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STUDY OF ANTIARRHYTHMIC ACTIVITY 2-(N-BUTYLPYRROLIDINE)-N-(2-BROMOPHENYL)CARBOXAMIDE HYDROCHLORIDE

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ИЗУЧЕНИЕ АНТИАРИТМИЧЕСКОЙ АКТИВНОСТИ 2-(Н-БУТИЛПИРРОЛИДИН)-N-(2-БРОМФЕНИЛ)КАРБОКСАМИДА ГИДРОХЛОРИДА

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Objective. To study the efficacy of a new derivative 2-(alkylpyrrolidine)-N-(aryl)carboxamide with high antiarrhythmic activity.

Materials and methods. To study the antiarrhythmic activity of the compound, the experiment was carried out on models of arrhythmia caused by intravenous administration of aconitine and adrenaline. The effect was estimated by its ability to prevent the onset of arrhythmia, prolong the survival time of the animals or by the duration of an arrhythmia attack. In addition, the electrocardiogram of awake rats was analyzed. The studied compound and the comparison drug (lidocaine) were injected to the animals intravenously in effective antiarrhythmic doses.

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Results. In aconitine arrhythmia 2-(n-butylpyrrolidine)-N-(2-bromophenyl) carboxamide hydrochloride provides statistically significant limitation of the duration of arrhythmia attacks in experimental animals (1.7 times) in comparison with the control and also reduction of arrhythmia duration in comparison with lidocaine (2.5 times); besides, this compound guarantees animals' survival in 100 % of cases. When causing arrhythmia by adrenaline administration, the compound does not prevent the occurrence of cardiac rhythm disorder. The electrocardiogram readings of animals do not change significantly.
Conclusions. 2-(n-butylpyrrolidine)-N-(2-bromophenyl) carboxamide hydrochloride (compound K-23) shows visible activity in models of arrhythmia caused by administration of aconitine and calcium chloride, which may indicate its ability to impede the sodium flow through the cell membrane by slowing depolarization of cardiomyocytes.
Since the compound studied, demonstrates high antiarrhythmic activity without changing the ECG readings, the drug created on its basis may be effective.
Keywords. Arrhythmia, lidocaine, aconitine model of arrhythmia, adrenaline model of arrhythmia, electrocardiogram, effective antiarrhythmic dose.

Цель. Изучение эффективности нового производного 2-(алкилпирролидин)-N-(арил)карбоксамида, обладающего высокой антиаритмической активностью.
Материалы и методы. Для исследования антиаритмической активности соединения эксперимент проводился на моделях аритмии, вызванной внутривенным введением аконитина и адреналина. Эффект оценивали по его способности предупреждать возникновение аритмии, удлинять время выживания животных или по длительности приступа аритмии. Кроме того, был проведен анализ электрокардиограммы бодрствующих крыс. Исследуемое соединение и препарат сравнения (лидокаин) вводили животным внутривенно в эффективных антиаритмических дозах.
Результаты. 2-(н-бутилпирролидин)-N-(2-бромфенил)карбоксамида гидрохлорид в условиях аконитиновой аритмии обеспечивает статистически значимое по сравнению с контролем ограничение длительности приступов аритмии у экспериментальных животных в 1,7 раза и также снижение продолжительности аритмии по сравнению с лидокаином в 2,5 раза; кроме того, данное соединение гарантирует защиту животных от гибели в 100 % случаев. При создании аритмии, вызванной введением адреналина, соединение не предупреждает появление расстройства ритма сердца. Кроме того, показатели электрокардиограммы животных практически не меняются.
Выводы. 2-(н-бутилпирролидин)-N-(2-бромфенил)карбоксамида гидрохлорид (соединение К-23) проявляет выраженную активность на моделях аритмии, вызванной введением аконитина и хлорида кальция, что может свидетельствовать о его способности затруднять натриевый ток через клеточную мембрану, замедляя деполяризацию кардиомиоцитов.
Поскольку исследованное соединение демонстрирует высокую антиаритмическую активность, не изменяя при этом показатели ЭКГ, лекарственный препарат, созданный на его основе, может оказаться эффективным.
Ключевые слова. Аритмия, лидокаин, аконитиновая модель аритмии, адреналиновая модель аритмии, электрокардиограмма, эффективная антиаритмическая доза.

INTRODUCTION

For many decades, diseases of the cardiovascular system have occupied first place in the structure of morbidity and mortality in the world. Heart rhythm disturbances are one of the most common manifestations of these diseases. Among the factors influencing the increase in their prevalence, the increase in life expectancy of the population and the increase in morbidity of the circulatory system can be highlighted [1; 2]. The main methods of treating heart rhythm disor-

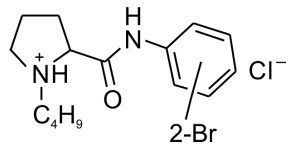
ders are surgical interventions and drug therapy with antiarrhythmic drugs. At the same time, pharmacotherapy remains the predominant method of treating patients with arrhythmias [3]. Consequently, the problem of choosing adequate antiarrhythmic therapy remains relevant and is of considerable interest.

The purpose of the research is to study the action of a new derivative 2-(alkylpyrrolidine)-N-(aryl)carboxamide, which has high antiarrhythmic activity and was synthesized at the Department of General and Organic Chemistry of the Perm State Pharmaceutical Academy (D.V. Kalinin – PhD in Chemistry, Head of the research group at the Institute of Pharmaceutical and Medicinal Chemistry, University of MŸnster, Germany).

MATERIALS AND METHODS

The biological activity of the compounds was assessed in experiments on animals obtained from the Andreevka nursery in the Moscow region. The animals management complied with the Good Laboratory Practice (GLP) and Order of the Ministry of Health of the Russian Federation No. 199n dated April 01, 2016 “Rules of Good Laboratory Practice”, as well as the provisions of the manual for conducting preclinical studies of medicines edited by. A.N. Mironov [4]. The studies were approved by the Bioethics Commission (protocol No. 8 dated January 20, 2023).

A compound was selected for the study, for which the antiarrhythmic index (AI) was initially calculated, depending on the values of the mean lethal and effective therapeutic doses 2-(n-butylpyro-lidine)-N-(2-bromophenyl)carboxamide hydrochloride (compound K-23).



Lidocaine produced by JSC BIOCHIMIC (Russia) was used as a reference drug.

To study antiarrhythmic activity, experiments were carried out on models of cardiac arrhythmia in awake animals caused by intravenous administration of chemicals; in particular, aconitine and adrenaline models of arrhythmia were used.

The study of the antiarrhythmic activity of the compound in a model of arrhythmia caused by the administration of aconitine was carried out on non-pedigree rats of both sexes, sexually mature, weighing 180–250 g. During the experiment, an electrocardiogram (ECG) was recorded in the animals in standard lead II using a single-channel electrocardiograph EK1T-1/3-07 "AXION" (manufactured by "Concern "Axion" LLC, Russia). The dose of aconitine that stimulated the formation of atrioventicular extrasystole was selected experimentally. Aconitine produced by Sigma-Aldrich (USA) was administered into the tail vein. As a result, a dose of 20 mcg/kg was chosen. The death rate of animals was

60 %[1], which made it possible to study the effect of the compound on the duration of arrhythmia in animals. Heart rhythm disturbances appeared 1–2 minutes after administration of aconitine. ECG was recorded for 20 minutes. The test compound was administered intravenously at an effective antiarrhythmic dose 2 minutes before the administration of aconitine. The activity of a substance was assessed by its ability to prevent the development of arrhythmia or increase the survival time of animals [4].

A study of the antiarrhythmic activity of the compound in a model of arrhythmia caused by the administration of adrenaline was carried out on awake chinchilla rabbits weighing 4.5–5 kg. Adrenaline was administered into the marginal vein of the ear at a dose of 15 mcg/kg to assess the heart reaction, which was recorded using an ECG in standard lead II using a single-channel electrocardiograph EK1T-1/3-07 "AXION" (manufactured by "Concern "Axion" LLC, Russia). After restoration of the heart rhythm, the test compound was administered intravenously at a dose equal to ED_{50}, and after 3 minutes, adrenaline was administered again [4]. The antiarrhythmic effect was assessed by the duration of the arrhythmia attack.

When studying the effectiveness of a new antiarrhythmic drug, it makes sense to evaluate its effect on heart function, since some antiarrhythmic drugs can cause changes in its physiological properties. Therefore, an analysis of the electrocardiogram of awake rats was carried out. The test compound, as well as the reference drug, were administered intravenously to animals in effective antiarrhythmic doses.

Statistical processing of the study results was performed using the Statistica 8.0 software package. The results were processed using variation statistics according to the Fisher–Student method [5].

RESULTS AND DISCUSSION

When studying the antiarrhythmic activity of 2-(alkylpyrrolidine)-N-(aryl)carboxamide derivatives, it was found that compounds of this group exhibit fairly high antiarrhythmic activity.

Among these compounds, a substance with maximum antiarrhythmic activity was found – K-23, the ED_{50} of which is 3.2 mg/kg, and the antiarrhythmic index is 13.6.

Thus, the relative activity of 2-(n-butylpyrrolidine)-N-(2-bromophenyl) carboxamide hydrochloride is 2.7 times higher than the activity of lidocaine[2]. In addition, the task was set to determine the degree of the heart protection from fibrillation that appears after disruption of blood flow in the coronary artery using this compound. Analysis of the ECG recorded during a study on a model of acute coronary occlusion in awake rats

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[2] Kalinin D.V., Pantsurkin V.I., Syropyatov B.Ya., Rudakova I.P., Vakhrin M.I. 2'-bromoanilide N-butylpyrrolidine-2-carboxylic acid hydrochloride, exhibiting antiarrhythmic activity. RF Patent No. 2504539; 2014.

revealed that the K-23 compound prevents the formation of ventricular fibrillation. This fact significantly distinguishes its effectiveness not only from control results, but also from the activity of lidocaine [6].

To be able to suggest a probable mechanism of the new compound action, its antiarrhythmic activity was studied in models using chemicals whose arrhythmogenic effect is associated with an effect on the cardiomyocyte membrane. Studies were carried out on a model of arrhythmia initiated by aconitine, which can interact with voltage-gated sodium ion channels of cardiomycytes, which leads to long-term depolarization. At the same time, the permeability of the membranes for potassium ions that leaves the cell, as well as for calcium ions, increases. An increase in calcium concentration in the cell stimulates the release of acetylcholine, which reacts with cholinergic receptors of postsynaptic membranes, opening sodium channels here and creating a new action potential, which leads to electrophysiological disturbances in myocardial cells [7; 8]. The results of the study are presented in Table 1.

The time of arrhythmia after administration of aconitine to control animals was 626.5 ± 95.2 s. Preliminary administration of the reference drug lidocaine not only does not reduce the duration of the arrhythmia, but also increases this time by 1.5 times in comparison with the control result. At the same time, compound K-23 works quite actively under conditions of aconitine arrhythmia, under its influence there is a statistically significant, compared with the control, limitation of the arrhythmia attacks duration in test animals to 366.7 s, as well as a significant decrease in the duration of arrhythmia, compared with the lidocaine effect (2.5 times). In addition, this compound guarantees protection of animals from death in 100 % of cases, while the use of lidocaine does not prevent their death. Mortality due to an arrhythmia attack when using lidocaine was 40 %, which differs little from the result in the control.

In addition, an arrhythmia model was used using adrenaline, which creates an arrhythmogenic effect caused by an increase in the activity of the sympathetic nervous system and the content of catecholamines, causing activation of slow transmembrane calcium channels mediated by the excitation of β-adrenergic receptors, which provokes the formation of ectopic activity of cardiac pacemakers [9; 10]. From the results of the experiment it follows that when creating arrhythmia caused by the administration of adrenaline, the substance K-23 does not prevent the appearance of cardiac arrhythmia in awake rabbits. In addition, when using this compound, the time of adrenaline arrhythmia increases slightly compared to control data. The results are shown in Table 2.

Table 1

Effect of compound K-23 on the course of aconitine arrhythmia in rats, $M \pm m$

Compound / drug	Number of animals in the experiment	Dose (ED_{50}), mg/kg	Duration of arrhythmia, s	Death, %
Control	10	–	626.5 ± 95.2	70
K-23	10	3.2	366.7 ± 30.4 $p = 0.023$ $p' = 0.047$	0 $p = 0.0002$ $p' = 0.024$
Lidocaine	10	7.7	929.7 ± 263.5 $p = 0.248$	40 $p = 0.196$

N o t e: p is level of statistical significance of differences in comparison with control data; p' is level of statistical significance of differences in comparison with lidocaine.

Table 2

Effect of compound K-23 on the development of adrenaline arrhythmia in rabbits, $M \pm m$

Compound / drug	Dose, mg/kg	Number of animals	Prevention of arrhythmia, % of the number of experiments			Duration of arrhythmia, s	
			full	partial	total	control	experience
K-23	6.4	5	0	0	0	242.0 ± 17.4	297.0 ± 17.4 $p = 0.365$ $p' = 0.121$
Lidocaine	7.7	5	0	0	0	190.0 ± 14.5	152.0 ± 13.5 $p = 0.091$

N o t e: p is level of statistical significance of differences in comparison with control data; p' is level of statistical significance of differences in comparison with lidocaine.

Thus, the test compound exhibits a significant antiarrhythmic effect in the aconitine arrhythmia model. In accordance with the data obtained, the activity of K-23 in aconitine arrhythmia may characterize its ability to block sodium current, slowing down the depolarization of cardiomyocyte membranes.

When taking antiarrhythmic drugs of various groups, characteristic changes often appear on the ECG. In this regard, changes in the heart function of experimental animals were assessed based on an analysis of the ECG of rats after intravenous administration of the K-23 compound in an effective antiarrhythmic dose. The results are presented in Table. 3.

As a result of the analysis of the ECG of awake rats, it was found that intravenous administration of a comparison drug to animals in an effective antiarrhythmic dose leads to individual ECG changes. Lidocaine has a negative chronotropic effect. It reduces the heart rate by 1.2 times. This change is statistically significant compared

Table 3

Effect of compound K-23 on ECG parameters in rats, $M \pm m$

Compound / drug	Number of animals in group	ECG indicator				
		heart rate per minute	PQ interval duration, s	QRS duration, s	QT interval duration, s	amplitude R, mV
Control (0.9 % NaCl solution)	10	442.8 ± 17.05	0.04 ± 0.004	0.03 ± 0.003	0.06 ± 0.003	0.26 ± 0.04
K-23	10	445.3 ± 7.0 $p = 0.614$	0.06 ± 0.005 $p = 0.018$	0.03 ± 0.01 $p = 0.849$	0.07 ± 0.01 $p = 0.614$	0.32 ± 0.04 $p = 0.347$
Lidocaine	10	373.8 ± 11.3 $p = 0.003$	0.05 ± 0.005 $p = 0.220$	0.02 ± 0.002 $p = 0.664$	0.06 ± 0.004 $p = 0.480$	0.49 ± 0.04 $p = 0.018$

N o t e: p is level of statistical significance of differences in comparison with control data.

to the control result. There is also a slight increase in the amplitude of the R wave. Deviations in ECG parameters with the introduction of K-23 compound are insignificant, in particular, there is a slight prolongation of the PQ interval compared to the control result, which indicates a slowdown in the conduction of excitation from the sinus node to the atrioventricular node, heart rate, QRS interval time, QT, wave height R do not differ from these indicators in the control series of experiments.

Conclusions

1. 2-(n-butylpyrrolidine)-N-(2-bromophenyl)carboxamide hydrochloride (compound K-23) shows visible activity in models of arrhythmia caused by administration of aconitine and calcium chloride, which may indicate its ability to impede the sodium flow through the cell membrane by slowing depolarization of cardiomyocytes.

2. Since the compound studied, demonstrates high antiarrhythmic activity without changing the ECG readings, the drug created on its basis may be effective.

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