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Research Article

# Evaluation of Negative Inotropic Effects of an Isoquinoline Alkaloid N-14

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## Abstract

In studies, the alkaloid 1-(2-Chloro-4,5-methylenedioxyphenyl)-2-hydroxyethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (N-14) had a negative inotropic effect on the activity of the papillary muscle contraction of the rat heart detected. $Ca^{2+}$ ions from SR play an important role in the process of contraction of the heart muscle. With this in mind, the negative inotropic effect of the N-14 alkaloid was investigated with the modification of the accumulation processes of $Ca^{2+}$ ions to SR. To clarify this, we examined the effects of the alkaloid being studied on SERCA2a and RyR2. To do this, the inhibitor of SERCA2a - cyclopiazonic acid (CPA) and RyR activator caffeine, which provide the accumulation of $Ca^{2+}$ ions in SR, were used.

**Key words:** isoquinoline alkaloid; cardiac muscle; ion channels

## Introduction

Alkaloids have a wide range of pharmacological activity, many of which are used in traditional or modern medicine or as a starting point for medicines. The most widely studied alkaloids from a pharmacological point of view today are isoquinoline, indole, and purine alkaloids [1]. Isoquinoline alkaloids have begun clinical use for the treatment of arrhythmias and hypertension. In general, drugs consisting mainly of isoquinoline alkaloids are effectively used today in the treatment of atherosclerosis, hypertension, myocardial infarction, cardiomyopathy, heart failure, and arrhythmias [2].

Recently, to find new effective antiarrhythmic agents a series of hydroxyethyl derivatives of 1-aryl tetra isoquinoline alkaloids were synthesized [3]. In previous studies on the effects of these derivatives on rat left ventricular papillary muscle contractility we found that among them the most potent negative inotropic activity exert 11- (2-Chloro-4,5-methylenedioxyphenyl)-2-hydroxyethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, designated as N-14 [4]. 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 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 of 5 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 signals from the force-displacement transducer amplified by transducer amplifier (SI–BAM21–LCB, WPI, Inc.) were converted to a digital form by Works computer interface (IX/228-S Data Acquisition System) and recorded to a computer using iWorx Labscribe 2 software. 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 was 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.

**Drugs and Reagents**. The derivative of isoquinoline alkaloids 11-(2-Chloro-4,5-methylenedioxyphenyl)-2-hydroxyethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (N-14) was synthesized by the Bischler-Napieralski cyclization with 3,4-disubstituted phenethylamine and aromatic acetic acid as starting materials in the Institute of Plant compounds Uzbek Academy of Sciences.

Cyclopiazonic acid and caffeine 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 Origin Pro 9.1 software (OriginLab Co., U.S.A).

## Results.

Among the chemical compounds with heterocyclic structure, the alkaloids isoquinoline are considered to have a wide range of physiological effects [5].

In particular, some of them have been described as antiarrhythmic and cardiotropic effects on diseases of the cardiovascular system. In our experiments, when we examined the dose-dependent effect of the N-14 alkaloid on rat heart papillary muscle contraction activity, it was found that this alkaloid had a negative inotropic effect at all concentrations. Fig. 1 shows the original record of the negative inotropic effect of the N-14 alkaloid in section A, showing that this alkaloid reduces papillary muscle contraction activity depending on the dose. Also shown in Fig.1, Part B, the N-14 alkaloid at all concentrations has a negative inotropic effect on rat heart papillary muscle contraction activity. In this study, the effect of the N-14 alkaloid at a concentration of 5 µM to a concentration of 30 µM was found to have a maximal effect at a concentration of 30 µM, reducing muscle contraction force by 72.6±4.1% compared to the control.

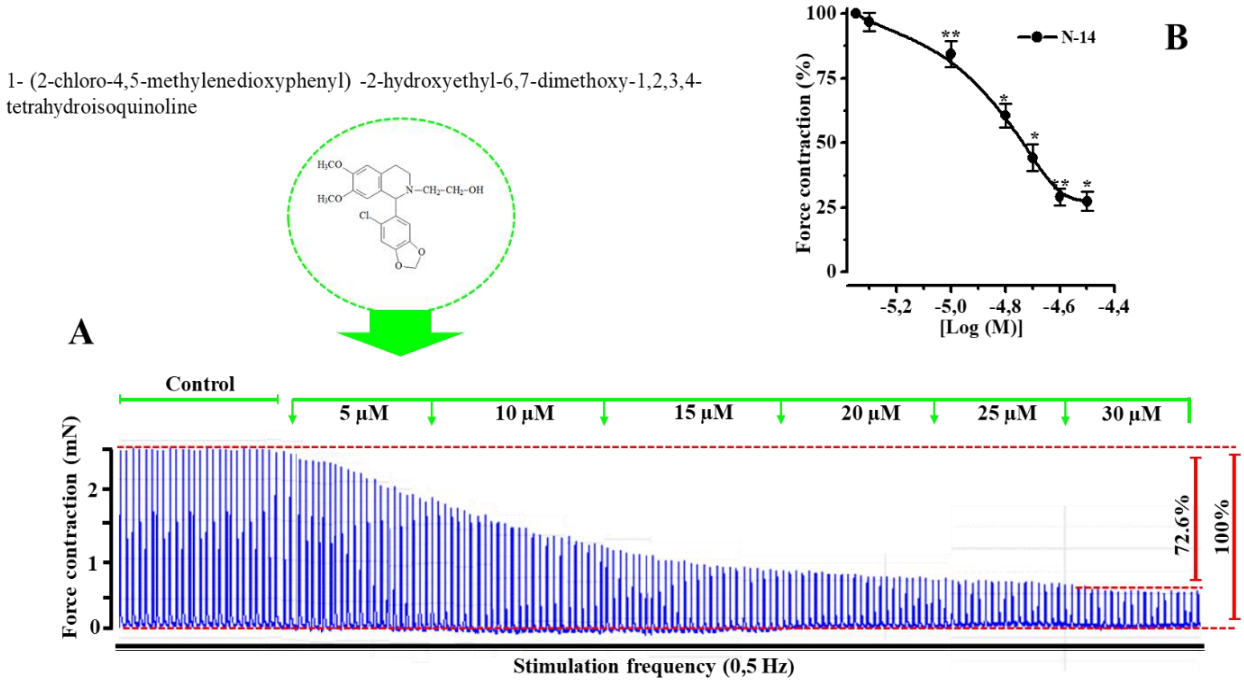


**Figure 1:** The effect of N-14 on the contraction force of the rat left papillary muscle

(A) Representative tracing shows the effect of N-14 on isometric contractions of rat papillary muscle, driven at 0.5 Hz. (B) Concentration-response curve for the negative inotropic effect of N-14 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.

It is known that one of the main reasons for the negative inotropic effect of many pharmacological agents lies in the decrease in the amount of intracellular ($[Ca^{2+}]_i$) ions in these cardiomyocytes. Some of these agents reduce the entry of $[Ca^{2+}]_i$ into the internal environment of cardiomyocytes from outside the cell, while others modify the $Ca^{2+}$-transport systems of the intracellular $Ca^{2+}$-deposition [6,7]. $Ca^{2+}$ ions from SR play an important role in the process of contraction of the heart muscle. In subsequent experiments, the effect of the alkaloid N-14 on SR $Ca^{2+}$-transport systems was investigated during post-rest potentiation. The process of post-rest potentiation is explained by a sharp increase in the initial contraction force when this heart muscle stimulation is stopped for 30 seconds and resumed after a certain period of rest [8]. After resting for 30 s, cardiomyocytes accumulate more $Ca^{2+}$ ions than in the previous physiological order of SR, and more $Ca^{2+}$- ions are released into the cytosol when re-stimulation is given. In this case, a sharp increase in the initial contraction force is observed. Post-rest potentiation is an adequate method that is very widely used in the study of changes in the $[Ca^{2+}]$ SR concentration in cardiomyocytes. At the same time, as a result of the contraction of cardiomyocytes, cardiomyocytes accumulate more $Ca^{2+}$ ions than in the physiological state due to $Ca^{2+}$ ions released from the SR, and after the recovery of excitation leads to the release of more $Ca^{2+}$-ions [9].

In our control experiments, it was found that when the papillary muscle stimulation was stopped for 30 s and returned to the previous excitation, the amplitude of the first contraction force increased by 94.4±5.6%. When we examined the effect of the N-14 alkaloid on the post-rest potential value under the influence of a concentration of 30 µM under these conditions, it was found that it decreased by 21.7±3.3% compared to the control (Fig.2.).

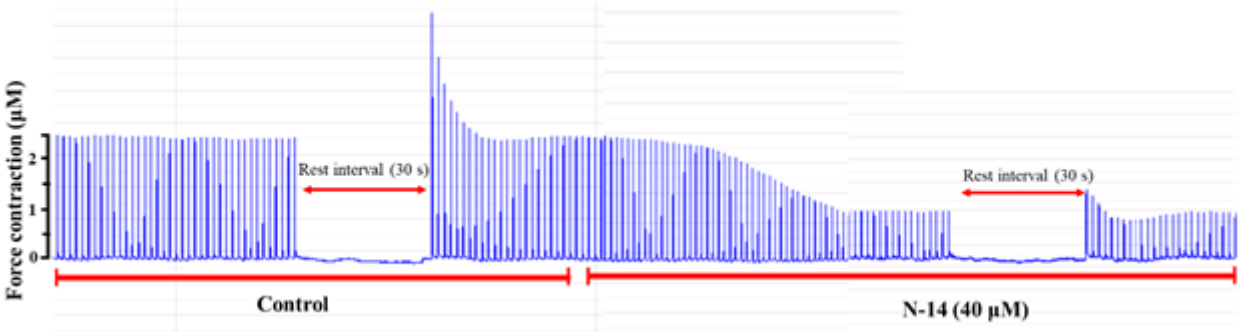


**Figure 2:** *The effect of N-14 on the post-rest potentiation of contraction in rat papillary muscle*

Data are reported as mean±SEM (n=5) and expressed as a percentage of control contraction, obtained in normal Krebs solution at 0.5 Hz before the addition of drugs, which was taken as 100%. P<0.05 vs baseline.

According to the analysis of the results of this experiment, the alkaloid N-14 affects the SR function, reducing the amount of $Ca^{2+}$ ions emitted from it, resulting in a decrease in the value of post-rest potentiation in the papillary muscle. The negative inotropic effect of the N-14 alkaloid is explained by its effect on SR by reducing the amount of $Ca^{2+}$ ions accumulated and excreted.

Based on the analysis of the results of this experiment, it can be concluded from the change in post-rest potentiation value that N-14 alkaloid affects SR function that the negative inotropic effect of this alkaloid on papillary muscle contraction activity is related to cardiomyocytes [$Ca^{2+}$] SR dynamics.

### Effect of N-14 alkaloid on RyR2 activity

In the next series of experiments, we studied the effect of the N-14 alkaloid on RyR2 activity. It was noted that [$Ca^{2+}$], a method that allows estimating the amount of SR - i.e., RyR2 activator - caffeine (20 mM) in the case of incubation, causes a single contraction without stimulation [10]. Under these conditions, no post-rest potentiation was observed during the 30-second pause after ~ 15 minutes, which is explained by the release of [$Ca^{2+}$] SR into complete cytosol under the influence of caffeine (20 mM). At the same time under the influence of caffeine (20 mM) in the absence of stimulation causes a phase single contraction due to the activation of $Ca^{2+}$ output from SR through RyR2. The increase in the concentration of [$Ca^{2+}$] induced by caffeine (20 mM) is normalized by the $Na^{+}/Ca^{2+}$ -change function [11]. In the incubation environment $[Na^{+}]_{out}$=0, the extracellular transport function of $Na^{+}/Ca^{2+}$-changeable $Ca^{2+}$ ions is blocked and, in turn, the concentration of [$Ca^{2+}$] in and the amplitude of the contraction force are kept stable under the influence of caffeine (20 mM). Experiments have shown that caffeine (20 mM) increases the force of papillary muscle contraction by 28±4.4% under control under $[Na^{+}]_{out}$=0 conditions without stimulation. In the presence of the alkaloid N-14 (30 µM) isoquinoline in an incubation medium, the amplitude of papillary muscle contraction force caused by caffeine (20 mM) decreased by 31.3±4.1%, respectively, relative to control (Fig.3).

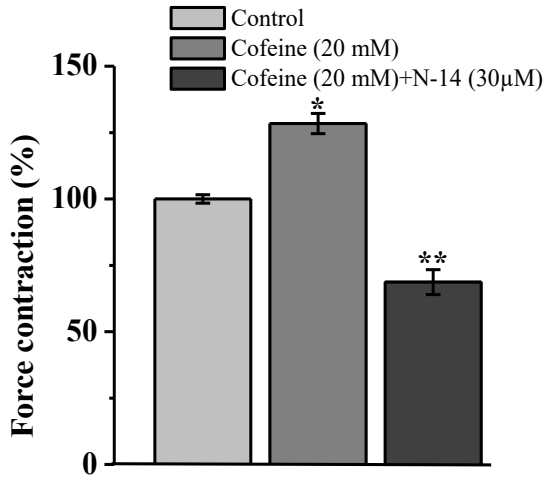


**Figure 3:** *CP RyR2 activator is the effect of the N-14 alkaloid in the incubation of caffeine (20 mM)*

On the ordinate axis - the force of contraction of the papillary muscle is expressed as a percentage of the maximum value assumed to be 100%. The stimulation frequency is 0.5 Hz (t=+36±0.5 ºC). Relative to the control * - p <0.05, ** - p <0.01 (n = 5).

The results show that the negative inotropic effect of the studied N-14 isoquinoline alkaloid causes a decrease in the concentration of $Ca^{2+}$ ions in the cytosol. Thus, in the absence of this alkaloid stimulation, caffeine-induced papillary muscle contraction was found to reduce the force of individual contraction.

This is due to the modulation of RyR2 under the action of the N-14 isoquinoline alkaloid, an increase in the concentration of [$Ca^{2+}$] in $Na^{+}/Ca^{2+}$ -blocking blockade, and, in turn, the stabilization of the concentration and amplitude of [$Ca^{2+}$] in the conditions of caffeine (20 mM). can be explained.

### Effect of N-14 alkaloid on SERCA2a activity

SERCA2a plays an important role in regulating the concentration of $Ca^{2+}$ ions in cardiomyocytes and plays a key role in cardiac muscle relaxation

by allowing $Ca^{2+}$ ions to enter SR [12]. Based on the results of the above experiment, the negative inotropic effect of the N-14 alkaloid may be related to the modification of the processes of accumulation of $Ca^{2+}$ ions to SR. To clarify this hypothesis, we examined the effect of the alkaloid being studied on SERCA2a. To do this, the inhibitor of SERCA2a, which initially provides the accumulation of $Ca^{2+}$ ions in SR, is cyclopiazonic acid (CPA) [13].

In control experiments, the papillary muscle contraction activity of CPA at an excitation frequency of 1 Hz was examined in a dose-dependent manner (1–10 μM). CPA was found to reduce the force of papillary muscle contraction by 80.7±4.8%. The half-maximum concentration of CPA for papillary muscle contraction force was $IC_{50}$-5.6 μM (Fig.4).

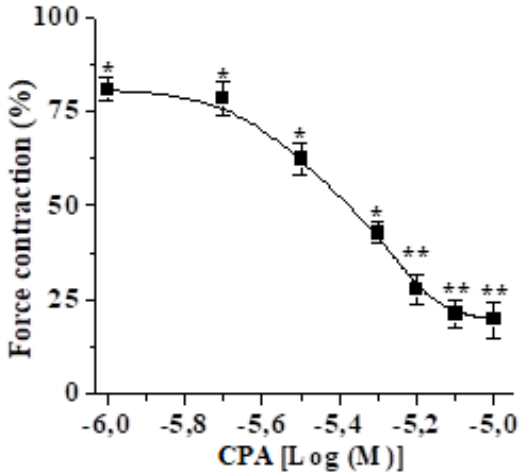


**Figure 4:** *Dose-dependent effects of CPA on papillary muscle contraction activity*

On the ordinate axis - the amplitude value of the force of contraction, expressed as a percentage (%) of the maximum, on the abscissa axis - the logarithmic concentration of CPA (μM) (* -p<0.05; ** - p<0.01). Stimulation frequency 0.5 Hz, t=+36±0.5ºC; n = 4.

In subsequent experiments, the effect of the alkaloid N-14 (30 μM) on the force of papillary muscle contraction under conditions of semi-maximum concentration of the inhibitor SERCA2a CPA was investigated. It was found that the force of contraction of the papillary muscle CPA ($IC_{50}$-5.6 μM) in the presence of 36.4±5.1% and a decrease (Fig.5).

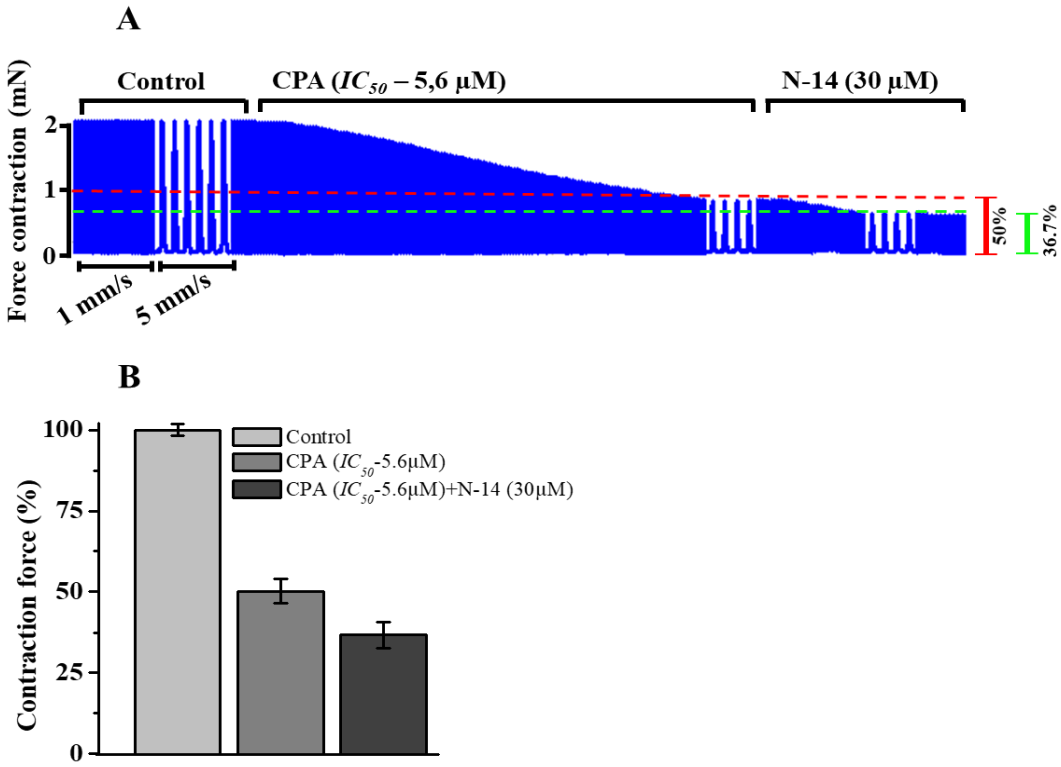


**Figure 5:** *Effect of N-14 (30 μM) alkaloid on papillary muscle contraction force in the presence of Ca2+-ATPase blocker-cyclopiazonic acid (IC50-5.6 μM) in the incubation environment*

On the ordinate axis - the force of contraction of the papillary muscle is expressed as a percentage (%). The stimulation frequency is 0.5 Hz (t =+36±0.5ºC; - $p<0.05$; n =5).

The results of this experiment suggest a partial role of $Ca^{2+}$-ATPase in the negative inotropic effect of the N-14 alkaloid. If we compare the effect of N-14 alkaloid on papillary muscle contraction activity in 72.4±3.7% of the control variant, the negative inotropic effect of N-14 alkaloid is explained by its effect on $Ca^{2+}$-ATPase.

The results of the experiments obtained show that the potentiating effect of the alkaloid is related to the process by which $Ca^{2+}$ ions accumulate in the SR.

## Conflict of Interest

The authors have declared that no conflict of interest exists.

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