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EVALUATION OF NEGATIVE INOTROPIC EFFECTS OF A ISOQUINOLINE ALKALOID DERIVATIVE, 1-(4-DIMETHYLAMYPHENYL)-6,7-DIMETHOXY-1,2,3,4-TETRAHYDROISOQUINOLINE.

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Abstract

This study evaluated the mechanism of the negative inotropic effect of an isoquinoline alkaloid derivative, 1-(4-dimethylamyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-24) using electrically stimulated rat left ventricular papillary muscle. F-24 produced a pronounced negative inotropic effect in a dose-dependent manner. This effect of F-24 was depended on the Ca^{2+} concentration in the bathing media and markedly reduced in the presence of nifedipine. Moreover, the negative inotropic effect of F-24 was decreased in the presence of lidocaine and in external media with high KCl (26 mM). Furthermore, F-24 significantly decreased contraction induced by low Na^{+} solution and ouabain, as well as, markedly inhibited post-rest potentiation of contraction. We conclude that the negative inotropic effect of F-24 may be mediated by multiple mechanisms involving either direct or indirect modulation of Ca^{2+} handling in cardiomyocytes resulting in a reduction in the amount of Ca^{2+} released from the SR and suppression of the contraction force.

Key words: alkaloid, muscle, papillary, ouabain, antiarrhythmic, cardiomyocytes

INTRODUCTION

Isoquinoline alkaloids constitute one of the largest groups of plant-derived compounds with a wide range of pharmacological activities are a promising candidate for developing new cardiovascular drugs. A significant number of isoquinoline alkaloids possess a potent antiarrhythmic activity mediated through multiple cellular mechanisms (Qian, 2002; Huang and Hong 1998). The antiarrhythmic effect of the well-known representative of these alkaloids tetrandrine are provided by blockage of L- and T-types calcium and sodium channels with slow recovery kinetics, thus terminating acute episodes of paroxysmal supraventricular tachycardia (Lau *et al*.,2001; Zhang *et al*., 2016). It also has been demonstrated that tetrandrine exerted a substantial negative inotropic and chronotropic effects associated with decreased intracellular Ca^{2+} due to the blockage of the voltage-operated Ca^{2+} channels and modulation of the sarcoplasmic reticulum Ca^{2+} loading and release functions (Wang *et al*., 1996; Wang and Zheng, 1997). Negative inotropic of tetrandrine, like most antiarrhythmic drugs, resulting in reductions in cardiac contractility and cardiac output is a serious adverse effect, limit its use in patients with already impaired left ventricular function (Liu *et al*., 1992; Chen *et al* .,2003).

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Recently, with the aim to find new effective antiarrhythmic agents a series of hydroxyethyl derivatives of 1-aryltetraisoquinoline alkaloids were synthesized (Zhurakulov *et al*., 2013; Zhurakulov *et al*.2014). In previous studies the effects of these derivatives on rat left ventricular papillary muscle contractility we found that among them the most potent negative inotropic activity exert 1-(4-dimethylamyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, designated as F-24 (Inoyat Jumayev *et al*.2020). Therefore, the aim of this study was further to characterize the negative inotropic effect of this new isoquinoline derivative and to define the mechanism of this action.

MATERIAL AND METHODS

All experimental protocol 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 drugs 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. In some experiments to inactivate the fast Na^+ channel, the K^+ concentration of the Krebs solution was raised to 26 mm by substituting KCl for equimolar NaCl. To test the drugs effect on Na^+/Ca^{2+} exchanger function a low Na^+ Krebs solution and ouabain were used. To prepare low Na^+ Krebs solution NaCl concentration was reduced to 36 mM by replacing the NaCl by equimolar choline chloride and nifedipine (0.1 μM) and ryanodine (4 μM) were added.

Drugs and Reagents. The derivative of isoquinoline alkaloids 1-(4- dimethylamyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-24) was synthesized by the Bischler-Napieralski cyclization with 3,4-disubstituted phenethylamine and aromatic acetic acid as starting materials in Institute of Plant compounds Uzbek Academy of Sciences. Nifedipine, phentolamine, propranolol, KB-R-7943, and ouabain were purchased from Sigma-Aldrich Chimie (Sigma, St. Louis, MO, U.S.A.).

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

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RESULTS AND DISCUSSION

Preliminary studies showed that the derivative of isoquinoline alkaloids 1-(4-dimethylamyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, designated as F-24, has a pronounced negative inotropic effect. As can be seen from Fig. 1, F-24 produced significant inhibition of the contraction force in a dose-dependent manner. The maximal reduction in the contraction force by 92,4 ± 3.1% of control value was obtained at 60 μM of F-24. The concentration of F-24 which produced half-maximal inhibition of the contraction force (IC_{50}) was of 15,1 μM.

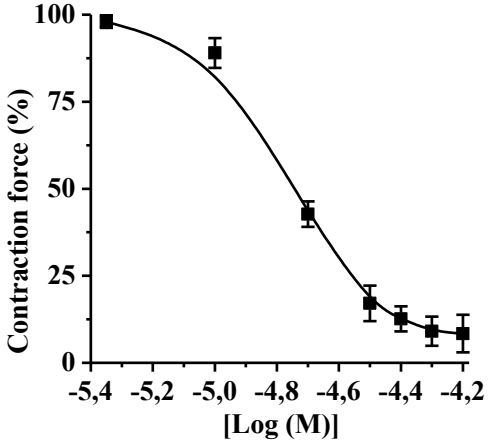

Fig.1. The effect of F-24 on the contraction force of the rat left papillary muscle. Concentration-response curve for the negative inotropic effect of F-24 in rat papillary muscle. Data are reported as mean ± SEM (n=5) and expressed as a percentage of control contraction, obtained in normal Krebs solution at 0.1 Hz before the addition of drugs, which was taken as 100%. $P < 0.05$ *vs* baseline.

Based on the data obtained we assumed that the negative inotropic action of F-24 may be similar to those found for class 1 antiarrhythmic drugs which are mainly related to impairment of Ca^{2+} handling in cardiac cells by alteration in the activity of Ca^{2+} transport system located in the sarcolemma and in the sarcoplasmic reticulum (SR) (Honerjager *et al*. 1986; Navada *et al*. 1994). Considering this to test the effect of F-24 on sarcolemmal Ca^{2+} transport systems for the first its action on the Ca^{2+}- contraction response curves in rat papillary muscle was examined. As shown in Fig.2,A cumulative addition of extracellular $CaCl_2$ from 0.5 mM to 2,5 mM caused stepwise increases in the contraction force. After pretreatment of papillary muscle with F-24 (60 μM), force development induced by increased $CaCl_2$ was significantly inhibited, indicating that the inhibition of Ca^{2+}-induced contraction by F-24 may be due to blockage of L-type Ca^{2+} channels. To verify further involvement of L-type Ca^{2+} channels in the inhibitory action of F-24 its effect was examined in the presence of nifedipine a typical L-type Ca^{2+} channel blocker. In this study treatment of papillary muscle with nifedipine resulted in a dose-dependent decrease in the contraction force, with IC_{50} value 0,01 μM. Subsequent administration of F-24 (60 μM) on top of nifedipine (0,01 μM) decreased the contraction force further from 50 ± 4,2% to 29,7 ± 3,3% (Fig. 2,B). As can be seen from Fig.2, B, under these conditions the effect of F-24 on the contraction force was less pronounced than in the absence of nifedipine, indicating that its inhibitory effect in the presence of nifedipine is markedly reduced.

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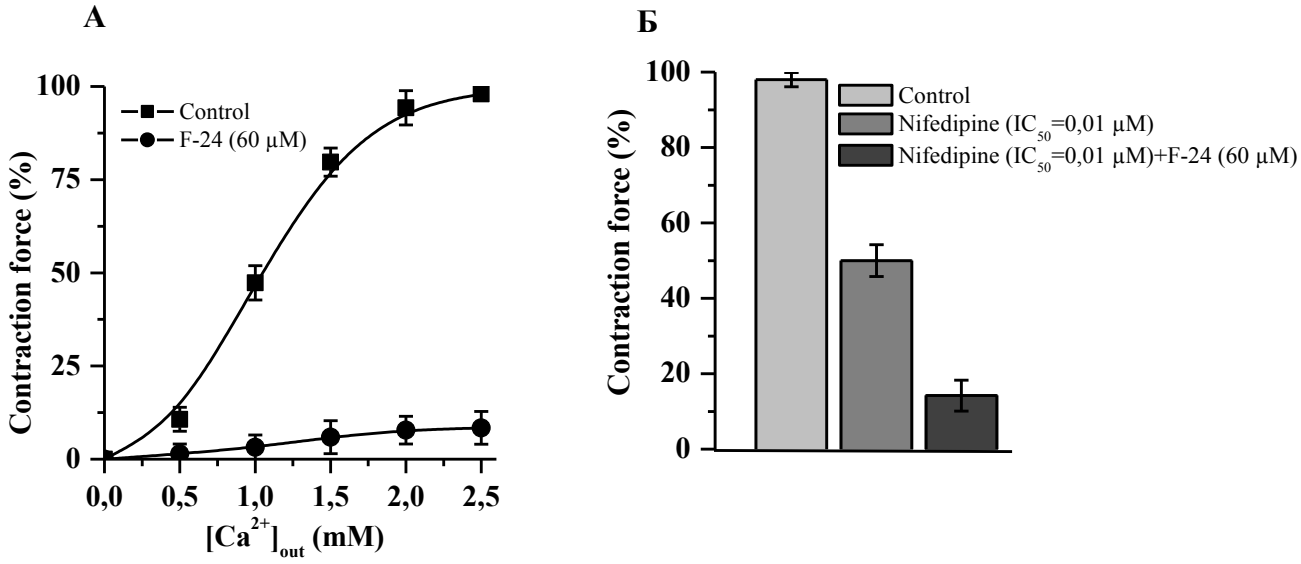

Fig.2. The effects of extracellular calcium concentration $[Ca^{2+}]_o$ and nifedipine on the negative inotropic effect F-24. (A) Effect of F-24 on the concentration-response curve of $CaCl_2$ in rat papillary muscle. (B) Effect of nifedipine (0,01 μM = IC_{50}) on the inhibitory action of F-24 on the contraction force of rat papillary muscle. Data are reported as mean ± SEM (n=5) and expressed as a percentage of control contraction, obtained in normal Krebs solution at 0.1 Hz before the addition of drugs, which was taken as 100%. $P < 0.05$ *vs* baseline.

Together, these results suggest that inhibition of Ca^{2+} influx through L-type Ca^{2+} channels may be a major determinant of the negative inotropic effect of F-24. However, F-24 administration on the top of nifedipine further decreased the contraction force suggesting that Ca^{2+} entering mechanism is not a major determinant of the negative inotropic effect of this alkaloids so that different mechanisms have to be involved.

It is well established that the negative inotropic effect of all the class 1 antiarrhythmic drugs strongly correlated with their inhibitory action on the fast Na^+ channels. By blocking the Na^+ channels and reducing $[Na^+]_i$, these agents lead to a fall in intracellular $[Ca^{2+}]_i$, via the Na^+/Ca^{2+} exchange system and thus suppress the contractile force of cardiac muscle (Heubach and Schule,1998; Wreight., 2001). Therefore, to evaluate the involvement of the fast Na^+ channels in the negative inotropic action of F-24 its effect in the presence of Na^+ channels blocker lidocaine was examined. In these experiments pretreatment of papillary muscle with lidocaine significantly reduced the contraction force in a dose-dependent manner with an IC_{50} value of 15 μM. When F-24 (60 μM) was added on top of lidocaine (15 μM) the contraction force decreased further from 50,4 ± 3,7 to 24,6 ± 4,2% (Fig.3, A).

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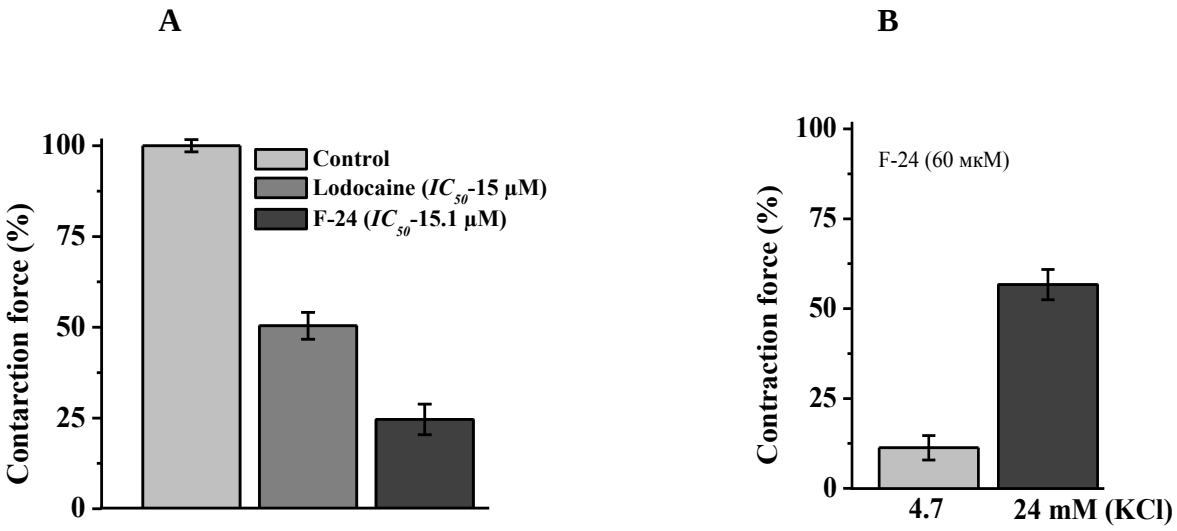

Fig.3. The effects of lidocaine and inactivation of fast Na^+-channels on the negative inotropic effect F-24. (A) Effect of lidocaine (15 μM = IC_{50}) on the inhibitory action of F-24 on the contraction force of rat papillary muscle. (B) Effect of F-24 on the contraction force of rat papillary muscle partially depolarized by high K^+ (24 mM). Data are reported as mean ± SEM (n=5) and expressed as a percentage of control contraction, obtained in normal Krebs solution at 0.1 Hz before the addition of drugs, which was taken as 100%. $P < 0.05$ *vs* baseline.

Although, in these experiments F-24 decreased the contraction force, its inhibitory effect under these conditions was less pronounced than in the absence of lidocaine, indicating that its effect in the presence of lidocaine is also markedly reduced. To further clarify the role of fast Na^+ channels in the inhibitory action of F-24 its effect was evaluated using preparations depolarized by increasing KCl (26 mM) to inactivate Na' channels. As can be seen from Fig.3, B in such preparation the inhibitory effect of F-24 also significantly reduced. These results indicate that inhibition of Na^+ influx through fast Na^+ channels also may be involved in the negative inotropic action of F-24. Together with data obtained suggested that the negative inotropic effect of F-24 was also mediated by a sodium-dependent mechanism and probably are related to the inhibition of Na^+ channels and subsequent impairment of the Na^+-dependent regulation of muscle contractility (Honerjäger *et al*.,1986; Sipido *et al*., 1995).

It is well known that there is a direct link between the activity of Na^+ channel, intracellular Na^+ concentration and Na^+/Ca^{2+}- exchanger activity that is one of the essential regulators of Ca^{2+} homeostasis in cardiomyocytes and thus an important modulator of the cardiac contractile function (Blaustein & Lederer,1999; Shigekawa & Iwamoto 2001; Khoshimov N.N et al 2020). Considering this we examined the effect of F-24 on papillary muscle contractions induced by low Na^+ solution and ouabain, which are mainly due to the Ca^{2+} entry via Na^+/Ca^{2+}-exchanger (Eisner &Sipido, 2004; Sheu & Fozzard, 1982). In these experiments replacement of the normal Krebs solution with modified low Na^+ (36 mM) solution containing nifedipine (0.1 μM) and ryanodine (4 μM) accompanied by an increase in the contraction force to 77.9 ± 4.1% of control. Under these conditions, application of F-24 (60μM) dose-dependently decreased the contraction force from 77,9 ± 2,3% to 12,7 ± 3,1 %. . Similarly, F-24 (60μM) decreased the contraction force induced by ouabain (20 μM) from 67.3±3.6% to 7,4 ± 2,6 % (Fig.4).

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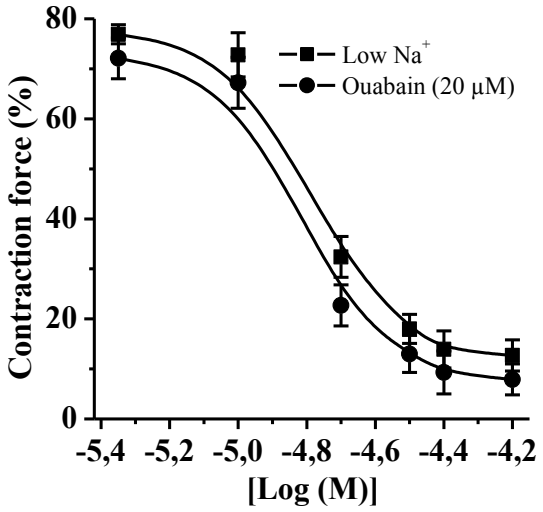

Fig. 4. The effects of F-24 on the contraction force of rat papillary muscle induced by low $[Na']_o$ solution and ouabain. The muscle was precontracted with low Na^+ solution (36 mM, filled square) and ouabain (20 µM, filled circle) in the presence of nifedipine (0,1 µM) and ryanodine (4 µM). Data are reported as mean ± SEM (n=5) and expressed as a percentage of control contraction, obtained in normal Krebs solution at 0.1 Hz before the addition of drugs, which was taken as 100%. $P < 0.05$ *vs* baseline.

The IC_{50} values of F-24 obtained from experiments with low Na^+ solution and ouabain were 37.8±2.7 µM, and 36.3 ± 3.4 µM, respectively. Since in these experimental conditions Ca^{2+} influx through L-type Ca^{2+}-channels and Ca^{2+} release from SR were blocked by nifedipine and ryanodine, the results indicate that this effect of F-24 on the contraction force induced by two different procedures is a result of direct blockage of Ca^{2+} influx through Na^+/Ca^{2+}-exchanger. Furthermore, from these results, it is evident that F-24 inhibited the contraction force induced by two different procedures almost with equal potency that provides the additional evidence that these effects of alkaloid are a result of direct blockage of Ca^{2+} influx through Na^+/Ca^{2+}-exchanger. Together, these results suggest that the inhibition of Ca^{2+} influx through Na^+/Ca^{2+} exchanger may also participate in the negative inotropic effect of F-24.

As already mentioned above the Na^+/Ca^{2+} exchanger as the main mechanism of calcium outflow from cardiomyocytes plays a key role in the regulation of $[Ca^{2+}]_i$ in cardiomyocytes. At the same time, there is evidence that in addition to $[Ca^{2+}]_i$ regulating function Na^+/Ca^{2+} exchanger working in synergism with L-type Ca^{2+} channels plays a central role in the modulation of the amount of Ca^{2+} in SR by indirect contribution to loading and release processes (Litwin *et al.* 1998; Bers 2001). Based on these data, we hypothesized that the modulation of the Na^+/Ca^{2+} exchanger function by F-24 may also affect the Ca^{2+} transport processes at the SR level. To test this hypothesis, we investigated the effect of F-24 on the post-rest potentiation of contraction, a phenomenon which reflects basic mechanisms involved in the Ca^{2+} fluxes through Na^+/Ca^{2+} exchanger and indicates the capacity of the SR to store and release Ca^2 (Shattock and Bers 1989, Bers and Christensen 1990). The post-rest potentiation of contraction was studied after a 30 s rest duration, and at an $[Ca^{2+}]_o$ of 0.5 mM because its evaluation is more sensitive at a low $[Ca^{2+}]_o$. In Fig.5, A original tracings illustrate the post-rest potentiation of contraction behavior of papillary muscles from control and treated with F-24.

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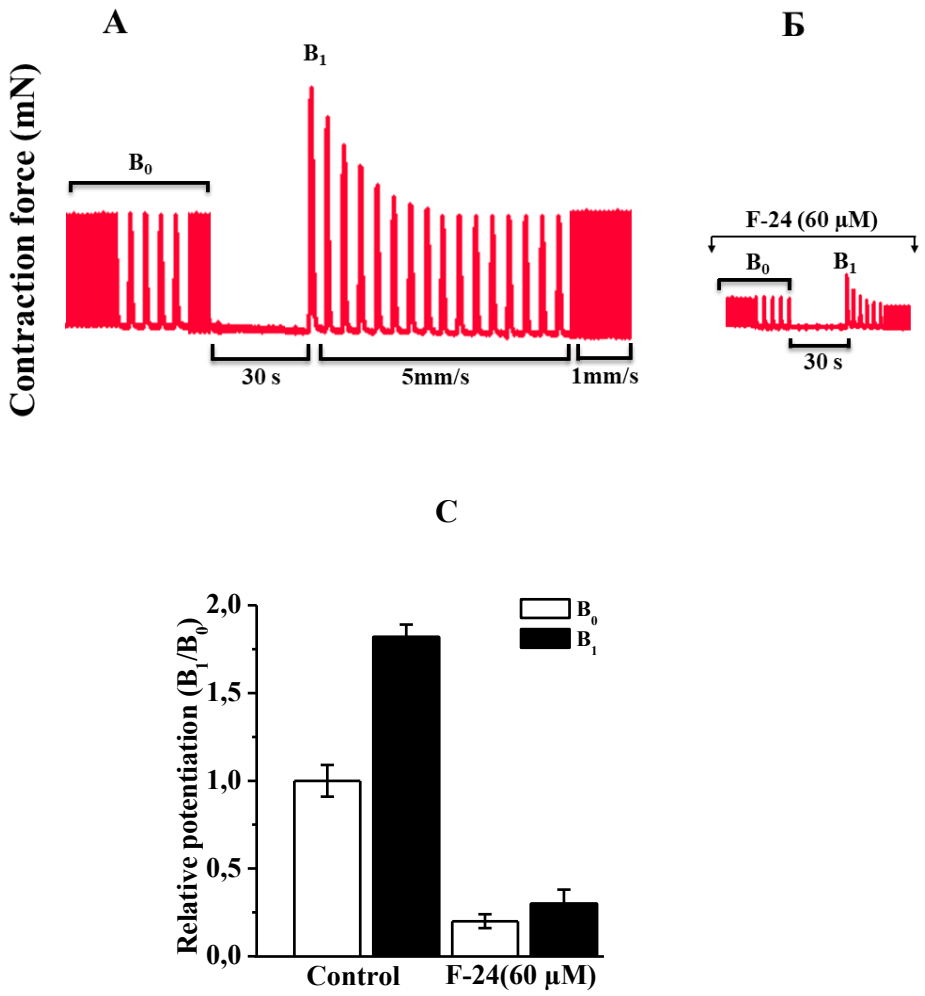

Fig.5. The effect of F-24 on the post-rest potentiation of contraction in rat papillary muscle. (A, B) Representative tracing shows the development of post-rest potentiation of contraction after 30 s rest period in the absence (A) and presence (B) of F-24 (60µM). (C) The relative potentiation of contraction (expressed as the ratio B1/B0) before and after administration of 60 µM F-24. Data are reported as mean ± SEM (n=5) and expressed as a percentage of control contraction, obtained in normal Krebs solution at 0.1 Hz before the addition of drugs, which was taken as 100%. $P < 0.05$ *vs* baseline.

The figure shows that in control conditions, the amplitude of first post-rest contraction (B_1) was significantly increased by 82,3±6,1 % of the last steady-state contraction before the rest (B_0).

There was also a significant decrease in the ratio B_1/B_0 from 1,8 to 1,5 (Fig.5, B). At the same time, as can be seen from Fig.5 C simultaneously with the decrease in the amplitude of B_1, the decrease in the amplitude of steady-state contraction (B_0) also occurs. This decrease in the amplitude of B_0 presumable is due to blockage of Na^+ channels that may secondarily alter Ca^{2+} transport so that a decreased Ca^{2+} influx would reduce $[Ca^{2+}]_i$ and thus suppress contractility (Pieske *et al.* 1999). If this effect of F-24 is mainly due to a decrease in the Ca^{2+} influx through L-type Ca^{2+}channels the decrease in B_0 should be accompanied by a similar decrease in the B_1, without significant changes in the ratio B_1/B_0. However, in the presence of F-24, the ratio B_1/B_0 significantly decreased from 1,8 to 1,5 indicating that depression of B_1 by F-

24 at least in part can be explained by its simultaneous inhibition of Ca^{2+}influx and impairment of loading or release function of SR.

CONCLUSION

The present results demonstrate that isoquinoline alkaloid derivative, 1-(4- dimethylamyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-24) exerts a pronounced negative inotropic effect mediated by multiple mechanisms involving either direct or indirect modulation of Ca^{2+} handling in cardiomyocytes. By direct blockage of L-type Ca^{2+} channels, F-24 can decrease Ca^{2+} influx in cardiomyocytes and thus reduce the Ca^{2+} content in SR. At the same time by blocking the fast Na^{+}-channels and decreasing intracellular Na^{+} concentration F-24 can alter Na^{+}/Ca^{2+} exchanger activity and thus also reduce the Ca^{2+} content in the SR. In both cases, these effects of F-24 are accompanied by a reduction in the amount of Ca^{2+} released from the SR resulting in suppression of the contraction force.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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