PHARMACOLOGY AND TOXICOLOGY

Interaction of Afobazole with σ_1-Receptors

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 7, pp. 53-55, July, 2009
Original article submitted December 3, 2008

In vitro radioligand assay revealed interaction of afobazole with σ_1-receptors (Ki=5.9×10^{-6} M). Translocation of σ_1-receptors from the endoplasmic reticulum to the outer membrane was demonstrated by confocal microscopy. Experiments were performed on the model of HT-22 immortalized hippocampal cells after incubation with afobazole in a concentration of 10^{-8} M.

Key Words: *afobazole; σ_1-receptor; radioligand binding; receptor translocation*

Previous studies demonstrated anxiolytic and neuroprotective properties of afobazole (5-ethoxy-2-[2-(morpholino)-ethylthio]benzimidazole hydrochloride) [1,2]. Prevention of stress-induced decrease in binding capacity of the $GABA_A$ receptor benzodiazepine region serves as a neurochemical target for afobazole [3]. However, there are no data on direct interaction between this compound and $GABA_A$ receptor. Analysis of model pharmacophores revealed stereochemical similarity between afobazole and (+)-pentazocine (prototypic ligand of σ_1-receptors) [4].

This work was designed to study ligand properties of afobazole in relation to σ_1-receptors. We evaluated the possibility of receptor translocation from the endoplasmic reticulum to the outer cell membrane upon interaction with afobazole. Published data show that this phenomenon is typical of σ_1-receptor agonists [8,12].

MATERIALS AND METHODS

The substance afobazole was synthesized at the V. V. Zakusov Institute of Pharmacology (Fig. 1). The compound was used in concentrations of 10^{-3}-10^{-9} M.

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The interaction of afobazole with σ_1-receptors was studied on the Jurkat cell line (neuroreceptor assay, in collaboration with the Seger Company) [7]. [^{3}H](+)-Pentazocine (8 nM) served as the labeled ligand. Haloperidol (IC_{50}=2.2×10^{-8} M, Ki=1.3×10^{-8} M, Hill coefficient (nH)=1.2) was used as the reference preparation. Nonspecific binding was studied in the presence of 10 μM haloperidol. Incubation with cells was performed at 22°C for 120 min.

The ability of afobazole to cause σ_1-receptor translocation to the outer neuronal membrane was studied by means of immunofluorescence with the immortalized mouse hippocampal cell line HT-22. Afobazole in a final concentration of 10^{-8} M was added to the culture medium. Previous studies on the model of oxidative stress and glutamate toxicity showed that this dose of afobazole produces a neuroprotective effect [2]. The cells were fixed with 4% paraformaldehyde (10-min treatment) 30 min and 1 h after addition of afobazole. These cells were washed 4 times with phosphate-buffered saline (10 min) and treated with 250 mM sucrose solution (4°C, 36 h) and 1 mg/ml BSA (room temperature, 2 h) [13]. Double immunofluorescence staining was performed with antibodies to σ_1-receptors (Santa Cruz Biotechnology) and antibodies to calnexin (endoplasmic reticulum membrane-associated protein, Abcam) in a solution of phosphate-buffered saline and

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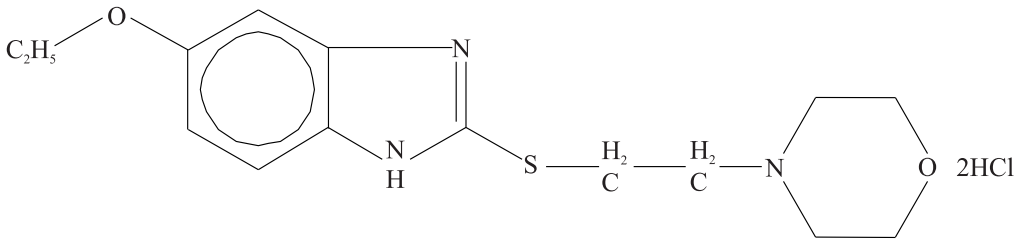

Fig. 1. Structure of afobazole.

BSA (1 mg/ml). The cells were incubated with antibodies to σ_1-receptors at 4°C for 12 h and antibodies to calnexin at room temperature for 1 h.

RESULTS

During displacement of σ_1-receptor agonist [^{3}H](+)-pentazocine (8 nM) from binding sites, IC_{50}, Ki, and nH for afobazole were 7.1×10^{-6} M, 5.9×10^{-6} M, and 0.9, respectively (Fig. 2). The results of radioligand binding indicate that afobazole can be considered as an σ_1-receptor ligand.

During the interaction with ligands, σ_1-receptor can migrate to the cell membrane (in the composition of lipid microdomains, lipid rafts) [9]. Radioligand study indicates that it is necessary to evaluate the effect of afobazole on intracellular localization of σ_1-receptors [13].

Immunofluorescence staining of mouse hippocampal cell line HT-22 with antibodies to σ_1-receptors and calnexin (protein marker of the endoplasmic reticulum) showed that addition of afobazole (10^{-8} M) to the culture medium is followed by intracellular redistribution of σ_1-receptors. Under control conditions, σ_1-receptors were revealed in the neuronal body, but not in axons. The location of σ_1-receptors was similar to that of calnexin (Fig. 3). Thirty minutes after addition of afobazole to the culture medium, σ_1-receptor staining was revealed not only in the neuronal body, but also in axons. These receptors were located near the cell plasma membrane (including the axons) 1 h after afobazole treatment. However, calnexin was associated with endoplasmic reticulum membrane in the neuron body.

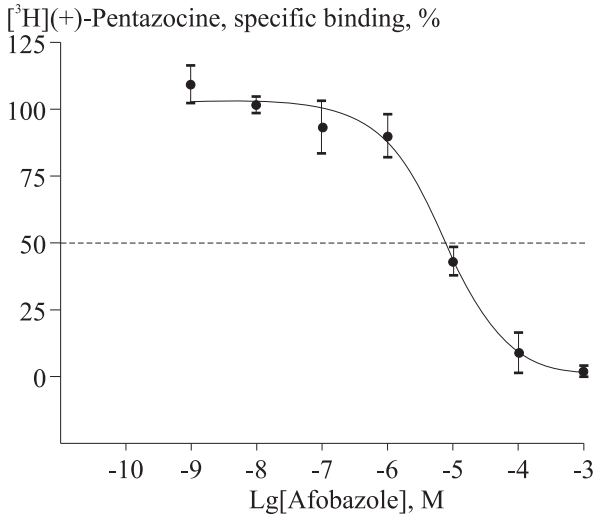

Fig. 2. Interaction of afobazole with the σ_1-receptor. Binding of afobazole to the σ_1-receptor was studied in 2 repetitions for each concentration of the compound.

σ_1-Receptors were discovered in 1976 and initially classified to the group of opioid receptors [11]. Cloning and evaluation of the amino acid sequence showed that these receptors should be considered as independent structures. σ_1-Receptors are mainly located on the endoplasmic reticulum and have two transmembrane domains [5]. σ_1-Receptors produce a regulatory effect on intracellular signal transduction (primarily on Ca^{2+} transport) and cell energy balance. Moreover, these receptors have a modulatory effect under conditions of induced changes in main neurotransmitter processes [10]. Specific binding to σ_1-receptors is typical of neurosteroids, some physiologically active neuropeptides, psychopharmacological products, and other compounds [14]. σ_1-Receptor translocation to the outer membrane after ligand-induced cell activation is of considerable functional importance [12]. This phenomenon and involvement of σ_1-receptors in signal transduction determine their role in prevention of cell dysfunction. We showed that afobazole serves as a ligand of σ_1-receptors. Moreover, afobazole causes σ_1-receptor translocation to the outer membrane. Our results and published data on the mechanism of cell damage [6] explain the neuroprotective effect of afobazole and recovery of $GABA_A$ receptor function under the influence of this compound. The data suggest that this anxiolytic holds much promise as a cytoprotective drug under various pathological conditions.

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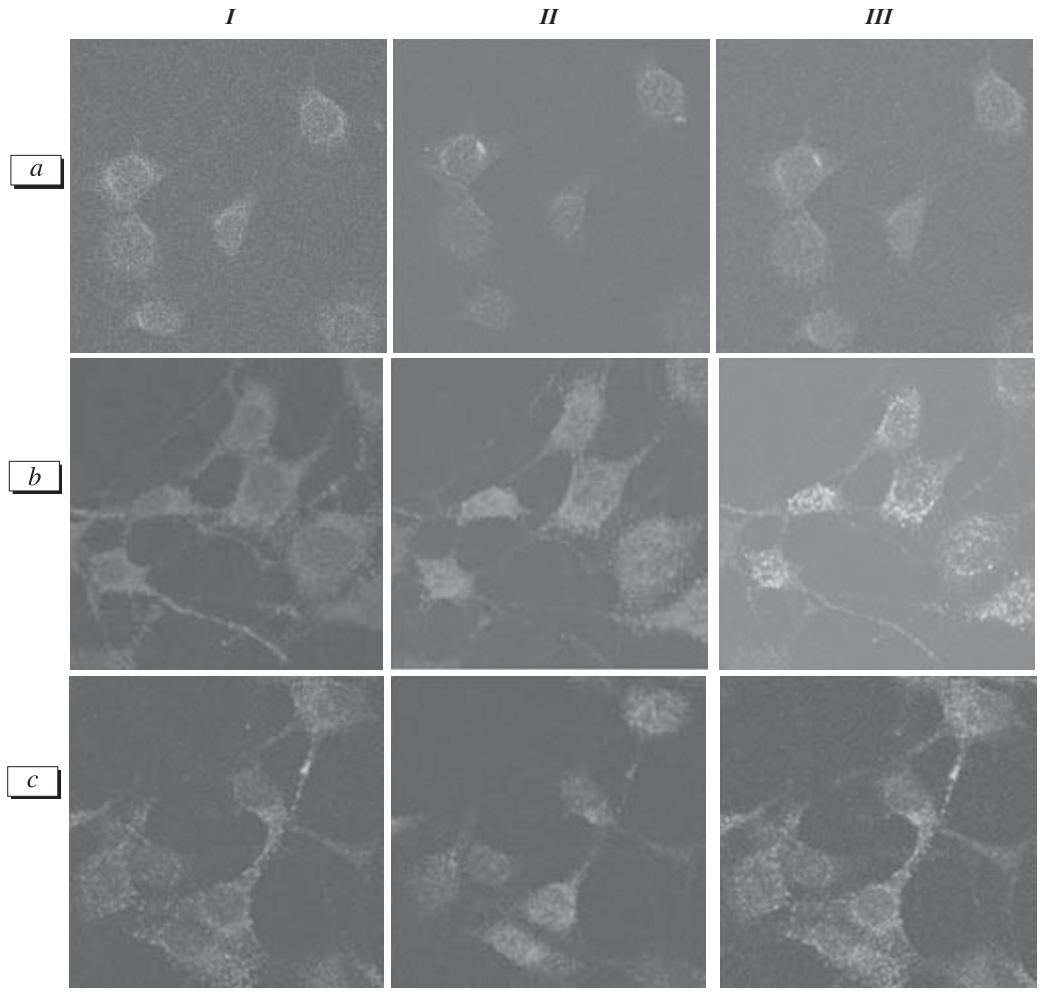

Fig. 3. Immunofluorescence staining of σ_1-receptors and calnexin in the immortalized mouse hippocampal cell line HT-22 (×400). Control staining (*a*); staining 30 min after addition of afobazole in a concentration of 10^{-8} M to the incubation medium (*b*); staining 1 h after addition of afobazole in a concentration of 10^{-8} M to the incubation medium (*c*). *I*, σ_1-receptors; *II*, calnexin; *III*, double staining.

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