BIOPHYSICS AND BIOCHEMISTRY

Effect of Afobazole on Mitochondrial Monoamine Oxidase A Activity *In Vitro*

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Selective anxiolytic afobazole (1 mM) inhibits monoamine oxidase A activity in mitochondria from rat brain and liver (IC_{50} 0.36 and 0.43, respectively). Effect of the compound does not depend on the time of preincubation with mitochondria. Triple washout of mitochondria is followed by complete recovery of initial enzyme activity.

Key Words: *afobazole; monoamine oxidase A; mitochondria, brain*

New selective anxiolytic afobazole (5-ethoxy-2-[2-(morpholino)-ethylthio]benzimidazole dihydrochloride; Fig. 1) was introduced into clinical practice in 2005 [2]. *In vivo* experiments showed a modulating effect of this substance on the GABAergic system [4]. According to the results of radioligand assay performed by Seger company (France), afobazole *in vitro* interacts with σ_1-receptors and MT_1 and MT_3 melatonin receptors and reduces binding of labeled inhibitor of type A monoamine oxidase (MAO A) [10]. The efficiency of afobazole interaction with molecular targets estimated by IC_{50} value was 9.9 nM for MT_3 receptors, 6.2 μM for MAO A, 7.1 μM for σ_1, and 27 μM for MT_1 [10]. These data indicate that afobazole produces a complex pharmacological effect due to simultaneous influence on several molecular targets.

The aim of the study was to investigate the effect of afobazole on MAO A activity in rat brain and liver mitochondria.

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MATERIALS AND METHODS

Afobazole was synthesized at the V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences.

Brain and liver mitochondria were isolated from albino outbred rats by differential centrifugation as described previously [7]. MAO A activity was measured using the radiometric method using 0.1 mM [^{14}C]serotonin creatinine sulphate [7] as the substrate. Reversibility of the effect of 1 mM afobazole was evaluated as described previously [3]. Statistical analysis was performed using the Student *t* test for dependent samples. The difference was considered significant at $p \leq 0.05$.

RESULTS

Afobazol decreased MAO A activity in mitochondrial from rat liver and brain (Fig. 2) and IC_{50} values were within the range of submillimolar concentrations $(3.6\pm1.5)10^{-4}$ M and $(4.3\pm1.1)10^{-4}$ M, respectively. Preincubation of afobazole with mitochondria did not potentiate its inhibitory effect on MAO A (Table 1). The absence of time-dependent potentiation of MAO A

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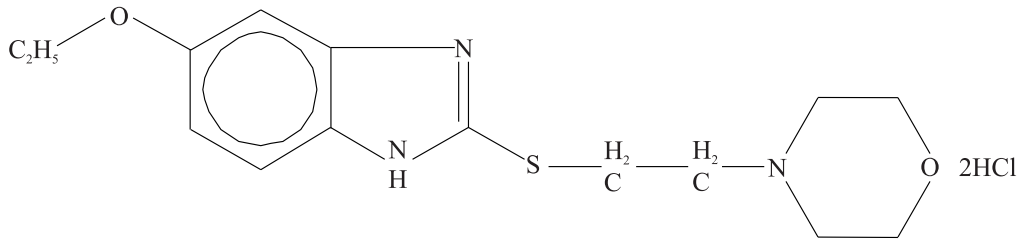

Fig. 1. Structural formula of afobazole.

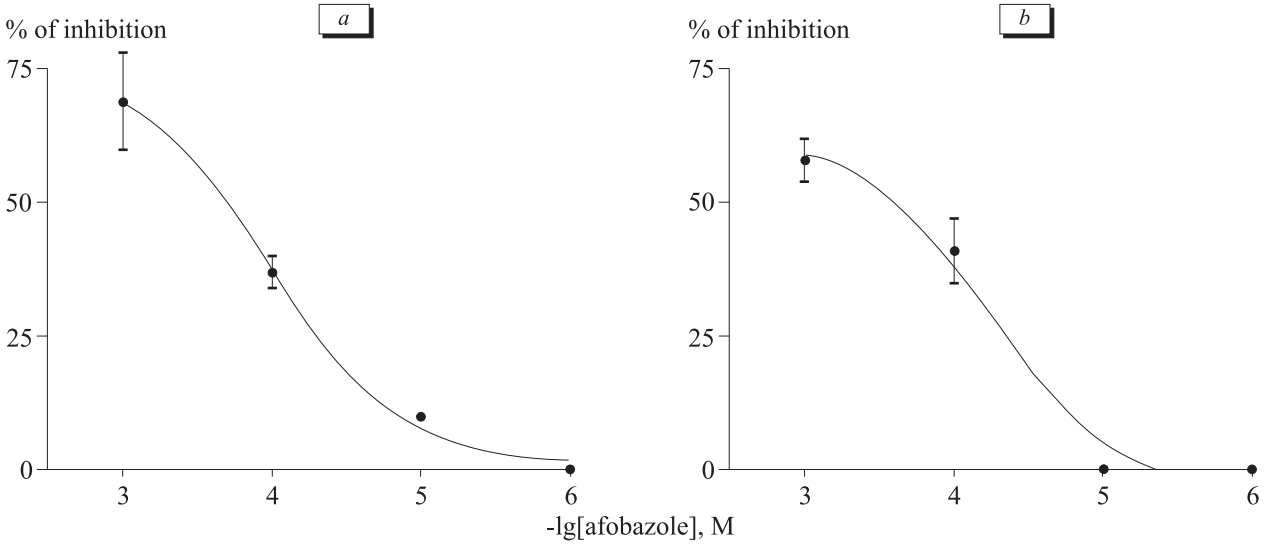

Fig. 2. Effect of afobazole on MAO A activity in brain (*a*) and liver (*b*) mitochondria. Effect of afobazole was studied without preliminary preincubation of mitochondria with the drug. The results of 3-6 independent experiments are shown.

TABLE 1. Effect of 30-min Peincubation of Mitochondria with Afobazole on MAO A Activity in Rat Liver Mitochondria (*M*±*m*)

Experimental conditions	Afobazole concentration	
	1 mM	0.1 mM
Without preincubation	58±4	41±6
30-min preincubation	46±4	36±10
p	>0.05	>0.05

Note. The results of 4-7 independent experiments are shown. Here and in Table 2: the results are expressed as percent of MAO A inhibition compared to the control (100%).

TABLE 2. Effect of Triple Washing of Liver Mitochondria on MAO A Activity (*M*±*m*)

Experimental conditions	% of initial activity
1 mM afobazole, 30-min preincubation	46±4
1 mM afobazole, 30-min preincubation+ triple washout mitochondria	105±6
p	<0.01

Note. Results of 3-4 independent experiments are shown.

inhibition attests to reversible interaction of afobazole with the enzyme. Indeed, MAO A activity fully recovered after 30-min preincubation of liver mitochondria with afobazole with subsequent triple washout of mitochondria (Table 2).

These results indicate that the drug acts as weak and easily reversible MAO A inhibitor. It remains unclear, whether afobazole interacts with MAO A *in vivo*. However, the data on region-specific increase in norepinephrine content (predominant MAO A substrate [11,12]) in the frontal cortex of C57Bl mice treated with afobazole [1] indirectly attest to this interaction.

It is worthy of note that MAO A inhibitors (including more potent inhibitors than afobazole) do not virtually exhibit their own anxiolytic activity after single administration [6]. Anxiolytic effect is usually observed after combined administration of MAO A and MAO B inhibitors [6] or after chronic administration of MAO A inhibitor (moclobemide) [5,8], when the effects not directly related to MAO A inhibition appear [9]. The interaction of afobazole with MAO A along with the data on the binding of this agent with σ_1 and MT_1 receptors prompt further investigation of its antidepressant effects.

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