A New Insight into Mechanisms of Age-Related Changes in Heart Rate

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Changes in cardiac rhythm induced by blockade of hyperpolarization currents with ZD 7288 depend on animal's age. The increase in cardiointerval duration is related to prolongation of *T—P* segment on ECG. It is hypothesized that the age-related changes in activity of hyperpolarization channels are determined by a modulating effect of the autonomic nervous system.

Key Words: *ontogeny; heart; hyperpolarization currents; rat*

The mechanisms of age-related bradycardia remain unknown. In light of classical notions on the antagonism between subdivisions of the autonomic nervous system (ANS), the age-related deceleration of the heart rate was explained by activation of parasympathetic and deactivation of sympathetic regulatory influences [1]. Some authorities consider the age-related bradycardia as a result of delayed activation of sympathetic regulation of the heart [9], which corroborates the hypothesis on accentuated antagonism between sympathetic-parasympathetic influences [8]. Bradycardia in rats can be induced either by activation of the parasympathetic system [2] or by inhibition of sympathetic influences via β-adrenoceptor blockade [4]. We previously demonstrated the absence of pronounced tachycardia in vagotomized rats of various age, which suggest the absence of tonic inhibitory activity in vagal parasympathetic fibers [3].

Recent studies demonstrated an important role of hyperpolarizing currents (Ih) in atypical and typical cardiomyocytes in the regulation of cardiac function [7]. Nonspecific Ih can depolarize membrane of sinoatrial node cardiomyocytes from -60 to -40 mV [10]. It was assumed that changes in activity of hyperpolarization (H) channels are responsible for age-related alteration of heart chronotropism [5]. However, H-channels can also be modulated by the sympathetic and parasympathetic systems [6].

Our aim was to study the effect of Ih block on cardiac function in rats at various stages of postnatal ontogeny.

MATERIALS AND METHODS

Experiments were carried out on 70 outbred albino rats aged 1, 3, and 20 weeks. The animals were intraperitoneally narcotized with 25% urethane (1000 mg/kg). Ih were blocked with a novel H-channel inhibitor ZD 7288 (4-N-ethyl-N-phenylamine)-1,2-dimethyl-6(methylamine)-pyrimidine chloride (Tocris). The drug was injected into the right femoral vein in doses of 0.7, 0.07, 0.021, and 0.007 mg/kg. The vagus nerves were stimulated with an ESL-2 electrical generator. Stimulation parameters were: pulse amplitude 5 V, duration 10-12 msec, delay 0.2-0.4 msec, and repetition rate 0.7-10 Hz. These parameters were chosen individually for each rat and remained constant throughout the experiment. Stimulation of the right vagus nerve was performed before and after injection of ZD 7288. The interval between stimulation sessions was 30 min.

The ECG was recorded and processed on-line. We used original software allowing to calculate 21 parameters of variational pulsogram and ECG. The following parameters of the heart rate variability reflecting autonomic homeostasis were analyzed: the duration of

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P and *T* waves and the length *P*—*Q*, *Q*—*T*, and *T*—*P* intervals. ECG was visually controlled with an S1-83 oscillograph.

The data were analyzed statistically using Student's *t* and Wilcoxon tests.

RESULTS

In all age groups, intravenous injection of H-channel blocker ZD 7288 modified the cardiac rhythm in a dose-dependent manner. In 7-day-old rats (newborn pups), the blocker produced the most pronounced increase in the mean cardiointerval duration (X_M). In doses of 0.007, 0.021, 0.07, and 0.7 mg/kg ZD 7288 increased *R*-*R* interval by 20, 56, 117, and 248%, correspondingly (Table 1), while injection of the same doses to 20-week-old rats increased X_M by 19, 13, 48, and 164% (Table 3). In 3-week-old rats this effect was less pronounced: X_M was increased by only 18, 26, 30, and 37% (Table 2). We previously showed that in 3-4-week-old rats the heart rate decreases with age.

After injection of ZD 7288, the development of bradycardia depended on the dose. In mature rats, bradycardia induced by ZD 7288 (0.7 mg/kg) peaked on minute 5 postinjection. When the dose was decreased 10-fold, X_M increased during 15 min. After decreasing the dose to 0.007 mg/kg the peak of bradycardia was attained on minute 30 postinjection.

In newborn pups, bradycardia induced by ZD 7288 in all specified peaked on minute 30 postinjection and was accompanied by pronounced arrhythmia confirmed by changes in variational range ΔX (Table 1). It can be hypothesized that mature rats, in contrast to newborns, possess mechanisms inducing a compensatory increase of the heart rate during pronounced bradycardia.

The shifts in X_M were accompanied by changes in variational pulsogram, which reflects the state of au-

TABLE 1. Effect of ZD 7288 in Various Doses on Variational Pulsogram, Mean Cardiointerval, and ECG Segments in 7-Day-Old Rats (*M*±*m*, *n*=5-6)

Index	Dose of ZD 7288, mg/kg			
	0.007	0.021	0.07	0.7
ARI, arb. units	938.0±51.4 / 940±43	945.0±42.5 / 497.0±35.5***	1140±24 / 178.0±7.7*	1501±59 / 1.26±0.20*
δ	1.13±0.50 / 1.37±0.20	0.75±0.20 / 2.10±0.67***	0.88±0.20 / 69.9±6.6**	1.06±0.20 / 562±39*
ΔX, msec	6.0±2.1 / 4.3±1.3	4.50±0.65 / 9.00±3.19	3.60±0.68 / 202±18*	4.3±1.1 / 1844±191*
ABI, arb. units	11,900±710 / 12,200±588	13,300.0±332.3 / 5080.0±183.1***	16,000.0±304.3 / 1480.0±760.3**	19,700.0±896.9 / 3.57±0.40*
MA, %	41.3±5.4 / 39.3±4.6	54.5±7.2 / 30.0±5.1	49.6±5.6 / 15.6±4.1***	46.00±7.06 / 6.70±1.23**
SI, arb. units	22,200±2500 / 20,300±1900	26,700±8670 / 8300±336***	28,800±6382 / 1890±107***	41,600±1980 / 3.83±0.79*
R—*R*, msec	293.00±19.17 / 349.00±29.69**	263.00±18.47 / 408.00±61.46***	291.00±17.46 / 525.0±44.7*	258.00±12.47 / 833.00±90.21*
P—*Q*, msec	90.00±9.82 / 100.00±12.02***	80.00±2.43 / 92.00±16.23***	89.00±7.81 / 127.00±17.66***	83.00±4.87 / 116.00±12.67**
T, msec	114.0±12.2 / 114.00±10.11	109.00±11.56 / 146.00±39.47***	94.00±5.07 / 123.00±9.46**	68.00±19.69 / 103.00±30.34***
T—*P*, msec	179.00±10.11 / 223.00±16.01**	162.0±15.9 / 284.0±51.8**	177.00±9.76 / 369.00±37.62*	156±7 / 694.00±83.47*

Note. Here and in Tables 2 and 3: initial and postinjection values are placed in nominator and denominator, respectively. MA: mode amplitude; ARI: autonomic rhythm index; SI: strain index . *p<0.001, **p<0.01, and ***p<0.05 compared to initial values.

TABLE 2. Effect of ZD 7288 in Various Doses on Variational Pulsogram, Mean Cardiointerval, and ECG Segments in 3-Week-Old Rats (*M*±*m*, *n*=6)

Index	Dose of ZD 7288, mg/kg			
	0.007	0.021	0.07	0.7
ARI, arb. units	2292±348 / 1846±419	2988±419 / 1289±397	4063±107 / 962±29**	1133±219 / 722.0±14.7
δ	0.57±0.20 / 0.97±0.40	0.50±0.03 / 2.20±0.06	0.67±0.20 / 2.40±0.06	1.13±0.20 / 1.8±0.4
ΔX, msec	3.00±0.52 / 4.83±0.20	2.25±0.20 / 7.50±2.21***	2.16±0.40 / 9.0±2.5***	4.30±0.67 / 7.00±0.21
ABI, arb. units	21,300±5362 / 18,200±5044	25,000±4847 / 6540±334***	33,400±8889 / 5830±233***	11,067±414 / 5859±129
MA, %	51.0±6.9 / 48.6±7.1	54.00±7.41 / 30.00±7.18	53.3±3.5 / 26.7±4.8***	36.00±5.51 / 29.00±3.37
SI, arb. units	63,600±16,331 / 50,900±14,824	88,800±20,532 / 21,200±1221***	114,000±34,163 / 15,900±661**	22,800±772 / 11,400±2766
R—R, msec	167.00±9.83 / 192.00±15.57***	145.00±6.73 / 177.00±14.99**	155.00±7.39 / 193.00±7.89**	244.00±15.36 / 288.00±20.83***
P—Q, msec	58.00±3.11 / 66.00±5.67	50.00±1.44 / 51.00±2.06***	53.00±1.87 / 53.00±2.71	67.00±3.99 / 73.00±3.26***
T, msec	73.00±5.11 / 83.0±4.3***	63.00±2.25 / 74.00±3.67	69.00±3.51 / 85.00±3.09**	65.00±4.16 / 76.00±5.25***
T—P, msec	96±6 / 111.00±8.92***	80.00±2.39 / 102.00±6.75	90.00±4.08 / 126.0±7.1**	142±10 / 175.00±15.76***

tonomic homeostasis. When the maximum dose of the blocker was injected to mature rats, a pronounced decrease in autonomic balance index (ABI) reflecting activation of parasympathetic control of cardiac function was observed only on minute 1 postinjection. Starting from minute 3 postinjection, ABI increased indicating activation of the sympathetic system. At the same time, after injection of ZD 7288 in the lowest dose ABI decreased for 15 min (Table 3).

Another reaction was observed in 7-day-old rats. After injection of ZD 7288 in a dose of 0.07 mg/kg ABI considerably decreased and remained at a low level throughout the entire observation period (Table 1). A paradoxical increase of ABI from 2.25±0.4 to 14.6±5.8 arb. units was observed during vagal stimulation after injection of the maximum dose of the blocker. When vagal stimulation was performed before injection of ZD 7288, ABI decreased from 9180±2513 to 37.5±2.9 arb. units. Decreasing the dose to 0.07 mg/kg gradually decreased ABI in 7-day-old pups during the entire observation period, while stimulation of the right vagus nerve further decreased this index from 8.97±3.61 to 3.12±0.78 arb. units.

The comparative analysis of changes in *R—R* interval and individual waves and segments on ECG showed that ZD 7288-induced X_M increase was accompanied by an insignificant prolongation of *P—Q* interval and *T* wave, and pronounced lengthening of *T—P* interval (Tables 1-3). Comparison of *T—P* intervals and X_M indicates that H-channels are involved in the development of spontaneous diastolic depolarization of cells in the cardiac conduction system.

Preinjection of ZD 7288 produced a dose-dependent effect on X_M increment under condition of electrical stimulation of the right vagus nerve. Injection of the blocker in the lowest dose moderated the effect of vagal stimulation in all age groups (Fig.1, *a-c*).

Injection of the blocker in a dose of 0.07 mg/kg potentiated bradycardia induced by vagal stimulation in newborn and mature rats, although did not affect the increment of X_M in 3-week-old pups. However, the maximum dose of the blocker (0.7 mg/kg) affected X_M

TABLE 3. Effect of ZD 7288 in Various Doses on Variational Pulsogram, Mean Cardiointerval, and ECG Segments in 20-Week-Old Rats (*M*±*m*, *n*=5-6)

Index	Dose of ZD 7288, mg/kg			
	0.007	0.021	0.07	0.7
ARI, arb. units	836±35 558±23	1244±454 515.0±14.3	1418.0±40.1 263.0±10.5**	1029±140 55.0±2.8*
δ	2.7±1.5 9.7±2.4***	1.58±0.40 3.3±0.9	1.3±0.2 9.9±1.5**	1.5±0.2 182.0±1.1*
ΔX, msec	11.6±3.6 17.6±6.3	6.2±1.4 12.6±3.3	4.8±0.9 53.8±3.4**	5.3±0.9 87.3±5.8**
ABI, arb. units	4965.0±279.6 2560.0±122.5	10 248.0±578.3 3339.0±142.1	10 500±270 1630.0±87.4**	6590±104 224±11**
MA, %	24.8±6.2 18.4±3.2	35.6±8.3 25.6±5.7	39.7±4.7 18.3±4.9	33.3±1.3 8.6±2.4
SI, arb. units	14 728±877 6578±331	29 181±385 8140±767***	29 800±930 3660±218**	17 200±245 295.0±14.7*
R—*R*, msec	199.00±16.22 235.00±21.68***	190.00±7.56 218.00±9.47**	192.00±3.65 282.0±24.4**	191.00±5.05 544±143**
P—*Q*, msec	63.00±3.46 72.00±3.73***	66.00±2.06 70.00±3.33***	56.00±4.13 67.00±4.97***	53.00±5.55 61.00±18.55***
T, msec	82.00±4.38 87.00±4.12	84.00±2.95 93.00±4.93***	49.00±3.03 76.00±7.37**	40.00±6.44 53.00±31.23***
T—*P*, msec	120.00±12.77 144.0±16.1***	110.00±5.57 132.00±6.81**	131±23 181.0±18.9***	108.00±5.84 440.00±167.39**

dynamics in newborn rats during vagal stimulation: in the control it increased X_M from 246.0±7.8 to 497.0±8.8 msec, while after injection of the blocker X_M decreased from 895±113 to 685±117 msec. This dose potentiated the stimulation-induced increment of X_M in 3-week-old pups, but slightly decreased it in mature rats (Fig. 1, *b*, *c*). It should be noted that against the background of pronounced bradycardia after injection of the maximum dose of ZD 7288 the increase in the heart rate in response to vagal stimulation in newborn pups can be due to heart pacing with this stimulator.

The observed changes in heart rate after injection of ZD 7288 in various during different periods of postnatal ontogeny reveal pronounced age-related peculiarities in the cardiac response to Ih blockade. The most pronounced changes were observed in newborn animals, which suggest that H-channels are most active during this period. The dynamics of variational pulosgram parameters and X_M attests to a possibility of H-channel modulation by the autonomic nervous system. This is also confirmed by the dynamics of cardiac rhythm variability indices during vagal stimulation performed against the background Ih blockade, as well as peculiarities of cardiac response to H-channel blockade in 3-week-old animals (period of the development of sympathetic innervation). The changes in the heart rate caused by vagal stimulation after Ih blockade also attest to the existence of various mechanisms reducing the heart rate.

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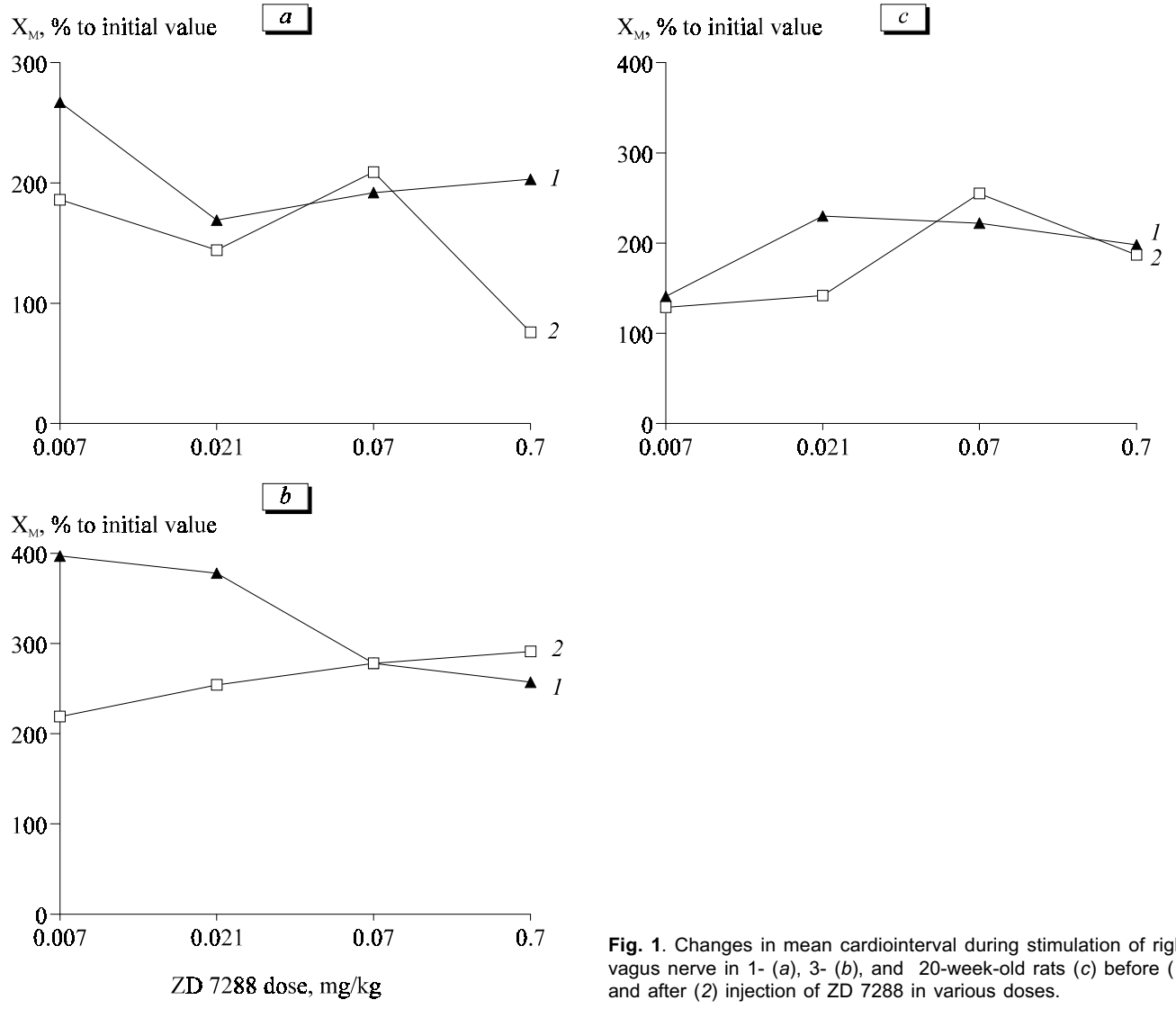

Fig. 1. Changes in mean cardiointerval during stimulation of right vagus nerve in 1- (*a*), 3- (*b*), and 20-week-old rats (*c*) before (*1*) and after (*2*) injection of ZD 7288 in various doses.

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