NO-Inhibiting and Vasotropic Activity of Some Compounds with Thioamidine Group


Using the method of electron paramagnetic spectroscopy we demonstrated that thiazine-thia- 
zoline compounds and amionethyl isothiourea containing the thioamidine group inhibit NO 
production in the liver of endotoxin-treated mice. Injection of these agents to anesthetized 
rats increased arterial pressure and enhanced respiration rate. This effect probably reflects 
inhibition of not only inducible, but also the constitutive synthesis of NO by compounds with 
thioamidine group.

Key Words: nitric oxide; thioamidine group; arterial pressure; respiration rate

Nitric oxide NO* remains in the focus of biological 
studies as a ubiquitous transmitter. The key role of this 
agent in various physiological reactions under normal 
and pathological conditions is widely known [4]. 
Many pathological states are associated with enhanced 
NO* release in the organism. This transmitter plays a 
protective role during infection by suppressing inva-
sion and reproduction of the pathogen [3], although in 
critical states, e.g. ischemia [7] or septic shock [6], 
excessive NO* production aggravates the state of the 
organism, in particular, by disturbing the regulation of 
the cardiovascular system. Therefore, synthesis of 
chemical compounds with NO-inhibitory activity is a 
perspective way for creation of new pharmacological 
agents regulating NO* functions in the organism.

Our aim was to study the effects of compounds 
with thioamidine group on NO* synthesis, arterial 
pressure, and respiration rate.

MATERIALS AND METHODS

Biological activity of the following compounds with 
thioamidine group (synthesized at the Department of 

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Chemistry, M. V. Lomonosov Moscow State University) was examined: S-(2-aminoethyl)isothiourea di-
hydrobromide (AET); 2-amino-2-thiazoline hydrobro-
mide (2-AT); 2-amino-5,6-dihydro-4H-1,3-thiazine 
hydrobromide (2-ADT); 4-oxo-2-amino-2-thiazoline 
(4-OAT). Nω-nitro-L-arginine (L-NNA, Sigma) was 
also used in this study.

NO-inhibitory activity of the test agents was stu-
died on 5-month-old albino random-bred male mice 
(initial genotype Swiss) weighing 27-30 g. The mice 
were kept under standard vivarium conditions with 
free access to standard food and water. Four hours 
before sacrifice (ether), the mice were injected infra-
peritoneally with LPS from E. coli 0111:B4 (Sigma, 
1.5 mg/kg dissolved in 0.9% NaCl). The test agents 
were injected intraperitoneally (in 0.9% NaCl, 0.5 ml/
mice) simultaneously with LPS or 3 h later.

NO* production was assayed as described pre-
viously [1]. Thirty minutes before sacrifice, the mice 
were injected with a spin trap consisting of sodium 
diethylthiocarbamate (500 mg/kg in 0.5 ml 0.9% 
NaCl, intraperitoneally) and iron citrate (50 mg/kg 
FeSO₄·7H₂O+250 mg/kg sodium citrate, 0.1 ml in 
each thigh). The liver was isolated, cut to fragments, 
and placed in metal tubes 4 mm in diameter to prepare 
10-mm columns. The specimens were frozen and sto-
red in liquid nitrogen and then used for electron paramagnetic resonance (EPR) spectroscopy. The amount of NO\(^{\bullet}\) in tissue was measured in an ESP-300E microwave spectrometer (Bruker). The content of NO\(^{\bullet}\) was calculated as described elsewhere [2].

The effects of test agents on systolic (SBP) and diastolic (DBP) blood pressure and respiration rate (RR) were studied on Wistar rats weighing 190-250 g (n=19). The rats were intraperitoneally narcotized with Nembutal (55 mg/kg). SBP and DBP were measured in the left carotid artery. After recording of the baseline values of RR, SBP, and DBP, the test substance dissolved in physiological saline (1 ml/100 g) was injected intraperitoneally. The physiological parameters and the state of the rats were recorded for 90 min postinjection. The control rats received the corresponding volume of physiological saline.

The data were processed statistically using Student’s t test.

**RESULTS**

The test substances had no effects on constitutive level of NO\(^{\bullet}\) production in the liver. LPS 30-fold increased NO\(^{\bullet}\) production in experimental mice. In these mice NO-inhibitory activity of the examined agents was significant (Table 1). 2-AT, 2-ADT, and 4-OAT injected 30 min before injection of spin trap significantly decreased NO\(^{\bullet}\) production in the liver. 2-AT and 4-OAT were most effective and decreased NO\(^{\bullet}\) production to 6 and 7% compared to LPS-induced production. The inhibitory effect of 2-ADT was less pronounced: it decreased NO\(^{\bullet}\) production to 18%.

Regulation of the blood flow via relaxation of blood vessels is the most important physiological functions of NO\(^{\bullet}\) [10]. Therefore, we studied the effect of hydrothiazine–thiazole derivatives and AET on some parameters of the cardiovascular system (Table 2). In control rats, injection of 0.9% NaCl produced no effect on SBP, DBP, and RR. In rats receiving the test agents SBP and DBP increased by 10-18% after 10 min and then changed insignificantly or decreased to baseline or below this level (Table 2).

In most rats, RR significantly increased 5-20 min after injection of the test agents and remained at this level to the end of observation (90 min). In rats receiving 2-ADT tachypnea was observed. The dynamics of the examined physiological parameters in rats treated with 4-OAT is shown in Fig. 1.

It should be noted that diethylidithiocarbamate, a component of the spin trap used in this study, is a potent inhibitor of Zn, Cu-superoxide dismutase (Zn,CuSOD). This enzyme catalyzes conversion of superoxide-anion (O\(_{2}^{\bullet}\)) into H\(_{2}\)O\(_{2}\). Inhibition of SOD by diethylidithiocarbamate results in accumulation of O\(_{2}^{\bullet}\) in cells. Since its affinity to NO\(^{\bullet}\) is limited only by diffusion, the removal of NO\(^{\bullet}\) by superoxide-anion can markedly decrease the amount of NO\(^{\bullet}\) detected by EPR [12]. Therefore, interpretation of EPR data should take into consideration possible stimulation of O\(_{2}^{\bullet}\) generation in cells, which will be recorded as inhibition of NO\(^{\bullet}\) production. In our study this is hardly possible, because many thiourea derivatives (L-S-alkylthiocitrulline, S-alkylisothiourea derivatives, etc.) demonstrate a direct inhibitory effect on NO-synthase, which synthesizes NO\(^{\bullet}\) and L-citrulline from oxygen and L-arginine [11].

Thus, our findings suggest that not only isothiourea (in particular, AET), but also their cyclic derivatives can inhibit NO\(^{\bullet}\) production. Of particular importance is the fact that this NO-inhibitory activity was observed under conditions of induction of NO\(^{\bullet}\) synthesis by endotoxin in vivo, i.e. on the model of septic shock. The agents 2-AT, 4-OAT, and AET inhibited NO\(^{\bullet}\) production in doses 2-5 times below the maximum tolerated dose and were as effective as standard

**TABLE 1.** NO-Inhibitory Activity of Compounds with Thioamidine Group

<table>
<thead>
<tr>
<th>Chemical agent</th>
<th>Dose, mmol/kg (mg/kg)</th>
<th>Relative NO(^{\bullet}) production, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-AT</td>
<td>0.18 (58)</td>
<td>7±3</td>
</tr>
<tr>
<td>4-OAT</td>
<td>1.72 (200)</td>
<td>6±2</td>
</tr>
<tr>
<td>2-ADT</td>
<td>0.30 (100)</td>
<td>18±4</td>
</tr>
<tr>
<td>AET</td>
<td>23 (150)</td>
<td>3±1</td>
</tr>
<tr>
<td>L-NNA</td>
<td>0.76 (167)</td>
<td>2±1</td>
</tr>
</tbody>
</table>

**Note.** Ms was calculated from the data of 3-4 experiments on 18-21 animals.

**TABLE 2.** Effect of Compounds Containing Thioamidine Group (100 mg/kg) on SBP, DBP, and RR

<table>
<thead>
<tr>
<th>Agent</th>
<th>t, min</th>
<th>SAP, %</th>
<th>DAP, %</th>
<th>t, min</th>
<th>RR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AET</td>
<td>10</td>
<td>20</td>
<td>15</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>2-AT</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>4-OAT</td>
<td>30</td>
<td>10</td>
<td>10</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>2-ADT</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>20</td>
<td>70</td>
</tr>
</tbody>
</table>

**Note.** t\(_{1}\) and t\(_{2}\) are the times corresponding to peak values of AP and RR.