Antiarrhythmic Activity of GABA Derivative TZ-50-2

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The GABA derivative TZ-50-2 exerted pronounced antiarrhythmic effects on a variety of arrhythmias (atrial, ventricular, and mixed). The drug was superior (or at least comparable) to quinidine, procainamide, lidocaine, verapamil, bonnecor, and other reference drugs in antiarrhythmic activity and therapeutic range, and showed no cardiotoxicity. The antiarrhythmic effects of TZ-50-2 were due to modulation of calcium and sodium channels.

Key Words: *GABA; cardiac arrhythmias*

Antiarrhythmic potency of local anesthetics and anticonvulsants with similar mechanisms of action attracts now considerable attention [10]. The novel GABA derivative hemisuccinate 4-oxi-3-benzylamino-N-benzylbutan amide (laboratory code TZ-50-2) exhibits both local anesthetic [9] and anticonvulsant properties [6]. This work was aimed at investigation of antiarrhythmic activity (AA) of TZ-50-2 on different models of atrial, ventricular, and mixed arrhythmias.

MATERIALS AND METHODS

Experiments were carried out on 446 outbred male albino rats (175-220 g), 48 mongrel rabbits (2.8-3.6 kg), 98 cats (2.6-4.2 kg), and 31 dogs (14-22 kg) of both sexes, as well as on isolated atria (trabecular and auricular preparations) of frogs (n=36) and guinea pigs (n=20).

Toxicity of TZ-50-2 and reference drugs (mean lethal dose, LD_{50}) was determined in experiments on rats with single intravenous injection followed by 48-h monitoring [3,7].

The ECG (*R, RR, QT, PQ, QRS*) were recorded in standard lead II with EKIT-04 electrocardiograph in experiments on dogs under pentobarbital anesthesia (40 mg/kg, intrapleurally) with drug administration by biological titration (1 mg/kg/ml) until cardiac arrest.

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Antiarrhythmic activities of TZ-50-2 and reference drugs were assessed on the following models: auricular arrhythmia caused by sinus node disruption with subsequent electric stimulation [16] in dogs or by application of aconitine and acetycholine [8] on sinoatrial area in cats; ventricular arrhythmias caused by reperfusion of coronary arteries in cats [15] and by myocardial infarction in dogs [13]; mixed arrhythmias caused by intravenous administration of adrenaline [11], aconitine [8], and calcium chloride [1] in rats; by infusion of aconitine [4] and strophanthin [2] in cats, and by injection of barium chloride [14] in rabbits.

Drug effects on the myocardial refractory period were studied on isolated auricles of guinea pig heart [8]. The effects on action potentials and transmembrane ionic currents were studied on isolated atrial trabeculas from the heart of *Rana ridibunda* [12].

The antiarrhythmic efficacy of the drugs was evaluated by comparing their potencies (mean effective doses, ED_{50}) and antiarrhythmic indices (LD_{50}/ED_{50}) characterizing the therapeutic window (TW) [3].

The data were analyzed statistically [3].

RESULTS

Intravenous infusion of TZ-50-2 had no effect on the ECG indices in the anesthetized dogs up to a total dose of 50 mg/kg (reached over 50 min). Further infusion (up to 60-120 mg/kg) caused respiration arrest

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(mean dose 66.4 mg/kg, individual doses ranged from 44.1 to 88.5 mg/kg), bradycardia, atrioventricular and inraventricular block, reduced myocardial contractility (*R*-wave depression), and led to cardiac arrest in a mean dose of 87.2 mg/kg (66.2-108.2 mg/kg). Therefore, TZ-50-2 administered in a therapeutic dose of 8 mg/kg (10% of the lethal dose) and in a 6-fold higher dose (50 mg/kg) had practically no effect on the heart rate, atrioventricular and intraventricular conduction and the effective refractory period, which indicates the absence of significant cardiotoxic effects characteristic of other antiarrhythmics [5].

TZ-50-2 was 3.7 times more potent than procainamide and 3.7 times less potent than cordarone in increasing the refractory period in the isolated auricle from guinea pig heart. Its potency was comparable with that of quinidine. It is important that verapamil (finoptine) in concentrations of 10^{-6}-10^{-5} M suppressed the contractility of isolated auricle without affecting the refractory period.

TZ-50-2 eliminated atrial flutter in dogs caused by electric stimulation of the atrium after sinus node destruction, being 5.7- and 2.1-fold superior to quinidine and ethmosine, respectively.

In modeled atrial arrhythmias induced by aconitine application on the sinus area in cats, TZ-50-2 was 2-fold superior to procainamide and comparable to lidocaine and ethacizine. In acetylcholine-induced arrhythmia TZ-50-2 was 3 times more efficient than procainamide.

TZ-50-2 exhibited 6 times lower potency than verapamil with respect to postreperfusion ventricular fibrillation, but showed a broader TW; the antiarrhythmic index of TZ-50-2 (17) was nearly twice as high as that of verapamil (17 and 9.4, respectively).

Under conditions of ventricular arrhythmias caused by myocardial infarction in dogs, TZ-50-2 (10 mg/kg) exerted a more pronounced antiarrhythmic effect (reduced the number of ectopic beats) than ethmosine (5 mg/kg). The most pronounced antiarrhythmic activity of the two drugs was observed during the first 5-10 min (Fig. 1). The effects of TZ-50-2 lasted for a longer period (30 min on average, n=5; 70 min in one experiment) than those of ethmosine (10 min on average, n=5; 15 and 20 min in 2 experiments). TZ-50-2 had practically no effect on heart rate during the first 5 min, while ethmosine induced statistically significant bradycardia which lasted for 30 min. It should be emphasized that TZ-50-2 was 6.8 times less toxic than ethmosine: under conditions of intravenous infusion to rats LD_{50} for TZ-50-2 was 82 (72-93) mg/kg *vs.* 12 mg/kg for ethmosine [10].

In arrhythmias of mixed origin (atrioventricular) caused by aconitine in rats (Table 1), TZ-50-2 exhibited no significant antiarrhythmic effects (its action lasted no longer than 1.5-3 min), but was effective on the same model in cats showing a 3.4-fold higher potency than procainamide, but a 2.8-fold lower potency than quinidine. Antiarrhythmic indices were 11.5 for TZ-50-2, 9 for quinidine, and 16.9 for procainamide.

In adrenaline-induced arrhythmia in rats TZ-50-2 was 6-, 3.6- and 3.4-fold superior to lidocaine, verapamil and quinidine, respectively, but 1.3 fold less effective than bonnecor. At the same time, in this model TZ-50-2 showed significantly broader TW than all reference drugs (Table 1). Procainamide was ineffective under these conditions.

TZ-50-2 eliminated mixed arrhythmias induced by intravenous strophanthin in cats. Its antiarrhythmic activity was 23.7-, 8-, and 5.3-fold higher than that of

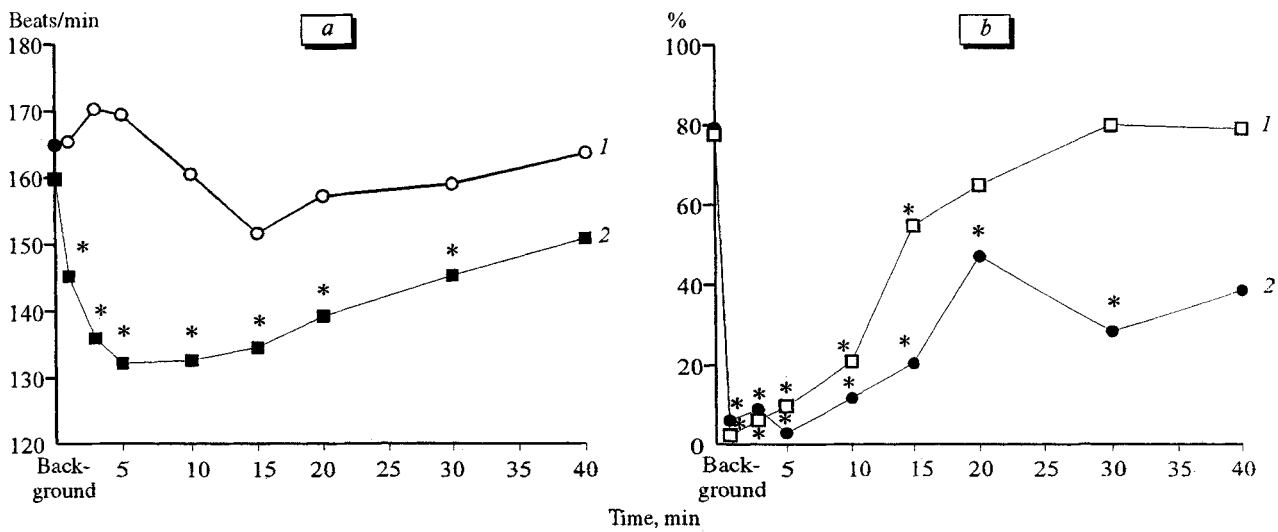

Fig. 1. Effects of intravenous infusion of TZ-50-2 (*1*) and ethmosine (*2*) on heart rate (*a*) and ectopic beats (*b*) in ventricular arrhythmia in dogs. *p<0.05 in comparison with background.

TABLE 1. Comparative Antiarrhythmic Activity and Therapeutic Window of TZ-50-2 and Reference Drugs on the Models of Arrhythmia Induced by Aconitine (Rats), Adrenaline (Rats), Strophanthin (Cats), Barium Chloride (Rabbits), and Calcium Chloride (Rats)

Drugs	ED50, mg/kg					LD50, mg/kg	LD_{50}/ED_{50}				
	aconitine	adrenaline	strophanthine	barium chloride	calcium chloride		aconitine	adrenaline	strophanthine	barium chloride	calcium chloride
TZ-50-2	5.4 (1.4)*	1.2 (6)	1.8 (5.3)	3.6 (0.6)	4.5 (2.3)	82.0 (0.3)	15.2	68.3	45.6	22.8	18.2
Verapamil	—	4.3 (1.7)	0.5 (19.2)	0.7 (3.3)	2.4 (4.3)	15.3 (1.8)		3.6	30.6	21.9	6.4
Quinidine	14.1 (0.5)	4.1 (1.8)	14.4 (0.7)	8.5 (0.3)	8.7 (1.2)	54.2 (0.5)	3.8	13.2	3.8	6.4	6.2
Procainamide	53.7 (0.1)	—	42.6 (0.2)*	15.6 (0.1)	12.5 (0.8)	166.0 (0.2)	3.1		3.9	10.6	13.3
Bonnecor	1.2 (6.2)	0.9 (8)	0.6 (16)*	0.7 (3.3)	1.5 (6.8)	10.8 (2.6)	9.0	12.0	18.0	15.4	7.2
Lidocaine	7.4 (1)	7.2 (1)	9.6 (1)	2.3 (1)	10.2 (1)	28.2 (1)	3.8	3.9	2.9	12.3	2.8

Note. The data normalized to lidocaine (taken as 1) are presented in parentheses. *indicates 1.5-3-min effect.

procainamide, quinidine and lidocaine, respectively, being, however, 3.6 and 3 times lower than that of verapamil and bonnecor. It is important to note that TZ-50-2 was superior to all reference drugs (lidocaine, quinidine and procainamide) with respect to the TW width.

Under conditions of barium chloride arrhythmia in rabbits, TZ-50-2 exhibited higher activity than procainamide and quinidine (5.7 and 1.8 times, respectively), but was less potent than verapamil, bonnecor (5.1 times) and lidocaine (1.6 times). Its TW in this model was comparable with that of verapamil, but was broader than that of quinidine (3.6 times), procainamide (2.2 times), lidocaine (1.9 times), and bonnecor (1.5 times) (Table 1).

TZ-50-2 more effectively prevented the development of calcium chloride arrhythmia (fibrillation) in rats than procainamide, lidocaine, and quinidine (2.8, 2.3 and 1.9 times, respectively). Its potency was, however, lower than that of verapamil and bonnecor (1.9 and 3 times, respectively). The antiarrhythmic index of TZ-50-2 was 6.5, 2.9, 2.8, 2.5, and 1.4 times higher than that of lidocaine, quinidine, verapamil, bonnecor and procainamide, respectively (Table 1).

Experiments on the isolated auricular trabeculas from frog heart showed that TZ-50-2 in concentrations of 10^{-6} and 10^{-5} M modulated transmembrane ionic currents showing the most pronounced effects in a concen-tration of 10^{-5} M. Alterations in the action potentials (a decrease in the amplitude and duration at the level of both its base and plateau) became noticeable after 20-30 sec perfusion and reached the peak after 2-3 min. The current threshold and membrane resting potential were unaffected.

The voltage clamp recording of transmembrane ionic currents revealed suppression of the inward sodium current (by 29%) and lowering of its reversal potential (by 8 mV) under the influence of TZ-50-2. The drug also dramatically (by 68%) reduced the slow inward calcium current and lowered its reversal potential (by 10 mV) leaving the dynamics of calcium channel activation unaffected. At the same time TZ-50-2 induced a 40-60% increase in the amplitude of the background ("instantaneous" component) outward potassium current, which was observed over the entire range of membrane holding potentials (20-100 mV).

With respect to the ability to suppress calcium and sodium currents, TZ-50-2 was comparable to verapamil, which reduced calcium current by 37% and 60%, when applied in 10^{-6} and 10^{-5} M concentrations, respectively. In these concentrations TZ-50-2 suppressed calcium current by 30% and 67%, respectively being 2-fold more effective than quinidine.

The observed membranotropic properties of TZ-50-2 (reduction of the amplitude and duration of action potentials, suppression of sodium and calcium

currents) allow us to assign it to classes I and IV antiarrhythmics.

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