

(*Received April* 22, 1968)