Positive Inotropic Effect of Tyrosine, Histidine, and Tryptophan in Experiments on Isolated Human Myocardium

K. N. Korotaeva, V. I. Tsirkin*, and V. A. Vyaznikov**

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Experiments on myocardial strips isolated from the right auricular myocardium of patients with heart failure showed that tyrosine, histidine, and tryptophan increased the amplitude of electrically stimulated contractions. The nature of this effect and clinical implications are discussed.

Key Words: *human myocardium; tyrosine; histidine; tryptophan; epinephrine*

It was previously found that blood serum can efficiently activate β-adrenoreceptors (β-AR) of uterine smooth muscles, blood vessels, trachea, and myocardium of experimental animals [4-6]. This phenomenon was explained by the existence of endogenous sensitizer of β-adrenoreceptors (ESBAR). It has been shown that this β-adrenosensitizing effect is produced by 3 amino acids: L-tyrosine, L-histidine, and L-tryptophan [3,5]. These amino acids are considered as a part of ESBAR, and all substances acting like ESBAR were named exogenous β-adrenergic sensitizers or ESBAR analogs [3-6]. Taking all these data into account, we studied physiological effects of tyrosine, histidine, and tryptophan including their impact on muscle contraction. Experiments on longitudinal strips of rat uterine horn demonstrated that histidine, tryptophan, and tyrosine showed ESBAR-activity and alone reduced the amplitude and frequency of spontaneous contractions in strips [5]. Experiments with rat myocardium demonstrated [4] that histidine in concentration of 6.5×10^{-6} M and 6.5×10^{-5} M potentiated the positive inotropic effect of epinephrine as well as increased the amplitude of electrically stimulated myocardial contractions. Tyrosine and tryptophan though demonstrated ESBAR-activity, did not increase the amplitude of myocardial contractions. Although the search for substances that modulate myocardial contractility is of great importance, there is no data on inotropic effect of amino acids on human cardiomyocytes. Here we studied the effects of tyrosine, histidine, and tryptophan on the amplitude of evoked contractions of myocardial strips of the right auricle from patients with heart failure.

MATERIALS AND METHODS

The study was performed on myocardial strips (n=20) of the right atrial auricle from 20 patients (mean age, 55±1 year). Biopsy samples were taken during the insertion of venous cannula during aortocoronary bypass surgery (18 samples) for angina pectoris (NYHA functional classes II and III) or cardiac valve replacement (2 patients with heart diseases of different etiology). Recording of evoked contractions was carried out according to [4]. A myocardial strip (5-8 mm length and 3-4 mm width, wet weight 44±10 mg) was placed in a 1-ml Miotsitograf working chamber (Noris) at 37°C and connected to a isometric force transducer (Honeywell); the signal was input through LA-70 analog-to-digital converter to a computer. The strip was stretched with a micromanipulator to a length corresponding to maximal contraction force and continuously perfused

Vyatka State Humanities University; *Kirov State Medical Academy; **Kirov Regional Clinical Hospital, Russia. ***Address for correspondence:*** tsirkin@list.ru. V. I. Tsirkin

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with oxygenated Krebs solution at a rate of 2.0 ml/min using a syringe doser. Throughout the experiment, contractions of the strip were induced by continuous electrical stimulation with ISE-01 electrostimulator via steel electrodes plunged into the chamber (1 Hz frequency, 5 msec pulse duration, and 25-30 V amplitude).

Six series of experiments on human myocardium were carried out. We studied the effect of three amino acids, tyrosine in concentrations of 5.5×10^{-5} and 5.5×10^{-4} M (series I and II), histidine, 6.5×10^{-5} and 6.5×10^{-4} M (series III and IV), and tryptophan, 4.9×10^{-5} and 9×10^{-4} M (series V and VI) on the amplitude of electrically-evoked contractions. Epinephrine in a relatively low concentration, 5.5×10^{-8} M (E8) was used as a reference drug with positive inotropic effect. Experiments were carried out according to the following protocol: Krebs solution (KS) → E8 → KS → amino acid. Amino acids and epinephrine were diluted with Krebs solution of the following composition (in mM): 136 NaCl, 4.7 KCl, 2.52 $CaCl_2$, 1.2 $MgCl_2$, 0.6 KH_2PO_4, 4.7 $NaHCO_3$, and 11 $C_6H_{12}O_6$ (pH 7.4). Epinephrine hydrochloride (Moscow Endocrine Plant), histidine (Sigma-Aldrich), tyrosine, and tryptophan (ACROS ORGANICS) were used.

The data are presented as *M*±*m*. The differences were estimated using Wilcoxon's test and considered significant at $p<0.05$ [1].

RESULTS

Contraction amplitude (or force) of human myocardial strips perfused with oxygenated Krebs solution before the introduction of substances (*i.e.,* background amplitude) was relatively constant in each experiment, but markedly varied in different series from 1.04 to 2.40 mN. We have shown that background amplitude of evoked contractions (A, mN) positively correlated with the left ventricular ejection fraction ($LVEFA_T$; ml) determined ultrasonographically before surgery and calculated using Teicholz's formula (correlation coefficient, 0.37; $p=0.0048$). Thus, the lower was contraction amplitude of cardiomyocytes, the lower was systolic blood volume in the patient before surgery. This indirectly suggests that contractility of isolated right atrial myocardium reflects the contractility of the left ventricular myocardium *in vivo*.

We have previously demonstrated on 7 myocardial strips that epinephrine (5.5×10^{-9}-5.5×10^{-4} M) dose-dependently increased the amplitude of electrically evoked cardiomyocyte contractions. Epinephrine dissociation constant was 1.1×10^{-6} M. Epinephrine in a concentration of 5.5×10^{-8} M, in series I-IV significantly increased the amplitude of evoked contractions (to 118.2-147.9% of the background level). In series V and VI, it had no effect on the contraction amplitude. Hence, epinephrine concentration used in series V and VI was below the threshold value. All these data indicate that myocardial strips were heterogeneous by their β-adrenoreactivity, which agrees with the assumption on reduced adrenoreactivity in heart failure [2,7,8].

Tyrosine (5.5×10^{-5} and 10^{-4} M), histidine (6.5×10^{-5} M), and tryptophan (4.9×10^{-4} M) had significant positive inotropic effect (Fig. 1, *a*; Fig. 2) comparable with that of epinephrine (5.5×10^{-8} M). Thus, 5.5×10^{-5} M tyrosine (series I) significantly increased the amplitude of evoked contractions to 148.4±13.0% of the background level; epinephrine, to 147.9±13.8%. Tyrosine in a concentration of 5.5×10^{-4} M (series II) increased the amplitude to 150.0±12.0% (epinephrine to 137.6±15.0%). Histidine in concentration of 6.5×10^{-5} M (series III) increased contraction amplitude to 133.1±14.0% (epinephrine to 118.2±6.6%), and 4.9×10^{-4} M tryptophan (series VI), to 116.7±6.9% (epinephrine to 112.3±7.4%). In series IV and V, histidine (5.5×10^{-5} M) and tryptophan (4.9×10^{-5} M) insignificantly increased contraction amplitude (Fig. 2).

Thus, we confirmed the data [4] on positive inotropic effect of histidine on the myocardium of warm-blooded animals and showed that tyrosine and tryptophan can provide a similar effect on the myocardium of patients with heart failure. The positive inotropic effect of tyrosine, histidine, and tryptophan can be explained by the fact that amino acids bind to β-AR receptor sites. We hypothesize that this site has high affinity for these three amino acids, so that they can improve the efficiency of β-AR activation by epinephrine. Therefore, the interaction of amino acids with this site results in activation of myocardial β-AR that increases the amplitude of evoked contractions. Our hypothesis is supported by the data that β-AR can be activated by some substances that are not their agonists [2]. In particular, antibodies against β-AR interacting with second extracellular loop of β_1-AR can activate this receptor in rats and produce a positive chronotropic effect similar to that of β_1-AR ago-

Fig. 1. Mechanogram of human isolated heart ***(myocardium)*** showing tyrosine-induced increase in the amplitude of evoked contractions (5.5×10^{-5} M, Tyr5). KS, Krebs' solution. Calibration, 1.5 mN, 1 min.

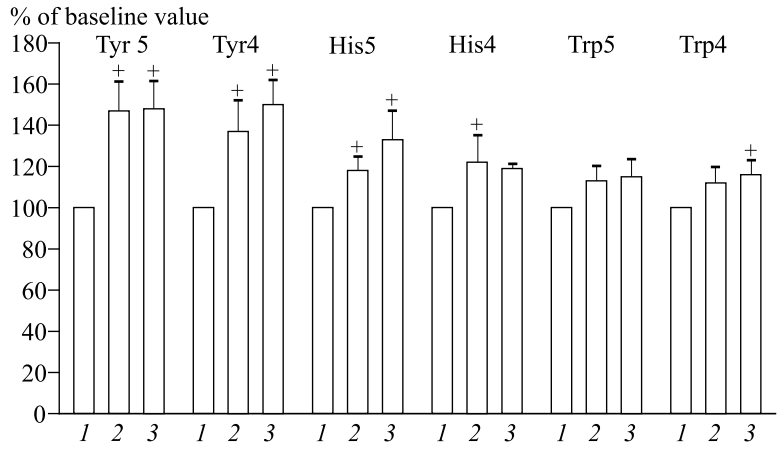

Fig. 2. Amplitudes of evoked contractions of the human right atrial myocardium in tests with tyrosine (5.5×10^{-5} M, Tyr5; 5.5×10^{-4} M, Tyr4), histidine (6.5×10^{-5} M, His5; 6.5×10^{-4} M, His4), and tryptophane (4.9×10^{-5} M, Trp5; 4.9×10^{-4} M, Trp4). Contraction amplitude in Krebs solution (*1*); under the influence of 5.5×10^{-8} M epinephrine (*2*) or amino acid (*3*). $^{+}p<0.05$ in comparison with baseline contraction amplitude (Wilcoxon's test).

nists. In our opinion, potentiation of contraction under the influence of amino acids depends on myocardial β-adrenoreactivity. This is seen from our data that even 6.5×10^{-4} M histidine (series IV) and 4.9×10^{-5} M tryptophan (series V) did not provide marked inotropic effect. The positive inotropic effects of the test amino acids can be also determined by other factors. For example, it was recently demonstrated [9] that L-histidine similarly to L-proline, L-serine, L-alanine, L-methionine, and L-glycine enhances Ca^{2+} entry into cultured rat enteric endocrine cells due to activation of sodium-coupled neutral amino acid transporter 2 (SNAT2). A similar mechanism, namely the increase in Ca^{2+} entry into cardiomyocytes, can underlie the interaction of amino acids and their transporters. The positive inotropic effects of tyrosine, histidine, and tryptophan have ambiguous clinical significance. We believe that our findings should be viewed primarily as applicable to patients with heart failure, because these amino acids administered with food or parenterally can significantly modulate myocardial function.

Thus, similarly to epinephrine, L-tyrosine (5.5×10^{-5} and 5.5×10^{-4} M), L-histidine (6.5×10^{-5} M), and L-tryptophan (4.9×10^{-4} M) increase the amplitude of evoked contractions in isolated atrial myocardium from patients with heart failure that should be taken into account in clinical practice.

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