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Effect of tetrabenazine and α-methyl-*m*-tyrosine on exploratory activity and brain catecholamines in rats

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Summary

1. Spontaneous exploratory locomotor activity of Wistar rats was measured in photocell activity cages, and brain noradrenaline (NA) and dopamine (DA) were determined fluorometrically after ion exchange purification.

2. Tetrabenazine (TBZ) (10 mg/kg) produced a fall in NA and DA concentrations in rat brain stems which was correlated with the fall in activity in female Wistar rats.

3. α-Methyl-*m*-tyrosine (αMMT) reduced the concentration of rat brain NA without affecting DA concentration or activity.

4. Pretreatment with αMMT did not stop TBZ from producing a marked reduction in activity and NA concentration, but partially protected DA from the depleting action of TBZ.

5. These results support a role for catecholamines in the control of motor activity, but they do not implicate NA more than DA and they emphasize that the mechanism by which drugs affect the concentrations of catecholamines may be more important than the gross concentrations attained.

Introduction

The role of monoamines in the central nervous system was brought into focus when it was found that reserpine depleted both 5-hydroxytryptamine (Brodie, Pletscher & Shore, 1955) and catecholamines (Holzbauer & Vogt, 1956). Shore, Pletscher, Tomich, Carlsson, Kuntzman & Brodie (1957) suggested that excess free 5-hydroxytryptamine (5HT) was responsible for reserpine sedation, whilst Carlsson (1959) favoured lack of noradrenaline (NA) as the responsible factor. In support of this, Matsuoka (1964) found that selective depletion of NA by oxypertine produced sedation.

Correlation of gross NA concentrations with the degree of sedation has not been obtained. Haggendal & Lindqvist (1964) found that with chronic reserpinization in rats this correlation could only be obtained after the stores of NA had been almost depleted, and Carlsson (1964) concluded that the storage function of the nerve granule was affected by reserpine and that this and not the gross concentration of NA was responsible for the maintenance of activity.

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In this work the problem has been approached by attempting to correlate a single parameter of behaviour with catecholamine content, when this was altered by drugs having different modes of action. The particular function of behaviour which appeared most suitable was that of spontaneous exploratory activity, as it is a primary drive mechanism responsible for the survival of the rat (Barnett, 1958) which is located in the reticular formation (Hebb, 1955) and easily quantitated. The particular drugs chosen were tetrabenazine (TBZ) and α-methyl-*m*-tyrosine (αMMT). TBZ depletes catecholamines, presumably by an action on the storage granule like reserpine, whose action it blocks (Quinn, Shore & Brodie, 1959). It has a short duration of action and its effect is almost entirely central. αMMT does not act on the storage granule, but selectively displaces NA by its decarboxylated product metaraminol (Shore, Busfield & Alpers, 1964).

Methods

Catecholamine assays

Rat brain stems, average weight $0{\cdot}58 \pm 0{\cdot}004$ g (S.E.) were homogenized in 0·1 N HCl. The proteins were precipitated with 0·4 N perchloric acid and the extracts purified through Dowex $50W \times 8$ (200–400 mesh) ion exchange resin. NA was eluted with 0·9 N HCl and dopamine (DA) with 2 N HCl.

Noradrenaline

NA was estimated by the trihydroxyindole method as used by Sharman, Vanov & Vogt (1962), using a Locarte Fluorimeter with a single sided monochromator. Activation wavelength was 365 nm (LF_2 filter) and fluorescence was read at 530 nm (Kodak Wratten No. 58 filter). Standard DL-noradrenaline bitartrate (Sigma) was used, and sample values were subject to correction by individual internal standards. Eighty-four per cent recovery was obtained.

Dopamine

DA was estimated according to the method of Laverty & Sharman (1965). DA was subjected to acetylation before ethylenediamine condensation and estimated in a Locarte fluorimeter activated at 436 nm (LF_3 filter) and fluorescing at 525 nm (monochromator). Eighty-eight per cent recovery was obtained.

Spontaneous locomotor activity

Spontaneous orientational locomotor activity was measured in individual photocell cages according to the method of Dews (1953). The cages were wire mesh cubes with internal dimensions of 26·5 cm. Two light beams at right angles to each other and directed across the cages 4 cm above the floor, intersected at the centre of the cage. At the point of intersection, a Perspex block 12·5 cm wide was fitted from floor to roof so that the rat could not interrupt both light beams at the same time. The animal was put into the cage through a swing door on one side. Interruptions of the light beams were relayed through photocells to a pair of digital counters.

Counts were made every 2·5 min for 10 minutes. A cumulative count after 10 min was also recorded and used as the quantitative measure of locomotor activity. Activity measurements were always made between the hours of 16.30–17.30 under

controlled environmental conditions. Only naive rats were used. Generally two cages and two counters were used together.

Animals

The experiments were carried out on female Wistar rats weighing 155 g–220 g.

Drugs

The drugs used were: tetrabenazine (Nitoman, Roche); α-methyl-meta-tyrosine (Merck, Sharpe and Dohme); DL-noradrenaline bitartrate (Sigma); 3-hydroxytyramine (dopamine, Sigma).

Results

Effect of tetrabenazine (10 mg/kg)

Rats were tested in activity cages 1 h after saline injection, and 3, 6 and 9 h after injection of 10 mg/kg TBZ intraperitoneally, after which the rats were killed and the brains removed for NA estimation. DA concentrations were estimated in another group of rats without activity measurements. They included six saline controls, six injected with 10 mg/kg TBZ for 1 h and five injected with 10 mg/kg TBZ for 6 hours.

The results (Fig. 1) show that both brain amine concentrations and spontaneous locomotor activity are reduced after TBZ.

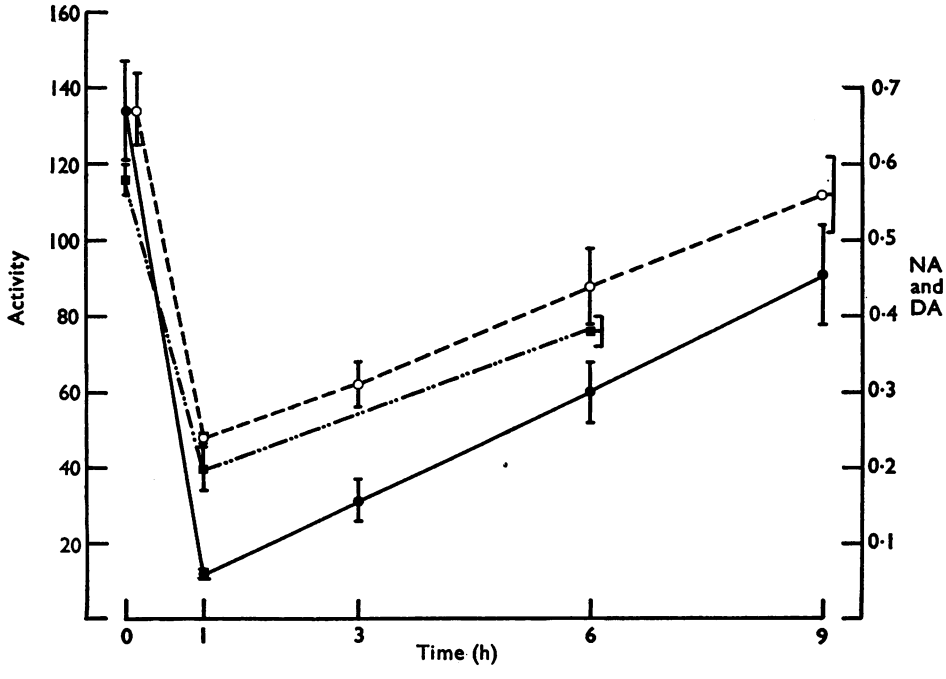

FIG. 1. Effect of tetrabenazine (10 mg/kg, intraperitoneally) on rat psychomotor activity (●——●), in c.p.m., and brain stem NA (○- - -○) and DA (■—··—■) concentrations in μg/g. $n=8$ for activity and NA, and 6 for DA.

Activity

Activity counts fell to 9% of normal 1 h after 10 mg/kg TBZ and gradually returned to within 68% of normal (at 9 h). There was a considerable variation in the score at any one time. The mean activity count of 23% of normal (at 3 h) was not significantly different from the 1 h level, but that at 6 h (45% normal) was, P (0·05).

An examination of the pattern of activity as seen in the score differentiated at 2·5 min intervals (Fig. 2) showed that, irrespective of the level of activity, the shape of the curves for the different recording periods did not vary much. The inference is that the same function was being measured.

Brain catecholamines

Brain NA was reduced to 33% of normal 1 h after 10 mg/kg TBZ. After 3 h, replenishment started to occur but the concentration then at 46% of normal was not significantly different from the 1 h concentration. After 6 h the concentration of NA had recovered to 66% of normal and was significantly different from the 1 h

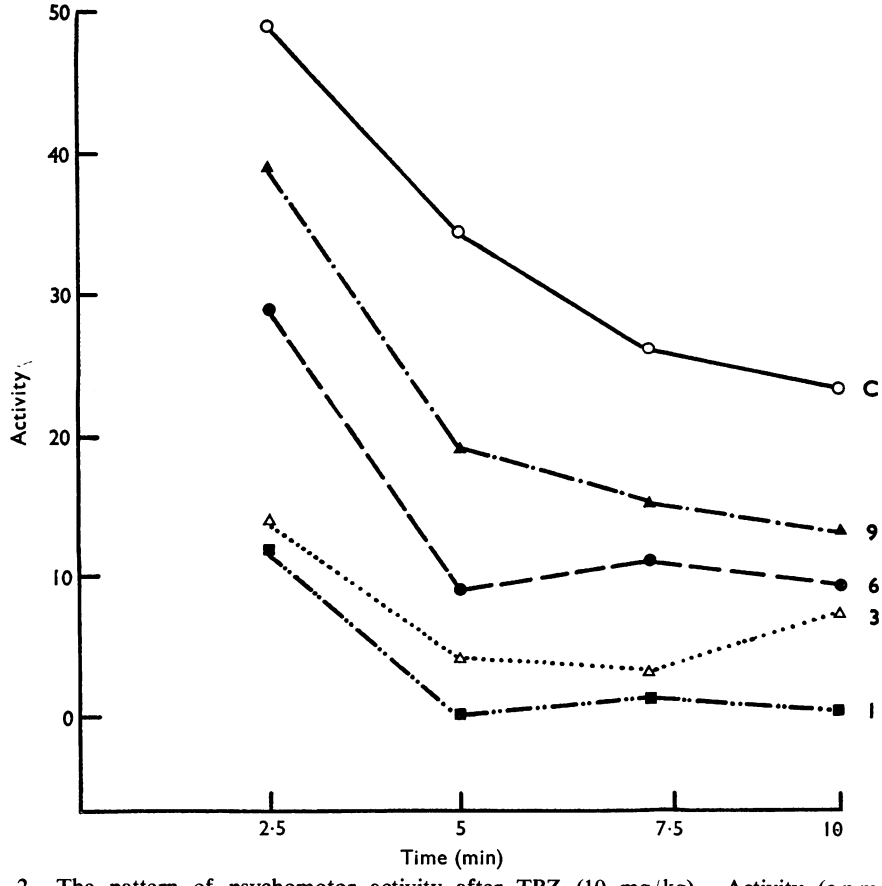

FIG. 2. The pattern of psychomotor activity after TBZ (10 mg/kg). Activity (c.p.m.) is differentiated for every 2·5 min of the 10 min recording period. Each point is the mean of eight rats. Results are plotted for control animals (○——○) and for varying times after TBZ. (■—··—■), 1 h; (△·····△), 3 h; (●– –●), 6 h; and (▲—·—▲), 9 hours.

concentration ($P<0{\cdot}05$) but not from the 3 h concentration ($P<0{\cdot}20$). After 9 h, 84% of normal NA concentration had been replenished. This was not significantly different either from normal concentrations or from 6 h concentrations.

A similar picture was seen for DA which fell to 34% of normal after 1 h and had reached 66% of normal in 6 h, a level which was significantly different from both 1 h ($P<0{\cdot}05$) and normal concentrations ($P<0{\cdot}01$).

Correlation

Figure 1 shows a good correlation of activity with brain amine content. A fall to one-third of normal values for both NA and DA resulted in a fall to one-tenth of normal activity. Replenishing concentrations of catecholamines were also accompanied by returning levels of activity. The correlation was highly significant for activity and NA content ($r=0{\cdot}77 : n=39$) ($P<0{\cdot}01$).

There was insufficient information to calculate the correlation coefficient for DA since this amine and activity were not measured in the same animals.

*Effect of α-methyl-*m*-tyrosine (40 mg/kg)*

In order to deplete NA but not DA, α-methyl-*m*-tyrosine (αMMT) (40 mg/kg) was used.

Spontaneous locomotor activity was recorded in thirteen female Wistar rats weighing 165–205 g, divided into three groups: three saline treated controls, four

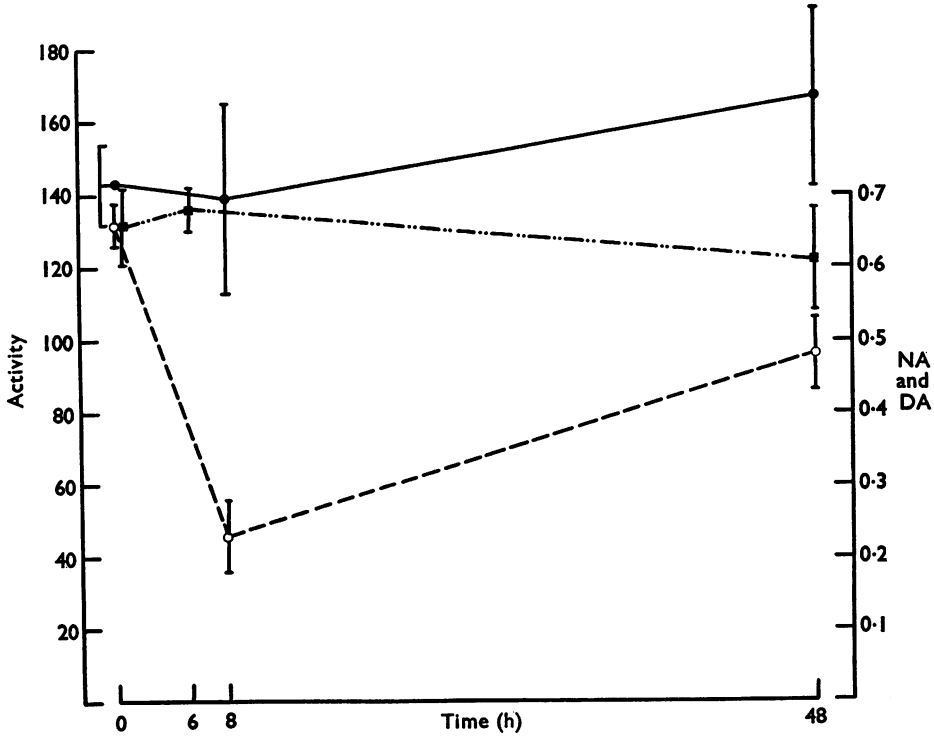

FIG. 3. Effect of αMMT (40 mg/kg intraperitoneally) on rat psychomotor activity (●——●) in c.p.m. and on the concentrations of brain stem NA (○- - -○) and DA (■—··—■) in μg/g. $n=9$ for controls, 4 at 8 h, 6 at 48 hours.

injected with 40 mg/kg αMMT intraperitoneally for 8 h, and six treated with αMMT for 48 hours. The rats were sacrified and brain NA estimated. Brain DA was estimated in another ten rats (175–195 g), five after αMMT treatment for 6 h and five after 48 hours. The results are shown in Fig. 3.

Activity

αMMT did not affect the activity scores. After 8 h activity was still 99% of normal. After 48 h it was 118% of normal. Although the photocell activity cage did not pick up anything significantly different, observation of the treated animals, especially at 48 h, showed hyper-excitability and hyper-aggressiveness.

Brain catecholamine

αMMT lowered NA concentrations to 35% of normal, a comparable effect to 10 mg/kg TBZ. They had recovered to 73% of normal after 48 hours. The concentrations at these two points were significantly different ($P<0{\cdot}05$). DA concentrations remained within normal limits at both 8 and 48 h after αMMT administration.

The result of administering 40 mg/kg αMMT to rats was thus markedly different from that obtained after TBZ. The concentration of NA was reduced selectively, with no effect on either activity or DA concentrations, suggesting that either DA or the metabolites of αMMT may be responsible for the maintenance of activity. Experiments were therefore performed to see if TBZ after αMMT would produce a reduction in DA which was correlated with depression of activity.

Effect of a combination of αMMT (40 mg/kg) and TBZ (10 mg/kg)

Spontaneous activity, and brain NA and DA concentrations were measured in female Wistar rats (thirty-seven) weighing 155–210 g divided into the following experimental groups: (a) Five saline controls, five rats receiving αMMT (40 mg/kg) for 7 h and four rats receiving αMMT (40 mg/kg) for 7 h plus TBZ (10 mg/kg) for 1 hour. Activity, NA and DA were measured. (b) Four saline controls, four rats receiving TBZ (10 mg/kg) for 1 h, and four rats receiving αMMT (40 mg/kg) for 7 h plus TBZ (10 mg/kg) for 1 hour. Only activity and DA concentration were measured. (c) Four rats with αMMT (40 mg/kg) for 7 h, three rats with TBZ (10 mg/kg) for 1 h, and four rats with αMMT (40 mg/kg) for 7 h plus TBZ (10 mg/kg) for 1 hour. Only DNA was measured.

The pooled results are presented in Fig. 4, which shows that administration of both αMMT and TBZ so that their peak effects coincide, depressed activity and NA concentration to the same extent as TBZ alone; but αMMT offered partial protection to the depletion of DA by TBZ.

Activity

The mean activity score for control rats was 170 ± 16, which was higher than in experiment 1. The effect of αMMT (40 mg/kg) was to raise the mean activity score to 181 ± 39 (106%), although most of this increase was due to one animal (count 366). This effect was completely obliterated by the administration of TBZ (10 mg/kg) when the mean activity was reduced to 8 ± 3 (5% normal). Compared

with the results obtained from TBZ treatment alone, there was no significant difference, suggesting a predominant TBZ effect.

Brain catecholamines

α-MMT (40 mg/kg) reduced NA concentrations by 50%, similar to TBZ (10 mg/kg) alone (experiment 1). However, the combined treatment resulted in an additive effect by which NA was further reduced to 20% of normal.

DA concentrations were not affected by α-MMT treatment (96% of normal) but fell significantly to 55% of normal after TBZ ($P<0.01$). The combined treatment resulted in a reduction to 67% ($P<0.05$), demonstrating that α-MMT pretreatment protected DA to some extent ($t=2.74$, $P<0.02$) from the full depleting effect of TBZ. This protective effect has also been reported by Carlsson & Lindqvist (1967). Thus, these results provide no further evidence that DA is correlated with activity.

Effect of αMMT and αMMT + TBZ on male rats

Twenty male Wistar rats 165–230 g were treated in the following manner and then sacrified for brain NA and DA concentrations: eight untreated control rats, three rats with αMMT (40 mg/kg) for 7 h, and nine rats with the combination of

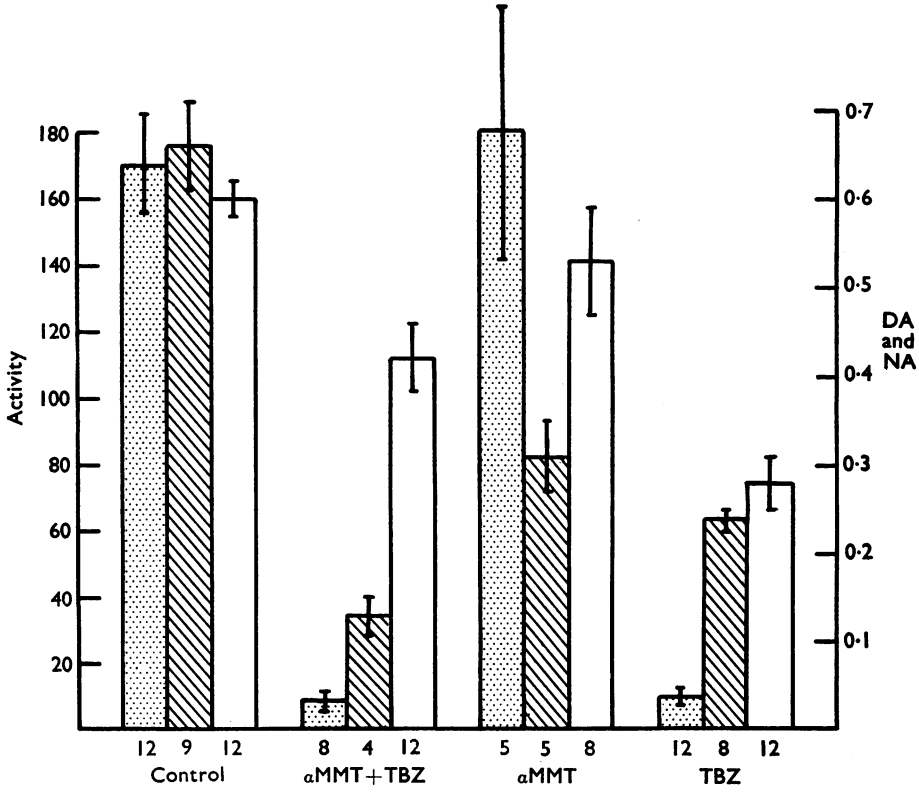

FIG. 4. Effect of αMMT and TBZ and the combination of both drugs on the rat psychomotor activity (dotted columns) in c.p.m. and on the concentrations of brain stem NA (hatched columns) and DA (clear columns) in μg/g. Value for *n* at base of each column. αMMT= 40 mg/kg αMMT for 7 h; αMMT+TBZ=40 mg/kg αMMT (7 h) and 10 mg/kg TBZ (1 h). TBZ=10 mg/kg TBZ for 1 hour.

αMMT (40 mg/kg) for 7 h and TBZ (10 mg/kg) for 1 hour. The effect of αMMT (40 mg/kg) on the brain catecholamine concentrations in male rats differed from that in females. NA concentrations were depleted to 5% of normal whilst DA, which remained unaffected in female rats, was depleted significantly to 57% of normal. Combination of αMMT and TBZ did not produce any further fall in DA concentrations. Thus male rats seemed to be more susceptible to the catecholamine depleting action of αMMT although the limited number of results precludes any firm conclusion.

Discussion

The results of these experiments indicate that there is no general correlation in rats between the gross concentrations of noradrenaline or dopamine in the brain and spontaneous locomotor activity, although with some drugs a correlation can be found.

Tetrabenazine, which has a reserpine-like action (Quinn *et al.*, 1959) and affects storage function, produced a highly significant correlation between noradrenaline concentrations and activity and there was also a suggestive correlation for dopamine concentrations. After α-methyl-*m*-tyrosine, the correlation between noradrenaline concentrations and activity was lost. Noradrenaline was markedly depleted, leaving activity and dopamine unaffected, suggesting that dopamine might be responsible for activity. However, α-methyl-*m*-tyrosine causes a selective depletion of noradrenaline due to displacement by stoichiometric amounts of metaraminol (Anden, 1964) and if this takes over the function of noradrenaline then activity could be maintained in the absence of noradrenaline due to metaraminol rather than dopamine.

In an attempt to separate these possibilities, tetrabenazine was given to rats pretreated with α-methyl-*m*-tyrosine, when it was found that although activity was reduced to a degree similar to that with tetrabenazine alone, there was a smaller loss of dopamine (67% of control instead of 50% after tetrabenazine alone), which might imply that a reduction in dopamine alone cannot account for loss of activity. This was also substantiated by the finding that in a few experiments performed on male instead of female rats, α-methyl-*m*-tyrosine caused a reduction in dopamine to 58% of normal without apparently affecting activity.

If the loss of dopamine with tetrabenazine after α-methyl-*m*-tyrosine is not sufficient to account for the marked reduction in activity, then the possibility remains that metaraminol was depleted by tetrabenazine. Certainly metaraminol can be released by noradrenaline releasing drugs like the sympathomimetic amines (Shore, *et al.*, 1964) but it should be noted that Carlsson (1964) has reported that metaraminol is resistant to depletion by reserpine, and therefore if tetrabenazine acts like reserpine it could be that it is reducing activity by some action other than by depletion of metaraminol. This can only be resolved by estimating brain metaraminol concentrations.

One possibility is that the marked reduction in activity with TBZ after αMMT pretreatment followed the additional fall in NA from 50% to 20% of normal. A similar correlation between activity changes and reduced concentrations of NA was also found by Haggendal & Lindqvist (1964) when they gave reserpine to rats after chronic reserpinization had already severely depleted the concentrations of NA, although the initial depletion produced no activity changes. This again

emphasizes that gross brain amine concentrations of NA are not indicative of activity but that storage function might be or that there are two pools of NA, a large non-functional entity and a smaller functional pool and that it is only when the latter is affected that activity is altered.

In conclusion it can be said that the mechanism by which a drug reduces the concentration of brain noradrenaline determines whether or not there is a correlation between noradrenaline concentrations and activity. α-Methyl-*m*-tyrosine depletes noradrenaline presumably by displacement with metaraminol whilst tetrabenazine affects storage function without producing a false transmitter. Whether or not tetrabenazine depletes the false transmitter when it reduces activity after α-methyl-*m* tyrosine remains to be determined. If it fails to do so then some other mechanism such as the control of a small functional pool of noradrenaline not related to gross amine concentrations might be the relevant factor, although the role of dopamine has not been fully eliminated. Also since 5HT has not been estimated its role cannot be assessed or completely ignored in the studies with tetrabenazine.

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