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Suprastin (Chloropyramine) Causes Proarrhythmic Deterioration of Excitation Conduction, Depolarization and Potentiates Adrenergic Automaticity in the Pulmonary Veins Myocardium

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A number of pharmacological drugs have side effects that contribute to the occurrence of atrial fibrillation, the most common type of cardiac rhythm disorders. The clinical use of antihistamines is widespread; however, information regarding their anti- and/or proarrhythmic effects is contradictory. In this work, we studied the effects and mechanisms of the potential proarrhythmic action of the first-generation antihistamine chloropyramine (Suprastin) in the atrial myocardium and pulmonary vein (PV) myocardial tissue. In PV, chloropyramine caused depolarization of the resting potential and led to reduction of excitation wave conduction. These effects are likely due to suppression of the inward rectifier potassium current (I_{K1}). In presence of epinephrine, chloropyramine induced spontaneous automaticity in the PV and could not be suppressed by atrial pacing. Chloropyramine change functional characteristics of PV and contribute to occurrence of atrial fibrillation. It should be noted that chloropyramine does not provoke atrial tachyarrhythmias, but create conditions for their occurrence during physical exercise and sympathetic stimulation.

Key Words: *chloropyramine; H1-receptor antagonists; atrial fibrillation; pulmonary veins; ectopic automaticity*

Supraventricular rhythm disorders such as atrial flutter and fibrillation (AF) affect 2.3 to 3.4% of the population [1,2]. A number of factors, including age and structural pathologies of the cardiovascular system, contribute to the induction of atrial tachyarrhythmias [1]. Moreover, increased uncontrolled use of pharmacological drugs with proarrhythmic side effects and drugs directly or indirectly causing myocardial remodeling also contributes to the occurrence and increase in the number of cases of AF [3]. Drugs of various groups including cholinomimetics, antihypertensives, antibiotics, immunomodulators, cytostatics, and antidepressants exhibit profibrillatory adverse effects [4,5]. The information on the antihistamines is contradictory. For instance, some H1-antihistamines of a first generation (antazoline) demonstrated the suppression of AF paroxysms [6,7], while others (cetirizine) induce tachyarrhythmias [8,9]. Both antazoline and chlorophenol-containing derivative of ethylenediamine compounds chloropyramine (CPM) are the antagonists of H1-histamine receptors. There is at least one report of direct induction of dysrhythmia by CPM in humans [10]. Ethylenediamine compounds structurally close to CPM are known to induce arrhythmias or cause proarrhythmic activity [10]. Numerous experimental studies have shown that CPM causes episodes of bradycardia and disturbances in the conduction of excitation wave in the atrial myocardium [11,12].

In both mammals and human, a wall of the pulmonary veins (PV) adjacent to the left atrium contains

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cardiomyocytes that form myocardial tissue, or myocardial sleeves of PV [13]. The myocardial tissue of the PV is characterized by specific electrophysiological properties and highly prone to spontaneous sinoatrial node-independent automaticity under adrenergic or sympathetic stimulation [14,15]. It has been shown that PV tissue is a suitable for studying proarrhythmic effects and serves as a source of ectopic activity leading to the initiation of supraventricular tachyarrhythmias in the majority of cases [16].

Due to intensive use of CPM, its structural similarity to compounds causing arrhythmias, and the ability to affect cardiac pacemaking, the purpose of this study was to investigate the effects of this drug on bioelectrical properties, as well as the ability to induce proarrhythmic changes in the PV myocardium.

MATERIALS AND METHODS

The study was carried out on 16-week-old male Wistar rats (n=11) weighing 350-400 g obtained from the Stolbovaya nursery (Scientific Center of Biomedical Technologies of Federal Medical-Biological Agency of Russia). Six and five rats were used in microelectrode and patch-clamp experiments, respectively. All experimental procedures on animals were approved by the Bioethics Committee of the Research Institute of Experimental Cardiology of the National Medical Research Center of Cardiology (Commission Decision No. LES/01.11.23).

PV were isolated according to the previously described protocol [15]. Effects of CPM on parameters of action potentials (APs) were simultaneously recorded in the myocardium of the distal (PVD) and the ostial (PVO) parts of the PV using the multichannel microelectrode technique described in details previously [15]. Pure preparations after adaptation were used as a control.

Glass microelectrodes (15-30 MΩ) connected to a KS-700 high-input impedance amplifier (WPI) were used to record APs. The amplified signal was converted by an E-154 analog-digital converter (L-Card), collected and analyzed using LGraph 2 software. The preparations were perfused with oxygenated (95% O_2, 5% CO_2) Tyrode's solution (in mM: 118 NaCl, 4.7 KCl, 2.2 NaH_2PO_4, 25 $NaHCO_3$, 1.8 $CaCl_2$, 10 glucose; pH 7.2-7.4) at 37°C with a flow rate of 10 ml/min. After a 30-min period of adaptation, during which electrically evoked APs were continuously recorded (S1-S1=300 msec, rectangular 2 msec stimuli with a double threshold amplitude), the tissue preparations were consecutively perfused with Tyrode's solution containing 2 mg/liter (6.9 μM) of CPM or 2 mg/liter of CMP and 1, 5, or 10 μM of epinephrine (EP) for 30 min. The resting potential (RP) level, AP amplitude, and AP duration at 90% repolarization ($APD_{90\%}$) were determined. The excitation conduction time was measured as the interval from the moment of the excitation stimulus application to the moment of a maximum rate of voltage change of the AP (dV/dt) in the PVO or PVD. The change in conduction time was calculated and presented as % of control (control was taken as 100%). We calculated the rate of the EP-induced spontaneous AP (period, msec) occurred in quiescent tissue PV preparations. The probability of excitation of atrial part of the preparations by spontaneous AP originated in PV in the paced preparations in presence of EP was estimated as well.

The inward rectifier potassium current (I_{K1}) was recorded in ventricular cardiomyocytes enzymatically isolated using collagenase type II (Worthington Biochemical Corp.) according to the previously described protocol [17]. After isolation, the cardiomyocytes with a capacitance of 155±6 pF (n=30) were placed into a Kraftbrühe (KB) solution (in mM): 3 $MgSO_4$, 30 KCl, 30 KH_2PO_4, 0.5 EGTA, 50 potassium glutamate, 20 HEPES, 20 taurine, and 10 glucose (pH 7.2) [18]. The I_{K1} current was measured at 24°C using borosilicate glass pipettes (2-3 MΩ), standard patch-clamp technique in a whole-cell configuration with aid of EPC-800 Patch Clamp Amplifier (HEKA Elektronik). To record the I_{K1} current, the cardiomyocytes were placed into 150 μl experimental RC-26 chamber (Warner Instruments) mounted in the table of a Diaphot 200 inverted microscope (Nikon). The cardiomyocytes in the chamber were perfused with a solution containing (in mM): 150 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 10 glucose, and 10 HEPES (pH 7.4). A pipette solution composition was as followed (in mM): 140 KCl, 1 $MgCl_2$, 5 EGTA, 10 HEPES, 4 MgATP, and 0.03 Na_2GTP (pH 7.2). The current value (pA) was normalized to the cardiomyocyte capacity (pA/pF). Clampfit 10.3 software (Molecular Devices) was used to collect and analyze the current.

Statistical analysis was performed using GraphPad Prism 7 software (GraphPad Software). The resting potential, AP amplitude, and $APD_{90\%}$ calculated in multicellular tissue preparations are presented as Me (Q1-Q3). The significance of the differences between groups was tested using Friedman's test or the Kruskal–Wallis test for repeated or independent measurements as a nonparametric alternative to ANOVA. The normality of the distribution of the I_{K1} current values was tested using the Shapiro–Wilk test. I_{K1} values are presented as M±SD. The statistical significance of differences in the IK_1 current analysis was assessed using one-way ANOVA followed by post hoc tests and Šidák correction for multiple comparisons. Differences were considered statistically significant at p<0.05.

RESULTS

In presence of 6.9 μM CPM, the RP in PVD shifted from -82 to -64 mV (p<0.001) accompanied by a decrease in the AP amplitude (p<0.001). At the same time, CPM did not affect the RP and AP amplitude in the atrial myocardium (Fig. 1, *a*, *b*). In the PVO, an increase in $APD_{90\%}$ in response to CPM was weak and statistically insignificant (Fig. 1, *c*). The CPM-induced RP shift in the PV was accompanied by a decrease in the AP upstroke velocity and a change in its configuration. When RP was shifted (depolarized) and AP amplitude was reduced to <20 mV, the $APD_{90\%}$ was not determined since the responses were considered as local. The CPM-induced RP shift was accompanied by an increase in the conduction time of the excitation wave in the PVD, which was determined by the moment of occurrence of the local response. CPM increased the time of excitation wave conduction to PVO and PVD by 14 and 23%, respectively (p<0.001; Fig. 1, *d*). Thus, CPM significantly affects the bioelectrical activity of the myocardial tissue in the PV, but has little effect in the working atrial myocardium. The depolarization, increased conduction time, and conduction blocks caused by CPM in the PV can be considered as proarrhythmic effects.

The addition of EP partially attenuated the CPM-induced shift of RP and the decrease in AP amplitude in PV. In particular, 1-10 μM EP restored RP and AP amplitude to the values characteristic of the atrial myocardium (Fig. 1, *a*, *b*). In contrast to the PVO, the administration of CMP in presence of 10 μM EP resulted in a significant increase of $APD_{90\%}$ in PVD (p<0.001; Fig. 1, *c*).

EP in concentrations of 1, 5, and 10 μM partially restored CPM-reduced conduction of the excitation

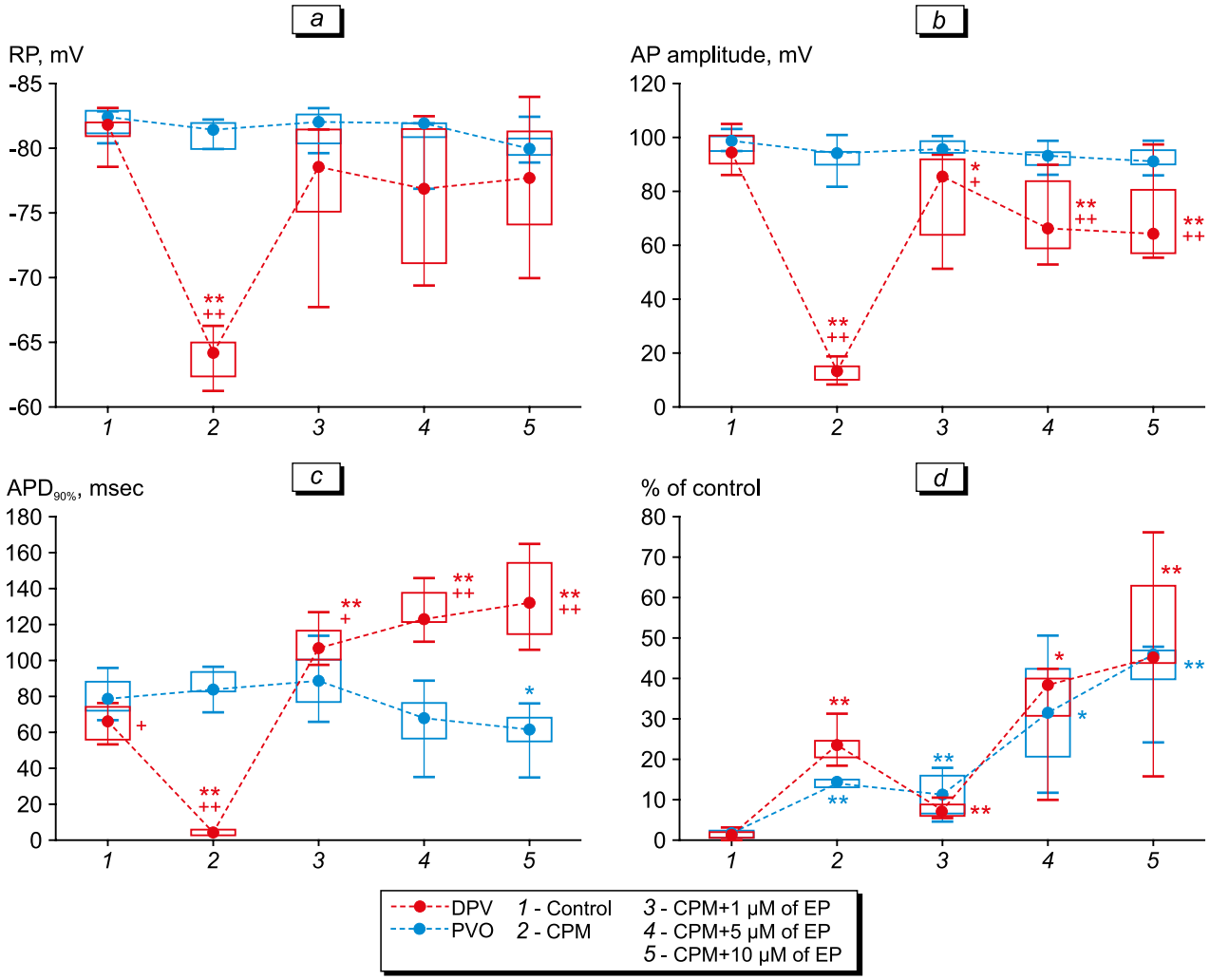

Fig. 1. The effect of CPM (6.9 μM) on the bioelectrical activity of the working myocardium of the PVO and the myocardial tissue of the DPV. *a-d*) The effect of CPM or EP against the background of CPM on PP (*a*), AP amplitude (*b*), $APD_{90\%}$ (*c*), and on the duration of the excitation wave in tissue preparations of the supraventricular region of the heart (*d*). There were 6 tissue preparations in each group. *p<0.05, **p<0.01 in comparison with the control; ^{+}p<0.05, ^{++}p<0.01 in comparison with PVO.

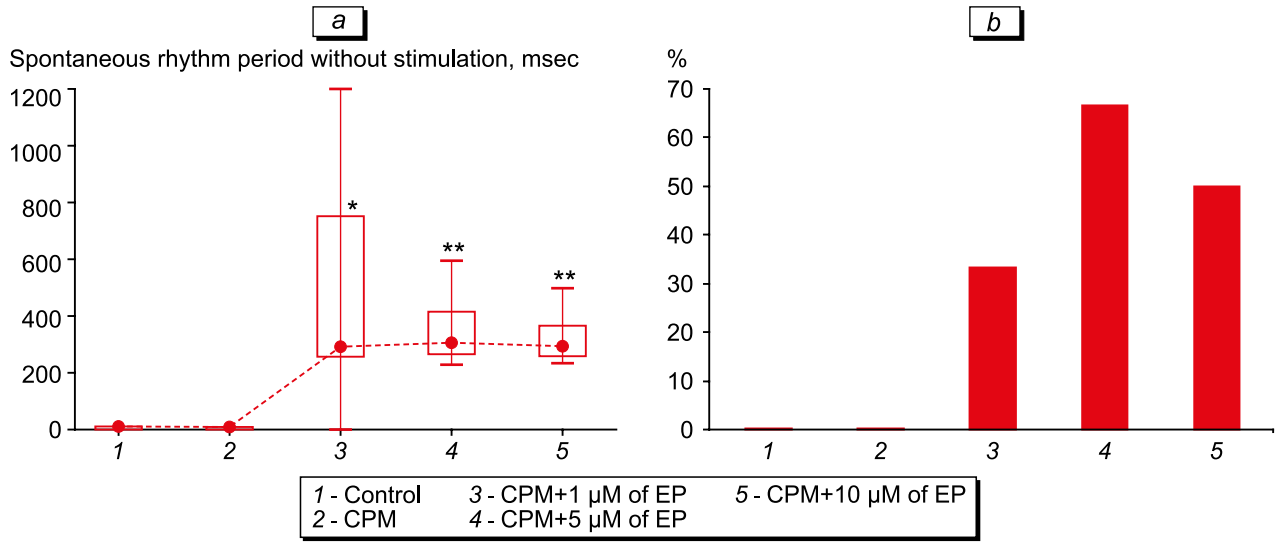

Fig. 2. Spontaneous activity in the myocardium of the PVD under the combined action of CPM and different concentrations of EP. *a*) The average period of spontaneous activity and the dynamics of changes in the period of spontaneous rhythm (dashed line). *b*) The probability of the natural rhythm leaving the PVD to the PVO. There were 6 tissue preparations in each group. **p*<0.05, ***p*<0.01 in comparison with the control.

wave in the PVD: this parameter increased by 7, 38, and 45%, respectively ($p<0.01$; Fig. 1, *d*). In presence of CPM, the same doses of EP induced spontaneous excitations in PV. These PV-derived spontaneous excitations dose-dependently captured the atria in 2, 4, and 3 out of 6 cases, respectively. The termination of electrical pacing resulted in an initiation of PV-derived automaticity (spontaneous rhythm), the periodicity of which depended on EP concentration (Fig. 2, *a*). Administration of CPM alone (without EP) did not induce any spontaneous activity either in PV or atrial myocardium.

Thus, EP facilitates proarrhythmic effects of CPM in the PV. On the one hand, EP partially restores normal atrial-like level of RP and attenuates reduction of conduction velocity which are altered by CPM in PV. On the other hand, EP in combination with CPM induces spontaneous rhythm, a dose-dependent increase in the time of conduction of the excitation wave in the atrium and PV and in a facilitation of reentrant conduction in the atrium. The last two effects result in a decrease of the excitation wavelength (by Wiener) and the appearance of ectopic foci in the PV and atrial re-entry, *i.e.* provide necessary conditions for AF.

A decrease in AP amplitude, depolarization (AP shift to less negative RP values), as well as the block conduction in the myocardial tissue of the PV under

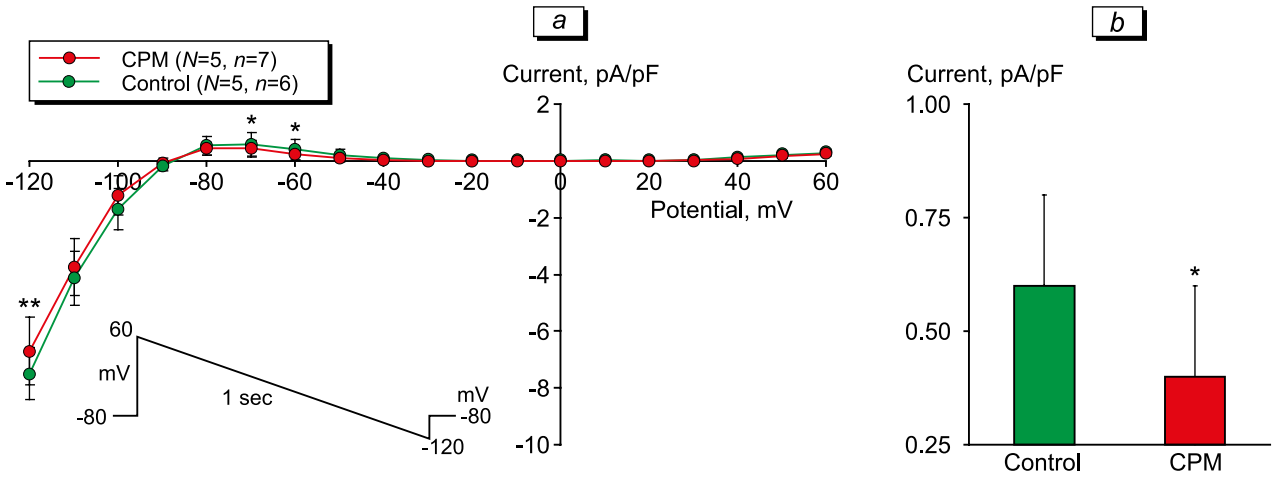

Fig. 3. The effect of CPM at a concentration of 10 μM on the current-voltage characteristic of the potassium current of abnormal rectification I_{K1} in enzymatically isolated cardiomyocytes of the rat heart ventricles (*a*) and the value of the outgoing component of the I_{K1} current at MP=-70 mV in the control and in the presence of CPM (*b*). *N* is number of animals, *n* is number of cardiomyocytes. The inset below is a "ramp" potential change protocol used to measure current density in the range from -120 to 60 mV (maintained potential -80 mV). **p*<0.05 in comparison with the control (ANOVA).

the action of CPM can be caused by several mechanisms, including suppression of I_{K1}. It is known that I_{K1} is the main ion current that determines the negative and stable RP in the cardiomyocytes of the working myocardium. It was previously shown that the myocardial tissue of both the pulmonary and caval veins is characterized by a reduced expression of the Kir2.x channels as well as a low density of the I_{K1} ion current [19,20]. Under conditions of reduced I_{K1} density, its weak suppression can lead to a significant shift in the RP, affect the ability of myocardial tissue to conduct an excitation wave, and facilitate spontaneous automaticity induced by norepinephrine. To test the hypothesis for the ability of CPM to affect I_{K1}, we used ventricular cardiomyocytes from male rats. The yield of viable cardiomyocytes enzymatically isolated from the ventricles is extremely high, and these cells exhibit a uniform and significant density of I_{K1}, which facilitates the detection of weak changes in this current under the influence of pharmacological compounds. In our experiments, CPM caused a change of I_{K1} current. At a concentration of 10 μM, CPM statistically significantly reduced the peak value of the outward functional component of I_{K1}: at -70 mV, the current density decreased by 26±3%. In addition, CPM statistically significantly reduced the inward component at -120 mV (Fig. 3, *a*, *b*). Thus, the suppression of I_{K1} and a decrease in its functionally significant outward component may underlie the prolongation of AP and the proarrhythmic effect of CPM in PV cardiomyocytes.

Our results suggest that, in cases of increased level of sympathetic input or increased level of circulating adrenaline, CPM *in vivo* may promote the induction of AF due to a proarrhythmic changes in bioelectrical activity in the arrhythmogenic substrate such the myocardial tissue of PV.

Long-term practice of CPM administration demonstrates that adverse, including proarrhythmic effects, rarely occur in conditions when there are no pronounced pathological changes in the heart or cardiovascular system. Nevertheless, the data obtained suggest that the CPM ability to potentiate proarrhythmic events leading to AF may manifest in pathologically altered (remodeled) myocardium. The side effects of CPM may be substantial in conditions of increased sympathetic/adrenergic input and in structurally altered cardiac tissue, for instance, in a result of ageing-associated fibrosis. The study of the CPM profibrillatory potential requires further experiments using *in vivo* models of cardiac pathologies affecting the bioelectrical activity of the myocardial branches of the thoracic veins.

In conclusion, CPM (Suprastin) does not cause atrial tachyarrhythmias, but can increase susceptibility of cardiac tissue to proarrhythmic stimuli during extreme physical exertion or elevated tone of the sympathetic nerves.

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Conflict of interest. The authors have no conflicts of interest to declare.

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