European Journal of Research volume 8 issue 8 2023 pages 9-15

Evaluation of the inotropic effects of the pirozaline alkaloid

Inoyat Z. Zhumaev[1]*, Sadriddin N. Boboev[1], Abdisalim A. Zaripov[1], Shavkat Yu. Rustamov[1], Eldor B. Ibragimov[1], Pulat B. Usmanov[1], Shahobiddin M. Adizov[2],

[1]Institute of Biophysics and Biochemistry of National University of Uzbekistan, Tashkent, Uzbekistan.

[2]Institute of the Chemistry of Plant Substances, Uzbek Academy of Sciences, Tashkent, Uzbekistan.

*Corresponding Author
Inoyat Z. Zhumaev, Laboratory of Cell Biophysics,
Institute of Biophysics and Biochemistry,
National University of Uzbekistan, Tashkent, Uzbekistan.
ORCID: 0000-0002-5027-0274
E-mail: inoyat8585@mail.ru

Abstract When studying the dose-dependent effect of pirozaline on the activity of papillary muscle contraction of the rat heart, it was found that this alkaloid has a positive inotropic effect on the activity of the papillary muscle contraction of the rat heart at concentrations from 5 μM to 70 μM. Partial reduction of the positive inotropic effect of pirozaline on papillary muscle contractile activity in experiments with nifedipine indicates that the positive inotropic property of this alkaloid partially affects $Ca^{2+}{}_L$-channels in cardiomyocytes. In the studies carried out in the presence of $NiCl_2$ (10 mM), it was found that the positive inotropic effect of pirozaline on the force of papillary muscle contraction is mainly related to the participation of Na^+/Ca^{2+}-exchange.

Keywords*:* papillary muscle, $Ca^{2+}{}_L$-channel, Na^+/Ca^{2+}-exchanger, alkaloid.

INTRODUCTION

Diseases of the cardiovascular system[1,2], which continue to occupy a leading position in the structure of mortality in developed and developing countries, represent a huge socio-economic problem in the modern world. According to modern concepts, the development of cardiovascular diseases is based on numerous pathophysiological processes[3,4,5], among which damage to the function of Ca^{2+} transporting systems and Ca^{2+} homeostasis of heart cells play a leading role. Quite complex mechanisms are involved in maintaining Ca^{2+} homeostasis of cardiac cells, which involve various Ca^{2+} transporting systems of the plasmalemma (Na^+-, $Ca^{2+}{}_L$-, and Na^+/Ca^{2+}-exchanger) and sarcoplasmic reticulum (RyR2 and Ca^{2+}-ATPase)[6].

ISSN 2521-3261 (Online)/ ISSN 2521-3253 (Print)
https://journalofresearch.eu/

Agents with a positive inotropic effect are substances that increase the concentration of $[Ca^{2+}]_i$ in the cardiomyocyte or increase the level of sensitivity of the myofilament to Ca^{2+}, as well as those that act based on both of these mechanisms (such as pimobendan)[7,8].

Substances with a positive inotropic effect (alkaloids, flavonoids, and glycosides) are mainly based on their protective effect on heart failure and ischemic heart diseases[9,10,11]. It is important that their cardiac cells effectively eliminate disturbances in the Ca^{2+} transport systems and bring the Ca^{2+} homeostasis to a normal state[12].

Among them, alkaloids have a wide range of pharmacological activities, many of which have been used in traditional or modern medicine or as starting points for medicines[13]. Today, the most studied alkaloids from a pharmacological point of view are isoquinoline[14,15], indole, and purine alkaloids. Some indole alkaloids have cardioprotective properties. Taking this into account, the inotropic effect of pirozaline indole alkaloid on the activity of papillary muscle contraction of rat heart was investigated.

MATERIAL AND METHODS

All experimental protocols and conditions for preoperative care were approved by the animal use committee of the Institute of Biophysics and Biochemistry. Adult male Wistar rats weighing 200–250g were anesthetized with sodium pentobarbital (50 mg/kg^{-1}, i.p.) and then sacrificed by cervical dislocation. The papillary muscles from the left ventricles of the rat hearts about 0,5-0,8 mm in diameter and 1-3 mm in length were dissected and mounted in a tissue bath (STEIRT, HSE, Germany) of 3 ml volume and superfused at a rate of 20 $ml\ min^{-1}$ with Krebs solution. The composition of the Krebs solution was (in mM) NaCl, 118; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; glucose 10; $NaHCO_3$, 24; $CaCl_2$, 2.54. The solution was continuously gassed with 95% O_2 and 5% CO_2 to give a pH of 7.4 and was maintained at 37°C. The preparation was mounted horizontally in the tissue bath with one end attached to a hook and the other end attached to an isometric force transducer (Type F30, HSE) connected to a (TAM-A, HSE) gain amplifier. Each preparation was stretched to a length at which maximum developed force was evoked and allowed to equilibrate for at least 1 h before the commencement of the experiments. The preparations were field-stimulated at a rate of 1 Hz by two platinum electrodes with rectangular wave pulses of 10 ms duration at twice the threshold voltage, delivered from an electronic stimulator (ESL-2, Russia**)**. The amplitudes of elicited maximal isometric contractions were used as the control (100%) and changes in the contractile force after drug action were expressed as a percentage of the maximal response. Contractions were recorded on a chart recorder (TZ 4620, Chech Rep.) and after conversion to digital form stored on a personal computer[16].

Data are expressed as mean±SD. Control values between groups were compared by analysis of variance. The Student's *t*-test was used to compare two means. A probability of less than 0.05

ISSN 2521-3261 (Online)/ ISSN 2521-3253 (Print)
https://journalofresearch.eu/

was taken as a statistically significant difference. Statistical analysis was performed using OriginPro 7.5 software (OriginLab Co., U.S.A).

RESULTS

In the experiments, the dose-dependent effect of pirozaline on the activity of papillary muscle contraction of the rat heart was studied. It was found that this alkaloid has a positive inotropic effect on the contraction activity of the papillary muscle of the rat heart at all concentrations. When studying the effect of pirozaline alkaloid from 5 µM concentration to 70 µM concentration, it was found that this alkaloid showed maximum effect at 70 µM concentration and increased muscle contraction force by 45.4±3.7% compared to the control (Fig. 1).

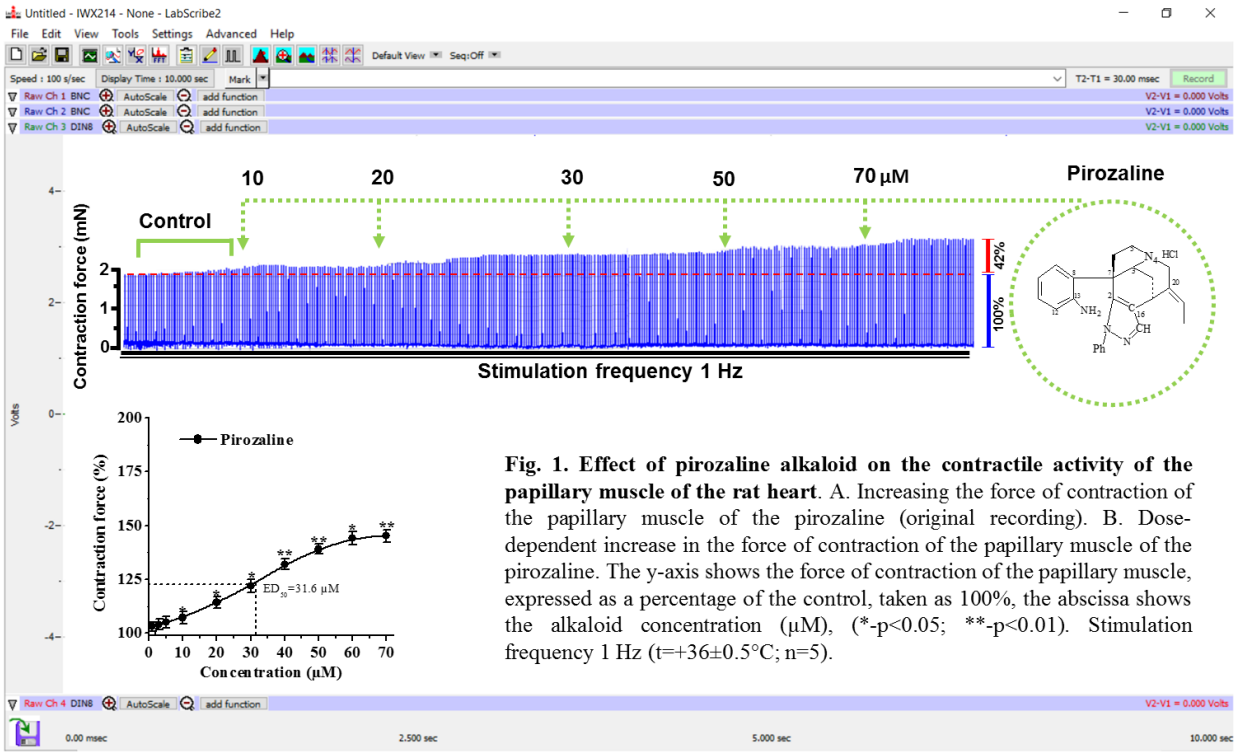

Fig. 1. Effect of pirozaline alkaloid on the contractile activity of the papillary muscle of the rat heart. A. Increasing the force of contraction of the papillary muscle of the pirozaline (original recording). B. Dose-dependent increase in the force of contraction of the papillary muscle of the pirozaline. The y-axis shows the force of contraction of the papillary muscle, expressed as a percentage of the control, taken as 100%, the abscissa shows the alkaloid concentration (µM), (*-p<0.05; **-p<0.01). Stimulation frequency 1 Hz (t=+36±0.5°C; n=5).

Calcium ions are universal cellular internal messengers that play an important role in the regulation of numerous biological processes in the cardiovascular system. Underlying this regulation is a dynamic change in the intracellular ($[Ca^{2+}]_i$) concentration of Ca^{2+} ions, which occurs in response to various effects of extracellular and intracellular physiological stimuli. For this reason, maintenance of Ca^{2+}-homeostasis or dynamic stability of Ca^{2+} ions in cardiac muscle cells is extremely important for the normal functioning of heart muscles, and its disturbance leads to the development of various pathologies in the cardiovascular system.

It is known that many agents with a positive inotropic effect cause an increase in $[Ca^{2+}]_{in}$ concentration in the cytosol by activating $Ca^{2+}{}_L$-channels located in the sarcolemma of cardiomyocytes. One of the main reasons for the positive inotropic effect is the modulation of the activity of $Ca^{2+}{}_L$-channels in cardiomyocytes, which in turn is associated with a change in the

ISSN 2521-3261 (Online)/ ISSN 2521-3253 (Print)
https://journalofresearch.eu/

amount of $[Ca^{2+}]_i$ in cardiomyocyte cells[17]. The positive inotropic effect of pirozaline alkaloid on the activity of papillary muscle contraction of the rat heart can be caused by the activation of $Ca^{2+}{}_L$-channels located in the membrane of cardiomyocytes. To clarify this assumption, the positive inotropic effect of pirozaline was studied in the presence of $Ca^{2+}{}_L$-channel blocker-nifedipine in an incubation medium. Nifedipine[18,19] showed a negative inotropic effect at concentrations ranging from 0.001 μM to 0.1 μM when the effect of nifedipine on papillary muscle contractile activity of rat heart was studied[20] and its half-maximal inhibitory effect concentration was IC_{50}=0.01 μM.

In further experiments, to clarify the nature of Ca^{2+} ions involved in the positive inotropic effect of pirozaline on papillary muscle contraction activity, $Ca^{2+}{}_L$-channel blocker nifedipine was studied. In the conditions of incubation of nifedipine (IC_{50}=0.01 μM) in the medium, it was found that the positive inotropic effect of pirozaline (70 μM) decreased by 23.4±3.3% compared to the control variant (Fig. 2).

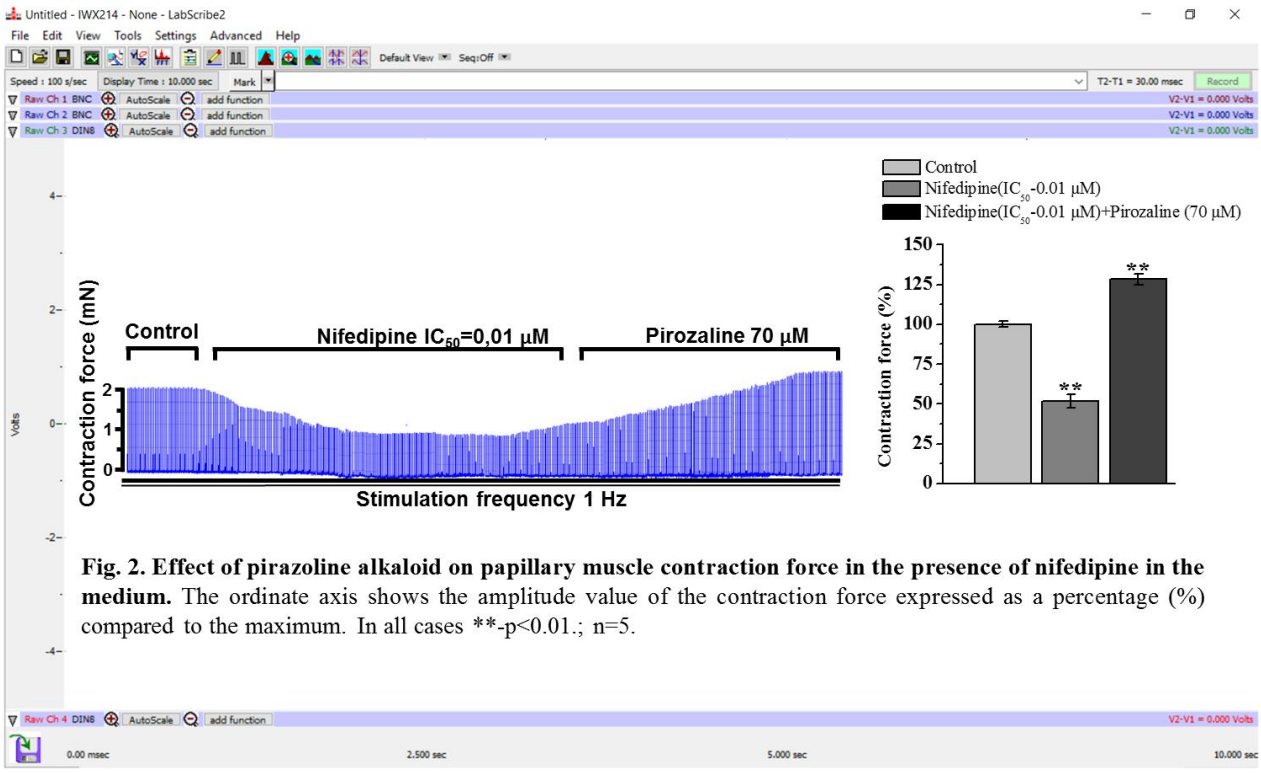

Fig. 2. Effect of pirazoline alkaloid on papillary muscle contraction force in the presence of nifedipine in the medium. The ordinate axis shows the amplitude value of the contraction force expressed as a percentage (%) compared to the maximum. In all cases **-p<0.01.; n=5.

The results of the conducted research show that, according to the analysis of the obtained results, in the presence of nifedipine, a partial reduction of the positive inotropic effect of pirozaline on papillary muscle contraction activity was determined. It can be concluded that the positive inotropic property of this alkaloid is explained by its partial effect on $Ca^{2+}{}_L$-channels in cardiomyocytes. SR Ca^{2+}-ATPase and Na^+/Ca^{2+}-exchange system function play a key role in cardiomyocyte relaxation. Increased function of SR Ca^{2+}-ATPase and Na^+/Ca^{2+}-exchange system leads to a decrease of $[Ca^{2+}]_i$ content in the cytosol of cardiomyocytes, this process, in turn, increases the relaxation rate[12]. It is known that the Na^+/Ca^{2+}-exchange system in cardiomyocytes in

ISSN 2521-3261 (Online)/ ISSN 2521-3253 (Print)
https://journalofresearch.eu/

normal physiological conditions is the main system that brings Na^+ ions into the cell and releases Ca^{2+} ions out of the cell[21]. Therefore, in the experiments, we studied the effect of these alkaloids on the papillary muscle Na^+/Ca^{2+}-exchange system. To evaluate the effect of pirozaline on papillary muscle contraction activity and the position of the Na^+/Ca^{2+}-exchange system located in the sarcolemma of cardiomyocytes, a concentration of 10 mM of its non-selective blocker - $NiCl_2$ was used. In the presence of $NiCl_2$ (10 mM) in the medium, the positive inotropic effect of pirozaline at a concentration of 70 μM on the force of papillary muscle contraction was 93.5±3.7% (Fig. 3).

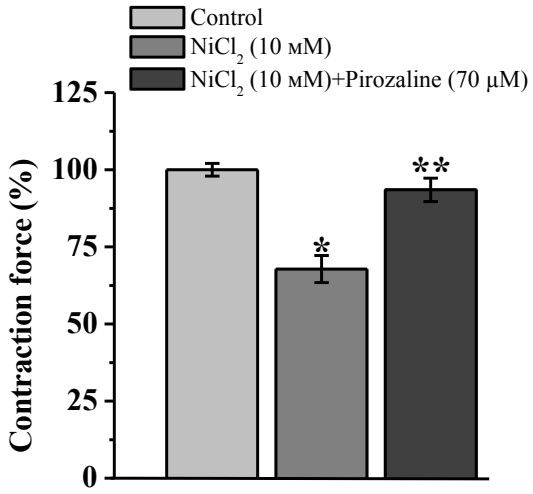

Fig.3. Effects of pirozaline alkaloid in the presence of $NiCl_2$. On the ordinate axis - the amplitude value of the contraction force is expressed as a percentage (%) compared to the maximum. The frequency of stimulation of the drug is 1 Hz. In all cases * - p<0.05; ** - p<0.01.; n=5.

The positive inotropic effect of this alkaloid on papillary muscle contractile activity was shown to decrease by 52.8±5.2% compared to the control condition. Based on this, this condition is evidenced by the participation of Na^+/Ca^{2+}-exchange in the positive inotropic effect of the alkaloid. The results of the conducted research show that, according to the analysis of the obtained results, in the presence of nifedipine, the positive inotropic effect of pirozaline alkaloid on papillary muscle contraction activity is partially reduced, and the positive inotropic property of the alkaloid is explained by the partial effect on $Ca^{2+}{}_L$-channels in cardiomyocytes.

To assess the position of the Na^+/Ca^{2+}-exchange system, pirozaline alkaloid is explained by the fact that its blocker $NiCl_2$ (10 mM) modulates the Na^+/Ca^{2+}-exchange by having a positive inotropic effect on papillary muscle contraction force.

ACKNOWLEDGMENTS. This work was supported by a grant F-OT-2021-154 from the Coordinating Committee for Development of Science and Technology under the Cabinet of Ministers of the Republic of Uzbekistan.

CONFLICT OF INTEREST. The authors have declared that no conflict of interest exists.

ISSN 2521-3261 (Online)/ ISSN 2521-3253 (Print)
https://journalofresearch.eu/

REFERENCES

1. Archana V, Indumathy R. In- vitro Antioxidant and Cardio-protective effect of Delonix elata (L.) Leaf extract against Doxorubicin induced toxicity in H9c2 Cardio-myocyte cell line. Research Journal of Pharmacy and Technology. 2021; 14(11):5635-1
2. Senthilkumar S, Dhivya K. Prognostic Potential of Serum Biomarkers as Predictors for Cardiovascular Complications and Disease Progression in Chronic Kidney Disease Patients. Research J. Pharm. and Tech. 2016; 9(3): 227-234.
3. S. Anuradha. Effect of Planned Teaching Programme regarding lifestyle modifications to prevent cardiovascular diseases among drivers at Sree Vidyanikethan Educational Trust (SVET), A. Rangampet-A.P. Int. J. Adv. Nur. Management. 2017; 5(3):246-250
4. Sneha R. Dubey. A Descriptive Study to Assess the Knowledge of Adolescents regarding Obesity as a Predisposing Factor for Cardiovascular Diseases with A View to Develop an Educational Pamphlet in Selected Urban Areas of Tumkur. International Journal of Advances in Nursing Management. 2021; 9(3):319-1.
5. Avinash Khairnar, Supriya Jamdade. Prospective Cross-Sectional Observational Study on Evaluation of Drug Utilization 90% Study in Cardiovascular Diseases. Research J. Pharm. and Tech. 7(9): Sept. 2014 Page 981-986.
6. David A. Eisner, Jessica L. Caldwell, Kornél Kistamás, and Andrew W. Trafford // Calcium and Excitation-Contraction Coupling in the Heart // Circ Res. 2017 Jul 7; 121(2): 181–195.
7. Scholz H. Inotropic drugs and their mechanisms of action // JACC. 1984. V.4(2). P.389–397.
8. Scholz H. Introduction: mechanisms of positive inotropic effects // Basic Research in Cardiology. 1984. V.84(1). P.3–7.
9. Jian SUN, Benny KH TAN, Shan-Hong HUANG, Matthew WHITEMAN, Yi-Zhun ZHU. Effects of natural products on ischemic heart diseases and cardiovascular system // Acta Pharmaco l Sin 200 2 Dec; 23 (12): 1142 -1151
10. Anubhav Dubey, Yatendra Singh. Medicinal Properties of Cinchona Alkaloids - A Brief Review. Asian Journal of Research in Pharmaceutical Sciences. 2021; 11(3):224-8.
11. Saripah Salbiah Syed Abdul Azziz, Mat Ropi Mukhtar, A. Hamid A. Hadi, Yuhanis Mhd Bakri, Ahmed Kareem Obaid Aldulaimi, Khalijah Awang, Yumi Zuhanis Has-Yun Hashim. Alkaloids from Popowia perakensis King and their potential as Dipeptidyl peptidase IV (DPP IV) inhibitors. Research J. Pharm. and Tech 2019; 12(1): 113-116.
12. Kussum Sharma, Monika Rangi. A Novel Ion-Selective Electrode Based on Pyrole-2-Carboxaldehyde-Semicarbazide for Potentiometric Determination of Ca (II) Ions. Asian J. Research Chem. 4(9): Sept, 2011; Page 1444-1446.
13. Michael Heinrich, Jeffrey Mah, and Vafa Amirkia. Alkaloids Used as Medicines: Structural Phytochemistry Meets Biodiversity-An Update and Forward Look // Molecules. 2021 Apr; 26(7): 1836.
14. Inoyat Jumayev, Pulat Usmanov, Shavkat Rustamov1 and Sherzod Zhurakulov. Comparative Inotropic Effects of the Some Isoquinoline Alkaloids // Biomedical & Pharmacology Journal, March 2020. Vol. 13(1), p. 325-333
15. Pulat B. Usmanov, Inoyat Z. Jumayev, Shavkat Yu. Rustamov, Abdisalim A. Zaripov, Adilbay T. Esimbetov, Sherzod N. Zhurakulov and Valentina I. Vinogradova. The Combined Inotropic and Vasorelaxant Effect of DHQ-11, a Conjugate of Flavonoid Dihydroquercetin with Isoquinoline Alkaloid 1-aryl-6,7-dimethoxy-1,2.3,4-tetrahydroisoquinoline // Biomedical & Pharmacology Journal, June 2021. Vol. 14(2), p. 651-661
16. Sh. S. Khushmatov, I. Z. Zhumaev, Sh. N. Zhurakulov, A. Sh. Saidov, and V. I. Vinogradova. Synthesis and Comparative Inotropic Effects of Several Isoquinoline Alkaloids // Pharmaceutical Chemistry Journal Vol. 54, No. 1, pp. 9 – 13, January, 2020.

ISSN 2521-3261 (Online)/ ISSN 2521-3253 (Print)
https://journalofresearch.eu/

17. Dirk von Lewinski, Sebastian Bruns, Stefanie Walther, Harald Kögler and Burkert Pieske. Insulin Causes $[Ca^{2+}]_i$-Dependent and $[Ca^{2+}]_i$-Independent Positive Inotropic Effects in Failing Human Myocardium // Circulation. 2005; 111:2588–2595

18. Naiya Devgan, Meenakshi Barsaul, Rashmi Sindhwani, Smiti Nanda , Manish Devgan. A Comparative Study of Nifedipine and Terbutaline as Tocolytic Agents in the Management of Preterm Labour. Research J. Pharmacology and Pharmacodynamics. 2013; 5(4). 232-236.

19. Hajare AA, Shetty YT, Mali MN, Sarvagod SM. Characterization of Melt (Fusion) Solid Dispersions of Nifedipine. Research J. Pharm. and Tech. 1(3): July-Sept. 2008; Page 230-234.

20. Benoit Vivien, Jean-Ste´phane David, Jean-Luc Hanouz, Julien Amour, Yves Lecarpentier, Pierre Coriat, and Bruno Riou. The Paradoxical Positive Inotropic Effect of Sevoflurane in Healthy and Cardiomyopathic Hamsters // Anesth Analg 2002;95:31–8

21. Atsushi Yao, Zhi Su, Akihiko Nonaka, Iram Zubair, Liyan Lu, Kenneth D. Philipson, John H. B. Bridge and William H. Barry. Effects of Overexpression of the Na^+-Ca^{2+} Exchanger on $[Ca^{2+}]_i$ Transients in Murine Ventricular Myocytes // Circulation Research. 1998;82:657–665.

22. Munekazu Shigekawa and Takahiro Iwamoto. Cardiac Na^+-Ca^{2+} Exchange Molecular and Pharmacological Aspects // Circulation Research. 2001;88:864–876

ISSN 2521-3261 (Online)/ ISSN 2521-3253 (Print)
https://journalofresearch.eu/