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Comparative Inotropic Effects of the Some Isoquinoline Alkaloids

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In this study, mechanisms of inotropic action of some isoquinoline alkaloids 1-(4-dimethylaminophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-24), 1-(2-chloro-4,5-methylenedioxyphenyl)-2-hydroxyethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (N-14) and 1-(2-chloro-4,5-methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-14) were studied. The F-24, F-14, and N-14 alkaloids have been shown to have a negative effect on papillary muscle contraction activity, IC50 value -16,8 μM, 14,03 μM and 12 μM. Ca^{2+}L-channel blocker - nifedipine, adenylate cyclase (AC) activator–forskolin, ß-adrenoreceptor (ß-AR) blocker – propranolol, protein kinase C (PKC) activator – phorbol 12-myristate 13-acetate and SR RyR2 activator caffeine were used. Inotropic effects of F-24, F-14 and N-14 isoquinoline alkaloids on cardiomyocytes were suggested, based on results obtained in experiments carried in cardiomyocytes ß-AR → [cAMP] → PKA → [Ca^{2+}]in ↑ cascade, PKC, RyR2 and Na+/Ca^{2+} modulation.

Keywords: Inotropic Effect; Isoquinoline Alkaloids; Papillary Muscle.

In the world, there has been a trend towards the spread of cardiovascular diseases in recent years, which continues to be a leader in general disease and death structure[1,2]. Worldwide, cardiovascular diseases are a major problem in the medical, social and economic context, leading to disability and premature deaths[3,4,5]. According to the World Health Organization's statistical data, about 17,300,000 people die of cardiovascular diseases each year and makes up ~31% of all deaths under general conditions[6,1].

Cardiovascular disease is associated with dysfunctional changes in cardiomyocytes [Ca^{2+}]in homeostasis, and myocardial rhythmic activity is provided by the function of Ca^{2+} transport system, located in the cardiomyocyte sarcolemma and sarcoplasmic reticulum (SR) membrane. Ca^{2+} entrance to potassium-activating L-type Ca^{2+} -channel ($Ca^{2+}{}_{L}$-channel) in the sarcolemma during the formation of Na+ -channel activation and action potential (AP) in Cardiomyocyte activates CR Ca^{2+} -channel (RyR2; ryanodine receptor type 2). Ca^{2+} affinity (Ca^{2+} -spark) type cardiomyocytes cytosol increases the concentration of Ca^{2+} and ions Ca^{2+} in troponin-C in myocardium. Myocardial contraction is resulted by increases of

Ca^{2+} concentration in Ca^{2+}–oscillation (Ca^{2+}–spark) type cardiomyocytes cytosol and further formation with troponin-? in the myofilament. During the diastole, Ca^{2+} are normalized by Ca^{2+} -ATPase (SERCA2a, Sarco (endo) plasmic reticulum calcium-ATPase type 2) in the sarcoplasmic reticulum membrane and Na+/Ca^{2+} -exchanger type 1 function. These ion-transport systems are at the center of the normal physiological function of myocardium. Breakage in function of these systems in cardiomyocytes leads to disruption of [Ca^{2+}]in dynamics and development of arrhythmia-type cardiopathology[7,8,9]. The effect of "target" on effective antiarrhythmic and cardiotropic drugs, currently used in clinical cardiology practice, is directed to the pharmacological correction of the function of these systems (Figure 1).

The disorders in the RyR2 structure have been reported to cause cardiac dysfunction and arrhythmias[10].

Specifically, increase in the spontaneous activation of RyR2, as well as increases of [Ca^{2+}]in concentration induced by hyperphosphorylation of RyR2 through ß–AR – PKA and the development of pathogenesis of post-depolarization-type arrhythmia were established[11].

The RyR2 functional activity is regulated mainly by three mechanisms: [Ca^{2+}]SR, [Ca^{2+}] in concentration changes, and by regulatory proteins and adaptive mechanisms regulated by RyR2 activation/inactivation[12].

In ß–AR– [cAMF] in-PCA reactions cascade, under the effect of cervical oscillation of RyR2-FKBP12.6, RyR2 activity increases; thereby the probability of formation of arrhythmia rises[13].

Adenylate cyclase (A?) and protein kinase A (PCA) system are important in the functional activity of cardiomyocytes.

In the case of ß–adrenoreceptor (B-AR) activation in cardiomyocytes sarcolemma, A? enzyme activation via guanine-dependent activating transmembrane Gs-protein (guanine nucleotide-binding proteins) and [?AMF] value increase. PCA is activated by cAMF, and functional protein molecules including PLB, troponin-I, Ca^{2+}L-channel are phosphorylated[14,15].

Regulating the functional activity of cardiomyocytes through the modulation of the above-mentioned mechanism is significant to establish mechanism of action of biologically active substances, creation of potential pharmaceutical preparations on their basis, and treatment/ prevention of cardiopathology.

Among chemical compounds, containing heterocyclic structure, isoquinoline alkaloids are characterized by a wide range of physiological spectra. Isoquinoline alkaloids possess antiarrhythmic and cardiotropic effects on cardiovascular system, spasmolytic, pain-relieving and anti-inflammatory effects[16,17]. Spasmolytic drugs included in the list of isoquinoline alkaloids, such as Papaverine and No-Shpa, are widely used in medical practice. Besides, papaverine analogs – salsolin, salsolidin, guanethidine, have a strong hypotensive effect[18].

The berberine (Berberine) isoquinoline alkaloid derived from Rhizoma Coptidis plant species has been reported to possess activity against pathogenic bacteria, ischemia, and thrombus formation, and hepatoprotective and antiarrhythmic effects[19].

Aim of this study was to establish

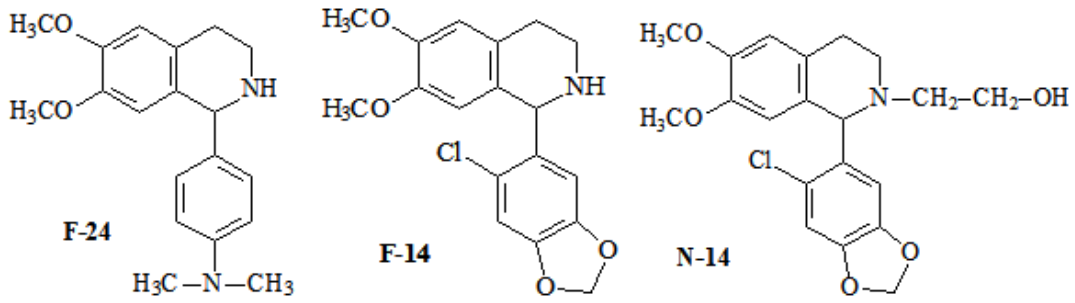

Fig. 1. Chemical structure of isoquinoline alkaloids

mechanism of action of 1-(4-dimethylaminophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-24), 1-(2-chloro-4,5-methylenedioxyphenyl)-2-hydroxyethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (N-14) and 1-(2-?hloro-4,5-methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-14) alkaloids on cardiomyocytes ion- transport systems, by registering papillary muscular contraction activity of rat heart, in vitro conditions.

MATERIAL AND METHODS

Solvents and Chemicals

All reagents, used in experiments, were of analytic–grade (NaCl, KCl, CaCl2, MgSO4, KH2PO4, glucose, NaHCO3). (±)-propranolol hydrochloride, nifedipine hydrochloride, forskolin, phorbol 12-myristate 13-acetate (PMA), caffeine were obtained from Sigma Chemical (St. Louis, Missouri, USA).

Isoquinoline alkaloids, the effects of which were studied, were synthesized by researchers group from the Institute for Plant Substances of Academy of Sciences of Uzbekistan. The isoquinoline alkaloids were synthesized on the basis of the Pictet -Spengler, and Bischler-Napieralski reactions. The chemical structures of the synthesized isoquinoline alkaloids were established using IR and NMR N1 spectroscopy.

Tissue Preparation and Measurement of Contractility

In the experiments, the standard mechanography was used to screen the inotropic effect of isoquinoline alkaloids.

The prepared papillary muscle was connected to a force transducer for signal recording. In experiments, the papillary muscle preparations were isolated from the right atrium of adult albino rats' hearts. The papillary muscles were 0.4–1.3 mm in diameter and 2.5–3.8 mm in length. The papillary muscles samples were prepared according to Sonnenblick, and the muscle was placed in a special horizontal tissue chamber (Type 813; Hugo Sachs Elektronik, March-Hugstetten, Germany), designed for in vitro study in standard pharmacological experiments for measuring contraction force response of papillary muscle preparations. The top of the system was open and it is provided with the organ chamber, volume

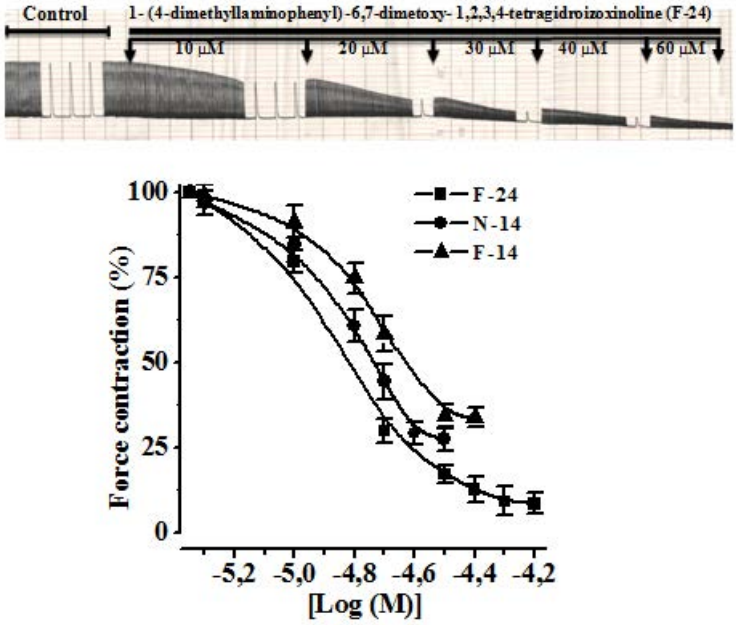

Fig. 2. Negative inotropic effect of isoquinoline alkaloids. The ordinate axis shows the pulsating force of the papillary muscle, expressed as a percentage of the maximum value of 100%. The stimulation frequency is 0.5 Hz (t=+36±0.5ºC). D<0.01 (n=3-4)

– 5 ml, the Thermo–circulator for flow heater physiological solution and the wire holder for the force transducer (Type F30/Model D-79232; Hugo Sachs Elektronik, March-Hugstetten, Germany), with a precision micrometer control. In the experiments, modified the physiological Krebs–Henseleit solution containing (in mM): 118 NaCl; 4.7 KCl; 2.5 CaCl2; 1.2 MgSO4; 1.1 KH2PO4; 5.5 glucose and 25 NaHCO3; pH 7.4 were used. This Krebs-Henseleit solution which was continuously bubbled with 95% O2 and 5% CO2 and kept at a temperature of +36±0.5 °C by means of water heating system controlled by temperature controller U8 (Bulgaria), and flowed in and out of the organ bath at a rate of 3-5 ml/min with the peristaltic pump LKB Bromma (Sweden).

The isometric force transducer F30 was connected to a transducer amplifier (Type TAM-A; Hugo Sachs Elektronik, Harvard Apparatus GmbH, Germany). The papillary muscle was lifted with electric impulses higher than a threshold (~20%), rectangular, electrical pulses of frequency 0.5 Hz; 5 ms and 5 V amplitude, delivered via a pair of platinum electrodes placed in the muscle-mounting organ chamber by using stimulator ESL-2 (Russia). Thus, wires of a pair of platinum electrodes were placed as parallel to the organ; the physiological solution of Krebs-Henseleit provided shortening the electrical contact distance between the electrodes and the preparation of the papillary muscles. After 60 min of incubation period, papillary muscles were stimulated by an initial electrical pulse of frequency 0.5 Hz, amplitude 5 V, and 5 msec pulses. The obtained signals were given from the transducer F30 to amplifier and sent to a computer by using a pen chart recorder (Type TZ 4620; Czech Republic) or a personal computer with analog-digital converter LabPro Logger Lite 1.2 software (Vernier Software & Technology, Beaverton, USA).

Data Analysis

Papillary muscle contractions were plotted as a percentage of the force before the drug application in each muscle. Data were analyzed by OriginPro 7.0 (MicroCal Software, Northampton, MA). Pooled data were given as means ±S.E.M. of observations (n). Concentration-response curves were fitted to the logistic equation: , where Emax – is the maximum effect, k – is a factor which represents the slope of the curve, and pD2 – is the drug concentration exhibiting 50% of the Emax expressed as negative log molar. Values are expressed as mean ±S.E.M. The values were considered as significantly different when $p<0.05$.

RESULTS AND DISCUSSION

Inotropic Action of Isoquinoline Alkaloids

The alkaloids of F-24 (10-60 µM), F-14 (5 to 40 µM) and N-14 (5 to 25 µM) isoquinoline in the

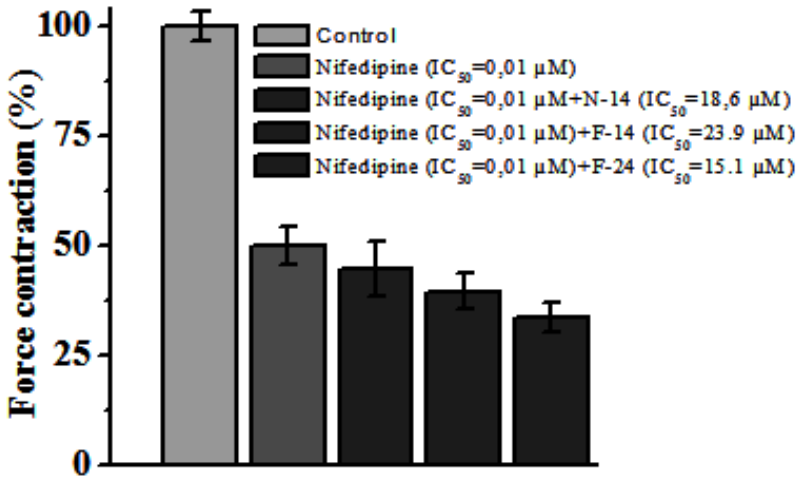

Fig. 3. Comparison of the inotropic effects of F-24, N-14, F-14 alkaloids and nifedipine on the contraction force of extracted rat papillary muscle. Stimulation: 0.5 Hz, 5 V, 5 msec, +36±0.5 °C, resting tension = 10 mN. *P<0.05* indicates value compared to control

experiments showed a negative inotropic effect on the papillary muscle contraction. Pupillary muscle contraction force decreased up to 92.4±3.8%, 72.7±4.4% and 66.5±3.3% respectively. Semi-maximum concentrations of F-24, N-14 and F-14 alkaloids (IC_{50}) were established to be 15.1 µM, 18.6 µM, and 23.9 µM, respectively (Figure 2).

The Role of Ca^{2+}L-Channels in the Inotropic Effect of Isoquinoline Alkaloids

Many negatively inotropic agents cause the Ca^{2+}L-channel blocking in cardiomyocytes to reduce the concentration of [Ca^{2+}]in the cytosol20. Therefore, in recent experiments, we have investigated negative inotropic effects of F-24, N-14, F-14 alkaloids in the current state of the Ca^{2+}L-channel blocker nifedipine (IC_{50}=0,01 µ?) in the incubation medium. The negative effect of isoquinoline alkaloids: F-24 (15.1 µM), N-14 (18.6 µM) and F-14 (23.9 µM) in nifedipine (0.01 µM) containing incubation medium compiled 44.7±6.3%, 39.7±4.1% and 33.7±3.3% respectively (Figure 3).

The results show, that negative effects of F-24, N-14, F-14 alkaloids, in partial correlation, blockade Ca^{2+}L-channel in cardiomyocytes

The Cascade of ß–AR–AC Reactions in the Inotropic Action of Isoquinoline Alkaloids

Proteinasease A (PCA) in cardiomyocytes is regulated by phosphorylation of functional protein macromolecules, for example, by the phosphorylation of PCA, RyR2 is activated and the value of [Ca^{2+}]in cytosol increases[21]. Cardiomyocyte protein kinase A – linked molecule (AKAP, A-kinase-anchor protein) ensures that signal transduction via PCA is performed correctly and the presence of mACAP (muscle-specific AKAP) molecules in the RyR2 phosphorylation22. The Beta-adrenergic receptors (ß-ARs) stimulation enhances contractility through protein kinase-A (PKA) substrate phosphorylation. This PKA signaling is conferred in part by PKA binding to A-kinase anchoring proteins (AKAPs). AKAPs coordinate multi-protein signaling networks that are targeted to specific intracellular locations, resulting in the localization of enzyme activity and transmitting intracellular actions of neurotransmitters and hormones to its target substrates. In particular, mAKAP (muscle-selective AKAP) has been shown to be present on the nuclear envelope of cardiomyocytes with various proteins including PKA-regulatory subunit (RIIa), phosphodiesterase-4D3, protein phosphatase-2A, and ryanodine receptor[23].

In further experiments, on the basis of inotropic effects on the papillary muscular contraction in the rats, we studied the putative effects of isoquinoline alkaloids (F-24, N-14, and F-14) on ß–?R–?C reactions cascade.

It has been revealed, that the positive inotropic effects of adenylate cyclase activator – forscolin (10 µM) decrease for 8.1±2.4%, 39.3±6.4% and 87.6±5.2% by F-24 (IC_{50}=15.1 µM), F-14 (IC_{50}=23.9 µM) and N-14 (IC_{50} = 18.6 µM) alkaloids, respectively (Figure 4).

The ordinate axis shows the pulsating force of the papillary muscle, expressed as a percentage of the maximum value of 100%. The stimulation frequency is 0.5 Hz (t=36±0.5ºC). * – p <0.05, ** – p <0.01 (n=4-5) for control.

The inotropic effect of isoquinoline alkaloids (N-14, F-14, and F-24) in the medium of ß-AR blocker propranolol (10 µM) + AC activator forskolin (IC_{50} = 3.4 µM) on AT-activation in cardiomyocytes. It was registered that under the effects of forskolin (I?50=3.4 µM), in the medium propranolol (10 µM) incubation, the ß-AR blocker,

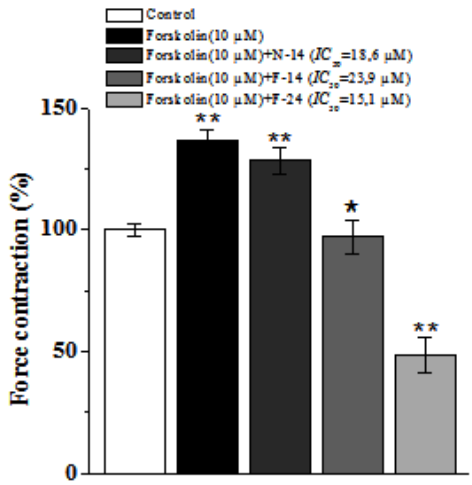

Fig. 4. Adenilate cyclase (AC) activator isotropic effect of isoquinoline sequence alkaloids (F-24, N-14, F-24) in current conditions of forscolin (1 µM). The ordinate axis shows the pulsating force of the papillary muscle, expressed as a percentage of the maximum value of 100%. The stimulation frequency is 0.5 Hz (t=36±0.5ºC). * – p <0.05, ** – p <0.01 (n=4-5) for control

papillary muscle contraction activity significantly increased by 23.2±3.5%, compared to control. The alkaloids N-14 (IC_{50}=18.6 μM), F-14 (IC_{50}=23.9 μM) and F-24 (IC_{50}= 15.1 μM) reduced propranolol (10 μM) + forskolin (IC_{50}=3.4 μM) influence by 2.8±2.3%, 13.5±3.4% and 69.5±6.2%, respectively (Fig. 5).

The ordinate axis shows the pulsating force of the papillary muscle, expressed as a percentage of the maximum value of 100%. The

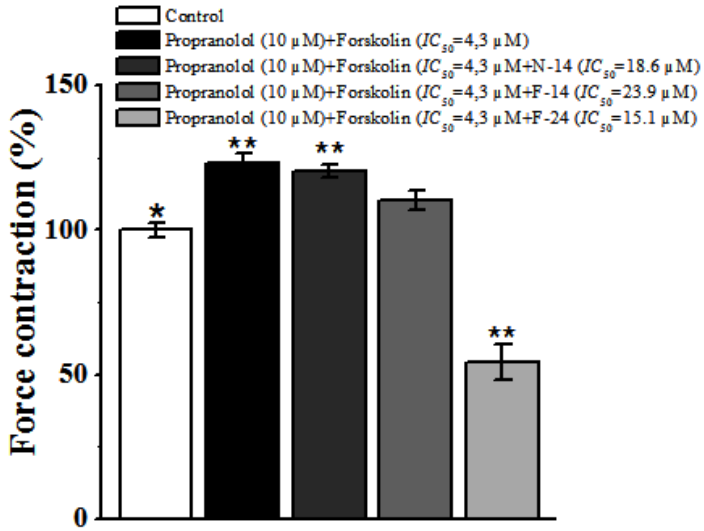

Fig. 5. The inotropic effect of isoquinoline alkaloids (F-24, N-14, F-24) in the conditions of incubation of â-ÀÐ blocker propranolol (10 iM) + forskolin (IC_{50}=3,4 iM). The ordinate axis shows the pulsating force of the papillary muscle, expressed as a percentage of the maximum value of 100%. The stimulation frequency is 0.5 Hz (t =36±0.5ºC). * –for control ** – δ<0.01 (n=5-6)

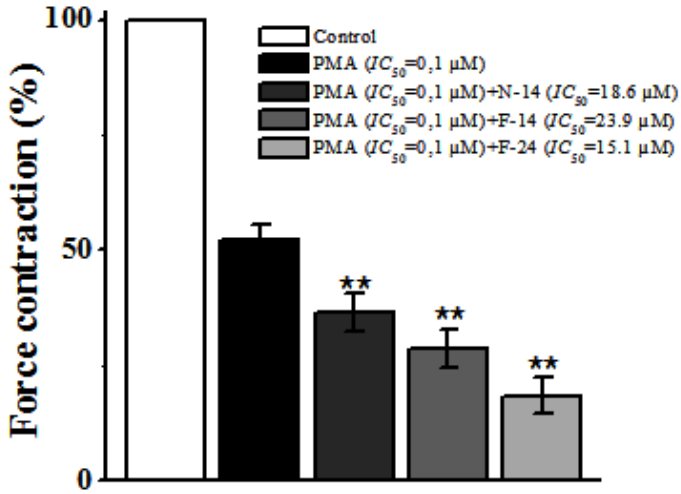

Fig. 6. The inotropic action of isoquinoline alkaloids (N-14, F-14, F-24) in incubation conditions of PKC activator - phorbol 12-myristate-13-acetate (PMA) (IC_{50}=0,1 μM). The ordinate axis shows the pulsating force of the papillary muscle, expressed as a percentage of the maximum value of 100%. The stimulation frequency is 0.5 Hz (t=36±0.5ºC). *– for control ** – δ<0,01 (n=4–5)

stimulation frequency is 0.5 Hz (t =36±0.5ºC). * –for control ** – ?<0.01 (n=5-6).

In the case of incubation of propranolol (10 µM) + forskolin (IC_{50} = 3,4 µM), the significant changes were detected by N-14, F-14 alkaloids, and there was no change by the negative inotropic effect of F-24 alkaloid. The results show that the negative inotropic effect of N-14 and F-14 alkaloids on the inhibition of inactivity is directly related to the modulation of AC activity.

Further, in order to clarify the mechanism of negative inotropic action, inotropic effects of the studied alkaloids were investigated in the incubation condition of phorbol 12-myristate-13 acetate (FMA), PKC activator. In the experiments, the heartbeat of the FMA concentration (0,01-1 µM) had a negative effect on papillary muscle contraction and its IC_{50} value equaled 0,1 µM. The negative inotropic effects of F-24 (IC_{50}), F-14 (IC_{50}) and N-14 (IC_{50}) in the FMA (EC50=0.1 µ?) incubation conditions compiled 30.7±6.4%, 21.4±3.5% and 14.6±4.2%, respectively (Fig. 6).

The ordinate axis shows the pulsating force of the papillary muscle, expressed as a percentage of the maximum value of 100%. The stimulation frequency is 0.5 Hz (t=36±0.5ºC). *–

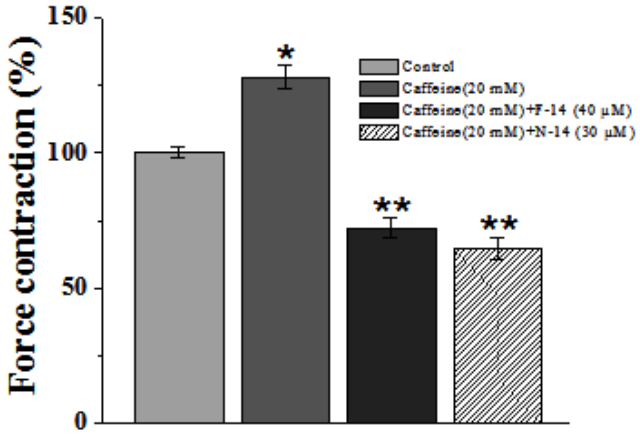

Fig. 7. Inotropic effect of isoquinoline alkaloids (F-14 and N-14) in incubation conditions of caffeine (20 mM). The ordinate axis shows force contraction of the papillary muscle, expressed as a percentage of the maximum value of 100%. The stimulation frequency is 0.5 Hz (t=36±0.5ºC). * – for control ** – δ<0.01 (n=3–5)

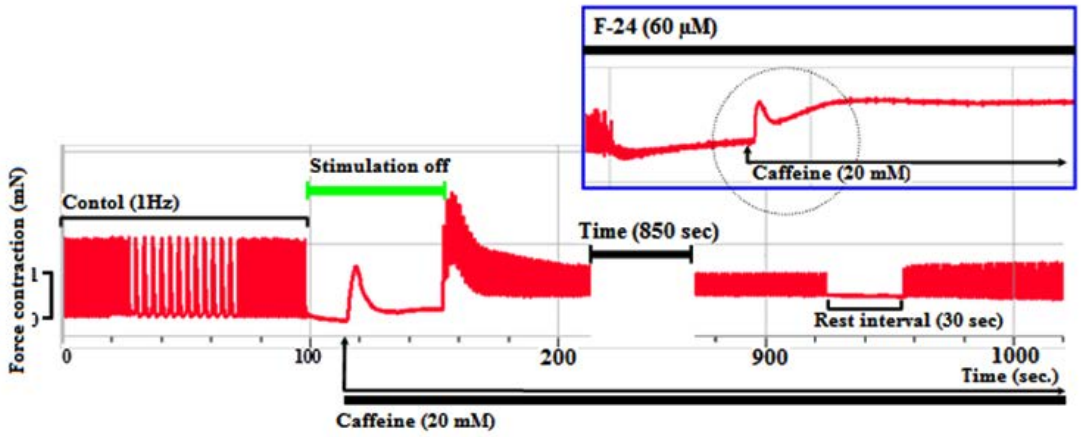

Fig. 8. Inotropic action of caffeine (20 mM) on papillary muscle contraction in the presence of F-24 (60 iM) alkaloid in the incubation environment (original image). The time caffeine (20 mM) added to the medium containing F-24 (60 µM) was indicated with a pointer. The frequency of Initial Stimulation is 1 Hz

for control ** – ?<0,01 (n=4–5).

Based on these results, it is suggested that the effects of negligible inotropic effects of the studied isoquinoline alkaloids were partially related to the change of [Ca^{2+}]in concentration by modulating PKC activity.

Effects of Isoquinoline Alkaloids on RyR2 Activity

In the series of subsequent experiments, we have studied the effects of isoquinoline alkaloids (N-14, F-14) on possible RyR2 activity. Methods, including RyR2 activator – caffeine (20 mM) in case of non-stimulus incidence of single contraction, allows us to estimate the amount of [Ca^{2+}]SR24. Under these conditions, no post-rest potentiation is observed for about 30 seconds after 15 minutes, which is explained due to caffeine (20 mM) [Ca^{2+}]SR excretion into cytosol completely [Bouchard, 1990]. In this case, caffeine (20 mM) causes a single contraction due to Ca^{2+} excretion from SR through RyR2 in non-stimulating conditions.

The concentration increase of [Ca^{2+}] in formed by the action of caffeine (20 mM) is normalized by the Na+/Ca^{2+} exchange function[25]. In incubation condition when [Na+]out=0, Na+/Ca^{2+} exchanger exporting function is blocked, and subsequently [Ca^{2+}]in ions concentration and amplitude force of contraction are maintained in a stable state under caffeine (20 μM)[26].

In our experiments when [Na+]out=0, caffeine (20 mM), without stimulus, was found to increase papillary muscle force contraction by 28±4.4%, compared to control. The amplitude force contraction of the papillary muscle, generated by caffeine (20 mM) in the presence of F-14 (40 μM) and N-14 (30 μM) isoquinoline alkaloids in the incubation environment, decreased for 18±3.7% and 35.6±4.1%, in comparison with the control group, respectively (Figure 7).

The ordinate axis shows force contraction of the papillary muscle, expressed as a percentage of the maximum value of 100%. The stimulation frequency is 0.5 Hz (t=36±0.5ºC). * – for control ** – ?<0.01 (n=3–5).

The results show, that the concentrations of Ca^{2+} ions in SR under the influence of F-14 and N-14 isoquinoline alkaloids are negatively affected. The experiments also revealed, that in F-24 isoquinoline alkaloid (60 μM) incubation condition, single-contraction changes into tonic contraction and its amplitude remains constant by caffeine (20 mM) (Fig. 8).

The time caffeine (20 mM) added to the medium containing F-24 (60 μM) was indicated with a pointer. The frequency of Initial Stimulation is 1 Hz.

This can be explained with the increase of [Ca^{2+}]in concentration through Na+/Ca^{2+} exchanger blockade together with the modulation of RyR2 under the influence of F-24, and constancy of [Ca^{2+}]in concentration and the contraction force amplitude with caffeine (20 mM)27.

CONCLUSION

Thus, the negative inotropic effect of N-14, F-14 and F-24 isoquinoline alkaloids on the papillary muscle contraction were established to be in association with partial blockade of Ca^{2+}L-channel in cardiomyocytes. It has been determined that negative inotropic effect of N-14, F-14 alkaloids occur depending on PKC modulation. The negative inotropic effect of F-24 isoquinoline alkaloid was suggested to arise from Na+/Ca^{2+} exchanger blockade together with RyR2 modulation.

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