PHYSIOLOGY

Ability of L-Histidine to Decrease Desensitization of the Myometrium to Epinephrine

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Experiments were performed with 62 longitudinal strips of the uterine horns from 10 nonpregnant rats. The ability of continuously infused epinephrine in high concentration (10^{-6} g/ml, 30 min) to suppress spontaneous contractions due to activation of β-adrenoceptors progressively decreased, which was associated with receptor desensitization. Histidine in a concentration of 3×10^{-11} g/ml had no effect, while in concentrations of 3×10^{-8}, 3×10^{-7}, and 3×10^{-6} g/ml decreased the degree of desensitization. Our results indicate that histidine not only potentiates β-adrenoceptor activation, but also prevents the development of desensitization. These data should be taken into account during therapy with β-adrenoceptor agonists.

Key Words: *histidine; epinephrine; myometrium; desensitization*

Similarly to a variety of neurotransmitters and hormones, long-term and continuous treatment with epinephrine induces desensitization. These changes are related to phosphorylation of β-adrenergic receptors (β-AR) under the influence of β-AR kinase and β-arrestin [1,4,7,11,12]. Histidine (3×10^{-8}, 3×10^{-7}, 3×10^{-6}, and 3×10^{-5} g/ml), tryptophan (10^{-6} and 10^{-5} g/ml), tyrosine (2×10^{-6} and 2×10^{-5} g/ml), and their combinations improve the interaction of epinephrine with β-AR in smooth muscles of the uterus, coronary artery, and trachea [3,7]. Published data show that histidine increases β_1-adrenoreactivity of frog myocardium [6]. This effect of amino acids is associated with inhibition of β-AR phosphorylation and/or increase in the intensity of β-AR dephosphorylation due to activation of phosphatase [3,7]. It cannot be excluded that histidine, tryptophan, and tyrosine constituting the endogenous sensitizer of β-AR [3,7] increase the degree of β-AR activation and prevent the development of desensitization to catecholamines. This study was designed to evaluate the ability of histidine to modify desensitization of the myometrium to epinephrine. We studied the inhibitory effect of long-term treatment with epinephrine (10^{-6} g/ml, 30 min) on longitudinal strips of the uterine horns from nonpregnant rats. The influence of epinephrine infusion was assayed in a medium containing histidine in concentrations of 3×10^{-11}, 3×10^{-8}, 3×10^{-7}, and 3×10^{-6} g/ml.

MATERIALS AND METHODS

Experiments were performed on 62 longitudinal strips (length 6-8 mm, width 2-3 mm) of the uterine horns from 10 nonpregnant rats. The phase of metestrus or diestrus was estimated by examination of vaginal smears. Contractile activity (CA) was recorded at 38°C under passive aeration of 1-ml working chambers [8]. We used a 6-channel Myocytograph device constructed from an H-3020 automatic recorded, 6MX1C mechanotron, thermostatic system, and syringe injector. The strips were perfused with Krebs solution at a flow rate of 0.7 ml/min. The initial load was 4.9 mN. Krebs

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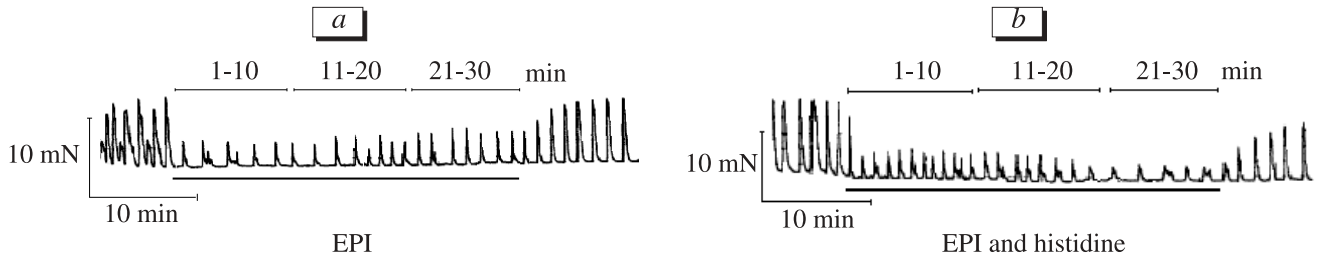

Fig. 1. Contractile activity of longitudinal strips from the uterine horns of nonpregnant rats after continuous 30-min infusion of epinephrine alone (EPI, 10^{-6} g/ml, *a*) or in combination with histidine (3×10^{-7} g/ml, *b*).

solution contained 136 mM NaCl, 4.7 mM KCl, 2.52 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.6 mM KH_2PO_4, 4.7 mM $NaHCO_3$, and 11.0 mM $C_6H_{12}O_6$ (pH 7.4). The study involved L-histidine (Renal) and epinephrine hydrochloride (Moscow Endocrine Plant).

We performed 5 series of experiments. Series I (control, n=16) recorded CA of strips during continuous treatment with epinephrine in a concentration of 10^{-6} g/ml for 30 min. In series II, III, IV, and V we studied CA of strips under conditions of continuous perfusion with 10^{-6} g/ml epinephrine (30 min) and histidine in concentrations of 3×10^{-11}, 3×10^{-8}, 3×10^{-7}, and 3×10^{-6} g/ml, respectively. Series II-V were performed in 6 repetitions. The results were analyzed by Student's t test and Fischer test. Differences were significant at $p<0.05$.

RESULTS

Longitudinal strips exhibited spontaneous CA (Table 1, Fig. 1). They generated 9.6-11.4 contractions over 10 min. The amplitude of contractions was 5.3-10.2 mN. Total CA was 55.1-95.7 mN/10 min.

Series I showed that 30-min infusion of epinephrine in a concentration of 10^{-6} g/ml produced on inhibitory effect (Table 1, Figs. 1 and 2). The frequency and amplitude of contractions and total CA decreased over the first (60.0±13.7, 32.0±7.7, and 18±4% of the baseline level, respectively) and last 10 min of epinephrine infusion (79.0±14.6, 66±20, and 40±10% of the baseline level, respectively, Table 1). The desensitization coefficient was calculated as the ratio between parameters recorded over the third and first 10-min intervals of epinephrine infusion. The desensitization coefficient for the frequency and amplitude of contractions and total CA was 1.3, 2.1, and 2.2, respectively. These data indicate that the effectiveness of epinephrine in inhibiting CA of the myometrium decreased during long-term treatment (30 min), which was related to desensitization.

Series II showed that histidine in a concentration of 3×10^{-11} g/ml did not modulate the inhibitory effect of epinephrine and development of desensitization (Table 1, Figs. 1 and 2). We estimated the frequency and amplitude of contractions and total CA over the first (49.0±26.7, 22.0±12.7, and 18.0±12.1% of the baseline level, respectively) and third 10-min intervals of treatment (67.0±22.3, 55±28, and 33.0±19.3% of the baseline level, respectively, Table 1). The desensitization coefficient remained unchanged under these conditions (1.4, 2.5, and 1.8, respectively).

Series III-V showed that the rate of desensitization to epinephrine decreased in the presence of histidine in concentrations of 3×10^{-8}, 3×10^{-7}, and 3×10^{-6} g/ml (Table 1, Figs. 1 and 2). Series III with histidine in a concentration of 3×10^{-8} g/ml evaluated the frequency and amplitude of contractions and total CA over the first (41.0±13.2, 31.0±16.2, and 19.0±9.1% of the baseline level, respectively) and third 10-min intervals of epinephrine treatment (34.0±11.6, 41.0±19.5, and 22.0±12.6% of the baseline level, respectively). In this series the desensitization coefficient was 0.8, 1.3, and 1.2, respectively. It should be emphasized that in series IV with 3×10^{-7} g/ml histidine the desensitization coefficient for the frequency and amplitude of contractions and total CA was 1.5, 1.4,

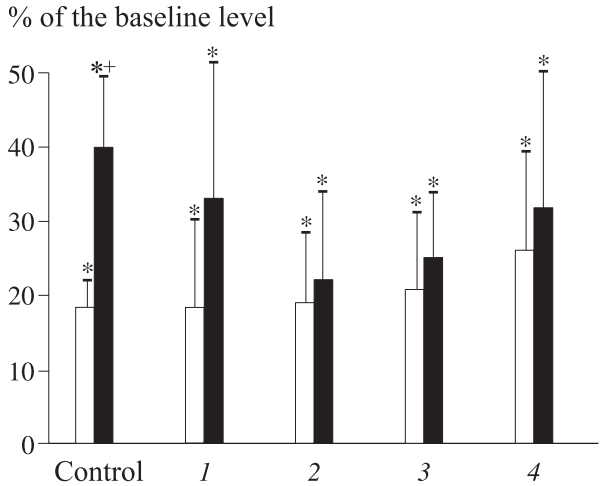

Fig. 2. Total contractile activity of longitudinal strips from the uterine horns of nonpregnant rats over the first (light bars) and third 10-min intervals (dark bars) of continuous 30-min infusion of 10^{-6} g/ml epinephrine alone (control) or in combination with histidine in concentrations of 3×10^{-11} (*1*), 3×10^{-8} (*2*), 3×10^{-7} (*3*), and 3×10^{-6} g/ml (*4*). $p<0.05$: *compared to baseline level; +compared to the first 10-min interval.

TABLE 1. Frequency, Amplitude, and Total CA (ΣCA) of Longitudinal Strips from the Uterine Horns of Nonpregnant Rats during Continuous Infusion of Epinephrine (10^{-6} g/ml, 30 min) Alone or in Combination with L-Histidine (*M*±*m*)

Parameters of spontaneous CA		Krebs solution, before treatment	Time, min		Krebs solution, after treatment
			1-10	21-30	
Epinephrine (control)					
frequency	abs., over 10 min	9.6±1.0	6.8±1.4*	8.6±1.4	8.0±1.6
	rel., %	100	60.0±13.7*	79.0±14.6	69.0±14.5*
amplitude	abs., mN	5.3±1.0	1.1±0.2*	2.3±0.4*+	2.4±0.7*
	rel., %	100	32.0±7.7*	66±20	60.0±17.7*
ΣCA	abs., mN/10 min	59.8±9.9	8.9±3.0*	18.0±3.7*	17.2±5.0*
	rel., %	100	18±4*	40±10*+	32.0±7.5*
Epinephrine and histidine (3×10^{-11} g/ml)					
frequency	abs., over 10 min	9.8±1.4	4.8±2.6	6.2±2.0	3.2±0.8*,**
	rel., %	100	49.0±26.7	67.0±22.3	34.0±7.8*,**
amplitude	abs., mN	5.5±1.9	0.6±0.3*	1.4±0.5	1.8±0.5
	rel., %	100	22.0±12.7*	55±28	56±26
ΣCA	abs., mN/10 min	55.1±18.9	6.4±3.6*	10.2±5.0*	7.3±3.4*
	rel., %	100	18.0±12.1*	33.0±19.3*	20±10*
Epinephrine and histidine (3×10^{-8} g/ml)					
frequency	abs., over 10 min	11.4±1.2	4.8±1.6*	4.2±1.6*	4.2±1.4*
	rel., %	100	41.0±13.2*	34.0±11.6*,**	38.0±11.9*
amplitude	abs., mN	9.9±3.5	2.6±1.9	3.7±2.7	4.8±2.8
	rel., %	100	31.0±16.2*	41.0±19.5*	77.0±45.6
ΣCA	abs., mN/10 min	95.7±25.6	15.1±9.4*	14.5±7.8*	22.2±10.4*
	rel., %	100	19.0±9.1*	22.0±12.6*	35.0±18.3
Epinephrine and histidine (3×10^{-7} g/ml)					
frequency	abs., over 10 min	11.0±1.4	4.2±1.2	5.4±1.0*	1.2±0.4*ox**
	rel., %	100	35.0±11.4*	51.0±11.4*	15.0±6.3*o**
amplitude	abs., mN	6.0±1.3	2.6±1.2	2.6±1.0	1.6±0.5*
	rel., %	100	48.0±15.7*	58.0±20.8	22.0±8.2*
ΣCA	abs., mN/10 min	66.4±16.3	18.0±9.5*	17.5±7.9*	2.9±0.9*,**
	rel., %	100	21.0±10.2*	25.0±8.9*	4.0±1.7*ox**
Epinephrine and histidine (3×10^{-6} g/ml)					
frequency	abs., over 10 min	11.4±1.2	8.4±2.4	7.4±2.6	4.0±1.4
	rel., %	100	69.0±18.8	61.0±21.5	41.0±16.2*
amplitude	abs., mN	10.2±2.9	2.1±0.8*	2.1±1.0*	1.5±0.8*
	rel., %	100	32.0±14.5*	41.0±21.7*	34±19*
ΣCA	abs., mN/10 min	104.0±23.8	19.9±10.0*	21.6±9.6*	7.9±4.3
	rel., %	100	26.0±13.9*	32.0±16.1*	13.0±7.2*

Note. $p<0.05$: *compared to baseline level (before treatment); **compared to the control; +compared to 1-10 min; ocompared to 21-30 min; xcompared to 51-60 min.

and 1.2, respectively. In series V with 3×10^{-6} g/ml histidine the desensitization coefficient was 0.9, 1.3, and 1.2, respectively.

Our results show that histidine prevents the development of desensitization to epinephrine in high concentration (10^{-6} g/ml). This effect was observed after treatment with histidine in concentrations exhibiting β-adrenosensitizing activity (3×10^{-8}, 3×10^{-7}, and 3×10^{-6} g/ml) [3,7]. Epinephrine-induced desensitization results from activation of β-AR phosphorylation. Therefore, histidine inhibits phosphorylation of β-AR and/or stimulates dephosphorylation of recep-

tors realized via phosphatase [1,4,7,11,12]. The data indicate that histidine inhibits β-AR phosphorylase and/or activates phosphatase, which prevents desensitization. Hence, histidine plays an important role in the regulation of internal organs, which is consistent with published data [2,3,5-7,9,10,13,14]. Previous studies showed that histidine produces a protective effect on myocardial mitochondria during cerebral ischemia [13], is involved in the maintenance of constant H^+ concentration in cardiomyocytes during anaerobic load [9], possesses antihypoxic [2], antioxidant [10, 14], and antiaggregant properties [13], and modulates β-adrenoreactivity of smooth muscles and myocardium [3, 5-7] and α-adrenoreactivity of vascular smooth muscles [14].

Probably, tryptophan and tyrosine can prevent desensitization induced by catecholamines. Similarly to histidine, these compounds exhibit β-adrenosensitizing activity [3,7].

It can be hypothesized that therapy of bronchial asthma or threatened preterm labor should involve combined treatment with β-adrenoceptor agonists and histidine, tryptophan, tyrosine, or their mixture to prevent receptor desensitization.

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