PHYSIOLOGY

Blockade of Hyperpolarizing Currents Produces a Dose-Dependent Effect on Heart Rate

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 3, pp. 256-259, March, 2001
Original article submitted November 27, 2000

Intravenous injection of ZD 7288, a new specific hyperpolarizing current blocker, dose-dependently reduces heart rate in adult rats. The autonomic nervous system modulates changes in heart rate caused by hyperpolarizing currents.

Key Words: *heart; H currents; nervous regulation; rats*

Hyperpolarization-activated nonselective ion channels (H channels) play an important role in pacemaker activity of some neurons and atypical cardiomyocytes [4]. Generation of spikes at certain threshold potentials triggers autooscillatory activity of cells. Hyperpolarizing currents (Ih) in H channels are produced by ion transfer (Mg^{2+}, Na^{+}, and Ca^{2+}); they are insensitive to Ba^{2+}, but can be blocked by Cs^{2+}. Ih depolarize cell membranes from -60 to -40 mV [7]. Blockade of H channels inhibits pacemaker activity of cells in the sinoatrial node due to prolongation of spontaneous diastolic depolarization.

On the basis of classical concept of heart rate (HR) regulation by sympathetic and parasympathetic mechanisms, it was hypothesized that the autonomic nervous system (ANS) modulates activity of H channels. Blockade of H channels attenuates norepinephrine- and isoproterenol-induced increase in HR, but has no effect on the increase in the strength of cardiac contractions caused by these adrenoceptor agonists [3]. The inhibitory effect of acetylcholine on Ih is related to decreased production of intracellular cAMP [6]. A new specific Ih blocker ZD 7288 was recently proposed [5]. Here we studied the effect of ZD 7288 administered intravenously in various doses on variability of heart rate in rats.

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MATERIALS AND METHODS

Experiments were performed on 20-week-old outbred albino rats (n=30) narcotized with 25% urethane (1 g/kg, intraperitoneally). ZD 7288 (4-(N-ethyl-N-phenylamine)-1,2-dimethyl-6(methylamine)-pyrimidine chloride, Tokris) in doses of 0.7, 0.07, 0.021, and 0.007 mg/kg was injected into the right femoral vein. Vagus nerves were stimulated using an ESL-2 device (5 V, 10-12 msec, 0.2-0.4 msec delay, and 0.7-10 Hz frequency). Parameters of stimulation were selected individually and remained unchanged during the experiment. The right vagus was stimulated before and after blocker administration at 30-min intervals and then intersected. The left vagus was cut 30 min after the right one.

ECG was continuously recorded and analyzed on a computer. We recorded 21 parameters of variational pulsogram and ECG reflecting changes in cardiac activity. Visual monitoring was performed using an S1-83 oscillograph.

The results were analyzed by Student's t and Wilcoxon tests.

RESULTS

Intravenous injection of 0.7 mg/kg ZD 7288 significantly decreased HR (p<0.001). One minute after ZD 7288 administration, the mean R-R interval (X_M) in-

0007-4888/03/1313-0213$25.00

creased from 191.00±5.03 to 325.0±18.9 msec and then progressively increased (Fig. 1); 5 min after ZD 7288 administration HR decreased by 285%, but slightly increased 15 min after injection of this blocker.

ZD 7288 in a dose of 0.7 mg/kg affected parameters of variational pulsogram reflecting changes in autonomic homeostasis. Their dynamics indicated that Ih blockade stimulated parasympathetic mechanisms regulating cardiac activity, but suppressed the effects of sympathetic factors. Variational range (ΔX), the parameter of variational pulsogram reflecting activity of parasympathetic regulatory mechanisms, peaked 5 min after ZD 7288 administration (increased 69-fold) and then decreased. Mode amplitude (A_{MO}) reflecting activity of sympathetic regulatory mechanisms sharply decreased 1 min after ZD 7288 administration and then remained unchanged.

Decreasing the dose of ZD 7288 to 0.07 mg/kg attenuated bradycardia: the decrease in HR was most pronounced 15 min after injection of ZD 7288. X_M increased by 45% (from 192.0±9.6 to 282.0±24.4 msec, $p<0.01$). ΔX attained maximum 15 min after ZD 7288 administration. A_{MO} decreased more slowly and attained minimum only by the end of observations; over the first 15 min this parameter decreased from 39.7±4.7 to 18.3±4.8%. This gradual decrease in the autonomic equilibrium index attested to predominance of the parasympathetic regulatory mechanisms.

Fifteen minutes after administration of ZD 7288 in doses of 0.021 and 0.007 mg/kg X_M increased by 13 and 19%, respectively. Other parameters of variational pulsogram underwent similar changes. ZD 7288 in a dose of 0.021 mg/kg slightly increased ΔX (from 6.2±1.4 to 9.4±3.8 msec). In a dose of 0.007 mg/kg, ZD 7288 increased ΔX from 11.6±3.6 to 16.0±5.2 msec. The dynamics of changes in the autonomic equilibrium index suggested a shift in autonomic homeostasis towards parasympathetic influences. This assumption was confirmed by gradual decrease in the index of strain and autonomic HR parameter.

Electrical stimulation of the right vagus decreased HR in intact 20-week-old rats by 98%. Preliminary intravenous injection of ZD 7288 a produced dose-dependent effects on the degree of HR deceleration during stimulation of the right vagus nerve (Fig. 2). These results indicate that preinjection of ZD 7288 caused opposite and dose-dependent effects on the decrease in HR caused by vagal stimulation. ZD 7288 in a dose of 0.07 mg/kg potentiated, but in low doses attenuated the effect of vagal stimulation. Parameters of variational pulsogram reflecting activity of sympathetic (A_{MO}) and parasympathetic (ΔX) regulatory mechanisms underwent less pronounced changes. The dynamics of changes in the autonomic equilibrium index suggested that activation of parasympathetic mechanisms caused by vagal stimulation was less pronounced after administration of ZD 7288, especially, in low doses.

Intersection of the right vagus after administration of 0.7 mg/kg ZD 7288 led to a gradual decrease in X_M, which was 82% of the initial value by the 30th minute of observations. Intersection of the left vagus in rats with left-sided vagotomy decreased X_M by 5%; by the 30th minute, HR increased by 9%.

Similar dynamics was observed after pharmacological blockade of sympathetic regulation. In these animals HR does not return to normal after short-term postvagotomic tachycardia [2]. It should be emphasized that the dynamics of changes in parameters of variational pulsogram after vagotomy and administration of this blocker in maximum dose attested to activation of parasympathetic regulatory mechanisms.

Intersection of the right vagus after administration of 0.07 mg/kg ZD 7288 did not change X_M, but the autonomic equilibrium index progressively increased to 62%, which indicated activation of sympathetic mechanisms in ANS.

Bilateral vagotomy did not change HR in adult rats injected with 0.021 mg/kg ZD 7288.

Intersection of the right vagus in rats injected with 0.007 mg/kg ZD 7288 slightly increased HR; by the 30th minute X_M decreased by 9% compared to the initial value. HR increased by 5% 1 min after intersection of the left vagus in animals with right-sided vagotomy; X_M remained unchanged over 30 min of observations. The dynamics of changes in parameters of variational pulsogram indicated predominance of sympathetic regulatory mechanisms.

Thus, intravenous injection of new specific Ih blocker ZD 7288 produces dose-dependent effects on

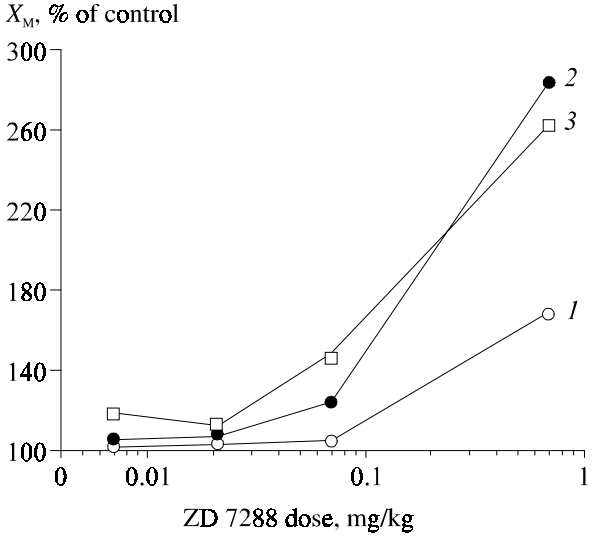

Fig. 1. Mean R-R interval (X_M) 1 (*1*), 5 (*2*), and 15 min (*3*) after administration of ZD 7288 in various doses.

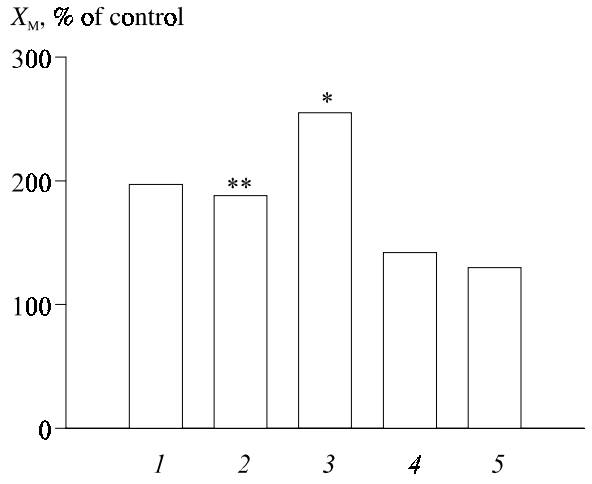

Fig. 2. Dynamics of changes in mean R-R interval (X_M) during electrical stimulation of the right vagus in intact rats (*1*) and after intraperitoneal administration of ZD 7288 in doses of 0.7 (*2*), 0.07 (*3*), 0.021 (*4*), and 0.007 mg/kg (*5*). *p<0.001 and **p<0.01 compared to the initial value.

adult rats. This preparation in the maximum dose suppresses the autonomic regulation of cardiac activity. Bradycardia induced by vagal stimulation was most pronounced after administration of 0.07 mg/kg ZD 7288. Our previous experiments on rats showed that the decrease in HR caused by stimulation of the vagus nerves is completely prevented by blockade of muscarinic cholinoceptors [1]. These data suggest that parasympathetic mechanisms regulating cardiac activity modulate functions of H channels in the heart. This assumption is confirmed by opposite and dose-dependent effects of Ih blockade on the vagal stimulation-induced decrease in HR. Experiments with successive vagotomies against the background of ZD 7288 administration suggest that the sympathetic mechanism is involved in the regulation of H channel activity. Ih blockade suppresses cardiac activity. The interaction of H channels and ANS requires further detailed investigations.

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