Antiarrhythmic Properties of a New Suphan-Based Combined Preparation and their Possible Mechanism of Realisation

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Considerable potentiation of antiarrhythmic and antifibrilation effects of two cardiotonic agents, Suphan and the Na-channel blocker L1, was found in the model experiments *in vivo* with early occlusion and reperfusion arrhythmias. Suphan in combination with L1 inhibited the rise of intracellular Ca^{2+} (by about 70%) in isolated cardiomyocytes incubated under hypoxic conditions.

Key words: *hypoxia; sodium channel blockers; antiarrhythmic drugs; Ca-exchange; cardiomyocytes*

Combined action of antiarrhythmic agents in the treatment of cardiac antiarrhythmia is not adequately investigated. When applied together, some antiarrhythmics, for example, tertiary derivative of Ajmaline (N-propylajmaline-bromide) and local anesthetic Trimecainum in combination with Ethmosine and Ethacisine (all named as Methacisine) potentiate therapeutic effects of each other [1,5]. Combined usage of antiarrhythmic agents with different mechanisms of action allows one to reduce substantially cardiotoxicity and the probability of adverse effects.

In this respect, suphan and L-1 (both preparations were synthesised in Russia) seem to be of particular interest. Suphan, di-potassium salt of N-succinyl-dl-tryptophan, is a nonglycoside cardiotonic with antianginal and antihypoxia activities. Its antiarrhythmic effect is due predominantly to its action on Ca^{2+} exchange in cardiomyocytes [2]. L-1, dimethyl-aminoethyl ether of para-butyl-aminobenzol acid, is a Na-channel blocker by mechanism of action, i.e., it belongs to IB class of antiarrhythmic drugs [8].

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We studied antiarrhythmic properties of L1, Suphan and their combinations in experiments *in vivo*, and the mechanism of their synergetic effect on intracellular calcium ($[Ca^{2+}]_{cyt}$) in isolated cardiomyocytes *in vitro*.

MATERIALS AND METHODS

Antiarrhythmic activity of the agents was assessed in model experiments with early occlusion and reperfusion arrhythmias on anesthetized cats *in vivo* in complete accordance with the methodical recommendations for experimental (pharmacological) investigation of preparations proposed for clinical trial as agents for cardiac arrhythmia treatment or prevention.

The procedures of isolating cardiomyocytes from the left ventricle, then loading with Fura 2-AM, and fluorescence recording were described previously [4]. Intracellular Ca^{2+} content was calculated from the following formula:

$$[Ca^{2+}]_{cyt} = K(R - R_{min})/(R_{max} - R),$$

where R_{min} and R_{max} are the ratios of fluorescence intensities at excitation wavelengths of 340 nm and

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380 nm (F_{340}/F_{380}) at zero and saturating Ca^{2+} concentrations, respectively, and the coefficient K is defined as $K_d(Fo/Fs)$; F_s is the fluorescence at 380 nm of Ca-free Fura 2-AM, Fr is the fluorescence at 380 nm of the probe complexed with Ca^{2+}. The equilibrium dissociation constant for the Ca^{2+}-Fura-2-AM complex (Kd) was determined in model experiments using a Fura 2-AM solution. Calculated by Scatchard analysis, it was equal to 140 nM.

To simulate hypoxia, cells were incubated for 30 min at 37°C in a medium containing 0.5 mM KCN and 10 mM 2-deoxyglucose. The results were statistically analyzed using Pharmacological Basic Statistics package. Confidence intervals for experimental values and the significance of differences between them were evaluated by non-parametric Wilcoxon-Mann-Whitney test (for data obtained from *in vivo* experiments) and by Student's *t*-test (for *in vitro* experiments), setting statistical significance at the 0.05 level.

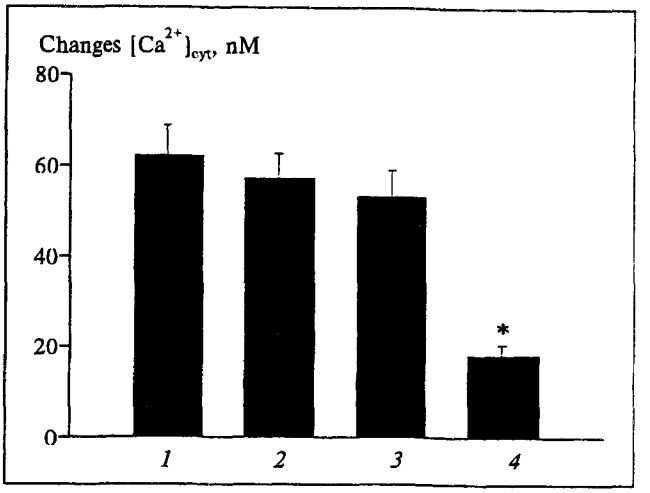

Fig. 1. Effect of Suphan (2), L-1 (3), and their combination (4) on $[Ca^{2+}]_{cyt}$ changes induced by hypoxia. 1) control (without preparations). *$p<0.05$ compared with the control.

RESULTS

The data on antiarrhythmic activity of L1, Suphan and their combinations obtained from *in vivo* experiments are summarized in Table 1. In control experiments, early occlusion arrhythmia was absent in 40% animals, whereas reperfusion arrhythmia developed in all cases. Moreover, in 67% animals it resulted in ventricular fibrillation. Antiarrhythmic and antifibrillation effects of the substances were dose-dependent. L1 in a dose of 0.08 mg/kg (1% of LD_{50}) did not prevent the development of either early occlusion or reperfusion arrhythmia, and ventricular fibrillation.

An increase in the preparation dose to 0.2 and 0.4 mg/kg (2.5 and 5% of LD_{50}, respectively) prevented both reperfusion arrhythmia (in 83% and 100% cats, respectively) and early occlusion arrhythmia (in 100% for both the doses); ventricular fibrillation did not occur at all.

Suphan in a dose of 20 mg/kg had no antiarrhythmic and antifibrilation effects. After its dose increased to 40 mg/kg, only antifibrilation effect had been observed (in 100% cats).

When administered together in subthreshold doses Suphan (20 and 10 mg/kg) and L-1 (0.1 and 0.05 mg/kg) potentiated antifibrilation effect of each other. After reducing Suphan and L-1 doses (to 5 and 0.025 mg/kg, respectively), we observed no antiarrhythmic and antifibrilation effects. Thus, combination of Suphan and L-1 leads to potentiation of their antifibrilation activity in case of acute transitory coronary failure (reperfusion).

Table 1. Efficacy of Preparation L-1, Suphan and Their Combinations in Early Occlusion and Reperfusion Arrhythmias

Preparations and their combinations	Dose, mg/kg	The nember of observations	Without early occlusion arrhythmia	Without reperfusion arrythmia	Without fibrilation
Control		15	6	0	5
L-1	0.08 (1.0)	6	1	1	2
	0.2 (2.5)	6	6*	5*	6*
	0.4 (5)	7	7*	7*	7*
Suphan	20 (13.5)	6	3	3	4
	40 (27)	7	4	5	7*
Suphan+L-1	20 (13.5)+0.1 (1/25)	6	5*	4	6*
Suphan+L-1	10 (6.8)+0.05 (0.6)	6	4	2	5*
Suphan+L-1	5 (3.4)+0.025 (0.3)	6	0	1	2

Note. In brackets — percentage, % of LD_{50}; *$p<0.05$, compared with the control.

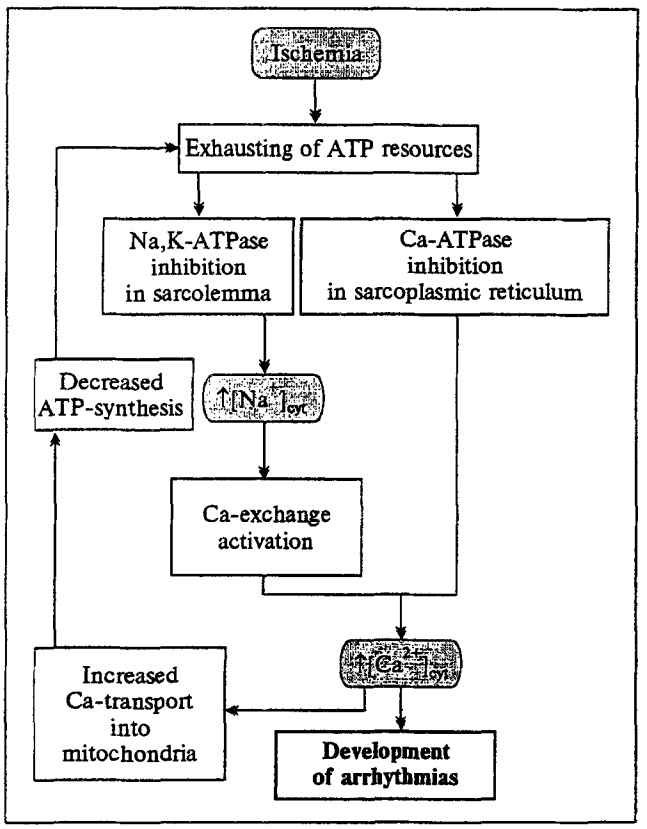

Fig. 2. Molecular mechanisms of myocardial ischemia.

In an attempt to investigate possible molecular mechanisms of synergistic action of Suphan and L-1, we examined the effects of these agents on $[Ca^{2+}]_{cyt}$ in cardiomyocytes under hypoxia, which is one of leading arrhythmogenic factors. Test concentrations of L-1 and Suphan were chosen taking into account the mean value of ED_{15} (the dose at which pharmacological effect is 15% of the maximum effect) obtained from the *in vivo* experiments. The final concentrations of L-1 and Suphan in the incubation medium were 12 and 7 mM/ml, respectively. The calculated concentration of Ca^{2+} in intact cardiomyocytes was equal to 134±11 nM. The determined basal Ca^{2+} level in nonoxygenated cells was significantly higher ($p<0.05$) than in the control ($\Delta Ca=62$ nM), which is consistent with the literature data on reduced activity of intracellular systems that maintain the diastolic $[Ca^{2+}]_{cyt}$ under conditions of myocardial hypoxia [6]. A factor, responsible for Ca^{2+} rise, may be a reduction in the intracellular contents of macroergic compounds, predominantly ATP. The addition of L-1 or Suphan to the cell suspension caused a small decrease (within 10-15%) in ΔCa, as recorded under hypoxia (Fig. 1.). Simultaneous addition of L-1 and Suphan significantly changed the diastolic $[Ca^{2+}]_{cyt}$; under the given experimental conditions ΔCa did not exceed 30% of the original level.

Analysis of the obtained results led us to the following explanation of mechanisms of mutual potentiation of Suphan and L-1. The course of events taking place in a cell that determine the development of arrhythmias is shown in Fig. 2. Exhaustion of ATP resources in a myocyte disturbs the function of energy-dependent transport systems: Na,K-pump and Ca-ATPase in sarcoplasmic reticulum. The following increase in free cytoplasmic Na^+ stimulates inversion of ionic flows related with electrogenic Na^+/Ca^{2+}-antiport. Rise of diastolic $[Ca^{2+}]_{cyt}$ leads to the development of arrhythmias [7]. L-1 inhibits $[Na^+]_{cyt}$ rise induced by hypoxia [3]. The unique pharmacological property of Suphan is its ability to activate Ca^{2+} uptake by the sarcoplasmic reticulum cisternal, thus reducing diastolic $[Ca^{2+}]_{cyt}$. This effect of Suphan is most pronounced under hypoxia. Thus, different links of pathogenesis of arrhythmias are the targets for L-1 and Sufan action. Our results point to advantages of combined antiarrhythmic preparations capable of selectively affecting both Na- and Ca-exchange in the myocardium.

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