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An investigation of α-methyl amino-acids and their derivatives on isolated tissue preparations

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Summary

1. The ability of α-methyl amino-acids and their corresponding amines to restore the sympathomimetic actions of tyramine, and the uptake of the amino-acids and the amines, were studied in isolated tissue preparations obtained from reserpine pretreated animals.

2. Tyramine relaxed isolated rat ileum preparations from non-reserpinized rats but not from reserpine treated animals. α-Methyldopa, α-methylnoradrenaline and lower concentrations of metaraminol restored the responses of reserpinized preparations. α-Methyldopamine, α-methyl-*m*-tyrosine, α-methyl-*p*-tyrosine and higher concentrations of metaraminol did not do so. The restoring effect of α-methyldopa was blocked by disulphiram. α-Methyl-*p*-tyrosine or α-methyl-*m*-tyrosine blocked the restoring action of α-methyldopa but not of α-methylnoradrenaline. Cocaine blocked the restoration of responses to tyramine by α-methylnoradrenaline but not by α-methyldopa. α-Methyldopa and α-methylnoradrenaline failed to restore responses to tyramine in the presence of sodium-free Tyrode solution.

3. Tyramine increased the perfusion pressure in isolated rabbit ear preparations obtained from non-reserpinized animals but was very much less active in preparations obtained from reserpine treated animals. α-Methyldopa, α-methyl-*m*-tyrosine, α-methylnoradrenaline, α-methyl-*p*-tyrosine and metaraminol restored the effects of tyramine. α-Methyldopamine did not do so. The restoring effect of α-methyldopa and α-methyl-*m*-tyrosine was blocked by disulfiram. α-Methyl-*p*-tyrosine blocked the restoring effect of α-methyl-*m*-tyrosine.

4. Tyramine produced positive inotropic effects in isolated rabbit heart preparations. This was either reduced or absent in preparations obtained from reserpine pretreated animals. α-Methyldopa, α-methylnoradrenaline, α-methyl-*m*-tyrosine and metaraminol restored the responses to tyramine. α-Methyldopamine and α-methyl-*p*-tyrosine did not do so.

Introduction

It has been suggested (Carlsson & Lindqvist, 1962 ; Day & Rand, 1963) that foreign amines derived from α-methyldopa and α-methyl-*m*-tyrosine may replace noradrenaline in its storage sites and be released by nerve stimulation as "false neurotransmitters." The metabolic fate of α-methyldopa and α-methyl-*m*-tyrosine being similar, the corresponding false neurotransmitters would be α-methylnoradrenaline (Muscholl & Maitre, 1963 ; Day & Rand, 1964) and metaraminol (Weissbach,

Lovenberg & Udenfriend, 1960; Lovenberg, Weissbach & Udenfriend, 1962; Crout & Shore, 1964) respectively. It has been demonstrated by Bejrablaya, Burn & Walker (1958), Burn & Rand (1958, 1960) and by numerous other investigators that dopa, dopamine and noradrenaline are able to restore the sympathomimetic action of tyramine in reserpine pretreated animals. Liberation of α-methylnoradrenaline by sympathetic nerve stimulation has been demonstrated on isolated tissue preparations such as the heart of rabbits previously treated with α-methyldopa (Muscholl & Maitre, 1963) and from isolated perfused cat spleen (Haefely, Hürlimann & Thoenen, 1967).

The object of the present study was to investigate in greater detail the ability of α-methyl amino-acids and their corresponding amines to restore the sympathomimetic action of tyramine in isolated tissue preparations obtained from animals pretreated with reserpine. Experiments were also designed to study the mechanism of uptake of the amino-acids and the amines.

Methods

Isolated rat ileum

Albino rats of either sex weighing 200–300 g were fasted with free access to water for 24 h before use. Pieces of terminal ileum 2 cm long were removed and suspended in a 33 ml organ bath containing oxygenated Tyrode solution (NaCl 8 g; KCl 0·2 g; $CaCl_2$ 0·2 g; $MgCl_2$ 0·1 g; NaH_2PO_4 0·05 g; $NaHCO_3$ 1 g; dextrose 1 g; distilled water to 1 litre, at $37 \pm 0{\cdot}5°$ C. The movements were recorded on a smoked drum with isotonic frontal writing lever giving 7-fold magnification and exerting 250 mg tension on the tissue.

The preparation was allowed to stabilize for 30 min before the experiment was begun. Relaxation responses were obtained with tyramine over a range of concentrations from 0·3 to 2·4 μg/ml, left in contact with the tissue for 3 min and applied at 10 min intervals.

In experiments with α-methyl amino-acids and their corresponding amines, the preparation was exposed to the drug for periods of 3–120 minutes. The drug was replaced, after washing, at 30 min intervals in experiments in which the period of exposure was longer than 30 minutes.

Experiments were also designed to test the interactions between α-methyl-*p*-tyrosine, α-methyl-*m*-tyrosine and α-methyldopa on the restoration of the responses to tyramine in tissue preparations obtained from reserpine pretreated rats. In these experiments ileum preparations were exposed to α-methyl-*p*-tyrosine or α-methyl-*m*-tyrosine for 10 min before the addition of α-methyldopa and remained in the bath for 30 min thereafter.

After each period of incubation the preparation was washed once or twice every 10 min until the base line was achieved. The preparation was now tested for its responses to tyramine.

Experiments with cocaine. Reserpinized ileum preparations were exposed to cocaine for 10 min followed by the addition of α-methylnoradrenaline for 3 min or α-methyldopa for 30 minutes. Responses to tyramine were tested after washing.

Experiments with sodium-free Tyrode solution. Experiments were carried out on rat ileum preparations obtained from reserpine treated animals to determine the effect of sodium depletion on the uptake of α-methyldopa and α-methylnoradrenaline.

Sucrose was used to maintain the osmotic pressure of the sodium-free Tyrode solution (sucrose 93·675 g; KCl 0·2 g; $CaCl_2$ 0·2 g; $MgCl_2$ 0·1 g; KH_2PO_4 0·056 g; $KHCO_3$ 1·19 g; dextrose 1·09 g; distilled water to 1 litre). Control responses to standard doses of tyramine were obtained in sodium-containing Tyrode solution. After exposing the tissue to sodium-free Tyrode solution for 30 min (washed every 10 min) it was treated with α-methyldopa for 30 min or α-methylnoradrenaline for 3 minutes. Following this the preparation was washed and sodium-containing Tyrode solution was replaced as the bathing fluid. The preparation was now tested for its responses to tyramine.

Isolated perfused rabbit ear

The isolated perfused rabbit ear was prepared and used as described by Burn (1952). The ear was perfused with oxygenated Ringer solution (NaCl 9 g; KCl 0·42 g; $CaCl_2$ 0·24 g; NaH_2PO_4 0·01 g; $NaHCO_3$ 0·5 g; dextrose 1 g; distilled water to 1 litre) at 32 ± 2° C and an initial pressure of 30 mm Hg (1 mm Hg ≡ 1·333 mbar) which was shown to give the most consistent performance. Perfusion pressure was recorded with a Statham pressure transducer (Model—P—23AA) and a Sanborn Twin-Viso recorder (Model 60-1300). Drugs were injected in a volume of 0·2 ml in the rubber tubing close to the cannula.

Constrictor responses were obtained with tyramine in a range of doses from 1 to 20 μg, each repeated twice at 10 min intervals.

Except for α-methylnoradrenaline which was given only once, the doses of all α-methyl amino-acids and their amines were injected four times at intervals of 15 minutes.

Isolated perfused rabbit heart

Hearts were obtained from young rabbits of either sex weighing 1·5–2·5 kg and were perfused with oxygenated Locke solution (NaCl 9 g; KCl 0·42 g; $CaCl_2$ 0·24 g; $NaHCO_3$ 0·15 g; dextrose 1 g; distilled water to 1 litre) by the method of Langendorff. The solution was maintained at 29° ± 1° C. Contractions of the heart were recorded by means of a Starling heart lever writing on a smoked drum. Drugs were injected in a volume of 0·2 ml in the rubber tubing close to the cannula.

After 30 min of stabilization, positive inotropic responses were obtained with tyramine in a range of doses from 10–200 μg each repeated twice at 15 min intervals.

Except for α-methylnoradrenaline which was given only once, the doses of all α-methyl amino-acids and their amines were injected four times at intervals of 15 min.

Pretreatment with reserpine

Rats were injected subcutaneously with reserpine 2 mg/kg daily for 8 successive days. Rabbits were given reserpine 1 mg/kg intraperitoneally at intervals of 48 h for 8 days. Animals were used 24 h after the last injection of reserpine. Rabbits were also injected intramuscularly with oxytetracycline 5 mg/kg daily to prevent infection during the course of reserpinization (Fleming & Schmidt, 1962).

Drugs

Tyramine hydrochloride, α-methyldopa, DL-α-methyldopamine hydrochloride, L-α-methylnoradrenaline, α-methyl-*m*-tyrosine monohydrate, α-mehyl-*p*-tyrosine,

metaraminol bitartrate, reserpine (Serpasil, Ciba), oxytetracycline (Terramycin, Pfizer), cocaine hydrochloride and disulfiram (Antabuse, Dumex). L-α-Methylnoradrenaline was dissolved in 0·025 N hydrochloric acid. All other drugs were dissolved in distilled water immediately before use.

The data were analysed for significance by Student's *t* test (Burn, Finney & Goodwin, 1952).

Results

Responses to tyramine of isolated preparations obtained from normal and reserpine pretreated animals

Rat ileum

Tyramine produced reproducible and dose related relaxation of the rat ileum preparations obtained from normal animals. Although the sensitivity of individual preparations to the amine varied somewhat, a linear dose-response relationship was obtained when percentage of maximal relaxation was plotted against the log dose of amine (Fig. 1).

Tyramine in the same doses, however, did not elicit relaxation in preparations obtained from reserpine pretreated rats (Fig. 1), though in some preparations contractile responses were elicited.

Rabbit ear

Tyramine elicited dose related increase in perfusion pressure of normal rabbit ear preparations (Fig. 2).

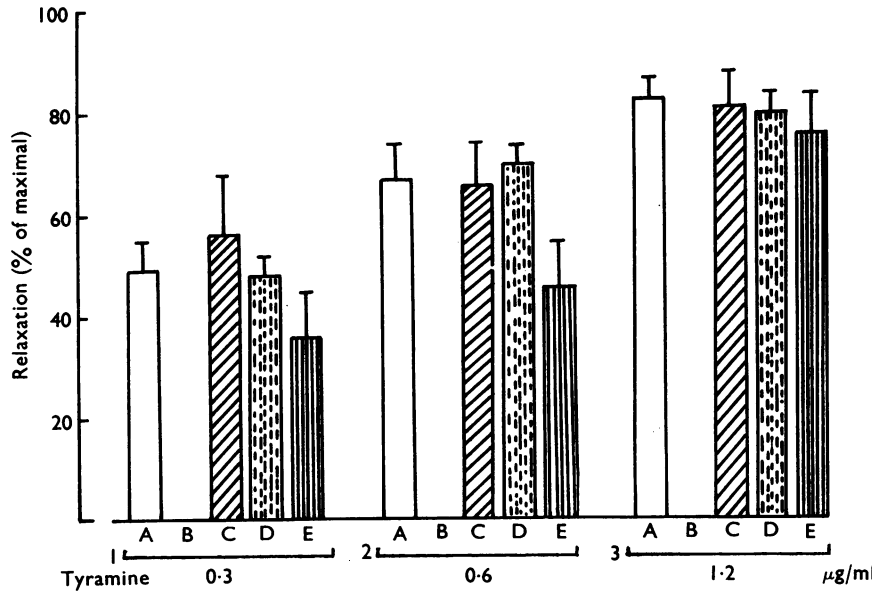

FIG. 1. Relaxation of isolated rat ileum by tyramine in the bath concentrations given under each panel. A, Control response of non-reserpinized preparation (ten values); B, control response of reserpinized preparation (eight values); C, D, E, responses of reserpinized preparations after incubation with: C, α-methyldopa, 10 μg/ml for 30 min (six values); D, α-methylnoradrenaline, 1 μg/ml for 3 min (twelve values); E, metaraminol, 1 μg/ml for 30 min (four values). The height of each bar and the vertical line projected on it represent the mean response and ±S.E., respectively.

In ears removed from rabbits pretreated with reserpine, tyramine elicited increase in perfusion pressure in almost all cases, though this was very much less than that obtained with preparations from normal rabbits (Fig. 2).

Rabbit heart

Tyramine elicited dose related positive inotropic responses of the perfused normal heart (Fig. 3), expressed as per cent of initial amplitude of contraction.

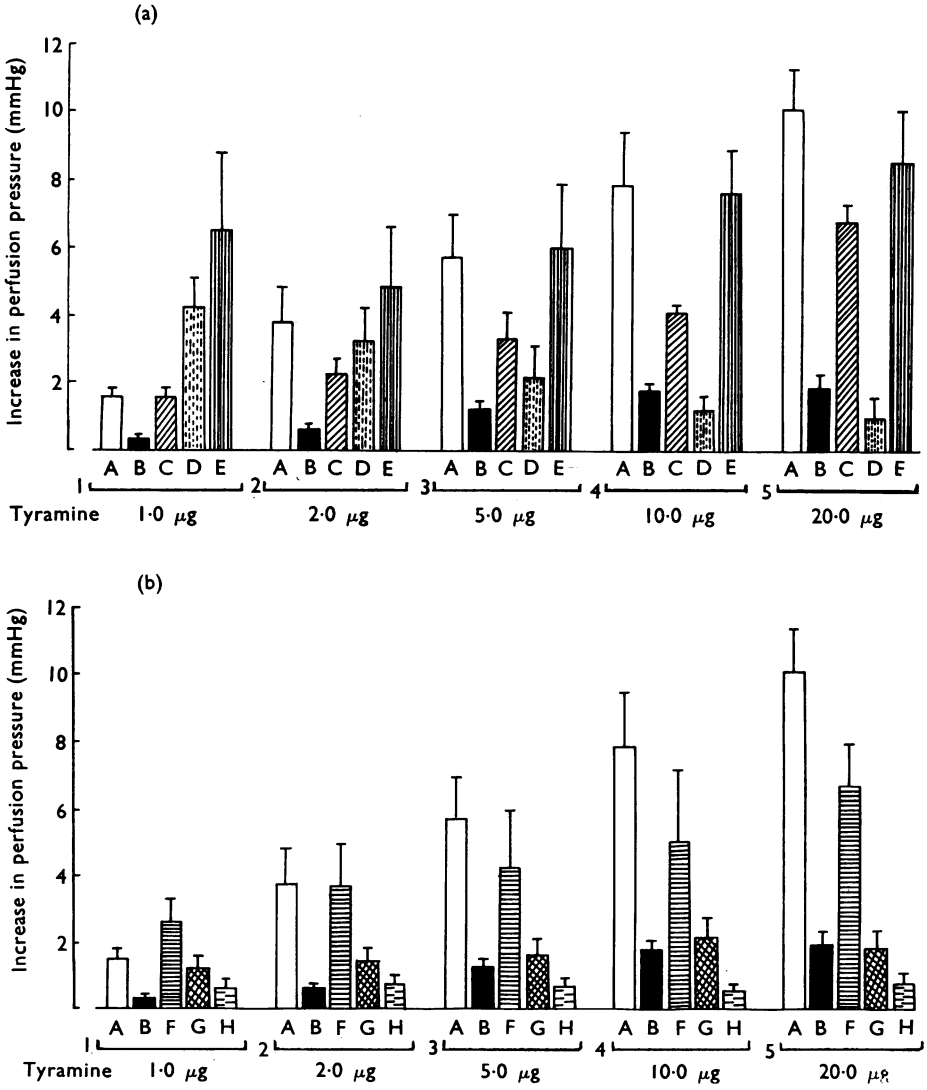

FIG. 2. Increase in perfusion pressure in isolated rabbit ear on injection of tyramine in the doses given under each panel. A, Control response of non-reserpinized preparation (eleven values); B, control response of reserpinized preparation (twenty-six values); C, D, E (Fig. 2a), F, G, H (Fig. 2b), responses of reserpinized preparations after injection of: C, α-methyldopa, 100 μg (three values); D, α-methylnoradrenaline 10 μg (three values); E, metaraminol, 10 μg (three values); F, α-methyl-*m*-tyrosine, 100 μg (four values); G, α-methyl-*p*-tyrosine 100 μg (six values); H, α-methyldopamine, 100 μg (seven values). The height of each bar and the vertical line projected on it represent the mean response and ±S.E., respectively.

Hearts obtained from reserpine pretreated rabbits did not show a positive inotropic response to a low dose of tyramine, though higher doses of tyramine did produce some positive inotropic effect in three of the thirteen preparations (Fig. 3).

Effect of α-methyl amino-acids and their corresponding amines

Rat ileum

α-Methyldopa, α-methyldopamine, α-methylnoradrenaline, α-methyl-*p*-tyrosine and metaraminol themselves relaxed the preparation. α-Methyl-*m*-tyrosine did not produce any effect of its own in the dose used.

α-Methyldopa and α-methylnoradrenaline restored the inhibitory responses to tyramine (Fig. 1). The minimum times required for α-methyldopa and α-methylnoradrenaline to restore responses to tyramine were 30 and 3 min respectively. Lower concentrations of metaraminol restored the responses to tyramine (Fig. 1) while higher concentrations (10μg/ml and 30 μg/ml for 30 min; nine trials) failed to

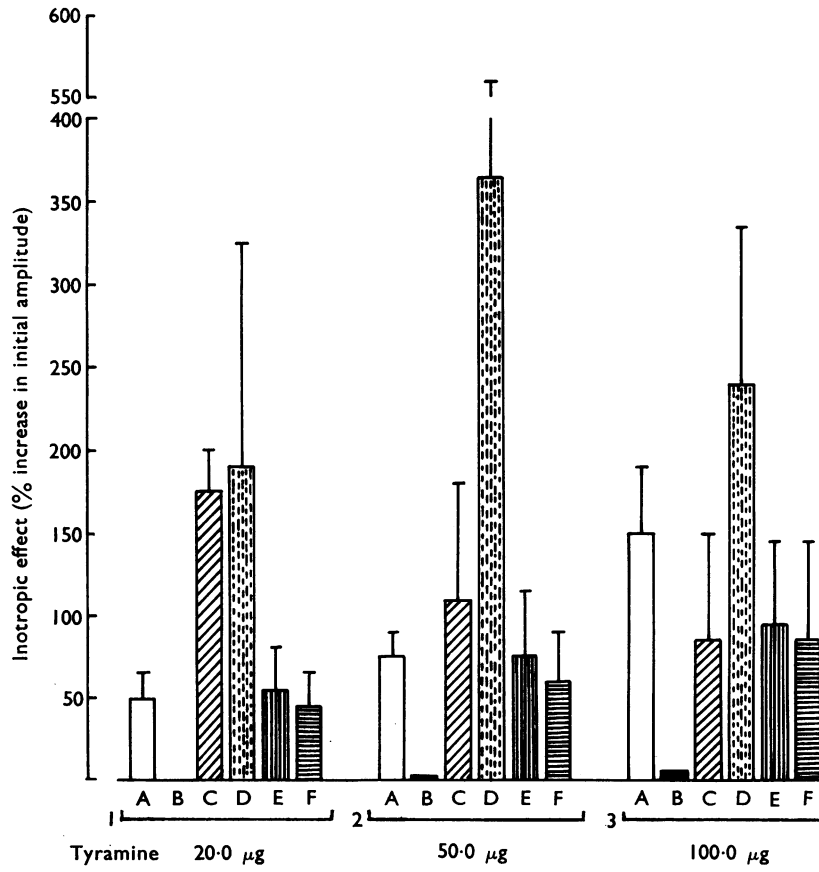

FIG. 3. Positive inotropic responses of isolated rabbit heart to tyramine injected in the doses given under each panel. A, Control response of non-reserpinized preparation (six values); B, control response of reserpinized preparation (thirteen values); C, D, E, F, responses of reserpinized preparations after injection of: C, α-methyldopa, 100 μg (three values); D, α-methylnoradrenaline, 10 μg (three values); E, metaraminol, 10 μg (three values); F, α-methyl-*m*-tyrosine, 100 μg (four values). The height of each bar and the vertical line projected on it represent the mean response and ±S.E., respectively.

restore the responses to the same doses of tyramine. This appeared surprising and, therefore, the influence of metaraminol in modifying the responses to tyramine and noradrenaline was studied in nine preparations obtained from normal rats. Higher concentrations of metaraminol completely blocked the relaxant effect of tyramine but the response to noradrenaline was not affected. Lower concentration of metaraminol, however, failed to modify responses to tyramine or noradrenaline.

The restoration of the responses to tyramine following α-methyldopa, α-methylnoradrenaline and metaraminol was substantial and the restored responses did not differ significantly ($P>0{\cdot}1$) from the responses of preparations obtained from normal animals (Fig. 1).

Treatment of the preparation with α-methyldopamine, α-methyl-*m*-tyrosine or α-methyl-*p*-tyrosine failed to restore the responses to tyramine; subsequent treatment of the same preparations with α-methyldopa produced restoration.

Rabbit ear

Injections of α-methyldopa, α-methylnoradrenaline, α-methyl-*m*-tyrosine or metaraminol produced an immediate increase in perfusion pressure which lasted approximately for 10, 45, 12 and 3 min respectively. α-Methyldopamine and α-methyl-*p*-tyrosine did not elicit any effect of their own.

In experiments with α-methyldopa and α-methyl-*m*-tyrosine, subsequent administration of tyramine led to dose related increase in perfusion pressure that was significantly greater ($P<0{\cdot}01$) than that in control preparations obtained from reserpinized animals. In preparations treated with α-methylnoradrenaline or α-methyl-*p*-tyrosine, though the increase in perfusion pressure for lower doses of tyramine was significantly greater ($P<0{\cdot}05$), the increase for the higher doses was not. The increase in perfusion pressure for different doses of tyramine following metaraminol though statistically significant ($P<0{\cdot}01$) was not strictly dose related. It is important to note (Fig. 2) that following metaraminol and α-methylnoradrenaline responses to the lowest dose of tyramine were not only restored but were highly potentiated and the restored responses were now significantly ($P<0{\cdot}05$) greater than normal responses.

Responses to tyramine after α-methyldopamine were not significantly different from the control response. However, subsequent treatment of these preparations with α-methyldopa (as described earlier) restored the tyramine induced increase in perfusion pressure. The data are depicted in Fig. 2.

Rabbit heart

α-Methyldopa, α-methylnoradrenaline, α-methyl-*m*-tyrosine and metaraminol elicited positive inotropic effects of their own lasting for 60–90 seconds. Responses to tyramine were now restored (Fig. 3). However, restoration by none of the drugs was dose-related.

α-Methyldopamine (100 μg) elicited a positive inotropic effect lasting for 2 min whereas α-methyl-*p*-tyrosine (100 μg) did not produce an effect of its own. No restoration of the responses to tyramine was observed following α-methyldopamine or α-methyl-*p*-tyrosine.

Effects of disulfiram

The conversion of dopamine to noradrenaline is accomplished by the enzyme dopamine β-hydroxylase (Hagen, 1956). A wide variety of phenylethylamine derivatives have been shown to act as substrates for this enzyme. Disulfiram has been shown to be a potent inhibitor of the enzyme dopamine β-hydroxylase (Goldstein, Anagnoste, Lauber & McKeregan, 1964) and to lower the levels of endogenous noradrenaline (Musacchio, Goldstein, Anagnoste, Poch & Kopin, 1966). Therefore, the effect of inhibition of dopamine β-hydroxylase by disulfiram on the restoration of the responses to tyramine following α-methyldopa and α-methyl-*m*-tyrosine was investigated with isolated perfused rabbit ear and rat ileum preparations.

The dose of disulfiram that by itself would not modify the responses of normal preparations to tyramine was found to be 0·2 μg for the rabbit ear and 1 μg/ml for the rat ileum. These were used throughout.

In three isolated perfused rabbit ear preparations obtained from reserpine pretreated animals, α-methyldopa or α-methyl-*m*-tyrosine in the presence of disulfiram failed to restore the increase in perfusion pressure to tyramine. After subsequent treatment with α-methylnoradrenaline or metaraminol in the presence of disulfiram, tyramine could elicit increase in perfusion pressure, although the increase was not dose related and reproducible.

In four isolated ileum preparations obtained from reserpine pretreated rats, α-methyldopa in the presence of disulfiram could not restore the responses to tyramine. In order to test the reactivity of the tissue, the preparations were then treated with α-methylnoradrenaline in the presence of disulfiram. The inhibitory responses to tyramine could now be elicited.

Experiments on the mechanism of uptake of α-methyl amino-acids and α-methylnoradrenaline

*Effect of α-methyl-*p*-tyrosine and α-methyl-*m*-tyrosine on responses to α-methyldopa*

When rat ileum preparations obtained from reserpine treated animals were incubated with α-methyldopa in the presence of α-methyl-*p*-tyrosine, responses to tyramine were restored in three out of five experiments. However, when the concentration of α-methyl-*p*-tyrosine was doubled, responses to tyramine could not be restored. α-Methyl-*m*-tyrosine blocked the restoration of responses to tyramine by α-methyldopa. α-Methylnoradrenaline kept in a bath for 3 min following exposure to α-methyl-*p*-tyrosine or α-methyl-*m*-tyrosine for 37 min restored the responses to tyramine.

Effect of cocaine

In the presence of cocaine, 3 μg/ml, α-methylnoradrenaline failed to restore the responses of reserpinized rat ileum preparations to tyramine. The same preparations were now exposed to cocaine for 10 min followed by α-methyldopa for 30 minutes. There was now restoration of the responses to tyramine.

Effect of sodium-free Tyrode solution

In four ileum preparations obtained from reserpinized rats, responses to tyramine in normal Tyrode solution could not be elicited. After treating the preparation with α-methyldopa in the presence of sodium-free bathing solution and subsequent wash-

ing, tyramine failed to elicit any response. Subsequent treatment with α-methyldopa in normal Tyrode solution, however, restored the responses to tyramine. Similar results were obtained with α-methylnoradrenaline.

*Effect of α-methyl-*p*-tyrosine on α-methyl-*m*-tyrosine and metaraminol treatment*

In preliminary experiments it was observed that α-methyl-*p*-tyrosine, 10 μg/ml, did not modify responses of normal rabbit ear preparations to tyramine. In four preparations obtained from reserpinized rabbits, α-methyl-*m*-tyrosine in the presence of α-methyl-*p*-tyrosine however, failed to restore the increase in perfusion pressure to tyramine. When these preparations were subsequently treated with metaraminol in the presence of α-methyl-*p*-tyrosine there was restoration of the increase in perfusion pressure to tyramine in three out of four cases.

Discussion

After pretreatment with reserpine, tissues lose their responsiveness to tyramine or other substances which act through the release of catecholamines from the postganglionic sympathetic nerve endings (Burn & Rand, 1958). Muscholl (1960) presented evidence that reserpine pretreatment almost abolishes the uptake of noradrenaline by various tissues. However, when such tissues are exposed to or perfused with noradrenaline or its precursors, their responsiveness to tyramine is restored (Burn & Rand, 1960; Trendelenburg & Pfeffer, 1964). Moreover, Trendelenburg & Crout (1964) have shown that isolated atria of reserpine pretreated guinea-pigs take up a small but measurable amount of noradrenaline. Since the precursors for synthesis, storage and release of neurochemical transmitter of the sympathetic nervous system are not entirely specific (Holtz, 1959; Lovenberg, Weissbech & Udenfriend, 1962), a number of structurally related substances may be taken up by the adrenergic nerve endings and subsequently processed to form amines resembling noradrenaline. These newly formed amines are stored by replacing noradrenaline from storage sites and are released by either nerve stimulation or indirectly acting sympathomimetic amines.

In the present investigation restoration of responses to tyramine of rat ileum, rabbit ear and heart preparations obtained from reserpine pretreated animals by α-methyldopa and α-methylnoradrenaline was qualitatively identical. Restoration following α-methyldopa could be due to release of one or more of the following: (i) retained α-methyldopa; (ii) α-methyldopamine synthesized by decarboxylation of α-methyldopa or (iii) α-methylnoradrenaline synthesized from α-methyldopa by decarboxylation and subsequent β-hydroxylation. Abolition of the restoring ability of α-methyldopa following disulfiram, a potent β-hydroxylase inhibitor, underscored the importance of β-hydroxylation for the restoration of responses to tyramine. These findings support the view that α-methylnoradrenaline must be formed from its precursor (Carlsson & Lindqvist, 1962; Jackson, Bhagat & Kopin, 1964; Musacchio, Goldstein, Anagnoste, Poch & Kopin, 1966). Further, with isolated rat ileum and rabbit heart preparations the time required for the restoration of the responses to tyramine following α-methyldopa (precursor) was 4–10 times greater than that required following α-methylnoradrenaline. This would suggest that time is required for the biotransformation of α-methyldopa to α-methylnoradrenaline. In close agreement with the present data are the results of Kroneberg & Stoepel (1963) and

Pettinger, Horwitz, Spector & Sjoerdsma (1963) obtained with cat nictitating membrane and human blood pressure, respectively.

In experiments with the isolated perfused rabbit ear the well sustained and prolonged vasoconstrictor response to α-methylnoradrenaline lasted for 45 minutes. Thus restoration of response to tyramine could not be studied during this period.

α-Methyl-*m*-tyrosine could restore responses to tyramine only in rabbit ear and heart preparations. Failure of α-methyl-*m*-tyrosine to restore responses to tyramine in rat ileum may be attributed to species and/or tissue differences, as suggested by Haefely, Hürlimann & Thoenen (1966). The amino-acid α-methyl-*m*-tyrosine undergoes a similar metabolic biotransformation to that of α-methyldopa, being decarboxylated to α-methyl-*m*-tyramine which is further β-hydroxylated to metaraminol (Carlsson & Lindqvist, 1962). In perfused rabbit ear preparations, α-methyl-*m*-tyrosine failed to restore the responses to tyramine in the presence of disulphiram. However, in these preparations metaraminol could restore the vasoconstrictor responses to tyramine. These results suggest that the restoration of the responses to tyramine following α-methyl-*m*-tyrosine was due to metaraminol resulting from decarboxylation and subsequent β-hydroxylation.

Metaraminol could restore the responses to tyramine of isolated rat ileum, perfused rabbit ear and heart preparations. Thus, like noradrenaline, metaraminol too can be taken up and released by tissues of reserpine treated animals. These findings are consistent with those of Carlsson & Waldeck (1965, 1966) who have reported that if amine storage is blocked by reserpine, tritiated metaraminol can still be concentrated in the neurone by the membrane pump.

It is of interest that when metaraminol was used in a higher dose, restoration of responses to tyramine was not seen. This could be due to high concentrations of metaraminol in the vicinity of the receptors decreasing sensitivity to noradrenaline which could in turn explain the diminished response to tyramine (Bhagat & Ragland, 1966). However, in the present study it was observed that in tissues from normal animals the dose of metaraminol that completely inhibited the responses to tyramine did not reduce sensitivity to noradrenaline. Thus the argument that the inability of higher dose of metaraminol in restoring responses to tyramine could be related to receptor occupancy by metaraminol is not tenable. The alternative that metaraminol in high doses blocks the uptake of tyramine by tissues seems most likely.

α-Methyldopamine did not restore responses to tyramine in tissues obtained from reserpinized animals though α-methyldopa and α-methylnoradrenaline could do so. α-Methyldopamine is probably not taken up by tissues obtained from reserpinized animals. This view is supported by the data of Buhs, Beck, Speth, Smith, Trenner, Cannon & Laragh (1964) ; of Musacchio, Goldstein, Anagnoste, Poch & Kopin (1966) and of Muscholl (1966).

According to Spector, Sjoerdsma & Udenfriend (1965), α-methyl-*p*-tyrosine unlike α-methyl-*m*-tyrosine is not converted to false transmitter. However, the restoration of the responses of perfused ear vessels to lower doses of tyramine following α-methyl-*p*-tyrosine supports the evidence of Maitre (1965) that small amounts of α-methyl-*p*-tyrosine do get converted to α-methylnoradrenaline. The negative results with rat ileum and perfused rabbit heart preparations may be due to tissue and species difference as suggested by Haefely, Hürlimann & Thoenen (1966).

If the enzyme systems involved in the biotransformation of α-methyl-*p*-tyrosine and α-methyl-*m*-tyrosine to α-methylnoradrenaline and metaraminol, respectively, were similar then the restoration of the responses to tyramine following α-methyl-*p*-tyrosine and α-methyl-*m*-tyrosine should be quantitatively and qualitatively comparable. In the rabbit ear preparation there was marked restoration of the responses to tyramine following α-methyl-*m*-tyrosine ; restoration following α-methyl-*p*-tyrosine was less. Possible explanations are that: (i) α-methyl-*p*-tyrosine serves as a weak substrate for the enzyme, tyrosine hydroxylase, leading to the formation of small amounts of false neurotransmitter (Maitre, 1965), (ii) the variation in the affinity of the carrier-transport mechanism which is presumably involved in the uptake of these amino-acids. It is not unreasonable to assume that the lesser potency of α-methyl-*p*-tyrosine in restoring the responses to tyramine could be due to its acting as a weak agonist for the carrier-transport mechanism for α-methyl-*m*-tyrosine. Blockade of the restoring effect of α-methyl-*m*-tyrosine and α-methyldopa by α-methyl-*p*-tyrosine in the rabbit ear preparations and of the effect of α-methyldopa by α-methyl-*p*-tyrosine or α-methyl-*m*-tyrosine in rat ileum preparations suggest that a common carrier-transport mechanism is involved in the uptake of these amino-acids.

Another noteworthy aspect of our study is the restoration of the responses to tyramine following α-methyldopa in the presence of cocaine and the failure of restoration following α-methylnoradrenaline. The elegant work of Axelrod, Weil-Malherbe & Tomchick (1959) and Whitby, Axelrod & Weil-Malherbe (1961) has shown that the bulk of the released noradrenaline is actively reabsorbed by the very nerve terminals that originally released it. Cocaine is reported to prevent this uptake of noradrenaline from the interstitial spaces into the nerve terminals (Muscholl, 1961 ; Hertting, Axelrod & Whitby, 1961). From the present experiments it would appear that different mechanisms operate for the uptake and transportation of the amino-acids and amines.

Recent studies of Bogdanski & Brodie (1966) ; Iversen & Kravitz (1966) ; Gillis & Paton (1967) and Horst, Kopin & Ramey (1968) have shown that sodium ions are essential for the uptake and storage of noradrenaline by sympathetic nerve endings. The results of the present study demonstrate that the uptake of the amino-acid, α-methyldopa and of the amine, α-methylnoradrenaline also require the presence of sodium ions. It may, therefore, be postulated that α-methyl amino-acids and amines are transported across the tissue membranes by independent transport mechanisms involving different carrier systems, the functioning of which are sodium dependent.

Potentiation of the vasoconstrictor response to a lower dose of tyramine following α-methylnoradrenaline and metaraminol and of positive inotropic response to tyramine following α-methyldopa may be due to release of increased quantities of the transmitter in the absence of retention by the storage granule-complex consequent on reserpine pretreatment. Thus the levels of the false transmitter that had been taken up would fall rapidly. This could further explain the present observations that the degree of restoration following α-methylnoradrenaline in perfused rabbit ear and following metaraminol in the perfused rabbit heart was reduced with increasing doses of tyramine.

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