EFFECT OF SODIUM NITRITE ON MONOAMINE OXIDASE ACTIVITY IN RAT LIVER AND BRAIN

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A number of monoamine oxidase (MAO) [EC 1.4.3.4. monoamine: oxygen oxidoreductase (deaminating)] inhibitors which were employed as a tool for studying the relationship between biogenic amines and function of MAO have been discovered (1, 2). As the result of studies on these compounds with respect to their special ability to inhibit MAO, it has been proved that there is a difference in enzymic properties of liver and brain MAO (3, 4) and mitochondrial and plasma MAO (5). However, a few number of works have been reported on the activation of MAO in which reagents have been employed (6–8). Since it has not yet been possible to prepare mitochondrial MAO in a soluble form, many studies have been carried out with crude preparation. The detailed mechanism of the enzymic oxidation of monoamines is still remained to be cleared. We have found that sodium nitrite (NaNO₂) is capable of activating MAO.

Freshly excised liver and brain of adult male Wistar rat were homogenized with a Potter-Elvehjem homogenizer in ice-cold 0.25 M sucrose and 0.01 M tris buffer pH 8.0. The homogenate was centrifuged 2 times at 600 × G for 10 minutes in a refrigerated centrifuge and the supernatant was retained and the residue was discarded. The supernatant was centrifuged again 2 times at 8500 × G for 20 minutes and the residue was retained and the supernatant was discarded. In case of liver, the residue was suspended in 3 volumes and in case of brain in 2 volumes of 0.1 M tris HCl buffer pH 8.0. MAO activity was determined by manometric assay of oxygen consumption at 38.0°C. The standard reaction mixture contained 0.1 M tris HCl buffer at pH 8.0, diluted enzymic preparations, distilled water and substrate solution in a total volume of 3.0 ml was equilibrated with oxygen gas for 10 minutes. Oxygen consumption was followed for 60 minutes, starting at 10 minutes after tipping in the substrate. Enzyme and NaNO₂ were pre-incubated for 15 minutes, before tipping the substrate. MAO activity was expressed as QO₂, which is the amount of oxygen consumption (μl) per hour per 1.0 ml of the mitochondria. Volumes of enzymic preparations were 0.4 ml in case of liver and 1.0–1.5 ml in case of brain, unless otherwise stated.

Effects of 0.1 M NaNO₂ on MAO activity in rat liver and brain mitochondria were studied using tyramine, serotonin, benzylamine, butylamine, tryptamine, amylamine, hexylamine and β-phenylethylamine as substrates. Table 1 indicated the effects of NaNO₂ on MAO activity in liver and brain. When tyramine and serotonin were used as substrates, in case of liver, MAO activity was inhibited by 20–30% at a concentration of 0.1
However, to the other substrates tested, NaNO₂ was found to increase MAO activity in liver remarkably. In case of benzylamine, butylamine, amylamine and β-phenylethylamine used as substrates, MAO activities in liver were 164.2, 236.8, 358.3 and 274.6% of the control, respectively. On the contrary, when tyramine and butylamine were used as substrates, MAO activity in brain increased remarkably in the presence of 0.1 M NaNO₂. However, no significant effect was observed when serotonin, benzylamine and tryptamine were used as substrates. When the other substrates mentioned above

| Substrate           | Liver |         | Brain |         |
|---------------------|-------|---------|-------|---------|
|                     | Control | NaNO₂ 10⁻³ M | % Control activity | Control | NaNO₂ 10⁻³ M | % Control activity |
| Tyramine            | 412.3  | 288.8   | 70.0  | 78.1    | 132.1     |
| Serotonin           | 118.3  | 98.8    | 83.5  | 91.5    | 90.2      |
| Benzylamine         | 164.0  | 269.3   | 164.2 | 21.7    | 17.5      |
| Butylamine          | 109.3  | 258.8   | 18.7  | 22.9    | 122.5     |
| Tryptamine          | 165.0  | 166.0   | 51.2  | 54.5    | 106.4     |
| Amylamine           | 71.3   | 255.5   | 6.5   | 8.3     | -         |
| Hexylamine          | 25.3   | 142.8   | 5.3   | 0       | -         |
| β-Phenylethylamine  | 59.0   | 162.0   | 4.3   | -       | -         |

CONCENTRATION OF SUBSTRATES WAS 1×10⁻⁵ M EXCEPT TYRAMINE AND SEROTONIN IN CASE OF BRAIN. CONCENTRATION OF TYRAMINE AND SEROTONIN WAS 5×10⁻⁴ M IN CASE OF BRAIN.

**Fig. 1.** Effect of various concentration of NaNO₂ on MAO activity in mitochondria of liver and brain.

Abscissa, number : neg. log. mol. concentration of NaNO₂. c : control

Ordinate, enzyme activity expressed as QO₂.
were used, MAO activity in brain was too small to determine.

The effect of various concentration of NaNO₂ on MAO activity in liver and brain is shown in Fig. 1. In case of tyramine used as a substrate, MAO activity was inhibited by 30% in the presence of 0.1 M NaNO₂, while in case of serotonin used as a substrate none of inhibition was observed at the same concentration of NaNO₂. On the contrary, in case of benzylamine and butylamine used as substrates, NaNO₂ was found to increase MAO activity at the same concentration, also it showed a slight increase of the activity at a lower concentration. Almost similar results were obtained in case of amylamine, hexylamine and β-phenylethylamine used as substrates. In case of brain, different results were obtained in comparison with the case of liver. When tyramine was used as a substrate, MAO activity was increased gradually with the increasing the concentration of NaNO₂. But in case of serotonin, benzylamine and butylamine used as substrates, MAO activity was slightly inhibited at a higher concentration of NaNO₂.

Presently, it has been shown that NaNO₂ demonstrated an increase of MAO activity in liver and brain when appropriate substrates were used. Fujimaki (4) have reported that there are two different types of MAO in brain and liver in respect to sensitivity to the inhibitors. When tyramine was used as a substrate, MAO activity in brain was increased, whereas in liver it was decreased by the administration of 0.1 M NaNO₂. These results may suggest the existence of multiple forms of MAO. The mechanism of this activation is now under investigation.

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PHARMACOLOGY OF A NEW ANTI-PARKINSONIAN DRUG: KAO-264

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Since Feldberg (1) suggested the possibility of a central acetylcholine-atropine antagonism to explain the usefulness of atropine in Parkinsonism, a number of centrally acting anticholinergic drugs have been introduced for the treatment of Parkinsonism (2,