# Evidence for a cholinergic mechanism in brain involved in the tetrabenazine reversal by thymoleptic drugs

The antagonism of the reserpine-like syndrome elicited in rats by tetrabenazine and other synthetic benzoquinolizines is used as a screening procedure for imipramine-like tricyclic antidepressants. The interest has been particularly focused on the antagonism of the reserpine-like behavioural depression (sedation, catalepsy) and its conversion into a "compulsive" locomotor hyperactivity (Bickel & Brodie, 1964; Matussek & Rüther, 1965; Sulser & Soroko, 1965; Sulser, Owens & others, 1968).

A similarity seems to exist between all the behavioural features shown by rats treated with thymoleptic agents of the imipramine group and tetrabenazine and rats treated with a single large dose of reserpine. In previous papers from this laboratory a "paradoxical" stereotyped behaviour consisting of locomotion, sniffing and rearing has been described in reserpinized rats (Schiørring & Randrup, 1968; Scheel-Krüger & Randrup, 1968). This behaviour, which occurred in several rats as periodic bursts of hyperactivity about 4–6½ h after reserpine, has been shown to be dependent on a cholinergic mechanism in brain (Scheel-Krüger & Randrup, 1968; Scheel-Krüger, in preparation).

However, in contrast the behaviour shown by the rats receiving a thymoleptic and tetrabenazine was observed to be more pronounced and more continuous.

The present experiments were made to establish whether a cholinergic mechanism in brain had any influence on the compulsive locomotor activity provoked by thymoleptics and tetrabenazine.

Male Wistar rats, 225–275 g, were kept in individual cages (floor area 21 × 27 cm and height 16 cm). Drug injections were given subcutaneously. In preliminary experiments 14 rats received 20 mg/kg of desipramine 2 h before 100 mg/kg of tetrabenazine. Another 14 rats received 40 mg/kg of nortriptyline 1 h before 100 mg/kg of tetrabenazine.

Eleven rats from the desipramine-tetrabenazine treatment and another 11 rats from the nortriptyline-tetrabenazine treatment showed characteristic compulsive activity, which began 1–3 h after the tetrabenazine injection in the desipramine group and 2-4 h after tetrabenazine in the nortriptyline group. The rearing (standing-up on hind-legs) occurred most often during the locomotion when the rat reached a corner of the cage.

Hyperactivity of this kind has been observed, in previous experiments for periods of more than 6 h at a time. When the activity had gone on for at least 1 h the rats were given 10 mg/kg of hyoscine. The locomotion was immediately continued when the rats were returned to the cages. However, from 5–10 min after hyoscine the locomotion, sniffing, rearing, and exophthalmus were completely inhibited for more than 1 h. The rats showed the characteristic hunched-back "bison" posture with ptosis. Catalepsy, tested for on a vertical wire netting, was present.

The inhibition was shown by all rats from the desipramine group (11/11) and all but one rat of the nortriptyline group (10/11). The 3 rats from each treatment group which did not show any reversal with hyperactivity even 3½ h after tetrabenazine, received 0·2 mg/kg physostigmine. From 5–15 min after this injection the catalepsy and ptosis were antagonized and all 6 rats began the compulsive locomotor activity with sniffing and rearing.

A quantitative evaluation of these behavioural activities was made in the following experiments (Table 1).

Table 1. *A cholinergic mechanism involved in the tetrabenazine-thymoleptic reversal of locomotion, sniffing and rearing.* All these rats from the desipramine-tetrabenazine groups showed continuous behavioural activities for at least 1 h before the injection of saline or hyoscine given 3½ h after tetrabenazine. The rats from the imipramine-tetrabenazine groups did not show any behavioural activities before the injections of physostigmine or saline given 2½ h after tetrabenazine. 5 min after the last drug injections (hyoscine, physostigmine or saline) the locomotion (L), sniffing (S) and rearing (R) of each rat were counted for the following hour. Maximal count is 30.

| Desipramine-tetrabenazine | | | | | | Imipramine-tetrabenazine | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Saline Behavioural counts for each rat | | | Hyoscine Behavioural counts for each rat | | | Saline Behavioural counts for each rat | | | Physostigmine Behavioural counts for each rat | | |
| L | S | R | L | S | R | L | S | R | L | S | R |
| 24 | 30 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 7 | 6 |
| 30 | 30 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 9 | 2 |
| 30 | 30 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 29 | 3 |
| 30 | 30 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 13 | 6 |
| 30 | 30 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 17 | 11 |
| 30 | 30 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 17 | 2 |
| 30 | 30 | 29 | 2 | 2 | 0 | 0 | 0 | 0 | 25 | 24 | 9 |
| 30 | 30 | 30 | 13 | 13 | 11 | 0 | 0 | 0 | 27 | 25 | 11 |
| 30 | 30 | 30 | 15 | 15 | 5 | 9 | 10 | 7 | 30 | 27 | 1 |
| 30 | 30 | 30 | 23 | 23 | 21 | 18 | 15 | 15 | 30 | 30 | 8 |
| | | | | | | 30 | 30 | 30 | | | |
| | | | *<0·01, <0·01, <0·01 | | | | | | *<0·05, <0·05, >0·05 | | |
| | | | †≦0·01, ≦0·01, ≦0·005 | | | | | | †≦0·005, ≦0·005, ≦0·005 | | |

* Values of *P* for the effect of hyoscine and physostigmine on the behavioural activities compared with the saline controls (The Rank test, Snedecor, 1956).

† Values of *P* for the corresponding comparison of the number of rats giving responses (Fischer's exact probability test, Siegel, 1956).

Compulsive hyperactivity was provoked in the rats by desipramine (20 mg/kg) given 2 h before tetrabenazine (100 mg/kg). Hyoscine (10 mg/kg) was injected 3½ h after tetrabenazine into 10 rats and another 10 rats received saline. Only rats previously showing continuous behavioural activities for at least 1 h before the injections of either hyoscine or saline were used.

Ten rats received imipramine (50 mg/kg) 2 h before tetrabenazine (100 mg/kg). Physostigmine (0·2 mg/kg) was injected 2½ h after tetrabenazine. Eleven rats received the same drug treatment but received saline instead of physostigmine. The rats from this imipramine treatment did not show any behavioural activities during 2½ h after the tetrabenazine injections. Preliminary experiments had shown that the reversal of the tetrabenazine sedation with imipramine was a very weak effect which only occurred in a few rats and usually about 4–5 h after tetrabenazine.

The locomotion, sniffing and rearing were counted by visual observation. After every 2 min period the presence of each of these behavioural features was recorded separately for each rat with 1 point for a positive response. The behavioural countings were made 5 min after the last drug injections (hyoscine, physostigmine or saline) and were continued for the following hour. The maximal count is thus 30 for each behavioural feature.

The results obtained for each of these 41 rats and comparisons of the drug treatments made by the Rank test (Snedecor, 1956) are given in Table 1.

The present experiments imply that a cholinergic mechanism in brain is involved in the tetrabenazine-reversal of locomotion, sniffing and rearing provoked by thymoleptic agents of the imipramine group. A close similarity thus seems to exist between this reversal and the "paradoxical" stereotyped behaviour shown by reserpinized rats. However, the metabolism of the catecholamines is probably implicated in the reversal of the tetrabenazine-sedation by thymoleptic agents into a locomotor hyperactivity since two conditions for the reversal have been previously considered necessary: first, a marked and rapid depletion of the catecholamine level in the brain (tetrabenazine action), and second, blockade of the amine-uptake mechanism at the level of the cell membrane (thymoleptic action) (Sulser, Bickel & Brodie, 1964; Matussek & Rüther, 1965; Sulser & Soroko, 1965; Sulser & others, 1968).

In addition, it seems to us that the possibility now exists that this reversal is closely connected with the depression of the central noradrenaline neurotransmission leaving a central cholinergic predominance. Thus, the reversal does not occur when the release rate of noradrenaline is greatest but when the level of noradrenaline seems most depressed (Sulser & others, 1964; Bartonicek, 1965; Sulser & Soroko, 1965; Matussek & Rüther, 1965; Häggendal, 1968).

The noradrenaline biosynthesis is strongly affected by reserpine, perhaps because reserpine (and tetrabenazine) inhibits the uptake of dopamine into the amine granules to which the enzyme dopamine-$\beta$-oxidase is bound (Dahlström, Fuxe & Hillarp, 1965; Kaufman & Friedman, 1965; Stjärne, 1966; Udenfriend, 1966; Rutledge & Weiner, 1967).

Further, the possibility seems to exist that thymoleptic drugs, by blocking the reuptake of extraneuronal-released catecholamines, increase the depletion induced by reserpine or tetrabenazine of a functionally imporant amine pool. Carlsson & Waldeck (1965) and Hamberger & Malmfors (1967) reported the increased disappearance of [$^3$H]metaraminol and $\alpha$-methylnoradrenaline by combined treatment with protriptyline and reserpine. High doses of thymoleptics decreased the endogenous levels of the catecholamines (Hamberger, 1967).

Carlsson, Fuxe & others (1966) found that both desipramine and protriptyline reduced the accumulation of noradrenaline after dopa in both central and peripheral noradrenaline neurons in rats pretreated with reserpine and nialamide.

A preliminary result of ours showed that a high dose of aceperone (10 mg/kg) which is an extremely potent noradrenaline antagonist with central effect (Janssen, Niemegeers & others, 1967) did not inhibit the compulsive locomotion, sniffing and rearing produced by desipramine and tetrabenazine (10 rats).

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