Peculiarities of Heart Rhythm Variability in Narcotized and Immobilized Wakeful Rats during Blockade of Calcium Channels

M. V. Grigor'eva, M. M. Fateev, and F. A. Kouzmin

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In narcotized rats, verapamil and cinnarizine modified some heart rate variability (HRV) indices and heart rate (HR) indicating up-regulation of parasympathetic tone in contrast to nifedipine that elevated activity of sympathetic subdivision of ANS producing no influence on HR. In wakeful stressed rats, the time-domain and geometric analysis established that verapamil decreased HR and up-regulated sympathetic tone; nifedipine elevated sympathetic tone and produced no effect on HR, while cinnarizine enhanced parasympathetic tone without any effect on HR. Spectrum analysis of HRV revealed probable activation of some other neurohumoral mechanisms by the employed calcium blockers.

Key Words: *heart rate variability; autonomic nervous system; calcium channel blockers; short-term immobilization stress*

Similar to β-adrenoceptor blockers and the inhibitors of angiotensin-converting enzyme, the calcium channel blockers are widely used to treat hypertension and ischemic heart disease (IHD) [3,7,8,10]. Calcium antagonists counteract the vasoconstrictor agents at the smooth muscle vasculature by attenuating the potential-dependent calcium entry and potentiating the vasodilator effects of NO [9]. By chemical nature, the blockers of calcium channels are subdivided into *1*) phenylalkylamines (verapamil, cilnidipine), *2*) benzodiazepines (diltiazem), *3*) dihydropyridines (nifedipine, amlodipine) and *4*) diphenylpiperazines (cinnarizine, flunarizine) [6]. The variety of calcium blockers are matched with a large family of potential-operated calcium channels termed as L, T, N, P/Q, and R, where the most salient members are L-type calcium channels [6,7]. L-type calcium channels are located in the transverse tubules (T-system) of skeletal, smooth, and cardiac musculature where they are involved in the electromechanical coupling between excitation and contraction. Activation of these channels strengthens myocardial contractility and induces vasoconstriction. T-type calcium channels are found in vascular smooth muscles and in the sinoatrial node; in the latter, they modulate cardiac automaticity, while the block of these channels induces bradycardia without concomitant changes in myocardial contractility. T-type channels emerge in the cardiomyocytes in hypertrophic myocardium. N-type calcium channels found in the neurons of central and peripheral nervous system play an important role in the control of membrane excitability; the P/Q-type channels are characteristic of Purkinje fibers in the heart conduction system [7,10]. To block the slow calcium channels, the cardiologists widely use the derivatives of phenylalkylamines, benzodiazepines, dihydropyridines, and the diphenylpiperazines referred to as the selective blockers of L-type channels [3,6,8]. The phenylalkylamines with their typical representative verapamil mostly affect the heart conduction system exerting some effect on vasculature; the dihydropyridines predominantly demonstrate the

Department of Normal Physiology, Yaroslavl' State Medical Academy, Russia. ***Address for correspondence:*** marigrig77@mail.ru. M. V. Grigor'eva

vasotropic activity with their basic drug nifedipine, while the diphenylpiperazines (exemplified by cinnarizine) selectively tune the arterial network in the brain [6].

This study is designed to examine the heart rate variability (HRV) in narcotized rats or in wakeful immobilized (stressed) rats under the effect of verapamil, nifedipine, and cinnarizine taken as the typical members of the above families of cardiotropic preparations differing in their structure and the mode of action.

MATERIALS AND METHODS

Two series of experiments have been carried out on male random-bred mature rats weighing 180-210 g.

In series I, three groups of rats (each comprising 15 animals) were narcotized with intraperitoneal Nembutal (40 mg/kg) and immobilized in a supine posture. In this series, the rats were subdivided into 3 groups and administered intragastrically via a tube with 1 ml solution of verapamil (1.7 mg/kg, group 1), nifedipine (3.0 mg/kg, group 2), or cinnarizine (0.8 mg/kg, group 3). The doses of the drugs were selected with due account for the recalculation coefficient 5.9 [2]. Immediately before administration and 1 h after it, ECG was recorded in rats, which were still narcotized and immobilized.

Series II experiments were performed on wakeful rats subdivided into 4 groups. Group 1 rats (control, n=30) the animals were administered intragastrically with 1 ml physiological saline. Three groups of experimental rats were given verapamil (group 2, n=15), nifedipine (group 3, n=15), and cinnarizine (group 4, n=15) at the same volume and doses as in series II. In 1 h after administration, the rats were restrained in a supine posture to record ECG.

ECG was recorded with a 2-channel electrophysiological system, which included an L-CARD E-440 digitizer and PC Pentium II. The sampling rate was 4.0 kHz. Recording was performed in the second standard lead during 4 min with the help of an L-GRAPH software supplied with the digitizer. Inspection and the primary processing of ECG were performed with an RRMatch software, while the final calculations and plotting HRV parameters were carried out in a CR-Graph software [5].

HRV was analyzed by *1*) time-domain parameters (HR, standard deviation of all normal-to-normal *R-R* intervals SDNN, coefficient of variation CV, root mean square of successive differences of *R-R* intervals RMSSD), *2*) geometrical parameters (variational range MxDMn, mode Mo, mode amplitude AMo, strain index SI, scatterplot area EllSq, scatterplot width-to-length ratio EllAs), and *3*) spectral parameters (low frequency wave power LF, middle frequency wave power MF, high frequency wave power HF, total spectrum power TP, relative LF power RLF, relative HF power RHF, and autonomic balance index ABI=LF/HF). To calculate the geometric parameters, the histogram step was set to 2 msec. In narcotized rats, LF and HF ranges were 0.02-0.15 Hz and 0.15-2.0 Hz, respectively. In wakeful stressed rats, the respective parameters were 0.02-0.75 Hz and 0.75-3.00 Hz [5].

The data were analyzed statistically using Statistica 6.0 software and presented as M±SEM.

Significance of differences was assessed by Student's t test at $p<0.05$.

RESULTS

Of the three studied calcium blockers, verapamil and nifedipine most strongly affected heart rhythm in narcotized rats (Table 1). Verapamil decreased HR by 15.7% ($p<0.001$) in comparison with initial level. The following HRV parameters increased significantly: RMSSD (by 36.6%), Mo (by 20.6%), EllAs (by 35.3), and RHF (by 60.4%), while RLF and ABI significantly decreased by 42.0 and 64.8%, respectively.

Nifedipine significantly decreased SDNN, CV, and RLF by 31.2, 28.0, and 46.4% respectively (relatively to the initial values) and increased AMo, SI, EllAs, and RHF by 27.5, 52.3, 38.8, 64.5%, respectively (Table 1).

In comparison with verapamil and nifedipine, cinnarizine produced a weaker effect on HR in narcotized rats. Only three HRV parameters changed significantly: MxDMn and ELLSq increased by 1.5 and 2.4 times, respectively, while SI decreased by 2.1 times ($p<0.05$).

Intercomparison of HRV parameters showed that HR values in the rats administered with nifedipine and cinnarizine were greater than HR in verapamil-treated rats by 20.2 and 16.1% respectively; in contrast, Mo values were smaller by 18.8 and 20.9%, respectively ($p<0.01$). In nifedipine-treated narcotized rats, SDNN and RMSSD were significantly smaller than in verapamil-treated animals by 37.3 and 32.2%, respectively ($p<0.05$), while AMo and SI were greater by 34.4% ($p<0.05$) and 98.6% ($p<0.01$), respectively (Table 1). In comparison to verapamil, cinnarizine exerted more stronger effect on such HRV parameters as SDNN, MxDMn, EllSq, LF, and TP, which increased by 1.8, 1.9, 4.1, 4.7, and 2.9 times, respectively ($p<0.05$); it was also true for AMo and SI, which decreased by 1.7 and 3.2 times, respectively ($p<0.05$).

In wakeful rats subjected to the short-term immobilization stress, verapamil exerted the strongest effect on HR, while the effects of cinnarizine and especially nifedipine were not pronounced (Table 2). In contrast

TABLE 1. Effect of Calcium Blockers on HRV Indices in Narcotized Rats (*M*±SEM)

Index	Initial state	Verapamil	Nifedipine	Cinnarizine
HR, min^{-1}	434.0±9.7	366.0±17.8***	440.0±9.4^{++}	425±10^{++}
SDNN, msec	1.860±0.143	2.040±0.304	1.280±0.135*$^{+}$	2.340±0.235^{o}
CV, %	1.320±0.098	1.190±0.141	0.950±0.104*	1.620±0.197
RMSSD, msec	1.340±0.087	1.830±0.185*	1.240±0.080^{+}	1.380±0.154
MxDMn, msec	11.50±0.94	12.70±1.66	9.00±1.27	16.80±1.33*^{o}
Mo, msec	140.00±3.32	168.8±8.1***	137.00±2.89^{++}	133.6±3.15^{++}
AMo, %	42.90±2.79	40.70±3.04	54.70±4.21*$^{+}$	31.4±2.7^{oo}
ABI	16,849±2170	12,918±2212	25,654±3679*$^{++}$	8111±1526*^{oo}
EllSq, $msec^2$	68.70±8.85	100.40±25.72	41.1±7.2	167.2±30.5*^{oo}
EllAs, %	43.00±3.71	58.20±6.94*	59.70±4.93*	36.90±7.59
LF, $msec^2$	0.220±0.052	0.120±0.045	0.070±0.047	0.330±0.123^{o}
HF, $msec^2$	0.230±0.038	0.290±0.078	0.180±0.042	0.400±0.098
TP, $msec^2$	0.450±0.076	0.410±0.119	0.250±0.067	0.730±0.171^{oo}
RLF, %	43.30±4.37	25.10±3.83*	23.20±6.54*	41.20±7.74
RHF, %	46.70±4.37	74.90±3.83*	76.80±6.54*	58.80±7.74
LF/HF, arb. units	1.080±0.204	0.380±0.080*	0.440±0.194	0.950±0.199

Note. ***$p<0.001$, *$p<0.05$ in comparison with the initial state; $^{++}p<0.01$, $^{+}p<0.05$ in comparison with verapamil; $^{oo}p<0.01$, $^{o}p<0.05$ in comparison with nifedipine.

to cinnarizine and nifedipine inducing tachycardia, verapamil reduced HR by 6.5% ($p<0.001$). This calcium blocker significantly decreased SDNN (1.6), CV (1.7), RMSSD (1.2), MxDMn (1.5), EllSq (2.3), LF (6.2), TP (3.6), RLF (1.4), and ABI (2.3), where the parentheses show the corresponding reduction factors. In addition, verapamil increased Mo, AMo, SI, and RHF by 1.1, 1.3, 1.5, and 1.4 times, respectively.

Nifedipine significantly decreased the following indices (reduction factors are given in parentheses): SDNN (1.6), CV (1.6), MxDMn (1.5), EllSq (2.3), LF (9.7), TP (4.3), RLF (1.7), and ABI (3.5). At the same time, it increased AMo, SI, and RHF by 1.3, 1.8. and 1.6 times, respectively. Similar to nifedipine, cinnarizine produced little affect on HR, but modified HRV parameters – although in the opposite direction. It significantly increased SDNN, CV, MxDMn, EllSq, LF, TP, RLF, and ABI by 1.5, 1.5, 1.5, 1.7, 2.2, 1.9, 1.2, and 1.7 times, respectively, and decreased Mo, AMo, SI, EllAs, and RHF by 1.04, 1.3, 1.9, 1.3, and 1.5, respectively (Table 2).

Comparison of the effect of verapamil and nifedipine revealed that the only significant difference between them was the effect on HR (in verapamil-treated rats, it was smaller than in nifedipine-treated ones by 8.0%). Both blockers similarly modified HRV parameters without any significant difference in paired comparison. However, when compared with cinnarizine, both nifedipine and verapamil demonstrated significantly different effects on some HRV parameters (Table 2). Cinnarizine resulted in greater values of SDNN, CV, RMSSD (only in comparison with verapamil), MxDMn, EllSq, LF, HF, TP, RLF, and ABI (compared to both other blockers) by 2.3, 2.5, 1.4, 2.2, 4.0, 16.3, 2.4, 7.4, 1.9, and 4.8 times, respectively. In contrast, it yielded smaller values of Mo (only in comparison with verapamil), AMo, SI, EllAs, and RHF (compared to both other blockers) by 1.1, 1.7, 3.1, 1.6, and 2.3, respectively.

Thus, the time-domain and geometric analyses showed that in narcotized rats, verapamil decreased HR and somewhat increased TP indicating elevation of the tone in parasympathetic branch of ANS, which agrees with the data obtained on humans [11]. In wakeful stressed rats, in addition to decreasing HR, it also decreased TP, elevated AMo and SI indicating enhancement in the tone of sympathetic branch of ANS.

In contrast to verapamil, nifedipine produced virtually no effect on HR in both series of experiments corroborating the published data, but changes in HRV indices attested to enhancement of the sympathetic tone that was especially pronounced in stressed rats [12].

Similarly to nifedipine, cinnarizine did not change HR, although it elevated TP and decreased SI (espe-

TABLE 2. Effect of Calcium Blockers on HRV Indices in Wakeful Immobilized Rats (*M*±SEM)

Index	Control	Verapamil	Nifedipine	Cinnarizine
HR, min^{-1}	480.0±3.9	449.0±7.7***	485.0±12.5^{+}	495.0±5.5^{+++}
SDNN, msec	2.260±0.196	1.440±0.114*	1.390±0.099*	3.310±0.417*$^{+++ooo}$
CV, %	1.800±0.148	1.070±0.089*	1.120±0.077*	2.720±0.339**$^{+++ooo}$
RMSSD, msec	1.840±0.119	1.520±0.188**	1.590±0.232	2.110±0.233^{+++}
MxDMn, msec	14.8±0.98	10.0±0.76*	9.70±0.71**	21.90±2.29**$^{+++ooo}$
Mo, msec	125.20±1.04	134.50±2.29***	125.20±3.33	120.50±1.47*$^{+++}$
AMo, %	39.50±1.99	49.60±3.22*	52.2±2.9**	29.80±3.73*$^{+++ooo}$
ABI	13 484±1360	20 741±3025*	23 771±2616***	7254±1483*$^{+++ooo}$
EllSq, $msec^2$	117.50±17.06	50.20±6.74*	51.50±8.96*	204.50±44.39*$^{++oo}$
EllAs, %	52.10±3.21	60.00±5.76	64.20±5.79	39.30±4.77*$^{+oo}$
LF, $msec^2$	0.870±0.181	0.140±0.028*	0.090±0.012*	1.870±0.521*$^{++oo}$
HF, $msec^2$	0.380±0.055	0.210±0.044	0.200±0.042	0.490±0.109^{+o}
TP, $msec^2$	1.250±0.222	0.35±0.06*	0.290±0.048*	2.360±0.604*$^{++oo}$
RLF, %	59.40±2.71	42.00±5.48**	35.20±3.88***	73.60±3.17**$^{+++ooo}$
RHF, %	40.60±2.71	58.00±5.48**	64.80±3.88***	26.40±3.17**$^{+++ooo}$
LF/HF, arb. units	2.150±0.341	0.92±0.21*	0.620±0.116*	3.680±0.644*$^{+++ooo}$

Note. ***p<0.001, **p<0.01, *p<0.05 in comparison with the control; ^{+++}p<0.001, ^{++}p<0.01, ^{+}p<0.05 in comparison with verapamil; ^{ooo}p<0.001, ^{oo}p<0.01, ^{o}p<0.05 in comparison with nifedipine.

cially in the stressed rats) indicating enhancement of parasympathetic tone.

The data of spectrum analysis were somewhat at odds with those of time-domain and geometric approaches. While in narcotized rats the spectral data on verapamil also indicated enhancement of parasympathetic tone, nifedipine and cinnarizine produced practically no effect on spectral parameters. In stressed rats, verapamil significantly decreased LF and hence, TP and ABI indicating enhancement of parasympathetic tone. In contrast, cinnarizine significantly increased LF, TP, and ABI indicating activation of sympathetic system during stress. Probably, the above discrepancy in the results of various analytical methods suggests that LF index reflects not only the level of sympathetic activity, but also some other neurohumoral mechanisms, which agrees with our previous data on the effect of β-adrenoceptor blockers on HRV parameters [4,5]. The opposite effect of cinnarizine (in comparison with verapamil and nifedipine) on LF is probably related to its ability to improve cerebral circulation.

Therefore, the study ranged the examined blockers by their effects on the heart rate in narcotized and wakeful stressed rats as follows: cinnarizine, verapamil, and nifedipine.

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