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Mechanisms of Histamine-Induced Increase of Calcium Level in Cardiomyocytes. A Relative Efficacy of Histamine Receptor Blockers

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Histamine causes a rapid and prolonged increase in the intracellular concentration of free Ca ions in cardiomyocytes. The initial increase in Ca content is determined by Ca release from intracellular depots; the second phase of Ca response (plateau phase) is due to Ca entry. The effects of antihistamine agents with different mechanisms of action on the histamine-stimulated increase in free Ca are examined. H_1-histamine receptor antagonists blocking both phases of cellular Ca response proved to be the most effective. The drug activities rank as follows: diazoline>dimebone>tavegil>pyrilamine. H_2-receptor blockers (ranitidine>cimetidine) suppressed only the plateau phase. Thioperamide, a selective H_3-receptor antagonist, has no effect on histamine-induced changes in Ca concentration.

Key Words: *histamine; calcium; histamine receptor blockers; cardiomyocytes*

The myocardium is highly sensitive to histamine, but the content of this biogenic amine in the heart is an order of magnitude lower than in the lungs, alimentary canal, and whole blood [3]. *In vivo* experiments on isolated perfused preparations of animal hearts showed that physiological concentrations of histamine increase myocardial contractility, excitability, and heart rate, enhance cardiac automatism, and improve atrioventricular conduction [5]. At the beginning of the 1980s, H_2- and H_1-histamine receptors were identified in cardiac atria and ventricles of mammals; these receptors mediate positive inotropic and chronotropic effects of histamine [6]. Molecular and cellular mechanisms underlying the histamine effect on the myocardium remain obscure.

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In this study we compared the effects of antihistamine agents on histamine-stimulated increase of the concentration of free Ca ions in the cytoplasm ($[Ca^{2+}]_{cyt}$) of cardiomyocytes isolated from the left ventricle of rat heart.

MATERIALS AND METHODS

Methods for preparing cardiomyocyte suspension, labeling of the cells with the fluorescent probe Fura 2-AM, fluorescence measurement, and evaluation of $[Ca^{2+}]_{cyt}$ were described previously [4]. The selective antagonists of H_1-histamine receptors pyrilamine, tavegil, diazoline, and dimebone [2], of the H_2-histamine receptor antagonists cimetidine and ranitidine, and the selective blocker of H_3-histamine receptors thioperamide were used.

The results were statistically processed using a Pharmacological Basic Statistics software. The con-

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TABLE 1. Effect of Antihistamine Preparations on Histamine-Induced $[Ca^{2+}]_{cyt}$ Increase (*M±mt*)

Experimental conditions	Cardiomyocyte Ca response ($[Ca^{2+}]_{cyt}$, nM)	
	first phase	second phase
Control, histamine, 5 μM	390±64	281±30
Blockers of H_1-histamine receptors		
Pyrilamine, 10 μM	73±4	85±6
Diazoline, 10 μM	47±8	71±6
Dimebone, 10 μM	56±7	75±5
Tavegil, 10 mM	62±9	79±8
Blockers of H_2-histamine receptors		
Cimetidine, 30 μM	96±9	72±10
Ranitidine, 30 μM	104±11	67±9
Blocker of H_3-histamine receptors		
Thioperamide, 100 μM	103±7	98±12

Note. Drug effects were calculated from the formula $E=Ca_e/Ca_c \times 100\%$, where Ca_c and Ca_e is the content of Ca ions in control and experimental (in the presence of the drug) samples, respectively.

fidence intervals of values and significance of differences were evaluated using Student's *t* test at 0.05 level of values.

RESULTS

The diastolic $[Ca^{2+}]_{cyt}$ in cardiomyocytes was 141±9 nM (n=16). Histamine (1 to 100 μM) rapidly (30-45 sec) increased the level of $[Ca^{2+}]_{cyt}$. The initial rise of Ca in response to histamine was dose-dependent: the minimal effective dose (ED) of histamine was 0.25 μM, ED_{50}=3.2 μM, the maximum ED was 40 μM. At the maximum ED of an inducer (40-100 μM), Ca content increased to 548±22 nM (n=10). Later (after 2-10 min) $[Ca^{2+}]_{cyt}$ decreased and persisted at the level of 210-270 nM. Study of the mechanisms underlying the histamine-induced increase in Ca^{2+} showed that the initial changes of $[Ca^{2+}]_{cyt}$ were not sensitive to elimination of Ca ions from the medium. At the same time, the second phase of cardiomyocyte Ca-response (the plateau phase) was not observed in Ca-free buffer (no $CaCl_2$, 0.2 mM EGTA). Treatment of cells with the inorganic blockers of Ca channels Ni^{2+} (1 mM) or Co^{2+} (1 mM) completely inhibited the second phase; nifedipine, an organic antagonist of potential-dependent Ca-channels, in a dose of 10 μM was ineffective. From these results we have concluded that the increase in $[Ca^{2+}]_{cyt}$ during the second phase is determined by Ca entry through receptor-regulated Ca channels of the cardiomyocyte plasma membrane.

The role of the sarcoplasmic reticulum (SPR) in the histamine-stimulated increase in $[Ca^{2+}]_{cyt}$ was investigated with the use of caffeine, a specific stimulator of Ca release from the SPR. The effect of histamine in doses of 0.1-1 μM changed considerably in the presence of 10 mM caffeine. The first peak of Ca was not expressed, and the maximum increase in $[Ca^{2+}]_{cyt}$ (about 240 nM) was observed 2-2.5 min after the addition of the inducer. Hence, Ca release from intracellular depots plays the main role in the formation of the first phase of cardiomyocyte Ca response to histamine (the inositol tris-phosphate-dependent caffeine-sensitive stage [1]).

In the second series of experiments we studied the effects of selective blockers of various types of histamine receptors on histamine-induced changes of Ca level in cardiomyocytes (Table 1). Thioperamide, a selective blocker of H_3-histamine receptors, induced no significant changes in $[Ca^{2+}]_{cyt}$. The H_1-histamine receptor antagonists inhibited both the first and second phases of Ca response of cells. The efficacies of the tested agents ranked as follows: diazoline>dimebone>tavegil>pyrilamine. It is noteworthy that at high concentrations (100 μM) all drugs suppressed Ca mobilization from SPR and the entry of extracellular Ca, but the sensitivity of the second component of Ca response to H_1-antagonists was always lower in comparison with the initial changes in $[Ca^{2+}]_{cyt}$.

The H_2-histamine receptor blockers cimetidine and ranitidine depressed only the second phase of the cardiomyocyte Ca response; the efficacy of cimetidine was lower than that of ranitidine (IC_{50} values were 34 and 22 μM, respectively). The combination of ranitidine (10 μM) and the H_1-blockers dimebone or diazoline in concentrations 1-10 mM (their minimal and mean ED) potentiated the inhibitory effects

of the agents. The effects of H_2-antagonists were not observed in the presence of the H_1-blockers in the maximum ED (40 μM). Thus, Ca-blocking activity of H_1-receptors is higher than that of H_2-histaminolytics. These differences can be explained by the specific features of cardiomyocyte Ca response mediated by histamine H_1- or H_2-receptors. Stimulation of H_1-receptors activated the membrane-bound phospholipase C and the formation of two second messengers: hydrophilic inositol triphosphate (IP_3) and lipid-soluble diacylglycerol from inositol-4,5-diphosphate [1]. IP_3 diffusing in the cytoplasm rapidly reacts with specific SPR receptors and causes Ca release from the depot. The effect of diacylglycerol is associated with activation of protein kinase C and subsequent phosphorylation of molecular targets of this enzyme: ionic channels, structural proteins, and membrane enzymes.

In contrast to H_1-histamine receptors, activation of H_2-receptors is not directly related to Ca metabolism. H_2-receptors are known as components of the adenylate cyclase complex, and their stimulation increases the intracellular level of cAMP. In cardiomyocytes cAMP acts as the Ca response modifier: on the one hand, an increase in cAMP level stimulates Ca flow into the cell as a result of cAMP-dependent phosphorylation of membrane Ca-channels; on the other hand, it increases Ca accumulation in SPR, the mechanism of this accumulation being mediated by phosphorylation of SPR protein phospholambane. Hence, the specific features of Ca metabolism in cardiomyocytes determine the leading role of H_1-receptor blockers in disruption of Ca response to histamine.

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