

# EFFECTS OF SOME ISOQUINOLINE ALKALOIDS ON CARDIAC MUSCLE CONTRACTILE ACTIVITY

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## Abstract

In studies, 1-(4'-methoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-4) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-36) cardioprotective properties of alkaloids were studied. In this case, cardiomyocyte  $Ca^{2+}$ -transport systems in the presence of their specific inhibitors were carried out under hypoxia conditions. Blockers of potential-dependent  $Ca^{2+}$ -channels, such as nifedipine and  $Na^+/Ca^{2+}$ - exchange blockers such as  $NiCl_2$  were used. The purpose of this study is to evaluate the cardioprotective properties of F-4 and F-36 isoquinoline alkaloids under hypoxia conditions in vitro and to expand the understanding of hypoxia-related molecular signaling pathways.

**KEYWORDS:** *papillary muscle, Ca-channels,  $Na^+/Ca^{2+}$ - exchange, hypoxia.*

## INTRODUCTION

Today, cardiovascular diseases are one of the main causes of death in developed countries<sup>1</sup>. According to the World Health Organization, 17.5 million people die each year from various heart diseases. The modern concept of prevention and treatment of these diseases and the identification of biologically active substances isolated from medicinal plants with cardioprotective properties are urgent problems of today<sup>2,3</sup>. Among them, it is important to find new drugs with cardioprotective properties, with high efficiency and few side effects, and to determine their mechanisms of action. Among the heterocyclic compounds, alkaloids have a wide range of pharmacological activities, and many of them have been used in traditional or modern medicine or as starting points for drugs<sup>4</sup>. Today, the most studied alkaloids from a pharmacological point of view are isoquinoline, indole, and purine alkaloids. Also, from the isoquinoline alkaloids, the pharmacology of berberine and its clinical use for its antiarrhythmic effects have begun to be used in the treatment of hypertension<sup>5</sup>. In general, drugs whose component consists mainly of isoquinoline alkaloids are effectively used today in the treatment of atherosclerosis, hypertension, myocardial infarction, cardiomyopathy, heart failure, and arrhythmias<sup>6</sup>. Taking this into account, the cardioprotective properties of F-4 and F-36 isoquinoline alkaloids were studied.

## MATERIAL AND METHODS

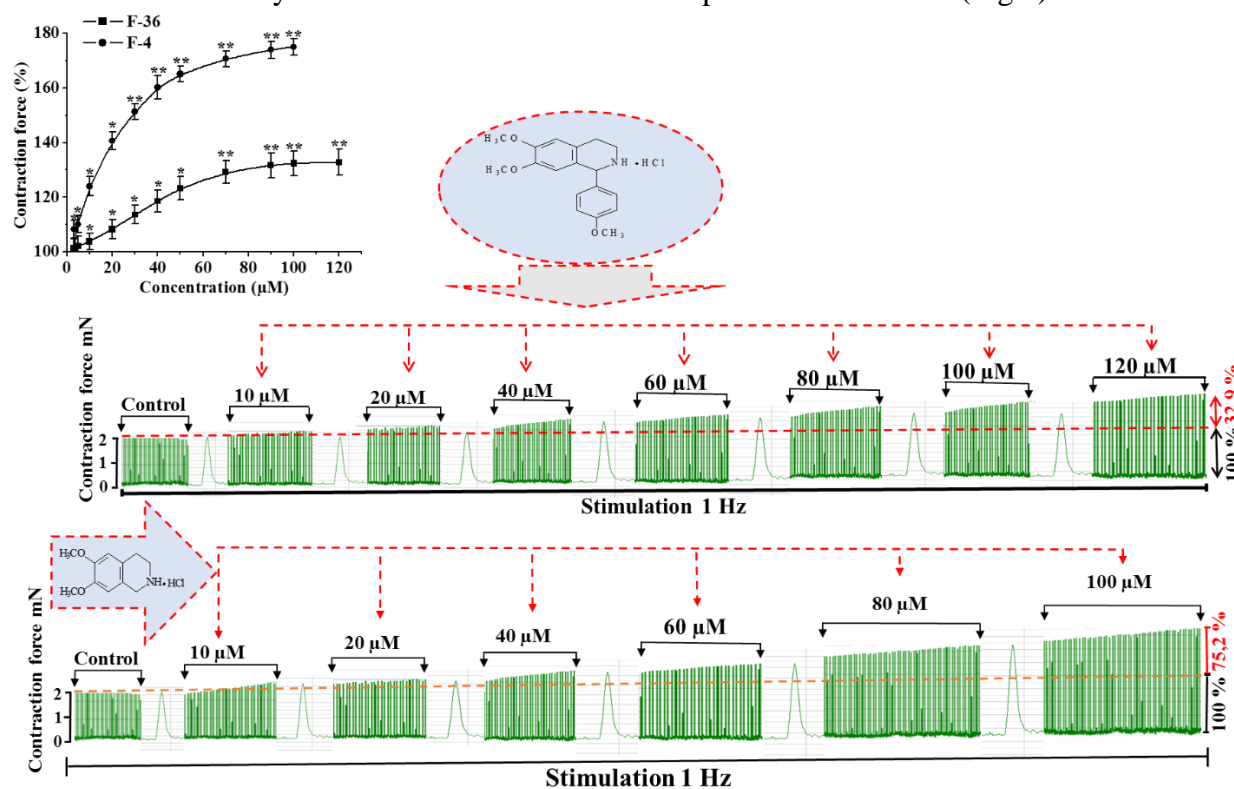
Purebred, white rats (100–250 g) were used in the experiments, and the international Declaration of Helsinki and the International Council of Medical Scientific Societies (CIOMS) (1985) rules for working with experimental animals were followed.

The mechanographic method (Mayflower Tissue Bath System, Hugo Sachs Electronic, Germany) and the hardware-software complex (LabScribe 2, World Precision Instruments, USA) were used to study the mechanism of action of biologically active substances on the functional activity of the myocardium of experimental animals in vitro. Oxygenated carbogen (O<sub>2</sub>–95% and CO<sub>2</sub>–5%) with the following composition is continuously perfused with Krebs saline: NaCl – 150; KCl – 4; CaCl<sub>2</sub> – 1,8; MgCl<sub>2</sub> – 1; NaHCO<sub>3</sub> – 14; NaH<sub>2</sub>PO<sub>4</sub> – 1,8; C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> – 11,5; 11 C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> (pH=7,4). A hypoxia model was called by substituting nitrogen (N–95% and O<sub>2</sub>–5%) for oxygen in the perfused Krebs saline solution described above.

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).

## RESULTS AND DISCUSSION

In preliminary studies, it was found that F-4 and F-36 isoquinoline alkaloids have a positive inotropic effect on the activity of papillary muscle contraction of the rat heart and increase the force of muscle contraction at all concentrations. In the experiments, it was noted that F-4 (10-100 μM) and F-36 (10-120 μM) alkaloids increase the force of cardiac papillary muscle contraction by 32.9±4.6% and 75.7±4.7% compared to the control (Fig.1).

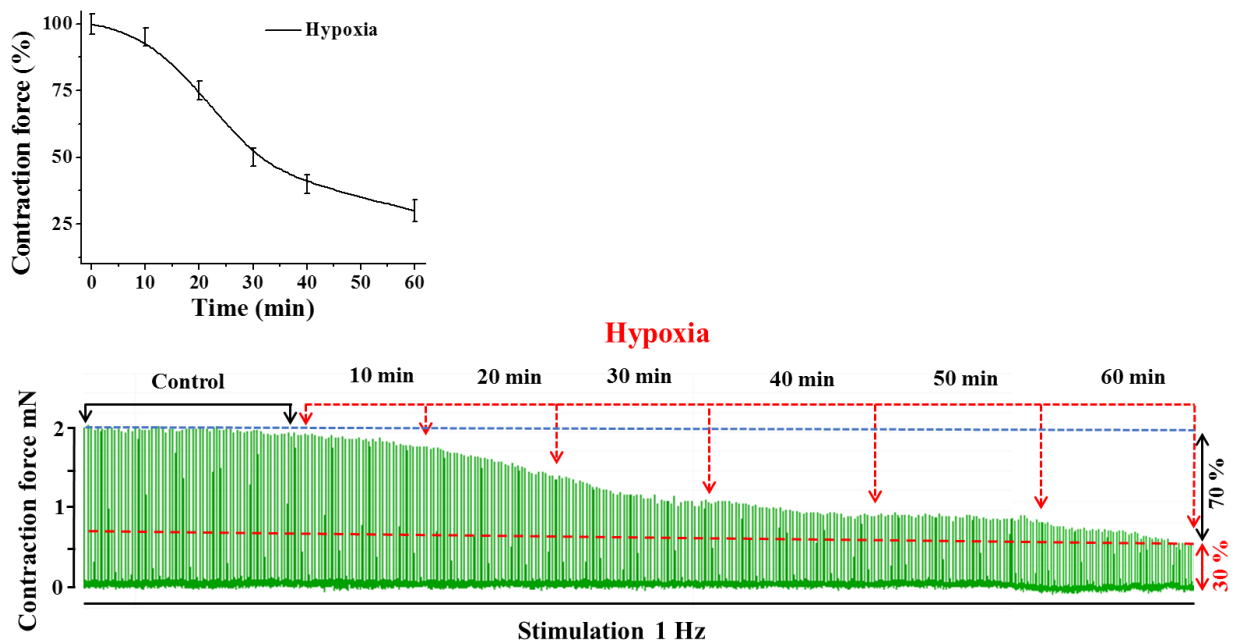


**Fig.1.** Dose-dependent effects of F-4 and F-36 isoquinoline alkaloids on the contractile activity of rat heart papillary muscle. The ordinate axis shows the amplitude

value of the contraction force expressed as a percentage (%) compared to the maximum. The frequency of stimulation of the drug is 1 Hz. In all cases \* -  $p < 0.05$ ; \*\* -  $p < 0.01$   $n = 6$ .

There are a large number of pathophysiological processes underlying the development of heart diseases, among which the dysfunction of  $Ca^{2+}$  homeostasis and  $Ca^{2+}$ -transport systems of cardiac muscle cells plays a leading role. Ischemia/hypoxia conditions play a key role in the development of these disorders, and the main energy source in cardiomyocytes is ATP and creatine phosphate products<sup>7</sup>. At the same time, in the conditions of ATP and creatine phosphate deficiency, the function of the  $Ca^{2+}$ -transport system, which is directly related to the energy consumption activity of macroergs, is disturbed. These disorders, in turn, negatively affect the functions of other  $Ca^{2+}$ -transport systems involved in maintaining  $Ca^{2+}$  homeostasis and circulation of  $Ca^{2+}$  ions in cardiac muscle cells<sup>8</sup>.

Taking into account the above, the hypoxia model was called by replacing 95% oxygen in the incubation medium with 95% nitrogen in the next experiments. It was found that when the perfused Krebs solution is aerated with nitrogen for 60 minutes, the force of papillary muscle contraction is reduced to  $27.6 \pm 3.1\%$  compared to the control (Fig.2. A, B.).

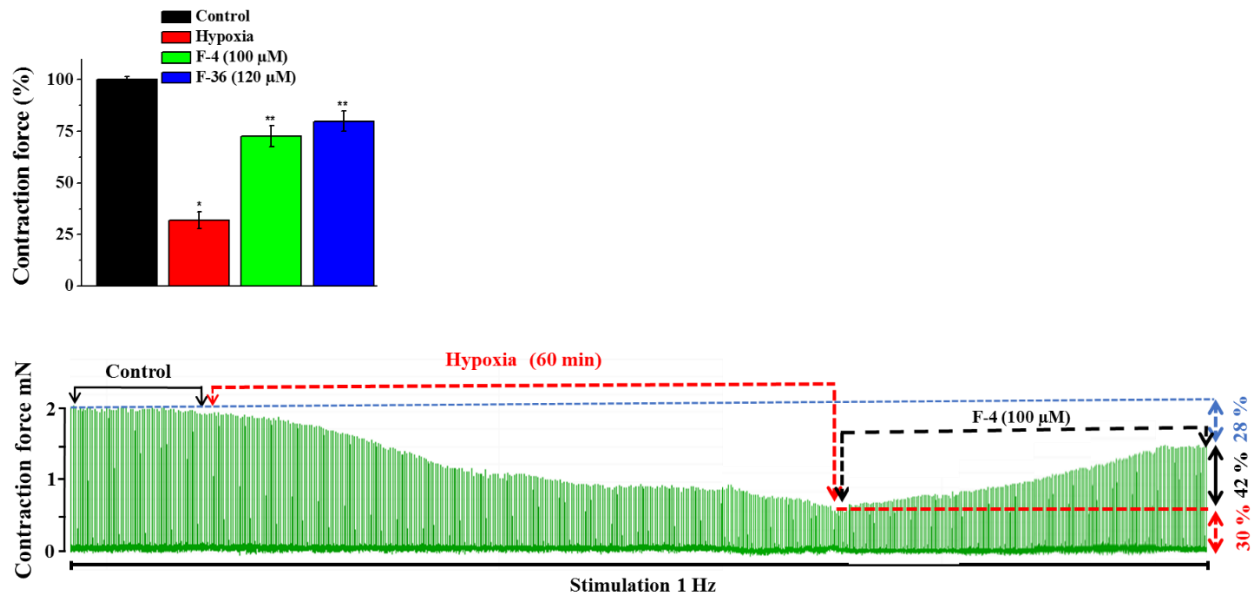


**Fig.2. A. Effect of hypoxia on the contractile activity of the papillary muscle of the rat heart.** On the ordinate axis - muscle contraction force, the control obtained under normal oxygenation of a physiological solution is expressed as a percentage and taken as 100%. On the abscissa axis is the deoxygenation time of the solution perfused with nitrogen. The frequency of stimulation of the drug is 1 Hz. In all cases \* -  $p < 0.05$ ; \*\* -  $p < 0.01$   $n = 5$ . **B.** Papillary muscle contractile activity under hypoxia (original entry).

Considering that the force of papillary muscle contraction is carried out with the participation of  $Ca^{2+}$  ions, the results of these experiments show that the decrease in muscle contraction force under hypoxia is accompanied by a decrease in  $[Ca^{2+}]_i$  ions<sup>9</sup>.

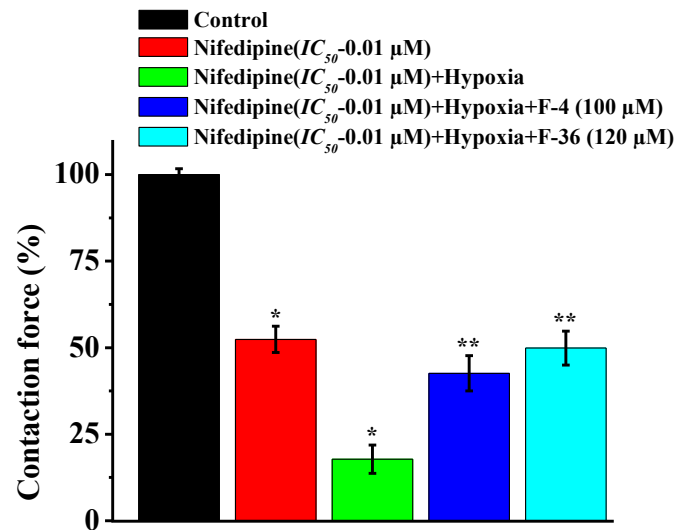
To evaluate the cardioprotective properties of the isoquinoline alkaloids mentioned above, the effect of hypoxia on the papillary muscle contraction activity of the rat heart was

studied. The effect of F-4 (100  $\mu$ M) and F-36 (120  $\mu$ M) isoquinoline alkaloids under hypoxia was  $72.6 \pm 5.1\%$  and  $79.9 \pm 4.9\%$ , respectively, compared to the control (Fig.3).



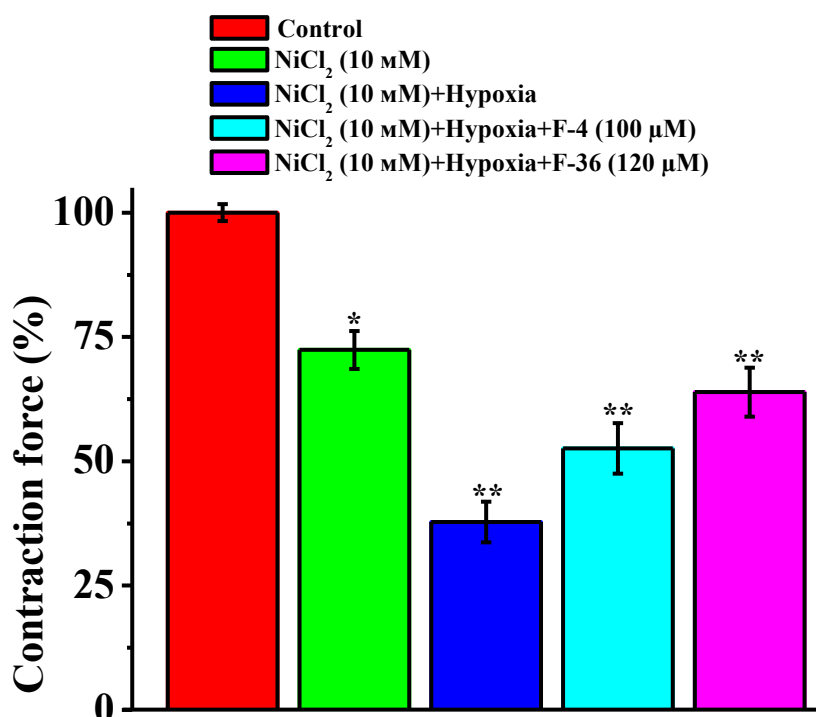
**Fig.3. Effects of isoquinoline alkaloids F-4 and F-36 under hypoxic conditions on the contractile activity of the papillary muscle of the rat heart.** On the ordinate axis, the amplitude value of the contraction force is expressed as a percentage (%) compared to the maximum. The frequency of stimulation of the drug is 1 Hz. In all cases \* -  $p < 0.05$ , \*\* -  $p < 0.01$ ;  $n = 4$ .

The obtained data show that the studied alkaloids have cardioprotective properties under hypoxia conditions in vitro and effectively eliminate the disturbances in the contraction activity of papillary muscle cells caused by hypoxia. One of the main causes of positive inotropic is the modulation of  $Ca^{2+}_L$ -channel activity in cardiomyocytes and, in turn, changes in  $[Ca^{2+}]_i$ <sup>10</sup>. In subsequent experiments, the positive inotropic effect of the studied substances was studied using nifedipine, a specific blocker of the potential-dependent activating  $Ca^{2+}_L$ -channel located in the cardiomyocyte sarcolemma under hypoxia. It was observed that nifedipine ( $IC_{50} = 0.01 \mu$ M) decreased papillary muscle contraction force by  $17.8 \pm 4.1\%$  compared to control in hypoxia-induced in vitro. In this case, the effect of F-4 (100  $\mu$ M) and F-36 (120  $\mu$ M) isoquinoline alkaloids on papillary muscle contraction force was  $42.4 \pm 5.1\%$  and  $49.9 \pm 4.9\%$ , respectively, compared to the control (Fig.4). It can be seen that the isoquinoline alkaloids F-4 and F-36 indicate the presence of  $Ca^{2+}_L$ -channels in the cardioprotective properties under hypoxia conditions.



**Fig. 4. Evaluation of the participation of  $Ca^{2+}_L$ -channels in the hypoxia of F-4 and F-36 isoquinoline alkaloids on the activity of papillary muscle contraction of the rat heart.** The ordinate axis shows the amplitude value of the contraction force expressed as a percentage (%) compared to the maximum. The frequency of stimulation of the drug is 1 Hz. In all cases \* -  $p < 0.05$ , \*\* -  $p < 0.01$ ;  $n = 5$ .

It is known that the  $Na^+/Ca^{2+}$ -exchange system in cardiomyocytes in the normal physiological condition is the main system that brings  $Na^+$  ions into the cell<sup>11</sup> and releases  $Ca^{2+}$  ions out of the cell. Therefore, in the experiments, we studied the effect of the studied substances on the cardiomyocyte  $Na^+/Ca^{2+}$ -exchange system under hypoxia conditions<sup>12</sup>. To evaluate the effect of isoquinoline alkaloids F-4 (100  $\mu$ M) and F-36 (120  $\mu$ M) on papillary muscle contraction activity, the location of the  $Na^+/Ca^{2+}$ -exchange system located in the sarcolemma of cardiomyocytes was used at a concentration of 10 mM of its non-specific blocker -  $NiCl_2$ . Under these conditions, papillary muscle contraction force was  $37.8 \pm 4.1\%$  and  $63.9 \pm 4.9\%$ , respectively, compared to the control (Fig. 5).



**Fig. 5. Evaluation of the role of Na<sup>+</sup>/Ca<sup>2+</sup>-exchange system of F-4 and F-36 isoquinoline alkaloids on papillary muscle contractile activity of rat heart under hypoxia.** The ordinate axis shows the amplitude value of the contraction force 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.

Experiments conducted with NiCl<sub>2</sub> under hypoxia revealed that the positive inotropic and cardioprotective activity of the studied isoquinoline alkaloids indicates that the Na<sup>+</sup>/Ca<sup>2+</sup>-exchange system is less involved.

In general, based on the results of the above research, we can conclude that F-4 and F-36 isoquinoline alkaloids have strong cardioprotective properties, and it was found that they effectively eliminate disturbances in the activity of muscle contraction in hypoxia-induced in the papillary muscle preparation of the rat heart under in vitro conditions. It can be concluded that this effect of alkaloids goes partly through Na<sup>+</sup>/Ca<sup>2+</sup>-exchange and mainly through modulation of Ca<sup>2+</sup><sub>L</sub>- channels.

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**Conflict of interest.** The authors have declared that no conflict of interest exists.

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