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

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Using the method of electron paramagnetic spectroscopy we demonstrated that thiazine-thiazoline compounds and aminoethyl 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 $NO^{\bullet}$ 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 $NO^{\bullet}$ release in the organism. This transmitter plays a protective role during infection by suppressing invasion and reproduction of the pathogen [3], although in critical states, *e.g.* ischemia [7] or septic shock [6], excessive $NO^{\bullet}$ 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 $NO^{\bullet}$ functions in the organism.

Our aim was to study the effects of compounds with thioamidine group on $NO^{\bullet}$ synthesis, arterial pressure, and respiration rate.

MATERIALS AND METHODS

Biological activity of the following compounds with thioamidine group (synthesized at the Department of Chemistry, M. V. Lomonosov Moscow State University) was examined: S-(2-aminoethyl)isothiourea dihydrobromide (AET); 2-amino-2-thiazoline hydrobromide (2-AT); 2-amino-5,6-dihydro-4H-1,3-thiazine hydrobromide (2-ADT); 4-oxo-2-amino-2-thiazoline (4-OAT). N_{ω}-nitro-L-arginine (L-NNA, Sigma) was also used in this study.

NO-inhibitory activity of the test agents was studied 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 intraperitoneally 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.

$NO^{\bullet}$ production was assayed as described previously [1]. Thirty minutes before sacrifice, the mice were injected with a spin trap consisting of sodium diethyldithiocarbamate (500 mg/kg in 0.5 ml 0.9% NaCl, intraperitoneally) and iron citrate (50 mg/kg $FeSO_4 \times 7H_2O$+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-

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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–thiazoline 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.

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

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

Note. $M\pm s$ was calculated from the data of 3-4 experiments on 18-21 animals.

It should be noted that diethyldithiocarbamate, a component of the spin trap used in this study, is a potent inhibitor of Zn, Cu-superoxide dismutase (Zn,Cu-SOD). This enzyme catalyzes conversion of superoxide-anion ($O_2^{\bullet -}$) into H_2O_2. Inhibition of SOD by diethyldithiocarbamate 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 2. Effect of Compounds Containing Thioamidine Group (100 mg/kg) on SBP, DBP, and RR

Agent	t_1, min	ΔSAP, %	ΔDAP, %	t_2, min	ΔRR, %
AET	10	20	15	80	50
2-AT	5	10	10	30	30
4-OAT	30	10	10	60	30
2-ADT	10	15	15	20	70

Note. t_1 and t_2 are the times corresponding to peak values of AP and RR.

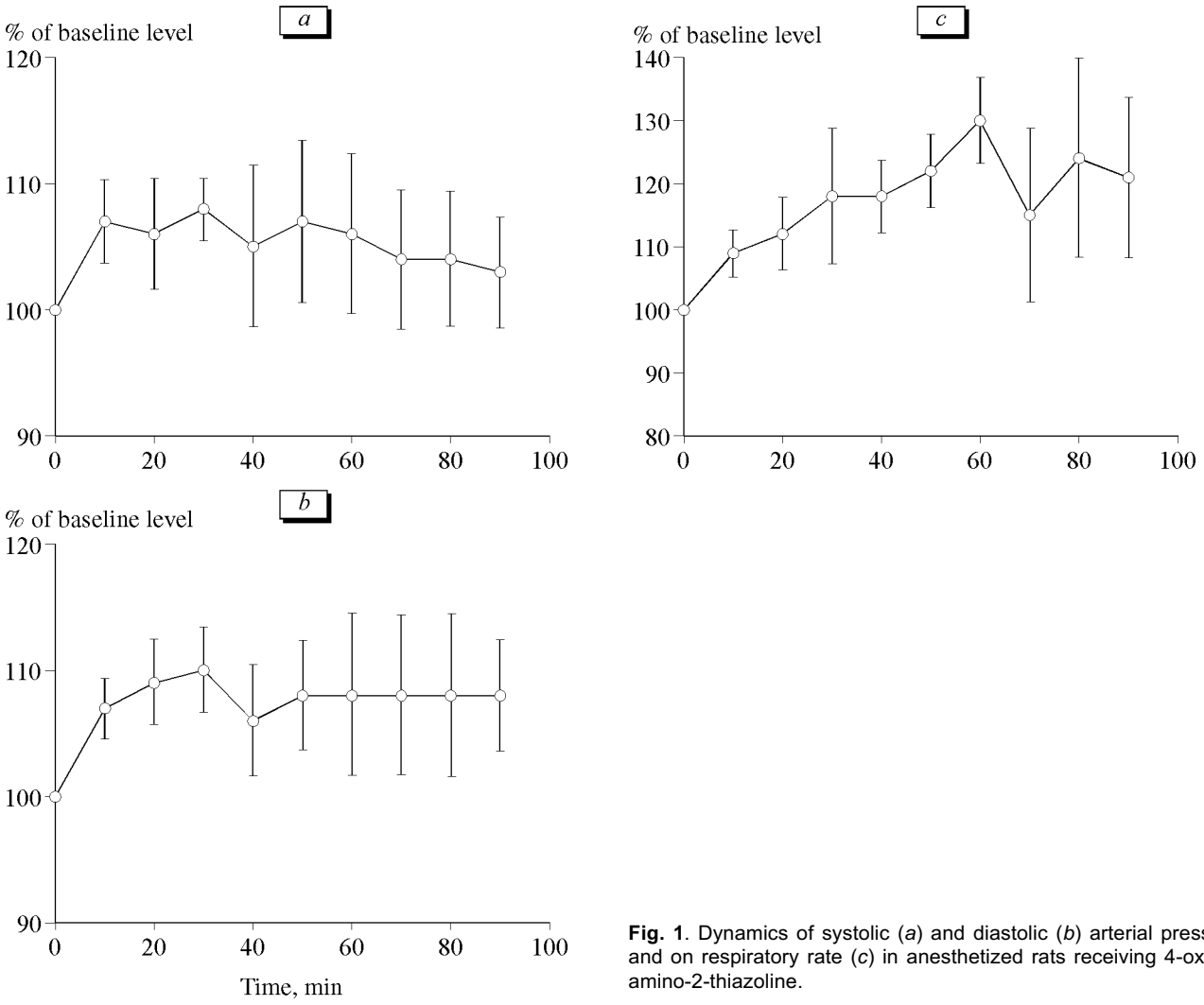

Fig. 1. Dynamics of systolic (*a*) and diastolic (*b*) arterial pressure and on respiratory rate (*c*) in anesthetized rats receiving 4-oxo-2-amino-2-thiazoline.

NO-synthase inhibitor L-NNA. It should be noted that 2-AT is the basic product of AET metabolism *in vivo* [5].

All test agents increased AP within 10 min post-injection. Since NO• is one of the most important endothelial relaxation factors, vasoconstriction can be induced by inhibition of NO• synthesis by the test agents. Further changes in AP can be explained by metabolism and dilution of the test agents, as well as their action on neurohumoral mechanisms of regulation of the cardiovascular system. Tachypnoe also attests to NO-activity of the examined agents. This effect is characteristic of many NOS-inhibitors of the arginine family and is related to their bronchoconstrictor effect [8], which is compensated by reflex tachypnoe.

Therefore, EPR-spectroscopy can test NO-inhibitory activity of chemical agents on the model of septic shock. The physiological reactions also attest to inhibition of NO• production by agents with thioamidine group. Comparison of biological activity of the examined substances with their chemical structure provides the basis for directed synthesis of antiinflammatory, antishock, and vasotropic pharmacological preparations [9,13,14].

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