In high concentrations (5-10 μM, corresponding to high pharmacological doses of glucocorticoids) HC elevates basal cAMP level suppressing the effector stages of the Ca-response activated by products of phosphatidylinositol tris-phosphate hydrolysis.

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Comparative Study of Na-Blocking Properties of Antiarrhythmic Drugs on Isolated Rat Cardiomyocytes

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The effects of the antiarrhythmic preparations lidocaine, flecainide, and rihlocaine on sodium concentration in cardiomyocyte cytoplasm are studied using the Na-sensitive fluorescent probe SBFI. The Na-blocking effect of lidocaine and rihlocaine depends on the frequency of electrical stimulation of cardiomyocytes (0.2-1.0 Hz). The data suggest the possibility of *in vitro* testing of novel antiarrhythmic drugs.

Key Words: *lidocaine; flecainide; hypoxia; sodium channel blockers; antiarrhythmic drugs; cardiomyocytes*

According to modern classification of antiarrhythmic drugs, sodium channel blockers (group I) are divided into three subgroups (Ia, Ib, and Ic) [8]. This division is made on the basis of the molecular mechanisms of interaction between antiarrhythmic agents class I (AAI) and voltage-dependent sodium channels (Na^+ channels) in the plasma membrane of electroexcitable cells [4]. There are three functional states of these channels: open, closed, or inactivated (refractory). AAI bind only to open or refractory channels and therefore block only **functioning** Na^+ channels; moreover, the higher heart rate, the stronger pharmacological effect of AAI [4], especially of agents of Ib subgroup (AAIb). These drugs (lidocaine and phenytoin) rapidly associate and dissociate with Na^+ channel, and their effect is completely reversible. Antiarrhythmic agents of subgroup Ic (flecainide, encainide, etc.) are characterized by a lower rate of dissociation and therefore exert a slower but prolonged effect (low-reversible). Apart from their Na^+-blocking activity, AAIc inhibit K^+ current through Ca^{2+}-insensitive K^+ channels [7]. AAIa such as quinidine and procainamide occupy an intermediate position between AAIb and AAIc.

The subgroups of AAI determine the peculiarities of clinical application of the given preparation and

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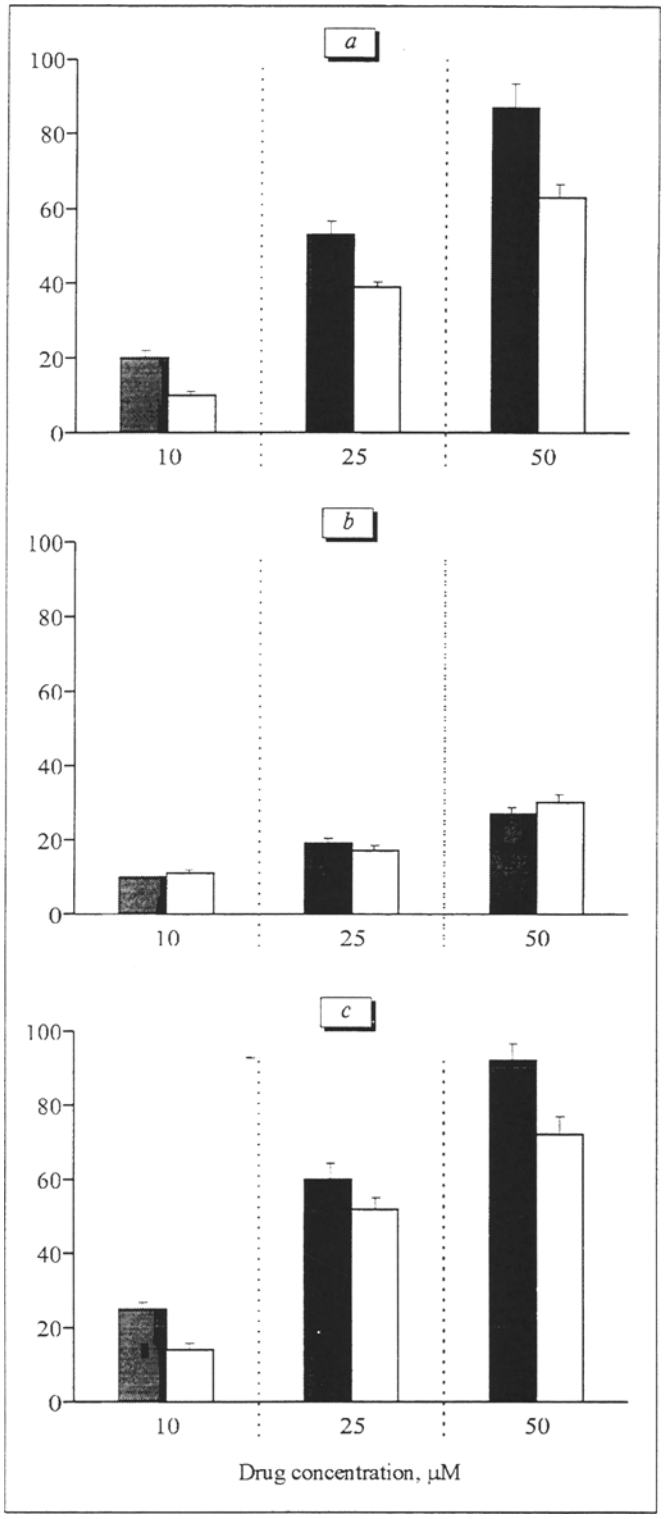

Fig. 1. Comparative efficiency of Na^+-blocking effect of lidocaine (*a*), flecainide (*b*), and rihlocaine (*c*) under conditions of experimental hypoxia. Cardiomyocytes were stimulated by electrical current of 1.0 Hz (dark bars) and 0.2 Hz (open bars) frequency. Ordinate: % of inhibition of $[Na^+]_{cyt}$ rise calculated from the formula: $[1—DNa_e/\Delta Na_c)]\times 100\%$, where ΔNa_e and ΔNa_c are changes in intracellular Na^+ concentration after a 30-min incubation in experimental (in the presence of Na^+ blockers) and control (no preparations) samples, respectively.

the probability of certain complications. For instance, AAIc exhibit a proarrhythmogenic effect, which considerably increases the risk of ventricular fibrillation in the postinfarction period [6]. Inhibition of action potential propagation in nerve cells underlies local anesthetic properties of AAI.

The aim of the present study was to compare the effects of lidocaine, flecainide, and rihlocaine, a local anesthetic with intrinsic antiarrhythmic activity on the concentration of Na^+ in cardiomyocyte (CM) cytoplasm.

MATERIALS AND METHODS

Isolation of CM from rat left ventricle was carried out as described previously [5] with some modifications. Experiments were carried out on male albino rats weighing 180-200 g. The animals were sacrificed under ether anesthesia, the heart was removed, and the apex formed by the left ventricle was cut out and placed to Ca^{2+}, Mg^{2+}-free Hanks' balanced salt solution (Sigma). The endo- and epicardial layers were removed, and the myocardium was gently minced with a blade, the medium was decanted and replaced with 2 ml Hanks' saline containing 0.5 mg/ml collagenase (Boehringer Mannheim). Tissue samples in 5-mm plastic Petri dishes were incubated at 37°C and constant shaking (no more than 100 rpm). After 15 min, the medium containing detached cells was decanted, transferred to 4-ml glass tubes, and centrifuged at 100*g* and room temperature for 1 min. The second incubation was induced by adding fresh portion of collagenase solution to the myocardium preparation. The supernatant obtained after the first centrifugation was used in the third incubation. CM isolated after 3-7 incubations were suspended and twice washed with isolation medium of the following composition (in mM): 113 NaCl, 4.6 KCl, 1.2 $MgCl_2$, 3.5 NaH_2PO_4, 21.9 $NaHCO_3$, and 5 glucose. $CaCl_2$ (2.45 mM) was added to the final suspension.

The intracellular concentration of sodium ions in CM was determined as described previously [1] using SBFI fluorescent probe. After calibration [2], $[Na^+]_{cyt}$ was calculation from the formula: $[Na^+]_{cyt}=K_d\times k\times (R-R_{min})/(R_{max}-R)$, where R is the ratio of fluorescence intensities at 340 nm (F_{340}) and 380 nm (F_{380}) excitation wavelengths; R_{min} and R_{max} are the same at minimal and saturating $[Na^+]_{cyt}$; k is the ratio of fluorescence intensity for free and bound probe at 380 nm (2.1±0.1); K_d is the steady-state dissociation constant of the probe—Na complex (20.8± 1.4 mM) [2].

Cell hypoxia was modeled using a 30-min incubation in the presence of 0.5 mM KCN and 10 mM 2-deoxyglucose at 37°C [3].

Electrical stimulation was performed using a MacLab Systems setup.

The data were processed using Pharmacological Basic Statistics software. The confidence intervals of experimental values and the significance of differences were assessed using the Student's t test at p=0.05.

RESULTS

Diastolic $[Na^+]_{cyt}$ in resting cardiomyocytes was 7.9± 0.5 mM (n=4). The rise of cell Na^+ concentration was induced by adding KCN and 2-deoxyglucose in cardiomyocyte suspension, and 30 min later a pronounced rise in the fluorescence intensity at 340 nm was recorded, accompanied by a decrease in F_{380}. The effect of the pharmacological agents on induced $[Na^+]_{cyt}$ rise was studied in two experimental series using resting CM and cell stimulated with electric pulses (0.2-1.0 Hz, 10 msec, and 60 mV).

Nonstimulated CM. The new elevated level of $[Na^+]_{cyt}$ under conditions of experimental hypoxia was 16.3±1.5 mM (n=6). Thus, the difference between intact and nonoxygenated cells in the basal Na^+ level (ΔNa) constituted about 8.4 mM. Preliminary addition of pharmacological agents to concentrations of 10, 25, and 50 μM reduces a dose-dependent decrease in ΔNa. All preparations exerted approximately equal Na^+-blocking effect: lidocaine, rihlocaine, and flecainide in a concentration of 50 μM considerably inhibited the rise of Na^+ by 24, 29, and 22%, respectively (p<0.05).

Stimulated CM. Experiments on stimulated CM revealed differences in the action of antiarrhythmic agents of different pharmacological groups.

Electrical stimulation during chemical hypoxia considerably changed $[Na^+]_{cyt}$ in comparison with both the control (ΔNa=15.9±2.3 mM) and nonstimulated CM (ΔNa=7.5±1.8 mM). The rise of $[Na^+]_{cyt}$ did not depend on the stimulation frequency: stimulation with 0.2 and 1.0 Hz produced the same effect.

The inhibiting effect of lidocaine on the rise of $[Na^+]_{cyt}$ depended on both the dose and stimulation frequency (Fig. 1). It should be noted that in stimulated cells the Na^+-blocking effect of lidocaine markedly surpassed that observed in nonstimulated cells at all concentrations used.

Flecainide (AAIc) less markedly inhibited the induced $[Na^+]_{cyt}$, the effect of the preparation did not depend on the stimulation frequency (Fig. 1).

It is known that AAIb selectively inhibit Na^+ channels in ischemized myocardium [4]. Hypoxia prolongs the refractory-to-closed channel transition, which determines the higher efficiency of lidocaine in comparison with flecainide.

The data presented in Fig. 1 suggest that the effect of rihlocaine on $[Na^+]_{cyt}$ is similar to that of lidocaine, and strongly depends on the stimulation frequency and, consequently, this drug can be assigned to antiarrhythmic agents of the Ib subgroup.

Our findings suggest that isolated CM provide an adequate experimental model for *in vitro* screening and studying the molecular mechanisms of action of novel antiarrhythmic agents of group I.

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