Coronarotropic Properties of Cyclic GABA Derivative TZ-146

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In the experiments on isolated contracting, fibrillating, and strophanthin K-arrested cat hearts, TZ-146, cyclic derivative of gamma aminobutyric acid enhanced the outflow of perfusate. When tested *in vivo*, this preparation increased the indices of phasic coronary blood flow in dogs, surpassing the effects of reactive hyperemia, which characterizes the dilatory capacity of coronary vessels. Pharmacological analysis and experiments with bilateral vagotomy in cats and dogs suggest that coronarotropic effects of TZ-146 can result from activation of adenylate cyclase and accumulation of cAMP.

Key Words: *GABA; coronary blood flow*

It has been shown that TZ-146 (cyclic GABA derivative, a structural analog of piracetam) prevents disturbances in both systemic and cardiac hemodynamics by enhancing coronary blood flow volume velocity (VVCF) in ischemized myocardium *in vivo* [2]. It is of interest, therefore, to study coronodilatory properties of TZ-146 in experiments on isolated hearts, its effect on phase coronary blood flow (CBF), the mechanisms of coronodilatory effects.

These investigations were also prompted by the fact that GABA derivatives can interfere with sympathetic extracardiac regulation [4,15]. It is known that changes in the sympathetic tone can cause both direct (true neurogenic) and indirect (mediated via metabolic processes in the myocardium) coronary vasomotor reactions [3].

MATERIALS AND METHODS

Experiments were carried out on 57 cats (body weight 2.6-3.5 kg), 20 dogs (15.5-21.5 kg), and 30 isolated cat hearts.

The effects of TZ-146 on coronary vessels were studied on contracting, fibrillating, and strophanthin K-arrested cat hearts (1:25,000) isolated according to Langendorff's technique [3].

The effects of TZ-146 on the phase CBF were studied in dogs. The following indices were analyzed: end-diastolic CBF, diastolic stroke and minute CBF, CBF per beat and per minute, coronary perfusion pressure and end-diastolic resistance; the systolic stroke CBF; systolic perfusion pressure and CBF index of (diastolic to systolic stroke CBF ratio) [8]. Additionally, the following indices were recorded: systemic blood pressure (SBP), reactive 20-sec hyperemia after intravenous or intracoronary drug injection [13], left ventricular pressure, the rate of its changes (dp/dt_{max} and dp/dt_{min}), and heart rate (HR). Contractile heart function was assessed by shifts in the maximum rate of rise and fall in the left ventricle pressure, by relaxation and Veragut's indices, and the myocardium-developed acceleration [7,14].

The coronodilatory effects of TZ-146 were analyzed with pharmacological agents [3,5,6,9,10,12] administered intravenously, intraperitoneally (in cats), and intracoronary (in dogs). The following drugs were used: noncompetitive and competitive $GABA_A$ receptor antagonists picrotoxin and bicuculline, (1 mg/kg each, intravenously); muscarinic cholinergic receptor antagonist atropine sulfate (2.5 mg/kg intravenously); β-adrenoblocker propranolol (Obsidan, 0.25 mg/kg, intravenously); α-adrenoblocker dihydroergotoxin,

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(2 g/kg, intravenously), phosphodiesterase and P_1-purinoreceptor antagonist caffeine (5 mg, intravenously); phosphodiesterase activator imidazole (5 mg, intracoronary); GR-755 benzofuran derivative blocking fast sodium channels [11] (1 mg/kg, intravenously); blocker of slow calcium channels verapamil (1 mg/kg, intravenously); adenosine (adenosine receptor agonist, 0.01-0.02 μg, intravenously or intracoronary); indomethacin (prostaglandin synthase inhibitor, 5 mg/kg, intravenously); reserpine depleting catecholamine stores (5 mg, intraperitoneally). Reactive hyperemia of 20-sec duration induced the release of endogenous adenosine [13]. TZ-146 was injected intravenously (in cats) and intracoronary (in dogs) in doses of 50 mg/kg and 5 mg, respectively.

Bilateral vagotomy in cats was performed at the level of the thyroid cartilage. The effect of TZ-146 on CBF was studied 15-20 min after denervation.

The data were analyzed statistically as described previously [1].

RESULTS

In experiments with isolated contracting cat heart, TZ-146 in concentrations of 10^{-6} and 10^{-5} g/ml significantly enhanced perfusate outflow by 5.7-6.3% and 10.1-16%.2, respectively. This effect persisted for 15 min (10^{-6} g/ml) and 45 min (10^{-5} g/ml). In the experiments on fibrillating and strophanthin K-arrested hearts, the same concentrations of TZ-146 only insignificantly increase the outflow.

The study of TZ-146 effects on the phase CBF in dogs revealed that 15 min after intravenous injection of 50 mg/kg the end-diastolic CBF increased by 30.8%, the diastolic stroke and minute CBF increased by 35.3% and 48.8%, respectively, CBF per minute by 57.2%, the systolic stroke CBF by 55%, Veragut index by 38.6%, relaxation index by 15.4%, and acceleration index by 28.7%. This dose of TZ-146 reduced the systemic pressure by 14.9%, lowered end-diastolic and end-systolic perfusion pressure by 16.5% and 14.5%, respectively, and decreased the end-diastolic resistance of coronary vessels by 17.4%. No significant changes were observed in CBF per beat, left ventricular pressure, CBF index, HR, dp/dt_{min}, and dp/dt_{max}. These effects persisted for 30-45 min.

When comparing the changes in coronary and hemodynamic indices during reactive hyperemia and after TZ-146 injection, we found that reactive hyperemia significantly increased the end-diastolic CBF (17.6%), diastolic stroke and minute CBF (20 and 39%, respectively), CBF per beat and per minute (40 and 52.5%, respectively), and systolic CBF (75%). It reduced the systolic perfusion pressure in coronary vessels by 8.8% and the end-diastolic pressure by 11.4%. TZ-146 increased CBF per minute by 7.1% and Veragut and relaxation indices by 17.5% and 16.4%, respectively. It reduced the left ventricular pressure by 18.3% and end-diastolic resistance of coronary vessels by 13.6%. Other indices remained practically unchanged.

Under the influence of TZ-146, the Veragut and relaxation indices surpassed the peak values in reactive hyperemia by 17.5% and 16.4%, respectively, the end-diastolic CBF and CBF per beat increased by 11.2% and 7.1% respectively, the left ventricular pressure decreased by 18.3% and end-diastolic resistance of coronary vessels by 13.6%. Thus, TZ-146 effect surpassed by a number of indices the parameters of reactive hyperemia characterizing the dilatory reserve of coronary vessels activated by endogenous adenosine and to some extent by myocardium contractions [13].

It is important to note that TZ-146-induced enhancement of phase CBF occurred against the background of relatively stable SBP and HR showing only slight tendencies to decrease (SBP) or increase (HR). This indicates that TZ-146 primarily modulate the tone of coronary vessels.

Possible mechanisms of TZ-146 dilatory effect on coronary vessels were analyzed using pharmacological agents and bilateral vagotomy.

Intravenous injection of TZ-146 to cats under conditions of $GABA_A$ receptor blockade with picrotoxin or bicuculline increased VVCF and decreased the myocardial oxygen consumption without affecting SBP.

Intravenous atropine and dihydroergotoxin did not significantly change the stimulating effect TZ-146 on VVCF.

Under conditions of propranolol-induced blockade of β-adrenoreceptors TZ-146 had no (in 2 out of 5 cats) or minor effect on VVCF. After reserpine-induced depletion of tissue catecholamine stores, TZ-146 increased VVCF and reduced myocardial oxygen consumption without affecting SBP.

When fast sodium channels in cats were blocked with GR-755, administration of TZ-146 gradually increased VVCF, decreased myocardial oxygen consumption, enhanced myocardial contractility, and normalized SBP.

In cats, ino-, chrono- and dromotropic effects were observed after intravenous injection of verapamil, a slow calcium channel blocker which reduced VVCF, myocardial oxygen consumption, and SBP. Subsequent intravenous injection of TZ-146 restored the myocardial contractility, increased VVCF, and normalized SBP.

In the experiments on cats and dogs, TZ-146 potentiated the dilatory effect of adenosine and endogenous adenosine-induced postischemic heart hyperemia.

In dogs, intracoronary administration of TZ-146 after inhibition of phosphodiesterase and caffeine-

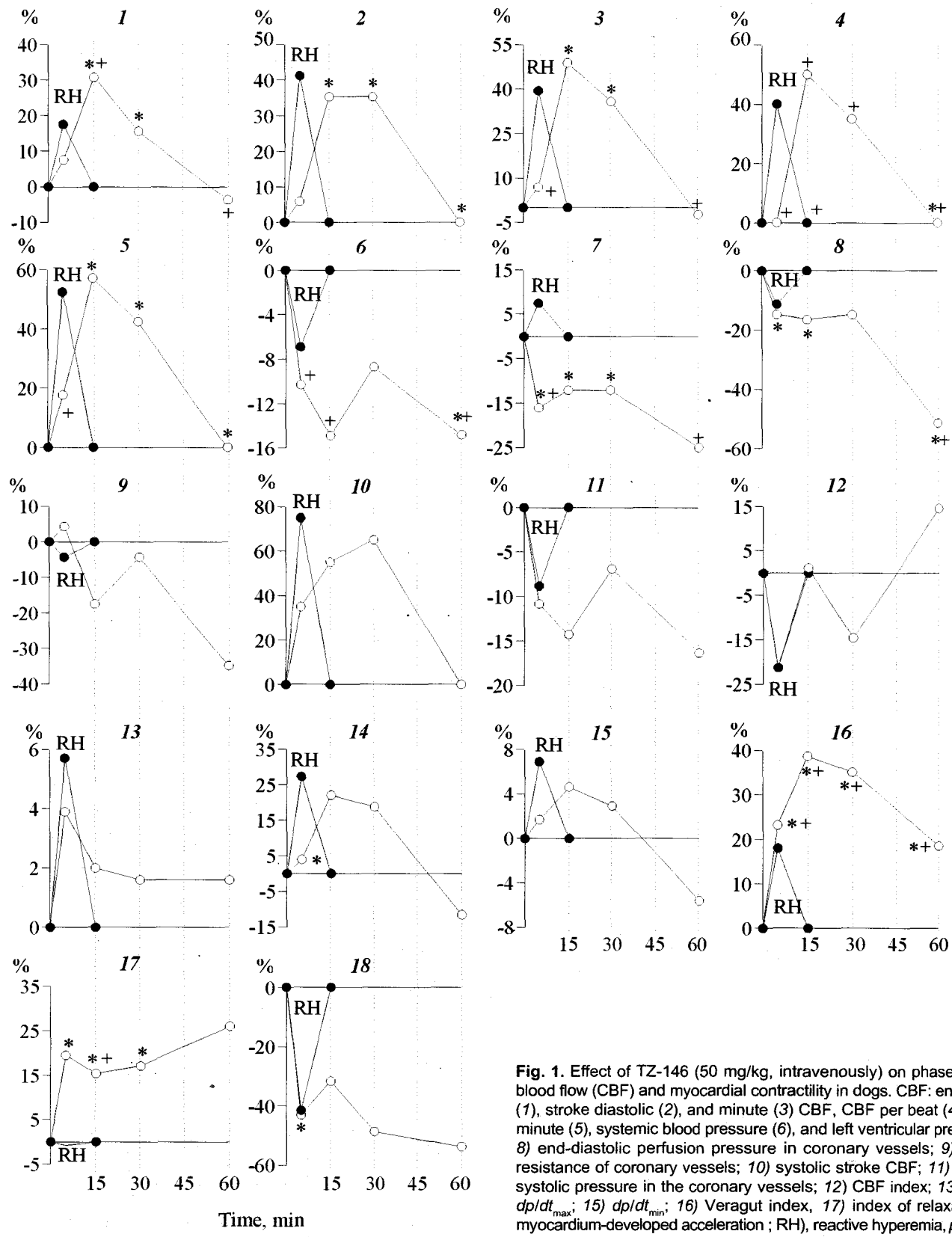

Fig. 1. Effect of TZ-146 (50 mg/kg, intravenously) on phase coronary blood flow (CBF) and myocardial contractility in dogs. CBF: end-diastolic (*1*), stroke diastolic (*2*), and minute (*3*) CBF, CBF per beat (*4*) and per minute (*5*), systemic blood pressure (*6*), and left ventricular pressure (*7*); *8)* end-diastolic perfusion pressure in coronary vessels; *9)* diastolic resistance of coronary vessels; *10)* systolic stroke CBF; *11)* perfusion systolic pressure in the coronary vessels; *12*) CBF index; *13)* HR; *14)* dp/dt_{max}; *15)* dp/dt_{min}; *16)* Veragut index, *17)* index of relaxation; *18)* myocardium-developed acceleration ; RH), reactive hyperemia, $p<0.05$: *in comparison with baseline; +in comparison with peak value during RH.

induced inhibition of adenosine production caused a more pronounced increase in VVCF than caffeine alone.

The combination of intracoronary TZ-146 and imidazole, a phosphodiesterase activator, increased VVCF to a significantly greater extent than imidazole alone.

The inhibition of prostaglandin synthase by indomethacin sharply reduced VVCF, while TZ-146 restored this index.

The experiments on cats with bilateral vagotomy revealed no significant changes in the ability of TZ-146 to increase VVCF.

Thus, experiments with pharmacological analyzers and bilateral vagotomy showed that TZ-146-induced activation of CBF is not mediated through $GABA_A$, muscarinic, and α-adrenoreceptors, and prostaglandin synthesis. It can be suggested that this effect is realized through activation of adenylate cyclase with subsequent accumulation of cAMP, or through accumulation of adenosine due to inhibition of its degradation or uptake by vascular smooth muscle cells.

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