STUDIES ON MONOAMINE OXIDASE (REPORT 17)
EFFECTS OF NaNO₂ ON MAO FROM
RAT LIVER MITOCHONDRIA

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There have been few reports on monoamine oxidase (MAO) [EC 1.4.3.4. monoamine:oxygen oxidoreductase (deaminating)] activators and at present it is only known that reserpine (1-3), in vivo and 4-diazo-imidazole-5-carboxamide (4), in vitro cause increases in MAO activity.

A previous report from this laboratory showed that sodium nitrite (NaNO₂) activated MAO with certain substrates (5). Namely, using benzylamine, butylamine, amylamine, hexylamine or β-phenylethylamine as substrate, MAO activity in rat liver mitochondria was increased by NaNO₂, while when using tyramine or serotonin it was inhibited. In rat brain mitochondria, MAO activity was increased by NaNO₂ when tyramine or butylamine were used as substrates, however, it was inhibited when other substrates were used. These results suggested the presence of multiple forms of MAO in mitochondria.

This paper describes the effects of NaNO₂ on the substrate specificities, pS maxima, pH optima and Michaelis constants of MAO in rat liver mitochondria.

MATERIALS AND METHODS

1) Materials

Adult male rats of the Wistar strain were used throughout. The mitochondrial fraction in rat liver was used to prepare the enzyme as reported previously (5).

2) Measurement of MAO activity

MAO activity was determined by manometric measurement of oxygen consumption at 38°C using the standard Warburg's method as described by Kinemuchi (6). The reaction mixture, containing enzyme solution, 0.1 M Tris-HCl buffer, pH 8.0 distilled water and substrate solution in a total volume of 3.0 ml, was equilibrated with oxygen gas for 10 min. A small filter paper soaked in 3 M KOH was placed in the center well to absorb any carbon dioxide evolved. The reaction was started by tipping the substrate solution from the side arm into the main well of the vessel. Oxygen consumption was followed for 60 min, starting 10 min after addition of the substrate. Enzymic oxidation of the substrate usually proceeded linearly during the periods of observation. Corrections were made for non-enzymic oxida-
RESULTS

1) Effects of NaNO₂ on MAO activity

The effects of NaNO₂ on mitochondrial MAO in rat liver were studied manometrically, using tyramine, benzylamine, butylamine, serotonin, amylamine, β-phenylethylamine or hexylamine at a concentration of 1 × 10⁻² M as substrate. Results are summarized in Fig. 1 and Table 1.

Using tyramine as substrate, NaNO₂ had no effect on MAO activity at concentrations below 1 × 10⁻⁴ M, but at higher concentrations the rate of oxidation of tyramine decreased linearly with increase in the concentration of NaNO₂. At a concentration of 1 × 10⁻¹ M it resulted in approx. 30% inhibition of activity. Similar effects were observed with serotonin.

![Graphs showing effects of NaNO₂ on MAO activity with various substrates.]

**Fig. 1.** Effect of NaNO₂ on MAO activity of rat liver mitochondria with various substrates.

**Table 1.** Effect of NaNO₂ (1 × 10⁻¹ M) on MAO activity in rat liver mitochondria with various substrates.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>MAO activity μlO₂</th>
<th>% Control activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyramine</td>
<td>164.9</td>
<td>115.5</td>
</tr>
<tr>
<td>Benzylamine</td>
<td>65.6</td>
<td>107.7</td>
</tr>
<tr>
<td>Butylamine</td>
<td>43.7</td>
<td>103.5</td>
</tr>
<tr>
<td>Amylamine</td>
<td>28.5</td>
<td>102.2</td>
</tr>
<tr>
<td>β-Phenylethylamine</td>
<td>23.6</td>
<td>64.8</td>
</tr>
<tr>
<td>Hexylamine</td>
<td>10.1</td>
<td>57.1</td>
</tr>
<tr>
<td>Serotonin</td>
<td>47.3</td>
<td>39.5</td>
</tr>
</tbody>
</table>
as substrate, and activity decreased with increase in the concentration of NaNO₂. A concentration of $1 \times 10^{-1}$ M NaNO₂ resulted in approx. 20% inhibition of activity. On the contrary, when benzylamine, butylamine, amylamine, β-phenylethylamine or hexylamine were used as substrates, MAO activity increased markedly with increase in the concentration of NaNO₂. Thus, using benzylamine, butylamine or β-phenylethylamine as substrate, activity increased 164.2%, 236.8% and 275.6%, respectively, on addition of $1 \times 10^{-1}$ M NaNO₂. With amylamine or hexylamine as substrates, the effect of NaNO₂ was even greater and oxygen consumption increased 3-5 times.

2) Effects of NaNO₂ on pS maxima

The effects of NaNO₂ on the pS maxima of MAO activity were studied using tyramine, benzylamine or butylamine as substrates. Oxygen consumption could not be measured at substrate concentrations below $1 \times 10^{-4}$ M, therefore the pS maxima were determined at substrate concentrations of $1 \times 10^{-4}$ M to $1 \times 10^{-1}$ M.

With tyramine as substrate, maximum activity was observed at a concentration of $1 \times 10^{-2}$ M and the activity-pS curve for enzymic oxidation was a typical bell-shape. At the substrate concentration for the pS maximum, addition of $1 \times 10^{-2}$ M NaNO₂, resulted in a slight inhibition while concentrations of substrate below $5 \times 10^{-3}$ M, resulted in a slightly increased activity (Fig. 2).

Using benzylamine as substrate, the rate of oxidation increased linearly with increase in the concentration of substrate from $1 \times 10^{-2}$ M to $5 \times 10^{-1.5}$ M. The activity-pS curve for enzymic oxidation was sigmoidal and maximum activity was observed at concentrations of

Fig. 2. Effect of NaNO₂ ($1 \times 10^{-1}$ M) on the pS-activity curve of MAO for tyramine.

Fig. 3. Effect of NaNO₂ ($1 \times 10^{-1}$ M) on the pS-activity curve of MAO for benzylamine.
substrate above $1 \times 10^{-12}$ M. At any concentration, benzylamine added to $1 \times 10^{-2}$ M NaNO₂ caused marked increase in activity, however, with $1 \times 10^{-3}$ M NaNO₂ the activity-pS curve became bell shaped and maximum activity was observed at a substrate concentration of $1 \times 10^{-8}$ M (Fig. 3).

Similarly, with butylamine as substrate, the rate of oxidation increased linearly with increase in substrate concentration from $1 \times 10^{-4}$ M to $1 \times 10^{-12}$ M. The activity-pS curve was sigmoidal and maximum activity was obtained at substrate concentrations above $1 \times 10^{-14}$ M. Addition of $1 \times 10^{-4}$ M NaNO₂ increased the activity at all concentrations of substrate, especially at high concentrations. Unlike the cases using tyramine or benzylamine no inhibition of activity was observed at high concentrations of substrate. The rate of oxidation increased with increase in the concentration of substrate (Fig. 4).

3) **Effects of NaNO₂ on pH optima**

The effects of NaNO₂ on the pH-activity curves of MAO with various substrates were studied using tyramine, benzylamine and butylamine as substrates at a concentration of $1 \times 10^{-5}$ M. In this experiment, McIlvaine buffer (pH 5.0), phosphate buffer (pH 6.0 to 7.5), Tris-HCl buffer (pH 7.0 to 9.0) and borate buffer (pH 10.0) were used.

As shown in Fig. 5 oxygen consumption could be measured between pH 5.0 and pH 9.5 and maximum activity was obtained at pH 7.5, with tyramine as substrate. Addition of $1 \times 10^{-1}$ M NaNO₂ caused slight inhibition of activity at each pH tested, but the difference between oxygen consumption in the presence and absence of NaNO₂ at each pH was negligible.

When benzylamine was used as substrate, the maximum activity was obtained at pH 8.5, and oxygen consumption decreased slowly at lower pH values and rapidly at higher pH.

**Fig. 4.** Effect of NaNO₂ ($1 \times 10^{-4}$ M) on the pS-activity curve of MAO for butylamine. **Fig. 5.** Effect of NaNO₂ ($1 \times 10^{-4}$ M) on the pH-activity curve of MAO with tyramine.
values. Addition of $1 \times 10^{-4}$ M NaNO$_2$ increased activity at all pH values and especially between pH 7.2 and pH 8.5. NaNO$_2$ shifted the pH optimum from pH 8.5 to pH 7.5 (Fig. 6).

Using butylamine as substrate, activity was maximal at pH 8.2. Addition of $1 \times 10^{-4}$ M of NaNO$_2$ increased activity at all pH values, and oxygen consumption was increased 3-fold at between pH 6.5 and 8.0. In the presence of NaNO$_2$, the optimal pH shifted from pH 8.2 to pH 7.5 (Fig. 7).

**Fig. 6.** Effect of NaNO$_2$ ($1 \times 10^{-4}$ M) on the pH-activity curve of MAO with benzylamine.

**Fig. 7.** Effect of NaNO$_2$ ($1 \times 10^{-4}$ M) of the pH-activity curve of MAO with butylamine.

**TYRAMINE**

**Fig. 8.** Effect of NaNO$_2$ on the Km value of MAO for tyramine. Km values were determined from Lineweaver-Burk's double reciprocal plot of activities in the presence and absence of NaNO$_2$ ($1 \times 10^{-4}$ M).
4) Effects of NaNO₂ on the Km values

The Michaelis constants were determined from Lineweaver-Burk's double reciprocal plots of values obtained manometrically. The effects of NaNO₂ on the Km values of MAO for tyramine, butyramine and benzylamine were studied.

With tyramine as substrate, the Km value was $6.8 \times 10^{-3}$ M and on addition of $1 \times 10^{-1}$ M NaNO₂ it was reduced slightly to $3.6 \times 10^{-3}$ M. As shown in Fig. 8 the two obtained lines crossed at the ordinate.

**Fig. 9.** Km values of MAO for benzylamine.

![Benzylamine Km Values](image1)

**Fig. 10.** Effect of NaNO₂ ($1 \times 10^{-1}$ M) on the Km value of MAO for benzylamine.

![Benzylamine with NaNO₂](image2)
**FIG. 11.** Km value of MAO for butylamine.

**Fig. 12.** Effect of NaNO₂ (1 x 10⁻¹ M) on the Km value of MAO for butylamine.

**TABLE 2.** Effect of NaNO₂ (1 x 10⁻¹ M) on the Km values of MAO for tyramine, butylamine and benzylamine.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Km (Control)</th>
<th>Km (+ NaNO₂ 1 x 10⁻¹ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyramine</td>
<td>6.8 x 10⁻² M</td>
<td>3.6 x 10⁻² M</td>
</tr>
<tr>
<td>Butylamine</td>
<td>6.9 x 10⁻² M</td>
<td>6.4 x 10⁻² M</td>
</tr>
<tr>
<td>Benzylamine</td>
<td>5.0 x 10⁻³ M</td>
<td>5.6 x 10⁻³ M</td>
</tr>
</tbody>
</table>
The Km values using benzylamine and butylamine as substrates, with and without NaNO₂ are shown in Figs. 9, 10, 11 and 12. The Km values were 5.0 x 10⁻³ M for benzylamine and 6.9 x 10⁻³ M for butylamine, and on addition of 1 x 10⁻³ M NaNO₂ they changed to 5.6 x 10⁻⁴ M and 6.4 x 10⁻⁴ M, respectively.

These results are summarized in Table 2 and show that addition of NaNO₂ caused marked reduction of the Km values for butylamine and benzylamine, but negligible reduction in the case of tyramine.

5) Reversibility of the effects of NaNO₂

Reversibility of the effects of NaNO₂ on MAO activity was determined using benzylamine and butylamine as substrates.

Equal volumes of an enzyme preparation were mixed with NaNO₂ at a final concentration of 1 x 10⁻³ M with and Tris-HCl buffer, respectively. With butylamine as substrate, oxygen consumption with NaNO₂ increased to 357.2% of the control value, while with benzylamine it increased to 338.8%. The mixtures containing NaNO₂ were then dialysed against 0.001 M Tris-HCl buffer for 12 hr in a cold room and then oxygen consumption was re-determined. After dialysis the oxygen consumptions were 121.3% and 124.9% of the control values using butylamine and benzylamine, respectively, as substrate (Table 3).

<table>
<thead>
<tr>
<th>TABLE 3. Effect of dialysis after activation of MAO by NaNO₂ (1 x 10⁻³ M) with butylamine and benzylamine as substrates.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
</tr>
<tr>
<td>Butylamine</td>
</tr>
<tr>
<td>Benzylamine</td>
</tr>
</tbody>
</table>

DISCUSSION

A previous report from this laboratory (5) showed that NaNO₂ activated MAO in rat liver mitochondria using benzylamine or butylamine as substrate, but inhibited MAO when tyramine or serotonin were used as substrate. On the other hand, MAO in rat brain mitochondria was activated by NaNO₂ when tyramine or butylamine was used as substrate, and inhibited when serotonin or benzylamine was used as substrate. This suggested the presence of multiple forms of MAO in rat liver and brain mitochondria.

This paper describes detailed studies on the effect of NaNO₂ on MAO in rat liver mitochondria. Addition of NaNO₂ inhibited MAO with tyramine as substrate and activated MAO with benzylamine, butylamine, amylamine or β-phenylethylamine as substrate, but had no effect with serotonin as substrate. Namely, the effect of NaNO₂ on MAO in rat liver mitochondria varied with different substrates. The active effect of NaNO₂ was especially strong with substrates for which MAO showed low activity.

Ogawa et al. (7) reported that nitroglycerine and related compounds inhibited MAO activity. Krantz et al. (8) reported that amyl nitrite, glyceryl trinitrate and isobutylglycol nitrate inhibited ATP-ase activity, while NaNO₂ had no effect on activity. These results
indicate that the effects of NaNO₂ on MAO are not non-specific, rather the present results suggest that they could be specific.

When tyramine was used as substrate, NaNO₂ decreased MAO activity at the concentration required for the activity-pS maximum, but slightly increased activity at lower substrate concentrations. It also caused a decrease in the Km value for tyramine. Using benzylamine or butylamine as substrate, NaNO₂ caused increase in MAO activity at all substrate concentrations tested and also changed the pS maximum. Moreover, high concentrations of benzylamine became inhibitory on addition of NaNO₂. The Km values for benzylamine and butylamine decreased from 5.0 x 10⁻³ M to 5.6 x 10⁻⁴ M and 6.9 x 10⁻³ M to 6.4 x 10⁻⁴ M, respectively, in the presence of NaNO₂. On addition of NaNO₂, MAO activity with butylamine as substrate increased with a substrate concentration increase. The effects of NaNO₂ on MAO in rat liver mitochondria differed with tyramine, benzylamine and butylamine as substrate. Addition of NaNO₂ changed the pH optima for benzylamine and butylamine from pH 8.5 to 7.5, respectively, but use of tyramine showed no effect. These results also show that the effect of NaNO₂ on MAO in rat liver mitochondria differs with different substrates.

There have been many reports on differences in the enzymic characters of MAO in different species and different organs (9, 10). Recently existence of multiple forms of MAO in one organ has been suggested (6, 11), however it is uncertain whether these multiple forms have any physiological significance. The present work shows that there are several kinds of MAO in rat liver mitochondria, such as those which oxidize tyramine, benzylamine and butylamine, respectively.

**SUMMARY**

Enzymic studies were made on the effects of NaNO₂ on the pS maxima, pH optima and Michaelis constants of MAO of rat liver mitochondria. The following results were obtained.

1. NaNO₂ inhibited MAO activity in rat liver mitochondria with tyramine as substrate, had no effect with serotonin as substrate and activated MAO with benzylamine, butylamine, amylamine, β-phenylethylamine or hexylamine as substrate.

2. With tyramine as substrate, NaNO₂ inhibited MAO activity at the substrate concentration for the activity-pS maximum but slightly increased activity at lower substrate concentrations. NaNO₂ did not change the activity-pS maximum for tyramine. With benzylamine or butylamine as substrate, NaNO₂ increased MAO activity at all concentrations of substrate. NaNO₂ changed the activity-pS maximum for benzylamine from 1 x 10⁻³ M to 1 x 10⁻⁴ M.

3. NaNO₂ did not change the pH optimum for tyramine, but changed those for benzylamine and butylamine from pH 8.5 to pH 7.2 and from pH 8.2 to pH 7.5, respectively.

4. NaNO₂ decreased the Km for tyramine slightly from 6.8 x 10⁻⁴ M to 3.6 x 10⁻⁵ M. It decreased the Km values for benzylamine and butylamine considerably from 6.9 x 10⁻³ M to 6.4 x 10⁻⁴ M and from 5.0 x 10⁻³ M to 5.6 x 10⁻⁴ M, respectively.

5. The active effect of NaNO₂ on MAO activity was reversible.
REFERENCES