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Effect of reserpine on the monoamine oxidase (MAO) activity in rat liver and brain

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A variety of drugs affect the concentration of serotonin within the nervous system; prominent among these is reserpine. Reserpine depletes tissues of serotonin and other amines. Kimbal and Vallejo [1] found that reserpine induced headache in migraine sufferers. Experiments performed in this direction showed that a subcutaneous (s.c.) injection of reserpine produced typical unilateral headache 6 hr after injection [2–5]. Reserpine releases serotonin from its major stores, i.e. brain, intestine and platelets. Further studies have shown that reserpine and also other related Rauwolfia alkaloids deplete stores of catecholamines in addition to serotonin in the brain and other organs. Most of the pharmacological effects of these alkaloids have been attributed to this action. The depletion and restoration rates are different in different organs. In view of these observations it was thought worthwhile to study the metabolism of neurotransmitter monoamines. Since these are the substrates for the enzyme monoamine oxidase (MAO), (monoamine: 0_2 oxidoreductase; E.C. 1.4.3.4), experiments were undertaken to study the *in vivo* effect of reserpine on the activity of MAO.

Norwegian rats weighing between 200–250 g. were given an intramuscular (i.m.) injection of a single dose of 0.2 mg reserpine/100 g wt of the rat. The drug Reserpine used in the experiments was a preparation of CIBA–Geigy, India, bearing the trade name Serpasil. The rats were sacrificed by stunning and decapitation, 24, 48, 72, 96 and 120 hr after injection. The liver and brain were removed immediately and homogenized in freshly prepared 0.5 M phosphate buffer pH 7.4. The crude homogenates were then centrifuged in an International Refrigerated Centrifuge at 0–4° at 2500 rpm for 15 min. The pellet consisting of nucleus and other cell debris was discarded. The supernatant was made up in volume with 0.5 M phosphate buffer pH 7.4 to 20 ml/g wt of liver and 40 ml/g wt of brain and this was used as the enzyme preparation.

The MAO activity in the different enzyme preparations was determined by using serotonin as well as tyramine as substrates in the different incubation mixtures. The incubation mixture contained 1 ml enzyme preparation, 0.4 ml of 0.5 M phosphate buffer pH 7.4 and 1 ml of substrate (600 μg/ml serotonin or 260 μg/ml of tyramine). The incubation was terminated at the end of 1 hr by the addition of 0.6 ml of 25% trichloro acetic acid (TCA) and immediate chilling. Control tubes were prepared by pretreating with TCA before the addition of the enzyme preparation, in order to ascertain the enzyme activity at zero hr. Serotonin was estimated in the supernatant by the Method of Udenfriend, Weissbach and Clark [6] and tyramine by the method of Udenfriend and Cooper [7]. The protein (of the enzyme) precipitated as the result of addition of TCA at the termination of incubation, was dissolved in 0.1 N NaOH and estimated by the method of Sutherland *et al.* [8]. Normal rats were used as controls and were decapitated immediately after an i.m. injection of reserpine.

The effect of reserpine on the MAO activity in rat liver and brain is shown in Figs. 1 and 2 respectively. The MAO activity was expressed in terms of sp. act. i.e. μg of substrate utilized/mg of protein. Administration of a single dose of reserpine produced an increase in the MAO activity in the rat liver with both serotonin and tyramine as substrates, reaching its maximum on day 3 (72 hr). The activity declined on the subsequent days and reached the normal level (control) on day 5 (120 hr). While the MAO activity showed a more than 2-fold increase with serotonin, the increase with tyramine was slightly less than 2-fold. The MAO activity in brain also showed an increase, reaching its maximum in 3 days (72 hr) with serotonin and in 4 days (96 hr) with tyramine. In both the cases the normal level was reached on day 5 (120 hr). The activity with serotonin showed a 2-fold increase and that with tyramine was less than 2-fold. In both the tissue preparations, the sp. act. with serotonin as substrate was higher than tyramine, throughout.

DISCUSSION

Results clearly indicate that administration of reserpine brings about considerable increase in the activity of MAO, thereby altering the metabolism of serotonin and tyramine in the liver and brain of rats. Increase in the activity of the enzyme will lead to higher rate of degradation or utilization of these amines. Eventually this will bring about a depletion of their stores in the liver and brain. It is widely believed that alteration in the neurotransmitter responses is involved in the precipitation of migraine attacks. Biochemical agents which alter the metabolism of these neurotransmitter amines include reserpine [9]. Some workers have observed that i.m. injection of reserpine in migrainous and normal

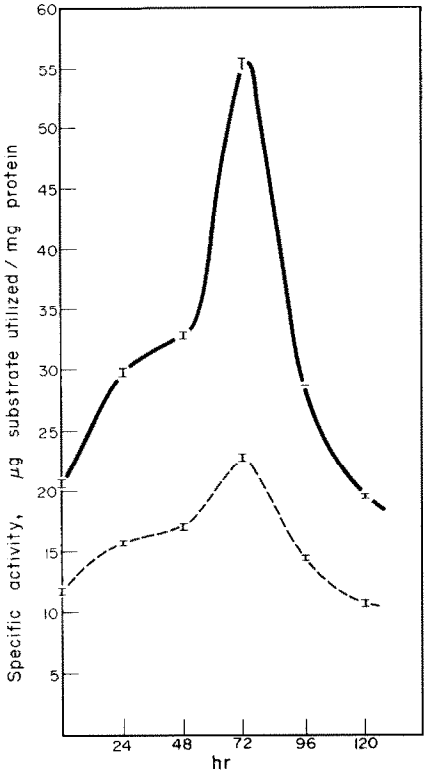

Fig. 1. MAO activity in rat liver after reserpine injection: —— Serotonin substrate. ----- Tyramine substrate. Each point represents the mean ±S.E.M. (bar) of six experiments.

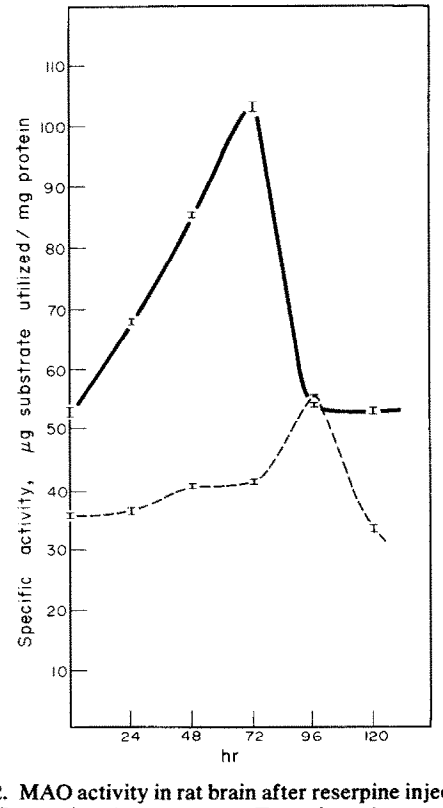

Fig. 2. MAO activity in rat brain after reserpine injection: —— Serotonin substrate. ----- Tyramine substrate. Each point represents the mean ±(bar) of six experiments.

subjects has brought about a fall in plasma serotonin [10]. It was also observed that all of them developed typical headache 1–5 hr after the injection. They also observed that slow i.v. injection of serotonin after the onset of either spontaneous or reserpine-induced migraine headache, produced amelioration of the headache in each instance. Results indicate that the effect of reserpine-induced headache may be due to increase in the activity of MAO which will lead to a depletion of serotonin and tyramine.

SUMMARY

Several studies have shown that reserpine given i.m. or s.c. produce typical unilateral headache. Further studies have shown that reserpine depletes tissues of serotonin and other catecholamines. Administration (by injection) of a single dose of reserpine (0.2 mg/100 g wt of rat) produced an increase in the activity of MAO both in liver and brain. The substrates used were serotonin and tyramine, and the increase was observed in both the cases. The maximum enzyme activity was observed on day 3 (72 hr) in liver, with serotonin as well as tyramine as substrates, and in brain with serotonin as substrate. Where tyramine was the substrate, the maximum enzyme activity was observed on day 4 (96 hr) in brain. In all the instances the activity returned to normal level on day 5 (120 hr). Our results suggest that depletion of serotonin as well as tyramine can be accounted by an increase in the activity of MAO.

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