Histidine Increases β-Adrenoreactivity of Myocardium in Frogs

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 8, pp. 144-147, August, 2004
Original article submitted October 15, 2003

Histidine (3×10^{-5} g/ml) had no effect on contractility and chronoinotropic relationships in frog myocardium, but rapidly and reversibly increased myocardial β-adrenoreactivity (increased myocardial response to 7×10^{-8}, 3×10^{-7}, and 4×10^{-6} g/ml epinephrine) and potentiated the positive effect of epinephrine (7×10^{-8}, 3×10^{-7} g/ml) on chronoinotropic relationships in the myocardium. Histidine is considered to be a component of endogenous sensitizer of β-adrenoceptors in human blood modulating the function of cardiomyocytes.

Key Words: *myocardium; epinephrine; histidine; β-adrenoceptor modulators*

It is known that 50-, 100-, 500-, and 1000-fold diluted human venous blood serum, umbilical blood serum, liquor, and amniotic fluid rapidly and reversibly increase β-adrenoreactivity of longitudinal myometrial strips from the uterine horn of nonpregnant rats [4,6,8, 10,11]. The 100-fold diluted solution of blood serum produced a similar effect on the smooth muscle of porcine coronary artery and bovine trachea [6,8,9,11] and on frog myocardium [7]. This fact is explained by the presence of endogenous sensibilizer of β-adrenoceptors in the blood [4-11]. Histidine (3×10^{-8}, 3×10^{-7}, 3×10^{-6}, and 3×10^{-5} g/ml), tryptophan (10^{-5} g/ml), and tyrosine (2×10^{-6} and 2×10^{-5} g/ml) also increase β-adrenoreactivity of smooth muscles in rat myometrium, porcine coronary artery, and bovine trachea [4,5, 8], which indicates a possible role of these amino acids as basic components of endogenous sensibilizer of β-adrenoceptors. The potency of these amino acids (histidine included) to rise β-adrenoreactivity of cardiomyocytes was not studied. However, taking into consideration high incidence of cardiovascular pathologies, cardiac insufficiency, and hypertension [12], which reduce the efficiency of adrenergic influences [2,13], this problem is of great importance.

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Our aim was to study the effect of histidine in a concentration of 3×10^{-5} g/ml, which corresponds to its content in the plasma of healthy humans [1], on β-adrenoreactivity of isolated frog heart.

MATERIALS AND METHODS

Experiments were carried out on isolated hearts of Rana Ridibunda (n=30). β-Adrenoreactivity of the heart was assessed by its responses to epinephrine (7×10^{-8}, 3×10^{-7}, and 4×10^{-6} g/ml in groups I, II, and III, respectively). Contractile activity of the heart and histidine-induced (3×10^{-5} g/ml) changes in its β-adrenoreactivity was measured at 16-22°C in a Myocytograph Complex consisting of a 6MX1C mechanotron, H-3020 recorder, and a syringe dispenser to perfuse a 1-ml working camber with Ringer solution (114.4 NaCl, 1.6 KCl, 1.80 $CaCl_2$, 2.4 $NaHCO_3$, pH 7.4, 0.7 ml/min perfusion rate). The heart was ligated to eliminate automatic contractions. The induced contractions were triggered with 30-sec trains of rectangular voltage pulses (1 Hz, 5 msec, and 5-10 V) generated with an ESL-1 stimulator. The experimental protocol consisted of seven 10-min stages: Ringer→epinephrine→Ringer→histidine→histidine+epinephrine→Ringer→epinephrine. At each stage, the stimulation trains were applied at the ends of the first and second 5-min intervals.

The data were processed statistically using parametric and nonparametric Fisher—Student's and Wilcoxon tests at $p<0.05$ [3].

0007-4888/04/1382-0123

TABLE 1. Effect of Epinephrine and Histidine (3×10^{-5} g/ml) on Evoked Contractile Activity of Isolated Frog Heart ($M\pm m$, %)

Parameter	Stage of experiment						
	stage 1 (Ringer)	stage 2 (epinephrine)	stage 3 (Ringer)	stage 4 (histidine)	stage 5 (histidine+ epinephrine)	stage 6 (Ringer)	stage 7 (epinephrine)
Series I (n=10)							
First stimulation train							
A_{min}	100	101.7±8.8	114.8±13.8	119.3±9.9	106.2±8.0	111.6±16.8	105.5±18.0
A_{max}	100	100.1±2.1	101.9±3.4	108.7±3.7[c]	117.5±2.5[c,d,b]	106.0±3.9	106.8±2.5[f,e]
TA_{max}	100	85.3±3.3[a]	75.7±5.5[a]	104.0±4.5	95.6±8.0	105.5±4.1	119.5±12.3[b]
Second stimulation train							
A_{min}	100	118.2±7.9[a]	122.5±7.5[a]	91.9±11.9	174.8±28.1[c,d]	79.5±3.9[c]	138.0±8.7[f,b]
A_{max}	100	100.1±1.9	102.5±3.4	105.8±5.6	130.3±5.6[c,d,b]	98.0±2.6[c]	110.7±2.1[f,b,e]
TA_{max}	100	103.5±4.4	89.7±5.0	100.3±10.9	123.0±17.1[c]	107.4±4.8	120.3±5.9[f,b]
Series II (n=10)							
First stimulation train							
A_{min}	100	144.9±13.2[a] (I)	136.7±15.3[a]	92.3±11.9[c]	215.0±20.4[c,d,b] (I)	122.3±13.8	120.0±17.9[e]
A_{max}	100	163.9±19.3[a] (I)	145.4±18.8[a] (I)	108.5±11.9	184.7±35.7[c,d]	98.5±13.4	135.2±12.0[f] (I)
TA_{max}	100	104.1±8.5	118.7±16.2 (I)	111.6±9.8	82.4±7.8[c,d]	99.5±7.1	97.7±5.3
Second stimulation train							
A_{min}	100	123.3±13.2	110.8±15.8	127.0±16.6	199.7±23.0[c,d,b]	125.0±22.7	103.5±11.7[e] (I)
A_{max}	100	154.8±17.7[a] (I)	134.1±13.9[a] (I)	106.6±6.6	196.4±39.6[c,d]	103.1±17.6	123.9±16.1
TA_{max}	100	96.5±6.7	108.2±6.9 (I)	89.1±7.7	95.2±8.3	93.3±8.5	91.7±2.9[f] (I)
Series III (n=10)							
First stimulation train							
A_{min}	100	193.1±35.4[a] (I)	170.2±33.8	48.8±10.5[c] (I, II)	238.3±22.9[c,d] (I)	89.9±18.9	178.3±27.4[f] (I)
A_{max}	100	141.8±10.3[a] (I)	109.6±11.4	77.5±8.0[c] (I, II)	179.0±14.8[c,d] (I)	103.5±10.0	142.0±9.2[f,e] (I)
TA_{max}	100	119.7±22.9	105.0±10.3 (I)	93.1±11.1	117.5±7.7[c,d] (II)	103.1±7.4	126.2±22.2
Second stimulation train							
A_{min}	100	187.9±24.5[a] (I, II)	113.0±27.8	77.5±13.9 (II)	241.3±26.1[c,d,b]	110.3±29.7	252.9±26.7[f] (I, II)
A_{max}	100	155.8±13.6[a] (I)	105.4±12.9[a]	95.1±5.7	177.2±14.3[c,d] (I)	86.2±5.5[c]	193.1±14.7[f] (I, II)
TA_{max}	100	124.4±16.5	105.3±8.5	77.0±6.8[c]	146.0±21.2[c,d] (II)	89.5±4.4[c] (I)	138.9±16.8[f] (I)

Note. n is the number of animals; A_{min}, minimum contraction amplitude; A_{max}, maximum contraction amplitude; TA_{max}, time of attaining maximum contraction amplitude. The parameters of stages 2 and 3 (4, 5, and 6) are given in percentage of the respective parameters measured in stage 1 and stage 3, respectively. The parameters of stage 7 are the percents of

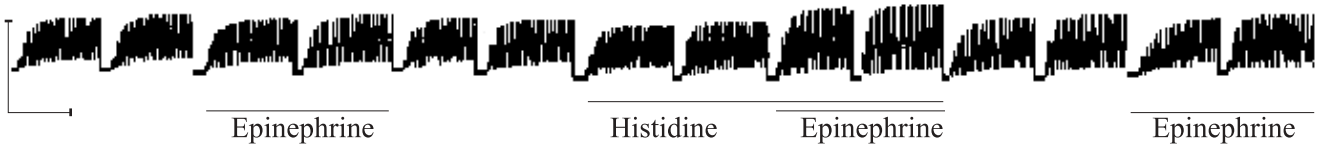

Fig. 1. Cardiac sensibilization to epinephrine induced by histidine (3×10^{-5} g/ml). Mechanogram of frog isolated heart. The horizontal lines show periods of epinephrine and/or histidine application . Each contraction was elicited by a single electrical pulse (duration 5 msec, amplitude 5 V, repetition rate 1 Hz). The calibration bars correspond to 10 mN (ordinate) and 15 sec (abscisa).

RESULTS

When the heart was perfused with Ringer solution, each stimulus applied during the first and second stimulations triggered contraction in all three series (Table 1, Fig. 1). The amplitude of the first contraction was always minimum. During the first and second electrostimulations, it was 2.99±0.60 and 2.94±0.51 mN (series I), 0.88±0.14 and 1.23±0.17 mN (series II), and 2.30±0.76 and 2.45±0.55 mN (series III), respectively. Than the amplitude of contractions increased and in series I, II, III peaked during the first stimulation after 9.4±1.2, 13.2±2.0, 12.2±1.0 sec, respectively; during the second stimulation after 7.2±0.5, 12.1±1.3, and 12.2±0.9 sec, respectively. The amplitude maxima for the first and second stimulation in series I, II, III were 7.89±0.66 and 8.28±0.79 mN, 3.97±0.66 and 4.36± 0.62 mN, and 7.60±0.91 and 7.30±0.94 mN, respectively. In all cases, the differences between the respective parameters recorded during the first and second stimulations were insignificant ($p>0.5$), which attests to a stable character of contractions of the heart perfused with Ringer solution.

In the first test, epinephrine (7×10^{-8} g/ml, series I) produced no effect on the minimum and maximum amplitudes of contractions, while in higher concentrations (3×10^{-7} g/ml, series II; 4×10^{-6} g/ml, series III) reversibly increased it in a dose-dependent manner by 123.0-144.9% and 154.8-163.9% (series II), and by 187.9-193.1% and 141.8-155.8% (series III), respectively, compared to baseline level (Fig. 2). In all series, epinephrine strengthened the chronoinotropic interrelations: in series I, it significantly shortened the time of attaining the maximum contraction amplitude to 85.3% baseline value, while in series II and III, it did not change this parameter, but markedly increased

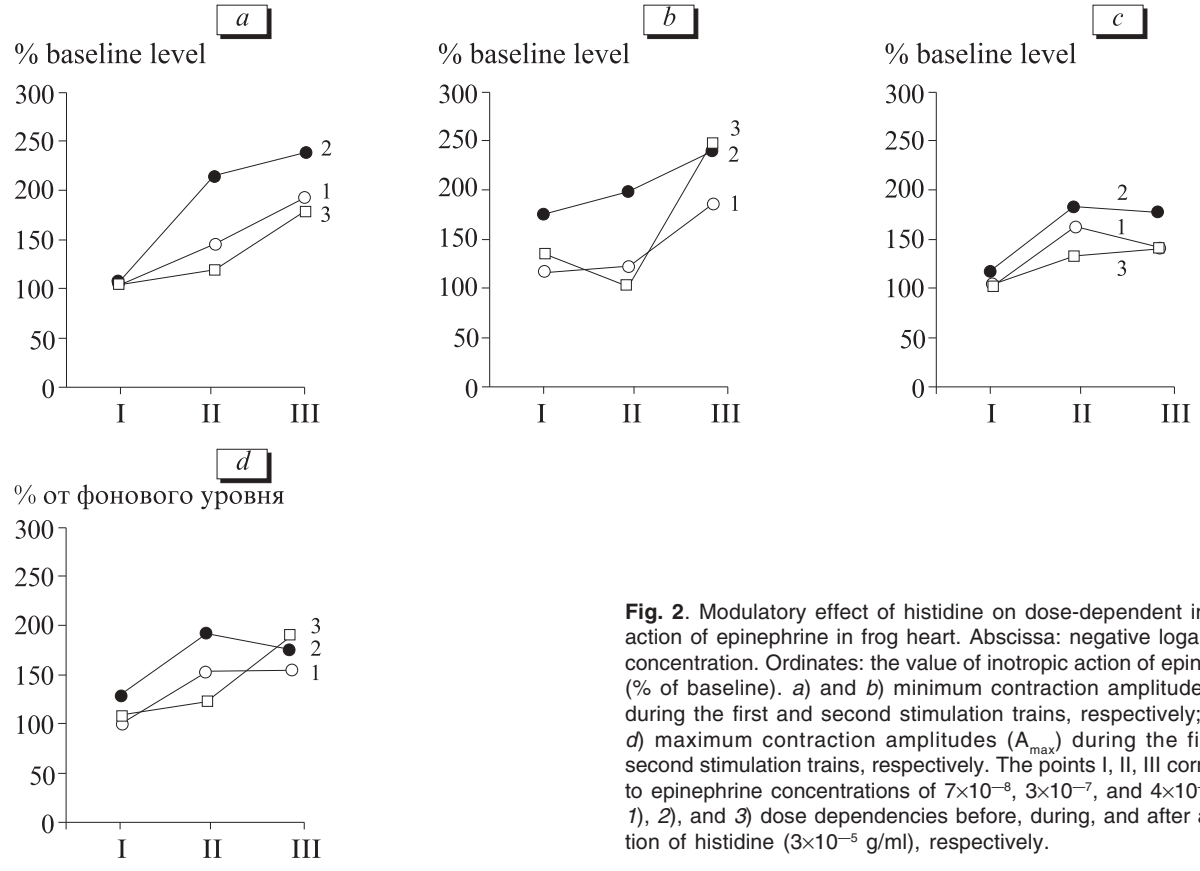

Fig. 2. Modulatory effect of histidine on dose-dependent inotropic action of epinephrine in frog heart. Abscissa: negative logarithm of concentration. Ordinates: the value of inotropic action of epinephrine (% of baseline). *a*) and *b*) minimum contraction amplitudes (A_{min}) during the first and second stimulation trains, respectively; *c*) and *d*) maximum contraction amplitudes (A_{max}) during the first and second stimulation trains, respectively. The points I, II, III correspond to epinephrine concentrations of 7×10^{-8}, 3×10^{-7}, and 4×10^{-6} g/ml. *1*), *2*), and *3*) dose dependencies before, during, and after application of histidine (3×10^{-5} g/ml), respectively.

contraction amplitude. Histidine (Fig. 1) had no effect on contraction amplitude, but significantly and reversibly potentiated the positive inotropic action of epinephrine in concentrations of 7×10^{-8} and 3×10^{-7} g/ml and to a lesser degree, in a concentration of 4×10^{-6} g/ml. In other words, histidine sensitized the myocardium to β-adrenergic influences in 60-100%, 50-90%, and 60-80% cases in series I, II, and III, respectively. Specifically, during combined application with histidine, epinephrine demonstrated a positive inotropic effect in low concentration (5×10^{-8} g/ml), and this effect increased in concentrations of 5×10^{-7} g/ml and to a lesser degree, 5×10^{-6} g/ml. For example, during the first stimulation train in series I, the minimum contraction amplitudes in tests 1, 2, and 3 were 144.9±13.2, 215.0± 20.4, and 120.0±17.9%, respectively (in comparison with the baseline level, $p<0.05$). Histidine shifted the dose-response curve for the inotropic effect of epinephrine to the left and upward (Fig. 2) and 2.7-fold decreased epinephrine dissociation constant from 243 to 94.6 ng/ml, which attests to increased affinity of β-adrenoceptors to epinephrine. Histidine potentiated the chronoinotropic effects of epinephrine (7×10^{-8}, and 3×10^{-7} g/ml). Specifically, while increasing the inotropic effect of epinephrine in series II (first stimulation, second test), histidine significantly shortened the time of attaining the maximum amplitude to 82.4± 7.8% baseline ($p<0.05$). This shows, that similar to blood serum [7], histidine sensitizes β-adrenergic receptors not only in smooth muscles of the coronary artery, trachea, and uterus [4,5,10], but also in the myocardium.

Thus, histidine (3×10^{-5} g/ml) does not affect contractility of frog myocardium, but increases β-adrenoreactivity of cardiomyocytes, potentiates the positive inotropic effect of epinephrine (in concentrations of 7×10^{-8}, 3×10^{-7}, and to a lesser degree, 4×10^{-6} g/ml) and augments its potency to affect the chronoinotropic relations (in concentrations 7×10^{-8} and 3×10^{-7} g/ml). Being a blood ingredient, histidine can be considered as a basic component of endogenous sensitizer of β-adrenoceptors capable of increasing cardiac adrenoreactivity.

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