Br. J. Pharmac. (1971), **41**, 700–708.

Importance of noradrenaline found in a functional pool in maintaining spontaneous locomotor activity in rats

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Summary

1. Spontaneous locomotor activity (activity) in male Wistar rats was compared with the concentrations of brain noradrenaline (NA), dopamine (DA) and metaraminol.

2. α-Methyl-*m*-tyrosine (αMMT) (400 mg/kg) reduced the concentrations of DA as well as NA but activity remained high in the presence of metaraminol formed from the αMMT. When tetrabenazine (TBZ) was given after αMMT pretreatment there was a fall in the levels of activity and in the concentrations of NA, DA and metaraminol.

3. α-Methyl-*p*-tyrosine (αMT) produced a fall in activity which was correlated with falls in the concentrations of NA and DA. 5-Hydroxytryptamine (5-HT) did not appear to be affected.

4. After depletion of NA and DA by αMT and TBZ, administration of L-dopa produced a return in activity which was significantly correlated with the concentration of NA but not DA. When αMMT was given to a similar group of pretreated animals there was no recovery of activity despite high concentrations of DA and metaraminol.

5. The dopamine β hydroxylase inhibitor, diethyldithiocarbamate (DDC), suppressed activity as well as the concentrations of NA and DA at high doses (750 mg/kg) but smaller doses (400 mg/kg) plus L-dopa gave high DA concentrations without activity.

6. It is concluded that NA and not DA is associated with activity but that it is only part of the total NA which is in the biosynthetic storage granule affected by drugs like αMT and TBZ, which controls activity. Drugs that do not affect this pool may lower NA concentrations but not reduce activity.

7. The replacement of NA by metaraminol in this functional pool does not restore activity.

Introduction

Our investigations (Chan & Webster, 1971) have suggested that the method by which a drug depletes the central nervous system of catecholamines may determine whether or not it reduces activity. Thus, although both α-methyl-*m*-tyrosine and tetrabenazine caused a comparable depletion of brain noradrenaline in the rat, at

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the doses studied, only tetrabenazine reduced psychomotor activity and the concentration of dopamine, an effect which it still retained when given after α-methyl-*m*-tyrosine. The simplest explanation of these findings is that dopamine and not noradrenaline is associated with the maintenance of activity despite the fact that when tetrabenazine was given after α-methyl-*m*-tyrosine it produced a similar reduction in activity but a smaller loss in dopamine than when given alone.

Another possibility is that metaraminol formed from α-methyl-*m*-tyrosine displaces noradrenaline from the nerve terminals and takes over its function to maintain activity irrespective of the concentration of dopamine. It is therefore important to determine whether tetrabenazine reduced activity after α-methyl-*m*-tyrosine by depleting the brain of metaraminol since reserpine, which produces central effects similar to those of tetrabenazine, apparently cannot produce such a depletion (Carlsson & Lindqvist, 1967). This possibility has been studied in the present experiments and the role of dopamine further evaluated by giving larger doses of α-methyl-*m*-tyrosine, which are believed to deplete dopamine (Anden, 1964) as well as noradrenaline, in an attempt to see if activity is also reduced.

Despite the apparent unimportance of noradrenaline in maintaining activity as reflected by gross brain concentrations, it remains possible that activity is associated with a small fraction (pool) of noradrenaline which is not altered by αMMT and that if this pool forms only a small part of total brain NA, then activity need not be correlated with absolute NA concentrations. This concept of two pools of catecholamines in the nerve ending has been proposed by several workers (Haggendal & Lindqvist, 1963, 1964 ; Weissmann, Koe & Tenen, 1966 ; Haggendal, Lindqvist & Roos, 1967 ; Rech, Carr & Moore, 1968).

The synthesis of noradrenaline and dopamine can be inhibited by α-methyl-*p*-tyrosine (αMT) and since this compound seems to produce some correlation between amine concentrations and behaviour (Rech, Borgs & Moore, 1966 ; Moore & Rech, 1967), it could also affect the functional pool and has therefore been tested. It has also been used together with TBZ to deplete all catecholamines from nerve endings in the central nervous system. Activity was then observed after selective replenishment of (1) noradrenaline by administration of dopa ; (2) metaraminol by giving α-methyl-*m*-tyrosine ; and (3) dopamine by using the dopamine β hydroxylase inhibitor diethyldithiocarbamate (DDC) to stop the synthesis of NA from DA. The results were compared with those obtained by the unmodified normal recovery from αMT plus TBZ treatment.

Methods

Male Wistar rats weighing 160–220 g were tested for spontaneous locomotor activity in photocell activity cages after various drug treatments. They were then killed for determination of noradrenaline and dopamine content in brain stems of average weight $0{\cdot}79 \pm 0{\cdot}01$ g. Methods were similar to those previously described (Chan & Webster, 1971). All drugs were injected intraperitoneally and animals were killed for brain extraction immediately after measurement of activity.

Metaraminol estimation

Metaraminol was estimated according to the method of Shore & Alpers (1964). HCl (0·9 N) eluates of rat brain extracts from Dowex 50W × 8 ion exchange columns

were used. Samples (1 ml) were added to 0·5 ml 0·5 M borate buffer at pH 9, reacted with 0·1 ml 1% o-phthalaldehyde in absolute methanol and acidified with 0·15 ml 3 N HCl for 7 minutes. The fluorescence of the sample transmitted at 500 nm was read in a Locarte fluorimeter activated at 365 nm.

Thin layer chromatography

The method of Carlsson & Lindqvist (1967) for the identification of catecholamines and metaraminol was adapted to thin layer chromatography. A solvent system of 1 N-HCl saturated *n*-butanol was used. Amines and metaraminol were developed with 0·5% diazotized *p*-nitroaniline in alkaline solution. The ascending chromatograms were run for 6 hours.

Drugs and chemicals

These included: DL-noradrenaline bitartrate (Koch-Light, PURE); dopamine hydrochloride (Koch-Light PURE); DL-metaraminol bitartrate (Merck, Sharpe and Dohme); L-dopa (Sigma); sodium diethyldithiocarbamate (BDH, reagent grade); tetrabenazine (inj. Nitoman, Roche); α-methyl-*p*-tyrosine (Koch-Light); α-methyl-*m*-tyrosine (Koch-Light). Also used were: cation exchange resin AG 50W × 8, 200–400 mesh (Bio rad); perchloric acid analytical grade (Fisons); redistilled isobutanol (2-methylpropanolol—BDH Analar); redistilled acetic anhydride (Hopkins and Williams, Analar); redistilled ethylenediamine (BDH, Analar).

Results

*α-Methyl-*m*-tyrosine (αMMT) and its combination with tetrabenazine (TBZ)*

Four rats were given αMMT (400 mg/kg) for 6 h; four others were given TBZ (10 mg/kg) additionally for 1 h and three rats were untreated.

Both DA and NA concentrations were reduced by αMMT (400 mg/kg) without significant effect on activity. Additional treatment with TBZ (10 mg/kg) reduced all parameters to very low levels (Fig. 1). Metaraminol was detected in αMMT treated rats by thin layer chromatography, but was greatly depleted by additional treatment with TBZ.

Activity

Activity was increased by αMMT (400 mg/kg) (after 6 h) to 115% of normal (not significant). The behaviour was qualitatively different from that of rats treated with αMMT (40 mg/kg) for 48 h (Chan & Webster, 1971). They were hyperactive, irritable and aggressive. Additional treatment with TBZ (10 mg/kg) made the rats inactive (8% of normal).

Catecholamines

NA and DA concentrations were considerably reduced by αMMT (400 mg/kg) after 6 hours. NA fell to 23% and DA to 29% of normal; this result agreed well with that of Anden (1964) for the same dose. By contrast a lower dose of αMMT (40 mg/kg) did not affect DA concentrations (Chan & Webster, 1971). Additional treatment with TBZ (10 mg/kg) resulted in almost complete depletion of NA and DA.

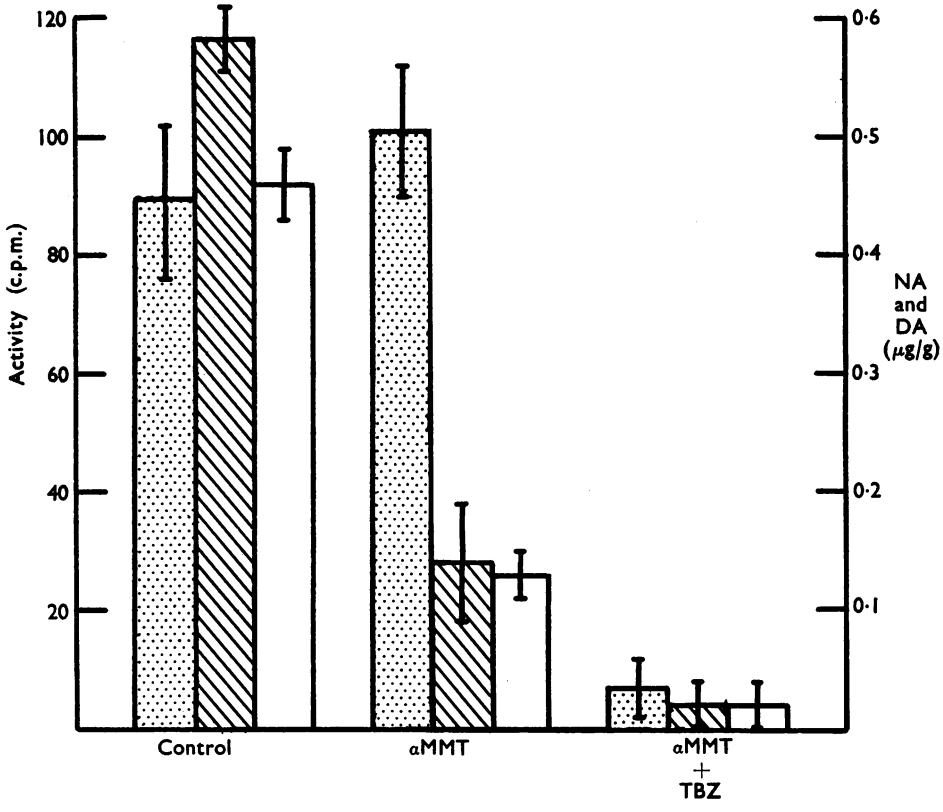

FIG. 1. Histogram of the effect of αMMT (400 mg/kg) and its combination with TBZ (10 mg/kg) on rat psychomotor activity (dotted columns) in c.p.m. and brain stem NA (hatched columns) and DA (clear columns) in μg/g. $n=3$ for control and 4 for treated groups.

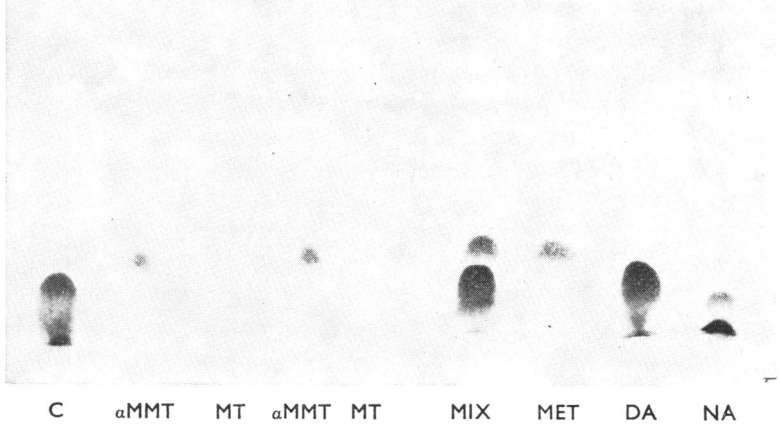

FIG. 2. Thin layer chromatogram of brain extracts of rats. MET=Metaraminol; NA=noradrenaline; DA=dopamine; C=untreated controls with DA and NA spots; αMMT=400 mg/kg for 6 h showing metaraminol spot; MT=combination of αMMT and TBZ (10 mg/kg) showing no spots; Mix=mixture of metaraminol, NA and DA reference standards; DA=dopamine reference; NA=noradrenaline reference. Solvent=butanol:1 N HCl (4:1). Spots developed with 0·5% diazotized *p*-nitroaniline.

Metaraminol

Brain extracts of rats treated with αMMT produced a pink spot (R_F=0·49) identical to that produced by standard metaraminol in ascending thin layer chromatograms (Fig. 2). However, rats treated with the combination of αMMT and TBZ did not show this spot. Brain extracts of untreated rats did not give the pink spot but produced spots identical with those given by standard NA (R_F=0·13) and DA (R_F=0·22).

*Effect of α-methyl-*p*-tyrosine (200 mg/kg)*

This drug was used to inhibit the synthesis of NA and cause depletion of NA without the formation of false transmitters. In this experiment four rats were given αMT (200 mg/kg) for 6 h, four were given the same dose for 24 h and four served as untreated controls. Activity and the content of brain NA and DA were reduced 6 h after αMT (200 mg/kg) to 26%, 22% and 24% of normal, respectively. At 24 h a significant recovery in all parameters had occurred, activity being 45% of normal when NA was replenished to 52% and DA to 60% (Fig. 3). There was a moderate correlation between activity and NA content (r=0·54 ; n=10 ; P<0·01) but no significant correlation with DA content (r=0·34 ; n=10 ; P<0·20). It should be noted, however, that the control values for NA were lower than normal and one sample (0·03 μg/g) seemed obviously faulty. Conversely, one very high value for DA (0·9 μg/g) elevated the control mean for this amine. Thin layer chromatograms of pooled brain extracts showed that, despite the reduction in activity, αMT treatment did not affect the presence of 5-HT (R_F=0·39) but caused the disappearance of NA and DA spots.

Effect of selective replenishment of catecholamines and metaraminol

All rats were treated with αMT (50 mg/kg) for 15 h before TBZ (5 mg/kg) to obtain a baseline depleted state. Eight rats (group C) were then allowed to recover

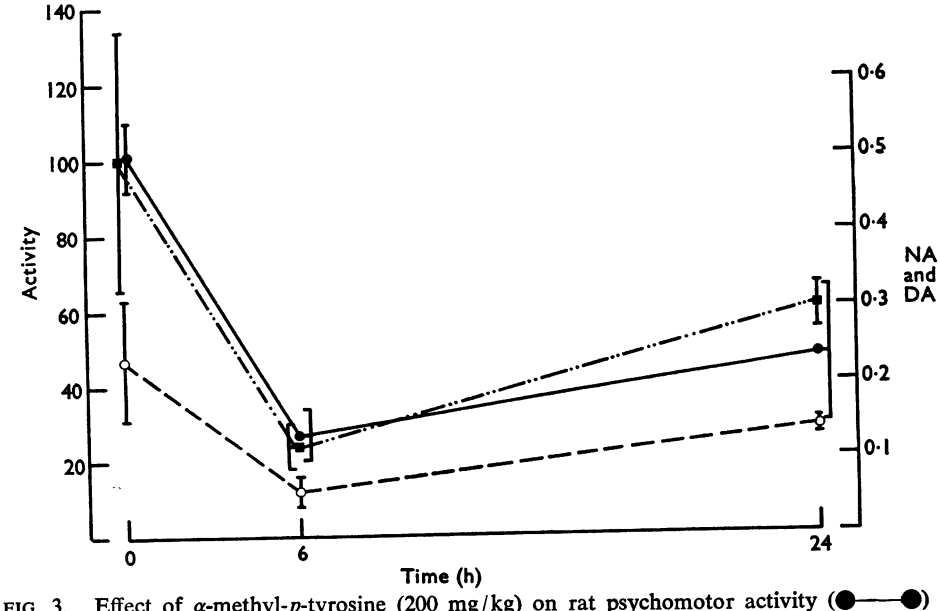

FIG. 3. Effect of α-methyl-*p*-tyrosine (200 mg/kg) on rat psychomotor activity (●——●) in c.p.m. and brain stem NA (○ – – ○) and DA (■ - - - ■) concentrations (μg/g). n=4.

without further drugs and 6·5 h after TBZ had attained levels of activity approaching normal with a greater replenishment of NA (0·48 ± 0·03 μg/g) than DA (0·19 ± 0·02 μg/g). Percentage values at 6·5 h for activity, NA and DA were 82, 80 and 38, respectively. The combination of αMT and TBZ produced a greater effect than either drug given alone and recovery was much slower. See Fig. 4.

Replenishment of dopamine

An attempt was made to replenish DA selectively by blocking the formation of NA with diethyldithiocarbamate (DDC). In group A (four rats) a total dose of DDC (750 mg/kg) (250 for 9 h and 500 for 3·5 h) inhibited the formation of DA as well as NA, and both amines remained at very low concentrations—that of NA was 38% of normal and that of DA was 18% of normal. Activity was reduced to flaccid immobility. The rats were kept alive by maintaining body temperature with a table lamp; they were not reactive to pain.

In group B (four rats) a smaller dose of DDC (400 mg/kg for 7·5 h), however, suppressed the replenishment of NA (23% of normal) but DA was increased by L-dopa (400 mg/kg for 5 h) to 0·32 ± 0·03 μg/g (64%). There was still only a little activity despite the increase in DA, but the rats were in much better physical condition.

Replenishment of noradrenaline

In group D, four rats were given L-dopa (400 mg/kg) for 5 h without DDC in the hope of restoring the concentrations of NA as well as DA. Activity had only recovered to 40% of normal when DA concentrations were above normal, but NA concentrations were lower at 0·38 ± 0·03 μg/g (63% of normal). All these concentrations were significantly different from that of the control group (C). Activity ($P<0·01$), NA ($P<0·05$) and DA ($P<0·01$).

L-dopa thus failed to antagonize the combined effect of αMT and TBZ although it reverses the effects of αMT (Hanson, 1965; Corrodi, Fuxe & Hokfelt, 1966; Moore & Rech, 1967). On the contrary, L-dopa seemed to depress the recovery

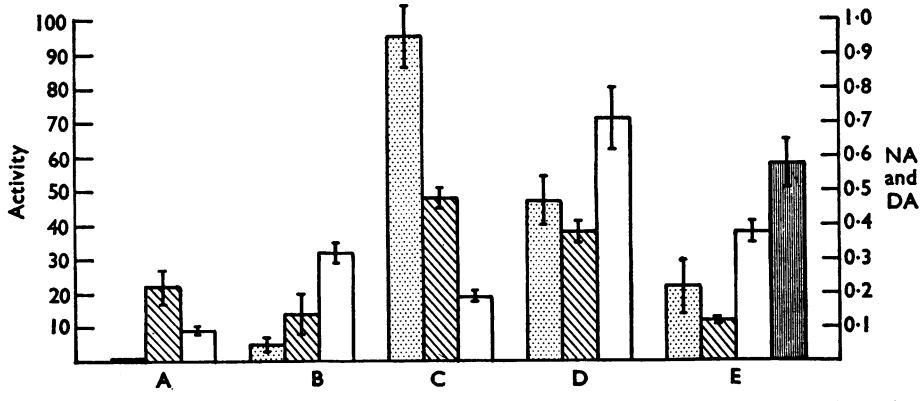

FIG. 4. Effect of selective replenishment of catecholamines by treatment with various drugs. All rats were treated with αMT (50 mg/kg) for 15 h and TBZ (5 mg/kg) for 1 h before additional drug treatments as follows: A=DDC (250 mg/kg) for 9 h, 500 mg/kg for 3·5×4 h; B=DDC (400 mg/kg) for 7·5×4 h and L-dopa (400 mg/kg) for 5 h; C=6·5×4 h after no other drugs; D=L-dopa (400 mg/kg) for 5 h; E=αMMT (100 mg/kg) for 6·5×4 hours. Rat psychomotor activity (dotted columns) expressed as c.p.m. over 10 min and NA (crossed hatched columns), DA (clear columns) and metaraminol (lined columns in E) as μg/g brain stem. $n=8$ for group C and 4 for all other groups.

of NA when compared with control group C, and this coincided with lower levels of activity as well. Dopamine, however, accumulated in excess (140% of normal) and this may have had a depressant effect on NA replenishment.

Replenishment with metaraminol

In order to build up a concentration of metaraminol instead of noradrenaline in the brain, rats were given αMMT (100 mg/kg). This not only prevented the replenishment of NA in rats pretreated with αMT and TBZ but also the recovery of activity. NA remained at a very low level (11% of normal) and although activity was also retarded at 19% of normal, DA was replenished to 76% of normal. The chromatographic demonstration of the presence of metaraminol was confirmed by fluorometric estimation, and was of the order of $0{\cdot}58 \pm 0{\cdot}07$ μg/g, almost the same as the normal concentration of NA. However, metaraminol did not seem to elevate the level of activity as was the case when αMMT was given alone without prior depletion of NA. Thus, in the absence of any NA, as in this instance, metaraminol did not act as an effective false transmitter. (See Fig. 4.)

Discussion

These results add to the view that there are several compartments of noradrenaline in the adrenergic nerve terminals of the central nervous system. The ' biosynthetic pathway ' disrupted by α-methyl-*p*-tyrosine and the ' storage granule ' fraction depleted by tetrabenazine seem to belong to a common ' biosynthetic storage granule ' fraction which is directly concerned with the maintenance of activity, as both these drugs produced a significant correlation between activity and brain noradrenaline. This fraction could be compared with the ' functional pool ' described by Weissman *et al.* (1966) and Rech *et al.* (1968).

On the other hand, α-methyl-*m*-tyrosine which depletes noradrenaline by displacement with stoichiometric amounts of metaraminol, did not produce any correlation between activity and noradrenaline, and could be said to affect the ' non-functional pool ' of noradrenaline. It seemed at first that metaraminol in fact acted as a ' false transmitter ' by displacing noradrenaline and taking over its function. The argument was further strengthened by the finding that depletion of metaraminol by tetrabenazine also reduced activity. However, when catecholamines were depleted from the functional pool by α-methyl-*p*-tyrosine and tetrabenazine and then replaced by metaraminol (instead of dopamine and noradrenaline) by administering α-methyl-*m*-tyrosine, activity remained depressed at 15% of normal, despite the fact the concentration of metaraminol in the brain was comparable to normal concentrations of noradrenaline.

We suggest therefore that when metaraminol merely displaces noradrenaline from the non-functional pool it does not affect the small functional fraction which maintains activity. Treatment with tetrabenazine depletes the functional fraction and activity is depressed for this reason rather than because of loss of dopamine or metaraminol. The fact that tetrabenazine, unlike reserpine (Carlsson, Dahlstrom, Fuxe & Hillarp, 1965) also depletes metaraminol is therefore coincidental. This means that although metaraminol was taken into the functional pool when formed from α-methyl-*m*-tyrosine after prior depletion of noradrenaline by α-methyl-*p*-tyrosine and tetrabenazine, it was unable to maintain activity, and this evidence puts into doubt the concept of its role as an effective false transmitter (Carlsson, 1964 ; Kopin, 1968).

Further experimental evidence was also obtained which indicates that noradrenaline rather than dopamine is involved in the maintenance of activity. The previous correlation between activity and dopamine after α-methyl-*m*-tyrosine (40 mg/kg) (Chan & Webster, 1971) was not maintained after 400 mg/kg, when dopamine concentrations were reduced without activity being decreased. Also, a comparison of the different groups of rats on a treatment regime of α-methyl-*p*-tyrosine and tetrabenazine to deplete all catecholamines showed no correlation between activity and dopamine, but there was a highly significant correlation between activity and noradrenaline (r 0·82 ; n 16) in this instance. In addition to this, the dopamine-β-hydroxylase inhibitor (DDC), when given to such animals, potentiated the sedation produced by α-methyl-*p*-tyrosine and tetrabenazine despite high concentrations of dopamine. In these experiments noradrenaline was not reduced sufficiently to account for the low level of activity recorded and rats treated with diethyldithiocarbomate seemed to be under an additional depressant effect. High doses in fact suppressed the formation of dopamine as well as noradrenaline.

Thus it is concluded that any possible role of dopamine or metaraminol in animals treated with α-methyl-*m*-tyrosine, in maintaining activity is secondary in importance to that of noradrenaline and this agrees with the report of Randrup & Munkvad (1967) that noradrenaline is responsible for locomotion. However, gross concentrations of noradrenaline are not correlated with activity, which may be determined by a small biosynthetic storage fraction. Also the peripheral effects of the drugs used in these studies and their effect on the turnover of brain amines have not been investigated and cannot be ignored.

We wish to thank Professor Hannah Steinberg for the use of activity cages and Mr. I. M. Jones for valuable technical assistance and the preparation of figures.

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(*Received August* 24, 1970)