# EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

# Combined Effects of Blood Serum as a Source of Endogenous β-Adrenoceptor-Sensitizing Agent and Its Analogues Histidine, Tryptophan, Tyrosine, Mildronat, and Preductal

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Human blood serum exhibiting β-adrenoceptor-sensitizing activity does not prevent manifestation of similar activity of histidine, tryptophan, tyrosine, mildronat, and preductal. This opens prospects for the use of analogues of endogenous β-adrenoceptor-sensitizing agent in clinical practice.

**Key Words:** *myometrium; amino acids; β-adrenoceptors*

Previous experiments on longitudinal uterine horn strips from non-pregnant rats under conditions of contracture produced by hyperpotassium (60 mM KCl) Krebs solution (HKS) or against the background of spontaneous contractive activity showed that human blood serum (in dilutions 1:10, 1:50, 1:100, 1:500, and 1:1000) potentiates activation of β-adrenoceptor, which was explained by the presence of endogenous sensitizer of β-adrenergic receptors (ESBAR) in the serum [3,5,6,11]. Histidine, tryptophan, tyrosine (they are regarded as ESBAR components [3,5,7]), preductal, and mildronat exhibited similar activity [5,6]. Activity of ESBAR and its analogues, abovementioned amino acids and drugs, can be explained by their ability to restore disordered conformation of β-adrenoceptors or G-protein α-subunit, *i.e.* by their capacity to restore effectiveness of signal transduction from β-adrenoceptors to intracellular effectors [4,5]. At the same time, physiological role and the mechanism of action of ESBAR, nature of its components, and possibility of clinical application of ESBAR analogues remain to be studied.

The objective of this study was to assess the effects of combined exposure to 100-fold diluted (optimal in terms of β-adrenoceptor sensitizing effect) human blood serum (as the source for ESBAR) and its analogues on effectiveness of β-adrenoceptor activation with epinephrine in longitudinal muscles of uterine horn exhibiting high $\beta_2$-adrenoceptor reactivity in non-pregnant rats [5,8]. Because of instability of spontaneous phasic activity during long-term experiments, the experiments were carried out under conditions of tonic contraction produced by HKS.

## MATERIALS AND METHODS

Five experimental series (Table 1) were carried out on 53 longitudinal uterine horn strips (5-8 mm long and 2-3 mm wide) isolated from 18 proestrus and diestrus rats; the phase of the estrous cycle was determined by vaginal smears [2]. The rats were sacrificed in accordance to Rules for Work with Experimental Animals (1977). Strip contractions were recorded using method [9] at 38°C, 0.7 ml/min perfusion rate (Krebs solution),

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**TABLE 1.** Tonus of Longitudinal Uterine Horn Stripes (in mN and in % of KCl-Imduced) at Different Stages of the Experiment ($M \pm m$)

| Tonus | Stages of the experiment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| | KCl | KCl+Ep | KCl+Ep+ compound | KCl* | KCl+Ep | KCl+Ep+ S-100 | KCl* | KCl+Ep | KCl+Ep+S-100+ compound |
| Series I (histidine, $10^{-4}$ g/ml; $n$=12) | | | | | | | | | |
| mN | 16.4±0.9 | 13.2±0.7[1] | 6.4±1.0[1,2] | 18.7±0.9[2,3] | 17.3±1.0[2,3] | 16.0±1.2[3] | 18.5±1.2[2,3] | 17.1±1.2[2,3] | 9.6±1.4[1,2,4-8] |
| % | 100 | 81.6±4.0[1] | 39.2±6.1[1,2] | 100[2,3] | 92.8±3.0[1-4] | 85.0±4.4[1,3,4] | 100[2,3,5,6] | 93.0±2.2[1-4,7] | 53.5±7.1[1,2,4-8] |
| Series II (tyrosine, $10^{-4}$ g/ml; $n$=11) | | | | | | | | | |
| mN | 13.6±1.3 | 11.3±1.6 | 6.3±1.2[1,2] | 15.5±1.2[2,3] | 13.7±1.3[3] | 11.0±1.2[3,4] | 14.1±1.7[3] | 11.7±1.7[3] | 8.8±1.3[1,4,5,7] |
| % | 100 | 78.2±7.4[1] | 43.9±6.7[1,2] | 100[2,3] | 86.6±4.2[1,3,4] | 68.3±5.1[1,3-5] | 100[2,3,5,6] | 79.5±5.9[1,3,4,7] | 58.7±7.3[1,4-8] |
| Series III (tryptophan, $10^{-4}$ g/ml; $n$=9) | | | | | | | | | |
| mN | 13.9±1.7 | 12.1±1.5 | 9.8±1.3 | 13.6±2.1 | 12.6±1.9 | 11.5±1.8 | 13.5±1.9 | 12.4±1.7 | 9.8±1.2 |
| % | 100 | 86.5±2.3[1] | 69.7±4.0[1,2] | 100[2,3] | 92.9±0.8[1-4] | 84.2±2.4[1,3-5] | 100[2,3,5,6] | 92.3±0.8[1-4,6,7] | 74.6±3.3[1,2,4-8] |
| Series IV (mildronat, $10^{-6}$ g/ml; $n$=11) | | | | | | | | | |
| mN | 10.9±1.6 | 8.2±1.6 | 6.4±1.6 | 9.4±1.8 | 8.2±1.9 | 6.1±1.4[1] | 9.8±1.6 | 8.4±1.7 | 6.8±1.6 |
| % | 100 | 71.8±4.7[1] | 52.4±7.0[1,2] | 100[2,3] | 80.7±5.3[1,3,4] | 59.0±4.5[1,4,5] | 100[2,3,5,6] | 80.5±4.1[1,3,4,6,7] | 61.9±4.9[1,4,5,7,8] |
| Series V (preductal, $10^{-5}$ g/ml; $n$=10) | | | | | | | | | |
| mN | 10.7±1.0 | 8.2±0.8 | 5.8±0.9[1] | 9.9±0.8[3] | 8.3±0.6[3] | 6.3±0.4[1,2,4,5] | 10.8±1.3[3,6] | 9.9±1.2[3,6] | 6.9±0.9[1,4,7] |
| % | 100 | 76.4±2.3[1] | 53.0±5.3[1,2] | 100[2,3] | 85.1±2.4[1-4] | 64.7±2.6[1,2,4,5] | 100[2,3,5,6] | 90.9±0.8[1-7] | 64.0±2.1[1,2,4,5,7,8] |

**Note.** *Stripes were perfused with Krebs solution between stages 3 and 4 and 6 and 7. Superscript numbers: significant differences ($p$<0.05) from the corresponding stages. Ep: epinephrine, $10^{-8}$ g/ml.

passive aeration of the experimental chamber, and 500 mg (4.9 mN) initial load on a Myocitograph setup equipped with 6MX1B mechanotrones, H-3020 writers, TRM1A Oven temperature controlling device, and syringe batchers. Krebs solution (pH 7.4) 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 $NaHCO_3$, 11 mM $C_6H_{12}O_6$. Epinephrine hydrochloride (Moscow Endocrine Factory), histidine (Sigma-Aldrich), tyrosine and tryptophan (Acros Organics), mildronat (GRINDEX), preductal (ANPHARM A.O) were also used. Serum was isolated from venous blood of 11 non-pregnant women (donors) by 20-min centrifugation at 1000 rpm. After 30-min strip perfusion with Krebs solution, its tonic activity was increased using HKS (60 mM KCl), *i.e.* potassium contracture was induced (Table 1). The inhibitory effect of epinephrine ($10^{-8}$ g/ml) alone and then the effect of combined application of histidine or other test compounds were evaluated. The test agents were used in concentrations producing β-adrenoceptor-sensitizing effect [5,7,10] (stages 1-3). Then, the strip was again perfused with Krebs solution, potassium contracture was induced again using HKS, and first epinephrine and then epinephrine in combination with 100-fold diluted serum (S-100) were applied, *i.e.* the intensity of β-adrenoceptor-sensitizing activity of the serum was assessed (stages 4-6). At stages7-9, strip perfusion with Krebs solution and potassium contracture induction were followed by application of first epinephrine alone and then epinephrine in combination with S-100 and histidine or another ESBAR analogue, *i.e.* their combined effect was assessed. Strip tonus was measured in mN and in percent to potassium contracture level, *i.e.* at stages 1, 4, and 7. The results were processed statistically; the differences were assessed using Student's *t* test and were considered significant at $p<0.05$ [1].

## RESULTS

In all experiments, HKS produced relatively stable contracture (Fig. 1). In 5 series, its magnitude during

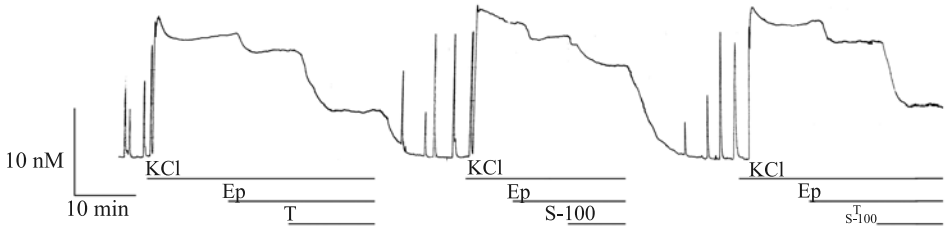


**Fig. 1**. Mechanogram of longitudinal uterine horn stripe from non-pregnant rat demonstrating adrenoceptor-sensitizing activity of tyrosine ($10^{-4}$ g/ml; T), S-100, and their combination against the background of KCl-contracture. Horizontal lines: time of application, including epinephrine application ($10^{-8}$ g/ml; Ep).

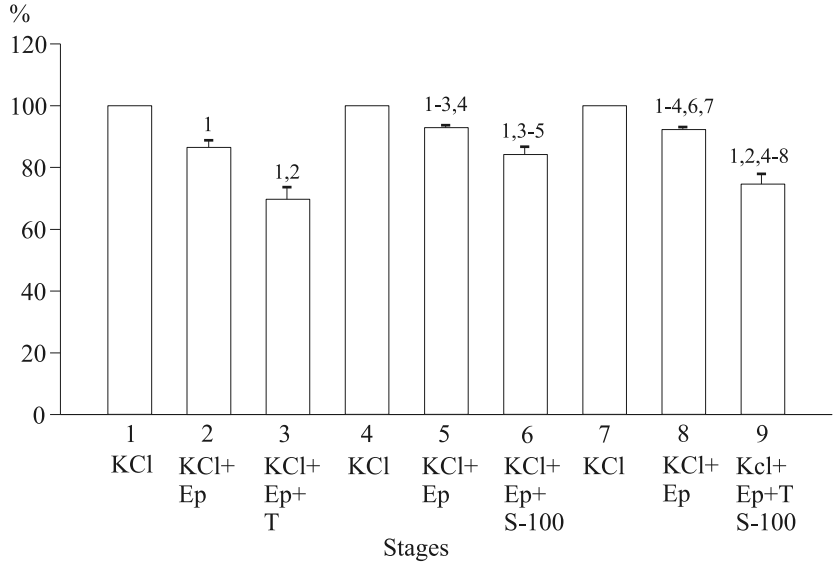


**Fig. 2.** Potassium contracture of longitudinal uterine horn stripes (in percent to stages 1, 4, or 7) after exposure to tryptophan ($10^{-4}$ g/ml; T), S-100, and their combination against the background of KCl-contracture and epinephrine ($10^{-8}$ g/ml; Ep). Numbers above the bars: significant differences ($p<0.05$) from the corresponding stages.

the first exposure to HKS varied from 10.7 to 16.4 mN, during second from 9.4 to 18.7 mN, and during third from 9.8 to 18.5 mN (Table 1). In all series, differences in potassium contracture were insignificant. which indicates its stability. In all series (stages 2, 5, and 8), epinephrine produced slight relaxing effect, which allowed observation of β-adrenoceptor sensitizing effect of ESBAR and its analogues. The first exposure to epinephrine significantly reduced potassium contracture to 86.5-71.8% from its initial level, during the second and third exposures it decreased to 92.8-80.7 and 93-79.5%, respectively. Low epinephrine relaxing effect during the second and third tests (series I, III и V) can be explained by desensitization to epinephrine. All ESBAR analogues in the studied concentrations (stages 1-3) exhibited β-adrenoceptor-sensitizing effect. Thus, potassium contracture during exposure to epinephrine and epinephrine with histidine was 81.6 and 39.2% of the initial level, respectively ($p<0.05$). This confirms the β-adrenoceptor-sensitizing effects of ESBAR analogues [5,6]. S-100 (stages 4-6) significantly potentiated the inhibitory effect of epinephrine (except series with histidine). For example, potassium contracture in tryptophan series after exposure to epinephrine and epinephrine with S-100 (Table; Fig. 2) was 92.9 and 84.2% of the initial value, respectively ($p<0.05$). These results confirmed β-adrenoceptor-sensitizing activity of S-100 [3,5,6], which can be explained by the presence of ESBAR. Combined exposure to ESBAR analogues and S-100 (stages 7-9) increased the inhibitory effect of epinephrine, but to a lesser extent than exposure to ESBAR analogue with epinephrine and to a greater extent than exposure to S-100 and epinephrine. Potassium contracture after exposure to epinephrine and epinephrine with S-100 and histidine was 93 and 53.5% of the initial value, respectively ($p<0.05$, Table), *vs.* 39.2 and 85% after exposure to epinephrine with histidine and epinephrine with S-100. These results indicate that S-100 does not prevent the β-adrenoceptor sensitizing effect of ESBAR analogues. At the same time, no mutual potentiation of ESBAR and its exogenous analogues was noted. It means that the mechanism of β-adrenoceptor sensitizing activity of S-100 is probably the same as in ESBAR analogues. It cannot be excluded that the point of application for ESBAR and ESBAR analogues is the same.

Our data that S-100 does not prevent manifestation of ESBAR effects indirectly suggest the possibility of using ESBAR analogues for improving the effectiveness of β-adrenoceptor activation, *e.g.* in bronchial asthma treatment and risk of premature birth.

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