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# The effect of monoamine depletors on metrazol induced convulsions and brain γ-aminobutyric acid (GABA) contents in rats

WADIE T. ABED, *Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan*

**Abstract**—The thresholds for metrazol (pentylenetetrazol) clonic convulsions and brain γ-aminobutyric acid contents were significantly reduced after treatment with the monoamine depletors reserpine, tetrabenazine and *p*-chlorophenylalanine. Moreover, α-methyltyrosine, and α-methyl-*m*-tyrosine also lowered metrazol threshold seizures, but had no effect on brain α-aminobutyric acid contents. Furthermore, neither 5-hydroxytryptophan nor tranylcypromine had a significant effect on metrazol threshold seizures or brain α-aminobutyric acid contents, but blocked the changes previously induced by *p*-chlorophenyl alanine and reserpine.

Disturbances in brain level of one biogenic amine are likely to alter the balance with other amines and may affect their level simultaneously. Subsequent alterations in enzymes necessary for the synthesis or metabolism of a particular amine, can lead to selective changes in tissue amine contents. This effect could be of value in understanding the interrelationship between various brain biogenic amines. The tyrosine hydroxylase inhibitor, α-methyltyrosine (α-MT) provides a means of depleting central stores of noradrenaline (NA) and dopamine (DA) without affecting 5-hydroxytryptamine (5-HT) concentrations (Spector et al 1965; Rech et al 1966), while the catecholamine releasing agent α-methyl-*m*-tyrosine (α-MMT) causes depletion of NA (Hess et al 1961; Porter et al 1961). Moreover, *p*-chlorophenylalanine (*p*-CPA) lowers brain 5-HT levels by blocking the synthesis of the amine at the rate-limiting tryptophan hydroxylase step (Koe & Weissman 1966). The present study was designed to explore the possible interrelationship between monoamines and GABA in the brain of rats, as well as measurements of metrazol (pentylenetetrazol) seizure thresholds after different treatments.

## Materials and methods

Wistar strain albino rats (170 to 200 g) of either sex were housed in groups of 4–6 with free access to food and water in a room maintained on a 12 h light/dark cycle at 23 ± 1°C.

The threshold for metrazol-induced clonic convulsions was determined by the method described by Hint & Richter (1958). Metrazol was dissolved in distilled water and a 3% solution was infused into the tail vein at a constant rate of 5 μL $s^{-1}$ by syringe pump. The duration of the infusion required to elicit generalized clonic convulsions was recorded, the clonic convulsion threshold being expressed as the mean ± s.e.m. of the dose of metrazol $kg^{-1}$. The animals were killed after the clonic convulsions and their brains removed and assayed for GABA according to Graham & Aprison (1966).

The monoamine depletors reserpine, tetrabenazine, α-MT, α-MMT and *p*-CPA were either dissolved in distilled water or suspended in 1% carboxymethylcellulose. All drugs were administered intraperitoneally (i.p.) (2 mL $kg^{-1}$). Control animals received the vehicle only. Since the effect of reserpine is partly central and partly peripheral (Brodie et al 1957), tetrabenazine which acts only centrally (Quinn et al 1959) was also used. Reserpine (5 mg $kg^{-1}$), tetrabenazine (50 $mg^{-1}$ kg) and α-MMT (300 mg $kg^{-1}$) were also administered i.p. in single doses and later challenged with metrazol at 6, 3 and 24 h, respectively. α-MT (50 mg $kg^{-1}$ i.p.) was administered at the interval of 4 h for three injections and the animals were challenged with metrazol 4 h after the last injection. *p*-CPA (100 mg $kg^{-1}$ i.p.) was administered for three consecutive days and the animals were tested 24 h after the last injection. In experiments with the combined effects of 5-hydroxytryptophan (5-HTP) and *p*-CPA, the animals received *p*-CPA for three consecutive days and 24 h after the last injection of *p*-CPA the rats received 5-HTP 100 mg $kg^{-1}$ and were tested 1 h later. For the combined effect of reserpine and tranylcypromine, rats were given reserpine (5 mg $kg^{-1}$ i.p.) 2 h before tranylcypromine (100 mg $kg^{-1}$ i.p.) and 4 h later were challenged with metrazol. Higher and lower doses of drugs as well as different times after administration were used.

The data obtained from the measurements of the threshold for metrazol-induced clonic convulsions as well as the measurements of brain GABA contents were assessed by Student's *t*-test.

## Results

The data in Table 1 show that the monoamine depletors

Table 1. Metrazol threshold seizures and brain GABA contents after different treatments with monoamine modulators in rats.

| Treatment | Dose (mg $kg^{-1}$) | Time (h) | PTZ threshold (mg $kg^{-1}$) | Brain GABA μg $g^{-1}$ |
|---|---|---|---|---|
| Control | — | — | 58·40 ± 2·35 | 146·25 ± 4·8 |
| Reserpine | 5 | 6 | 40·30 ± 3·16** | 118·36 ± 3·5** |
| Tetrabenazine | 50 | 3 | 41·58 ± 3·60** | 122·50 ± 2·8** |
| α-MT | 50 + 50 + 50 | 12 | 40·60 ± 3·60** | 144·80 ± 3·8 |
| α-MMT | 300 | 24 | 43·85 ± 4·10* | 148·60 ± 5·6 |
| *p*-CPA | 3 × 100 | 72 | 42·75 ± 3·15** | 124·60 ± 2·6** |
| Tranylcypromine | 10 | 4 | 56·60 ± 3·10 | 147·26 ± 2·6 |
| Reserpine + Tranylcypromine | 5 + 10 | 6 | 56·85 ± 2·80 | 145·30 ± 1·8 |
| 5-HTP | 100 | 1 | 58·65 ± 2·80 | 148·30 ± 2·8 |
| *p*-CPA + 5-HTP | (3 × 100) + 100 | 73 | 56·85 ± 3·10 | 146·60 ± 3·2 |

Each value is the mean ± s.e.m. of 10 or 12 values.
* $P < 0.01$ When compared with control group.
** $P < 0.001$ When compared with control group.

reserpine, tetrabenazine and *p*-CPA produced statistically significant effects on metrazol-induced clonic convulsion threshold ($p < 0.001$) and on brain GABA contents ($P < 0.001$). Furthermore, α-MT and α-MMT also caused significant lowering of metrazol-induced clonic convulsions ($P < 0.001$, $P < 0.01$, respectively), but had no effect on brain GABA contents. Table 1 also shows that treatment with tranylcypromine restored brain GABA and blocked the changes in metrazol threshold due to reserpine pretreatment. Similarly, treatment with 5-HTP also blocked brain GABA depletion and the reduction in metrazol threshold due to *p*-CPA pretreatment. However, neither tranylcypromine nor 5-HTP had any significant effect on metrazol threshold or brain GABA contents, when given to untreated animals.

## Discussion

The experimental data presented in this study show that monoamine depletors caused profound effects on metrazol-induced clonic convulsions and on brain GABA contents. Reserpine and tetrabenazine caused depletion of brain GABA, an effect which coincided with a decrease in the threshold of metrazol-induced clonic convulsions. α-MT, a selective depletor of NA and DA, and α-MMT, a specific depletor of NA, did not have any significant effect on GABA contents in the brain. However, the metrazol-induced clonic convulsion threshold was decreased in response to treatment with α-MT and α-MMT *p*-CPA, a potent depletor of 5-HT, produced a fall in brain GABA contents and subsequent reduction in the threshold of metrazol-induced convulsions. It is therefore, possible to speculate that the depletion of brain GABA seems to be due to depletion of 5-HT and not to depletion of NA or DA.

The results in this study show that the decrease in both brain GABA contents and metrazol-induced clonic convulsion threshold were effectively and completely blocked by pretreatment with 5-HTP. These results support the view that depletion of brain GABA might be related to depletion of brain 5-HT. Furthermore, 5-HTP failed to induce any changes in brain GABA contents or metrazol threshold when given alone. This effect can be interpreted on the basis that a balance exists between 5-HT and GABA, which when interrupted results in depletion of GABA, and pretreatment with 5-HTP restores GABA to its original level. Moreover, 5-HTP when given alone, and in the presence of a normal balance of biogenic amines, did not cause an increase in brain GABA contents. The decrease in brain 5-HT content induced by reserpine can be selectively blocked by pretreatment with tranylcypromine, without corresponding increase in NA (Green & Erickson 1962). Depletion of brain GABA and decreased metrazol clonic convulsion threshold due to reserpine treatment were reversed by pretreatment with tranylcypromine, but when given alone, tranylcypromine had no effect on the parameters studied (Table 1).

In conclusion and in light of data presented in this study it is therefore possible to suggest that the depletion of brain GABA and the decrease of metrazol clonic convulsion threshold are closely related to 5-HT behaviour in the brain.

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