On the Mechanism of Antifibrillatory Effect of Afobazole

S. A. Kryzhanovskyi, V. N. Stolyaruck, M. B. Vititnova, I. B. Tsorin, and S. B. Seredenin

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Effect of afobazole on the threshold of electrical fibrillation of the heart was studied on anesthetized rats with intact myocardium. It was shown that the drug considerably increased the threshold of electrical fibrillation of the heart, being not inferior to reference class I antiarrhythmic drugs (lidocaine and procainamide) according to V. Williamse classification. Against the background of preliminary injection of σ-receptor antagonist haloperidol, afobazole exhibited no antifibrillatory activity. These findings and analysis of published reports suggest that antifibrillatory activity of afobazole is determined by its antagonistic influence on σ_1-receptors localized in cardiomyocyte cytosol.

Key Words: *afobazole; threshold of electrical fibrillation of the heart; σ_1-receptors; haloperidol*

New original anxiolytic afobazole synthesized and tested at V. V. Zakusov Institute of Pharmacology is successfully used in clinical practice. The mechanism of the anxiolytic effect of the preparation is determined by prevention of stress-induced inhibition of binding in the benzodiazepine site of $GABA_A$ receptor [5]. At the same time, the interaction of afobazole with σ_1-receptors was demonstrated [1,4,6]. Apart from CNS, the σ_1-receptors were identified in other organs and tissues of the organism, including the myocardium [12,13]. Moreover, there are data that σ_1-receptor ligands exhibit antiarrhythmic and antifibrillatory activity [2,3]. Potent antifibrillatory effect of afobazole was previously shown by us on various models of heart rhythm disturbances [8].

Here we studied the contribution of σ_1-receptors to the realization of the antifibrillatory effect of afobazole.

MATERIALS AND METHODS

Experiments were carried out on anesthetized (urethane, 1300 mg/kg intraperitomealy) outbred male rats weighing 350-400 g. The animals were maintained under standard vivarium conditions according to Guidelines for Laboratory Practice (GLP) and Order of Ministry of Health of the Russian Federation, No. 267, June 09, 2003 "Concerning Approval of the Rules of Laboratory Practice". The animals were intubated, artificial ventilation was started, and sternectomy and pericardiotomy were carried out. Two gold-plated electrodes were implanted into the myocardium of the left ventricle at a distance of 0.5 cm from each other. The threshold of electrical fibrillation of the heart was determined by repeated scanning of the vulnerable period of the heart cycle with a series of 20 rectangular direct current pulses of increasing intensity (4 msec pulse duration, 50 cpm/sec frequency). The minimum current strength inducing ventricular fibrillation upon 2-fold repetition was taken as a fibrillation threshold. Animals with fibrillation threshold not exceeding 6 mA were used in further experiments. Electrostimulator HSE Stimulator II (Hugo Sach Electronik) was used in the experiments. ECG in standard lead II was recorded throughout the experiment with an electrocardiograph EK 4T-02. Visulal control of the recorded parameters during the experiment was carried our with a 4-channel Elema-Siemens oscilloscope. Afobazole was injected in a dose of 7.5 mg/kg at a constant rate

V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** sak-538@yandex.ru. S. A. Kryzhanovskyi

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and in a constant volume of 0.9% NaCl. Haloperidol (Gedeon Richter) was injected intravenously in doses of 0.1, 0.5, and 1.0 mg/kg at a constant rate and in a constant volume of 0.9% NaCl. A Syringe pump injector (Sage Instruments) was used for injection of the preparation.

The threshold of electrical fibrillation of the heart was determined 5 min after the end of intravenous injections. The interval between the testing stimuli was 2-3 min.

The results were processed statistically using dispersion analysis for repeated measurements and then using Newman–Keuls multiple comparison test, Student test, and paired Wilcoxon test.

RESULTS

In experimental series I, we evaluated the effect of afobazole on the threshold of electrical fibrillation of the heart in rats. It was shown that afobazole considerably ($p<0.05$) increased the threshold of electrical fibrillation of the heart (Table 1), being not inferior to reference class I antiarrhythmic drugs (lidocaine and procainamide) according to V. Williamse classification. These findings coincide with our previous findings obtained in experiments for evaluation of antifibrillatory activity of afobazole on cats with intact and denervated myocardium.

Published data suggest that ligands of σ_1-receptors, similarly to afobazole, exhibit antifibrillatory activity. Therefore, in the next experimental series were evaluated the antifibrillatory effects of afobazole against the background of preliminary administration of σ-receptor antagonist haloperidol. Although haloperidol is regarded as a nonselective σ-receptor antagonist, there are data that its affinity to σ_1-receptors is 170-fold higher than to σ_2-receptors.

Against the background of preliminary administration of haloperidol in a dose of 0.5 mg/kg, afobazole (7.5 mg/kg) exhibited no antifibrillatory effect (Table 2). Moreover, afobazole decelerated HR in intact animals, but not in animals receiving haloperidol ($p=0.046$).

Thus, these findings suggest that the antifibrillatory effect of afobazole can be related to its interaction with σ_1-receptors located in cardiomyocytes, because no antifibrillatory effect of afobazole was observed after blockade of these receptors.

The role of σ-receptors in the regulation of functional activity of the cardiovascular system is poorly studied. At the same time, experiments performed under supervision of V. P. Maslov demonstrated the antiarrhythmic and antifibrillatory effects of σ_1-receptor ligands [2,3,11]. It should be emphasized that these studies were performed on models similar to those used by us for evaluation of the antiarrhythmic and antifibrillatory effects of afobazole.

However, according to the hypothesis proposed by V. P. Maslov and co-workers, agonists but not antagonists of σ_1-receptor exhibit antiarrhythmic and an-

TABLE 1. Effect of Afobazole on the Threshold of Electrical Fibrillation of Heart Ventricles in Rats

No.	Baseline threshold	Afobazole, 7.5 mg/kg intravenously		
		HR		threshold
		before injection	after injection	
1	4	357	340	>100
2	3	375	375	>100
3	2	422	405	>100
4	4	416	333	>100
5	4	455	416	>100
6	1	384	300	>100
7	4	333	333	10
Mean value	3.14±0.46 Med 4.0 2.0-4.0	391.7±15.8	357.4±16.0* Med 100 100-100	87.1±12.9**

Note. *$p=0.046$, **$p<0.05$ compared to baseline.

TABLE 2. Effect of Afobazole on the Threshold of Electrical Fibrillation of Herat Ventricles in Rats against the Background of Preliminary Administration of Haloperidol

No.	Baseline threshold	Haloperidol, 0.5 mg/kg intravenously			Afobazole, 7.5 mg/kg intravenously		
		HR		threshold	HR		threshold
		before injection	after injection		before injection	after injection	
1	3	416	416	0.5	416	416	0.5
2	3	428	428	1	400	400	1
3	1	428	375	0.5	375	375	0.5
4	2	333	286	1	261	261	1
5	2	353	285	4	366	366	4
6	3	400	250	3	300	300	3
7	2	375	363	1	333	333	1
8	1	316	316	0.5	316	250	3
Mean value	2.19±0.29	381.1±15.4	339.9±23.1	1.44±0.47	345.9±18.6	337.6±22.1	1.75±0.48
	Med 2.0		Med 1.0		Med 1.0		
	1.5-3.0		0.5-2.0		0.75-1.3		

tifibrillatory activities. This assumption disagres with our findings, because there are strong evidence that afobazole is an agonist of σ_1-receptors [1,4,6].

To solve this contradiction we studied antifibrillatory effects of σ-receptor antagonist haloperidol; if the hypothesis of V. P. Maslov is true, haloperidol should exhibit antifibrillatory activity. From the theoretical point of view, it is unlikely that haloperidol possesses antifibrillatory activity; ample data, including clinical reports, suggest that haloperidol lengthens *QT* on ECG and provokes malignant heart rhythm disturbances (torsaide de pointes), *i.e.* increases the risk of ventricular fibrillation [9,14].

Analysis of our findings showed that intravenous administration of haloperidol in doses of 0.1-0.5-1.0 mg/kg (the same doses are administered to rats for studying the psychotropic effects of haloperidol, including the effects mediated by its interaction with σ-receptors [15]) does not increase the threshold of electrical fibrillation of the heart ventricles. Moreover, the threshold of electrical fibrillation tended to decrease after administration of haloperidol in all doses (Fig. 1).

The hypothesis of V. P. Maslov that σ_1-receptors agonists reduce the threshold of electrical fibrillation of the heart ventricles looks not very convincing. It was shown that endogenous σ_1-receptor agonists N,N-dimethyltryptamine blocks Na^+ current through cardiomyocyte membrane [8] (*i.e.* acts as a class I antiarrhythmic drug according to V. Williamse classification), thus exhibiting pronounced antifibrillatory activity.

Thus, it can be concluded that antifibrillatory, and probably antiarrhythmic activity of afobazole is to a greater or lesser extent determined by its interaction with σ_1-receptors in cardiomyocytes. It experimentally proven that afobazole in a dose of 7.5 mg/kg significantly increases the threshold of electrical fibrillation of the heart, while against the background of haloperidol (0.5 mg/kg) it does not affect this parameter

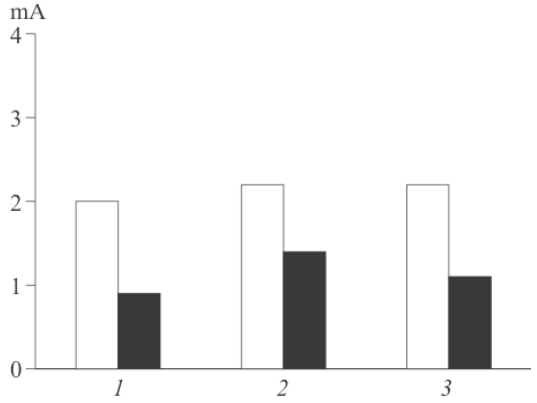

Fig. 1. Effect of haloperidol in doses of 0.1 (*1*), 0.5 (*2*), and 1.0 mg/kg (*3*) on the threshold of electrical fibrillation of heart ventricles. Ordinate: current strength. Light bars: initial fibrillation threshold; dark bars: fibrillation threshold against the background of haloperidol treatment.

in rats. Intravenous injection of σ_1-receptor agonist haloperidol in doses of 0.1, 0.5 and 1.0 mg/kg did not increase the threshold of electrical fibrillation of the heart in rats.

REFERENCES

1. L. G. Kolik, V. N. Zhukov, and S. B. Seredenin, *Eksp. Klin. Farmakol*., **72**, No. 1, 22-23 (2009).
2. Yu. B. Lishmanov, L. N. Maslov, A. V. Krylatov, *et al., Ibid*., **63**, No. 6, 24-27 (2000).
3. L. N. Maslov, A. V. Krylatov, Yu. B. Lishmanov, *et al., Ibid*., **60**, No. 2, 24-26 (1997).
4. S. B. Seredenin and M. V. Voronin, *Ibid*., **72**, No. 1, 3-11 (2009).
5. S. B. Seredenin, T. A. Voronina, G. G. Neznamov, *et al., Vestn. Ross. Akad. Med. Nauk.*, No. 11, 3-9 (1998).
6. S. B. Seredenin, G. M. Molodavkin, M. V. Voronin, and T. A. Voronina, *Eksp. Klin. Farmakol*., **72**, No. 1, 19-21 (2009).
7. V. N. Stolyaruk, M. B. Vititnova, and S. A. Kryzhanovskyi, *Psikhofarmakol. Biol. Narkol.*, **7**, Special issue (1998).
8. D. Fontanilla, M. Johannessen, A. R. Hajipour, *et al.*, *Science*, **323**, No. 5916, 934-937 (2009).
9. H. A. Hassaballa and R. A. Balk, *Expert Opin. Drug Saf.,* **2**, No. 6, 543-547 (2003).
10. A. Klouz, M. H. Loueslati, R. Daghfous, and D. Morin, *Therapie*, **56**, No. 5, 557-562 (2001).
11. Yu. B. Lishmanov, L. N. Maslov, N. V. Naryzhnaya, and S. W. Tam, *Life Sci*., **65**, No. 1, L13-L17 (1999).
12. M. Novakova, V. Bruderova, Z. Sulova, *et al., Gen. Physiol. Biophys*., **26**, No. 2, 110-117 (2007).
13. M. Novakova, C. Ela, W. D. Bowen, *et al., Eur. J. Pharmacol.*, **353**, Nos. 2-3, 315-327 (1998).
14. S. Rasty, N. B. Amin, H. N. Sabban, *et al*., *Chest*, **125**, No. 5, 1821-1829 (2004).
15. M. J. Robbins, H. M. Critchlow, A. Lloyd, *et al., J. Psychopharmacol*. **22**, No. 5, 536-542 (2008).