Effect of Class III Antiarrhythmic Preparation Nibentan on Extrasystolic and Post-Extrasystolic Contraction of Rat Papillary Muscle

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The effect of nibentan (2.5 and 25 μM) on extrasystolic and post-extrasystolic contraction of isolated and perfused papillary muscle was studied. The muscle contracted in an isometric regimen at a rate of external electrical stimulation of 0.5 Hz in a temperature-stabilized chamber (36.0±0.5°C). The extrasystolic contraction was induced with an extra electrical pulse applied 0.25 sec after the regular stimulus. Nibentan decreased the amplitude of extrasystolic contraction in a dose-dependent manner. At the same time, the effect of nibentan on extrasystolic contraction practically did not depend on its concentration. It is concluded that nibentan produces a dose-dependent effect on excitability of rat ventricular myocardium, and in parallel improves calcium-accumulating capacity of the sarcoplasmic reticulum in cardiomyocytes.

Key Words: *nibentan; extrasystolic stimulation; Ca^{2+}; papillary muscle*

The first data on clinical use of nibentan, a novel domestic antiarrhythmic drug, attest to its high efficiency in patients with various types of tachyarrhythmias [4-8].

Nibentan (similarly to other class III preparations) inhibits outward potassium currents and decelerates cardiomyocyte repolarization [2,3]. However, changes of action potential (*e.g.* prolongation for class III antiarrhythmic drugs) affect intracellular Ca^{2+} homeostasis, which in many respects is determined by the state of the sarcoplasmic reticulum (SPR) [12]. The latter effect can be responsible for cardiac rhythm disturbances observed during treatment with class III antiarrhythmic preparations [9]. There is evidence that the functional state of the sarcolemma and SPR in cardiomyocytes can be evaluated by the reaction of the papillary muscles to additional (extrasystolic) electrical stimulation [10]. Here we studied the effect of nibentan on extrasystolic and post-extrasystolic contraction of isolated rat myocardium.

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MATERIALS AND METHODS

The study was carried out on papillary muscle isolated from the left ventricle of intact male Wistar rats weighing 180-200 g. The animals were anesthetized with ether and immobilized by cervical dislocation. The heart was removed and placed in cold Krebs—Henseleit solution containing (in mM): 120.0 NaCl, 4.8 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20.0 $NaHCO_3$, and 10.0 glucose. The left ventricle was opened, and the papillary muscle was isolated and transferred into a perfused temperature-stabilized chamber. One end of the muscle was fixed to the chamber's wall, while the other was attached to an isometric transducer (6MX1C mechanotron).

The muscles were 5-6 mm in length and up to 1 mm in diameter. They were perfused at 36.0±0.5°C with oxygenated Krebs—Henseleit solution (95% O_2

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and 5% CO_2). The muscle was stimulated with rectangular electrical pulses (5 msec duration) delivered via two large silver electrodes placed in the perfusion chamber. The basic repetition rate of regular stimulation pulses was 0.5 Hz.

The extra pulse (extrasystolic stimulation) had the same parameters as the regular pulses and was applied 0.25 sec after the regular stimulation pulse.

Excitability of the sarcolemma was evaluated by changes in the contraction-relaxation cycle in response to extrasystolic stimulus. The capacity of SPR to accumulate Ca^{2+} ions entering the myoplasm during extrasystolic excitation was assessed by changes in post-extrasystolic contraction [10].

The experiments were performed on muscles developing tension of no less than $^1/_2$ calibration signal (1 V) at the end of 60-min adaptation period.

After adaptation of the muscle to perfusion regimen, the initial response to extrasystolic pulses was recorded. Then the muscle was perfused with physiological saline containing nibentan (2.5 and 25 μM) for 15 min and the extrasystolic stimulation was repeated. There is evidence that nibentan in a concentration of 2.5 μM significantly blocks potassium current, and the degree of this blockade significantly increases at 25 μM [2]. We used nibentan produced at the Experimental Medical and Biological Plant in Russian Cardiology Research-and-Production Complex, Ministry of Health, Moscow.

The contractile function of the papillary muscles was assessed by changes in the tension curve. The following parameters were determined: maximum tension (T_{max}), maximum rate of tension rise (dT/dt), and maximum rate of tension drop (-dT/dt) [1,11].

The data were analyzed statistically using Statistica 5.0 software and Wilcoxon test.

RESULTS

The extrasystolic stimulus applied 0.25 sec after the regular stimulus evoked an additional inotropic response with an amplitude of 38.6±1.17% of that induced by regular stimulus (Fig. 1).

Perfusion of the papillary muscles with nibentan had no significant effect on parameters of regular excitation-contraction cycle, although it modified their

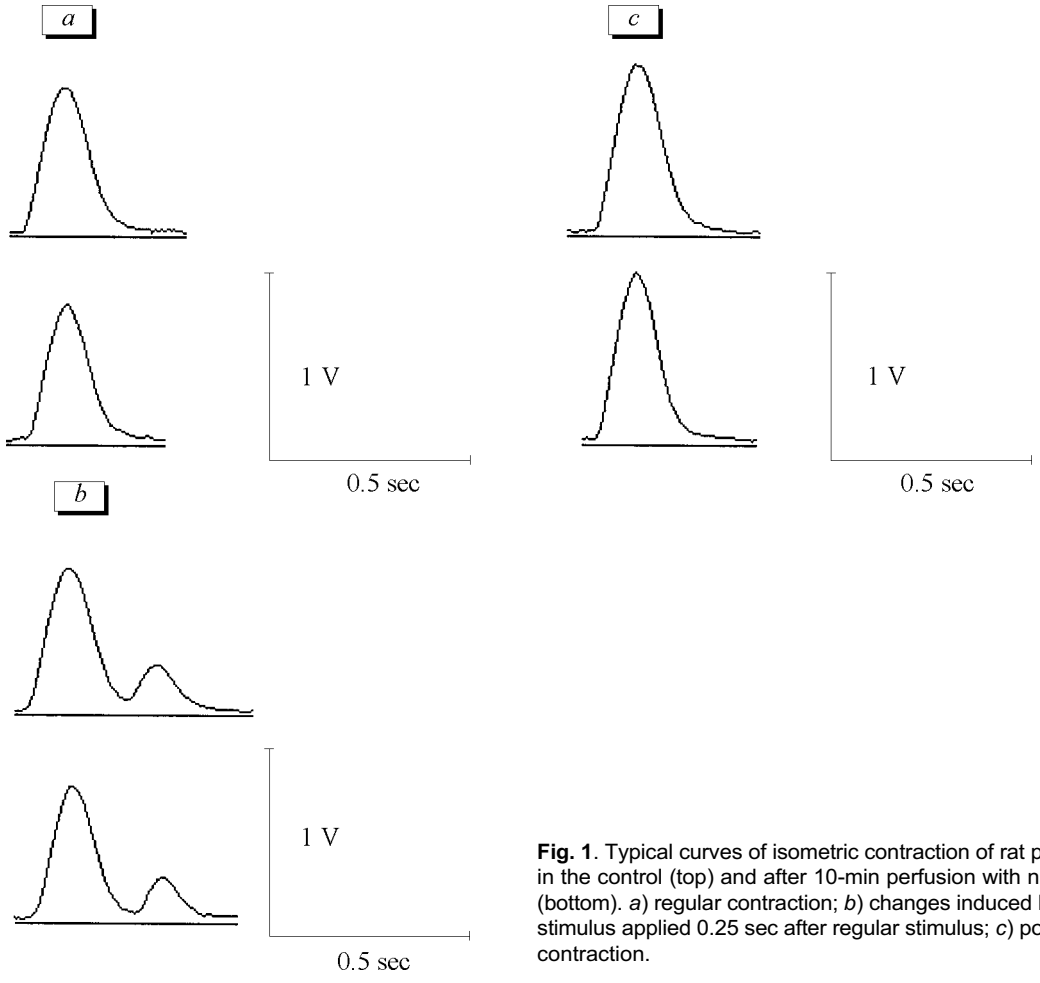

Fig. 1. Typical curves of isometric contraction of rat papillary muscle in the control (top) and after 10-min perfusion with nibentan 2.5 μM (bottom). *a*) regular contraction; *b*) changes induced by extrasystolic stimulus applied 0.25 sec after regular stimulus; *c*) post-extrasystolic contraction.

TABLE 1. Effect of Nibentan on Extrasystolic and Post-Extrasystolic Contraction of Rat Papillary Muscle (Percentage to the Corresponding Parameters of Regular Contraction; *M*±*m*, *n*=12)

Parameter	Control	Nibentan, μM	
		2.5	25
Extrasystolic contraction, T_{max}	38.60±1.17	35.70±1.68**	32.80±1.37*+
Post-extrasystolic contraction			
T_{max}	113.80±1.31	119.7±1.68*	121.00±1.99*
dT/dt	112.80±2.58	118.40±2.31**	121.50±3.02*
-dT/dt	114.40±1.51	121.50±3.02*	123.00±1.91*

Note. *p<0.001, **p<0.05 compared to the control; +p<0.05 compared to 2.5 μM.

reaction to extrasystolic stimulation. Nibentan produced a significant decrease in the amplitude of extrasystolic contraction (Table 1). This can be explained by inhibition of potassium current through the sarcolemma and widening the repolarization and refractoriness phases in cardiomyocytes [5]. The effect of nibentan on potassium channels is dose-dependent [2]. Our data agree with this evidence: 10-fold increase in nibentan concentration in the perfused saline (from 2.5 to 25 μM) produced a more pronounced decrease (p<0.05) in extrasystolic contraction (Table 1), which attests to decreased sensitivity of the papillary muscle to extrasystolic stimulation.

According to modern views on excitation-contraction coupling [10], the extra electrical stimulus initiates additional entry of Ca^{2+} ions to the myoplasm from the extracellular space. These ions are stored in SPR and participate in the first post-extrasystolic contraction-relaxation cycle. Functional manifestation of this phenomenon is post-extrasystolic potentiation (PESP) of inotropic response of the myocardium (Fig. 1). In the control, the amplitude of tension in the first extrasystolic cycle was greater than that in the regular cycle by 13.80±1.31% (Table 1). Repeated stimulation after application of nibentan significantly increased both the amplitude and rate of the post-extrasystolic cycle (Table 1). It should be noted that changes in the parameters of post-extrasystolic contraction virtually did not depend on nibentan concentration. Indeed, the increase in T_{max} produced by nibentan in concentrations of 2.5 and 25 μM was 19.70±1.68 and 21.00± 1.99%, respectively.

As was mentioned above, PESP is explained by accumulation of an extra amount of Ca^{2+} ions in SPR of cardiomyocytes [10], so nibentan-produced potentiation of PESP attests to ability of this drug to increase re-sequestering of Ca^{2+} into SPR of cardiomyocytes.

Therefore, our data show that nibentan, on the one hand, produces a dose-dependent decrease in myocardial excitability, and on the other hand, promotes re-sequestering of Ca^{2+} ions into SPR. The latter effect does not depend on nibentan concentration and develops in parallel with the major inhibitory effect of the drug on potassium current.

REFERENCES

1. S. A. Afanas'ev and V. Yu. Timofeev, *Kardiologiya*, No. 3, 46-50 (2000).
2. K. Yu. Bogdanov, T. M. Vinogradova, and L. V. Rozenshtraukh, *Ibid.*, No. 4, 28-33 (1997).
3. N. V. Kaverina, *Eksp. Klin. Farmakol.*, No. 5, 12-15 (1994).
4. E. B. Maikov, S. A. Bakalov, I. B. Britanov, *et al., Kardiologiya*, No. 4, 16-27 (1997).
5. E. B. Maikov, S. A. Bakalov, N. B. Krutanov, *et al., Ibid.*, No. 6, 38-50 (1996).
6. E. B. Maikov, S. P. Golitsin, M. Ya. Ruda, *et al., Ibid.*, No. 5, 10-18 (1995).
7. I. N. Merkulova, A. E. Tararak, K. L. Kotkin, *et al., Ibid.*, No. 4, 3-15 (1997).
8. M. Ya. Ruda, I. N. Merkulova, A. E. Tararak, *et al., Ibid.*, No. 9, 4-15 (1995).
9. A. E. Tararak, I. N. Merkulova, and M. Y. Ruda, *J. Mol. Cell. Cardiol.*, No. 6, A193 (1995).
10. F. D. Marengo, M. T. Marquez, P. Bonazzola, and J. E. Ponce-Hornos, *Am. J. Physiol.*, **276**, No. 1, H309-H316 (1999).
11. H. Reuter, C. Zobel, K. Brixius, *et al., Basic Res. Cardiol.*, **94**, 159-170 (1999).
12. M. Toyoaki, O. Mitsuhiro, T. Junji, *et al., Jpn. Circ. J.*, **64**, 65-71 (2000).