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Review Article

Endogenous sensitizer of beta-adrenergic receptors (ESBAR) and its analogs (review)

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Abbreviations: AR: Adrenoceptors; beta: ARIM: beta-adrenoceptor inhibitory mechanism; CA: Contractile Activity (of strips); CAU: Contractile Activity of the Uterus; LPC: Lysophosphatidylcholine; LS NPRUH: Longitudinal Strips of Nonpregnant Rat Uterine Horn

Abstract

The results of the 20 years studies of the presence in blood serum and other body fluids of endogenous modulators of adrenergic and M-cholinergic impact as a component of humoral link of autonomic nervous system. The article is devoted to the endogenous sensitizer of beta-adrenergic receptor (ESBAR) - water-soluble low molecular weight substances, analogs of which are histidine, tryptophan, tyrosine, mildronat and preductal. It is shown, that separate dilutions of human serum and animal (as a source of ESBAR) and analogs of ESBAR ways to enhance the effectiveness of activation of beta-adrenoceptors (AR) of smooth muscle (uterus, coronary and renal arteries, trachea, stomach), myocardium, erythrocytes and platelets (respectively influenced of histidine and tryptophan). It is reported that content of ESBAR in human serum (according to the titers of its dilution) depends on the sex and the presence of somatic diseases, and at women are also on the stage of reproduction and obstetric complications It is discussed possible mechanisms of ESBAR action, its physiological role, including as a component of beta-adrenoceptor inhibitory mechanism for myometrium, as well as the prospect of the use of analogs of ESBAR, including for the prevention of preterm labor, and for the treatment of bronchial asthma, coronary heart disease, hypertension and heart failure.

Introduction

Studying chemoreactivity of isolated myometrium of pregnant women and animals (rat, rabbit, pig), we have identified [1-5], that there are a variety of uterostimulatory, i.e. substances, which increase of contractile activity of the uterus (CAU), e.g., oxytocin, serotonin, histamine, but there is only one substance that able to inhibit spontaneous activity and /or induced (by uterostimulators) activity. It is likely, adrenaline, which interacts with beta$_2$-adrenoceptors (AR) of myometrium. Given the available by the time the data is relatively high effectiveness of beta$_2$-agonistin at pregnant women with threat of premature labor (TPL) [6], we have proposed a hypothesis about the functioning the so-called beta-adrenoceptor inhibitory mechanism (beta-ARIM) at pregnant women, the degree of influence on the CAU reduced only before labor [7-9]. According to this hypothesis, at pregnancy in uterine myocytes increased

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expression of beta$_2$-AR gene that leads to the dominance of the beta$_2$-AR above alpha- AR. Therefore catecholamine of blood serum and amniotic fluid, activating the beta$_2$-AR of uterine muscle cells, inhibit the spontaneous CAU and induced CAU, for example, by oxytocin, 5-hydroxytryptamine (serotonin) and histamine (with appropriate receptors). But contrary to this hypothesis, a circumstance known by that time well enough - a phenomenon of desensitization, i.e., loss of effectiveness of the activation of membrane receptors during continuous exposure to the agonist. It was demonstrated in our experiments with isolated myometrium of human and animals, including in relation to the interaction catecholamines with beta$_2$-AR [10]. But in that time the mechanism of desensitization does not have any detailed information about the process of phosphorylation of receptors associated with G-protein as the basis of desensitization and the existence of enzymes, it is now considered to be involved in this process, including kinases of alpha-AR or beta -AR [11], protein kinase A [12], and protein kinase C [13]. However, we have assumed that the effective functioning of the beta-ARIM addition to beta$_2$-AR agonist and it is necessary factor preventing desensitization of these receptors and thereby constantly maintain the effectiveness of their activation [7-9]. We hypothesized that this factor is contained in the blood of mother and possibly in the blood of the fetus. In this regard, we conducted an experiment with the longitudinal strips of nonpregnant rat uterine horn (LS NPRUH), in which assessed the efficacy of the effect of adrenaline on the CA of the strips three times - 1) initially, 2) against exposure to blood serum of pregnant women and 3) after its removal (Figure 1). It was assumed that the presence in the serum of the desired factor in certain dilutions of blood serum should rapidly and reversibly raise the efficiency of inhibition of spontaneous CA of these strips under the influence of adrenaline. Should take two fundamentally important in terms of methodological explanations. 1) LS NPRUH unlike the circular strips of of nonpregnant rat uterine horn mainly reflect the activity of the longitudinal layer of the myometrium, which is characterized by high expression of beta$_2$-AR, in connection with which the adrenalin even in very low concentrations inhibits of spontaneous CA of these strips; but in the circular layer of the uterus of nonpregnant rats no dominance beta$_2$-AR, in this connection, the adrenaline does not inhibit spontaneous CA of these strips, even in very high concentrations [14]. Therefore, to detect the presence of serum hypothetical modulator of beta$_2$-AR best suits LS NPRUH. 2) The threshold concentration of adrenaline, which causes the inhibition of spontaneous CA of LS NPRUH depends on the phase of the estrous cycle (maximum - in metaestrus) [4,14], and also depends on the season and other climatic factors [15]. Therefore, for the detection of the blood factors, that increases the effectiveness of beta$_2$-AR activation in experiments with LS NPRUH. Initially need to find the concentration of adrenaline, which is close to the threshold or slightly above it. All these factors taken into account when searching factor, which enhance the effectiveness of beta$_2$-AR activation. The result of this research was a series of your publications in 1997 year [16-18], which reported that the serum of pregnant women in a dilution of 1:100, 1:500 or even 1: 103 is able to increase the degree of inhibition of spontaneous CA of LS NPRUH under the influence

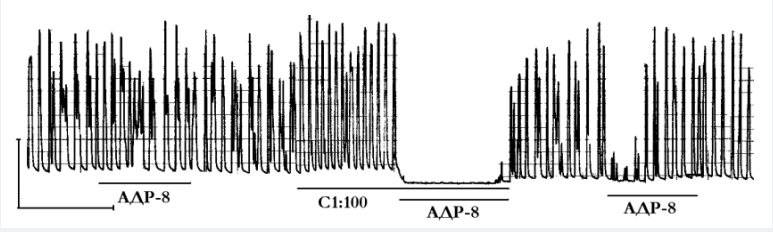

Figure 1: Mechanogram of longitudinal strips of nonpregnant uterine horn rats showing β- adrenosensitizing effect of 100-fold dilution of venous blood serum (C1: 100) of the pregnant woman. The horizontal lines under the mechanogram represent the time exposure of the serum (C:100) and adrenaline (8×10−8 g/ml; АДР-8). Calibration — 10 mN, 10 min. From [10]. Адр-8 — adrenalin, 10−8 g /Ml; C1:100 — serum of venous blood in dilution 1:100.

of a threshold concentration of adrenaline. Those (Figure 1), in the presence of blood serum of adrenaline in the concentration close to the threshold, he showed himself as adrenaline in a concentration close to the maximum [16-18]. In these articles, the first time a hypothetical factor that increases the effectiveness of the beta$_2$-AR activation, was named endogenous sensitizer of beta-AR, or ESBAR. They have also been reported [16-18], that the content of ESBAR, according to the blood serum dilution rate in which there is ESBAR- activity, depends on gender – men have content of ESBAR lower than that women, and women depends on the presence of pregnancy – at pregnancy content of ESBAR increases, which probably contributes to the formation of beta-ARIM. It was noted [16-18], that ESBAR was found in urine, saliva, in the cerebrospinal fluid, and at pregnant women - in the amniotic fluid. This meant that ESBAR can pass through various barriers - to the brain, kidneys, salivary glands, to the fetus, and from it - in the amniotic fluid. Thus, it has been proved the existence factor that enhances the efficiency of the beta$_2$-AR activation, i.e. ESBAR.

These publications preceded yet another of our paper [19], which was first reported that a 100-fold dilution of serum of umbilical cord blood of newborns improves beta- adrenoreactivity circular segments of the pig coronary arteries. If the initially adrenaline, even in very high concentration (10-6 g/ mL) did not reduce their tone, increased high potassium solution (25 mM KCl) Krebs, then the action of adrenaline along with 100-fold dilution of serums causes a very marked decrease in tone (Figure 2). This allowed to conclude that serum indeed contains ESBAR which can improve efficiency of beta$_2$-AR activating not only myocyte of uterus but myocytes of coronary artery. We believed that these works [16-19] are of interest to ESBAR. However, it took almost 20 years, during which time there and our other articles on ESBAR, but so far we have not seen a single work of other laboratories, which would confirm or refute our findings. Perhaps this is due to the fact that to date nature ESBAR is not defined, it is not isolated in pure form and is not set location (s) of its synthesis.

During these years, efforts have been made to clarify the physiological role of ESBAR, in particular, the ability of ESBAR to improve the efficiency of activation of beta$_2$-AR of smooth muscle from pig coronary artery [20,21], the arteries and veins of human umbilical cord [22-24], cow renal artery [25-28], cow trachea [21,29-31], rat stomach [32,33], and also myocardium of frog [34-37], rats [36-42] and humans [43-45] and human erythrocytes [46]. In addition, our efforts were made to find analogs of ESBAR [21,27-29, 35-37,39-43,45-60], to study the mechanism of action of ESBAR and its analogs [36,38-43,56-61], on the role of ESBAR in the pathogenesis of somatic diseases - coronary heart disease [62-64], arterial hypertension [25 26,65,66], bronchial asthma [30,31], and gastric acid-related diseases [33], as well as a number of obstetric complications as the weakness of labor activity, threat of premature labor, preeclampsia and placental insufficiency [15,67]. Preliminary analysis of these dates has been made in our two books [15,68].

Nature of ESBAR, analogs of ESBAR and sources of ESBAR production

We have attempted to study the nature of ESBAR. But to get pure ESBAR, determine its structure and molecular weight failed, including the lack of expensive equipment.

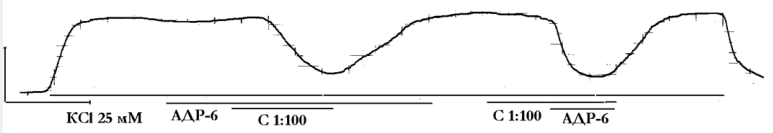

Figure 2: Mechanogram of circular strip of porcine coronary artery showing β- adrenosensibilizatotory activity of 100-fold dilution of umbilical cord blood serum (C 1:100) on the background of tone, induced by hyperpotassium (25 мМ KCl) Krebs solution. The horizontal lines under the mechanogram represent the time of exposure to substances, including hyperpotassium Krebs solution (25 mM KCl), adrenaline (10 -6 – g/ml, ADR-6) and the blood serum (C1:100). Calibration — 10 mN, 10 min From [9]. 25 мМ KCl — hyperpotassium (25 мМ KCl) Krebs.

At the same time determined that ESBAR-activity of blood serum a is relatively stable to 60 minutes of boiling and also for long-term storage, including up to 24 hours at 37°C, or up to 14 days at 4°C or 90 days at -20°C. It is also obvious, that ESBAR is the water-soluble substation as all blood serum dilutions, including exhibiting ESBAR-activity, preparing with Krebs solution [15,18,68]. Preconcluded that ESBAR is a low molecular weight substances, as an ultrafiltrate of blood serum of pregnant women, as well as its low molecular fraction wich obtained by gel filtration on Sephadex G10, retain ESBAR- activity in experiments with LS NPRUH [15,68]. In view of these data it has been searched substances capable exhibits ESBAR-activity. It was found [68-70], that among of the 37 different substances (of low molecular weight with a different physiological effect) including 20 amino acids, 30 substances did not have ESBAR- activity. Among them -antihipocsants (meksidol and emoksipin), acids of Krebs cycle and its salts (α-ketoglutaric acid, oxaloacetic acid, fumarate, succinate sodium), nicotinic acid, blocker Na+-K+-pump (strophanthin K), hormones (thyroxine, hydrocortisone), protein synthesis blocker (adrioblastin), a substance similar in structure to trimetazidine (piperazine) or histidine (imidazole), and 17 amino acids (β-alanine, L-arginine, L-asparagine, L-aspartic acid, L- glutamine, D,L-glutamic acid, L-lysine, L-leucine, L-cysteine, D, L-glycine, D,L-valine, D,L-isoleucine, D,L-methionine, D,L-proline, D,L-serine, D,L-threonine, D, L-phenylalanine. At the same time it was established [15,47,55,56,58,68,70], that ESBAR-activity exhibited 3 amino acids - L-histidine (3x10-8-10-5 g / mL), L-tryptophan (10 -5 g/mL) and D, L-tyrosine (2x10-6-10-5 g / mL), as well as used in cardiology metabolic drugs - trimetazidine (preductal) and trimethylhydrasine propionate (mildronat) [48-50,52,53,56,58] (Figures 3, 4). Furthermore, ESBAR-activity showed nitroglycerin (10-5 g/mL) and ethanol in high concentrations (9,6 x 10-3 g/mL) [68,69]. Data on the ability of the histidine, tryptophan, tyrosine, mildronat and preductal improve efficiency activate of $beta_2$-AR were obtained in experiments with the LS NPRUH, including background spontaneous CA and under tone caused by high potassium (60 mM KCl) Krebs solution (Figure 3) and against the background of an artificial reduction of the efficiency of the activation of $beta_2$-AR by ozonated (5x10-8 g/mL) Krebs solution [51-53], lysophosphatidylcholine [71] or non-selective beta-blocker propranolol [56,58,59]. When this has been shown

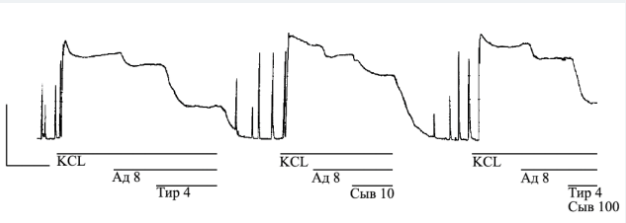

Figure 3: Mechanogram of longitudinal strip of nonpregnant rat uterine horn showing of β- adrenosensitizing effect of tyrosine, 10–4 g/ mL (tyrosine 4), 100-fold dilution of serum (serum 100), including at their combined effect on KCl-induced contracture. Horizontal lines indicate the time of exposure to substances, including adrenaline (10–8 g / ml; Ад 8. Calibration — 10 mN, 10 min. From [20]. KCl - hyperpotassium (25 мМ KCl) Krebs solution; Ад 8 — adrenaline, 10–8 g/mL Тир 4 — tyrosine,10–4 g / mL; Сыв 100 — serum of venous blood in dilution 1:100.

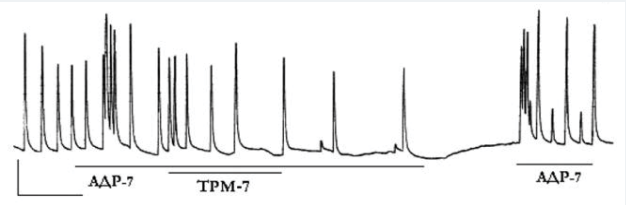

Figure 4: Mechanogram of myometrium strip of pregnant woman showing β- adrenosensitizing effect of trimetazidine. Horizontal lines under the mechanogram reflect the time of exposure to substances, including trimetazidine, 10–7 g / ml (TPM-7) and adrenaline, 10–7 g/ mL (АДР-7). Calibration — 10 mN, 10 min. From [38]. АДР-7 — adrenaline, 10–7 g/mL TPM-7 — trimetazidine, 10–7 g / ml.

relative selectivity analogs ESBAR - in experiments with LS NPRUH they did not exhibit this effect against acetylcholine response [72]. Given that the histidine, tryptophan, and tyrosine are natural components of blood serum, it was suggested that ESBAR is at least one of these three amino acids. Taking into account the concepts of beta-ARIM, the degree of influence on the myometrium which should increase during pregnancy [7-9], as well as our data about increasing of ESBAR-activity of blood serum at pregnancy [16-18], was studied blood levels of 20 amino acids, including histidine, tryptophan and tyrosine at pregnant women and parturient. These studies, however, have not confirmed our hypothesis [54,67]. Contrary to expectation levels of these three amino acids are not increased at pregnancy. Therefore, we hypothesized that ESBAR- activity of blood serum is determined not only by the content of the three amino acids, but also the presence in it other any substances [54,67].

Adrenergic synapses of the myometrium as a source of ESBAR

Given the fact that among the amino acids having ESBAR-activity was tyrosine, known as a precursor of the catecholamines, we hypothesized, that the adrenergic nerve fibers of myometrium can to be source of ESBAR. To prove this hypothesis a series of experiments held [15,18]. In this experiments part of the longitudinal strips of uterine horn of nonpregnant and pregnant rats, as well as strips of myometrium of nonpregnant or pregnant women served as a prospective source of ESBAR («donors» of ESBAR) and identification of ESBAR- activity of perfusate flowing from the strip – «donors», carried out on a test object, in which quality in all series used LS NPRUH. At the same time, the ability of the perfusate flowing from the «donor» strip, modify the test object response to adrenaline, which was used at concentrations close to the threshold (10-9, 10-8 or 10-7 g /mL). In experiments in which strips- donors were strips of nonpregnant uterine horn of rats, it was found that the perfusate collected from donor strip after the beginning of perfusion prior to onset of spontaneous CA (about 20 minutes) had ESBAR-activity, i.e. enhanced the inhibitory effect of adrenaline (10-9, 10-8 or 10-7 g /mL) in 38.6% of experiments. Against the background of spontaneous CA of strips-«donor» ESBAR- activity of perfusates was observed in 46.2% of experiments. Against the background of the 20-minute transmural electrical stimulation of the strips- «donor», which was held in a 30-second bursts of electrostimulation (inside – 3 impuls /s, duration - 0,5ms, voltage - 50V), going every 30 seconds, ie, just 10-11 packs, ESBAR -activity was noted in 33.3% of experiments, and after transmural electrostimulation - in 46.2% of experiments (respectively, P1-,P2-, P3- and P4-perfusates collected for 20 minutes each). If the "donor" is the longitudinal strips of uterine horn of pregnant rats, the values were, respectively, 21.4%, 42.8%, 57.3% and 36.4%. In similar experiments, in which «donors» were strips of myometrium nonpregnant women (obtained at hysterectomy about uterine fibroids), these values were, respectively, 42.9%, 28.6%, 0% and 14.3%, and in experiments in which the strips–«donor» were strips of myometrium of pregnant women (they are dissected with a planned caesarean section from the lower uterine segment) - respectively, 30.0%, 10.5%, 50.0% and 36.8% of experiments. These data allow us to conclude that: 1) the myometrium of rat and human can produce ESBAR; 2) in rats, when in strips generate of spontaneous CA it was increases of productions of ESBAR, and women (non-pregnant and pregnant women), by contrast, is reduced; 3) with transmural electrical products ESBAR at nonpregnant rats and (especially) at nonpregnant women is reduced, and in pregnant rats and pregnant women, on the contrary, increases. It is suggested that during pregnancy the so-called physiological sympathectomy of the uterus, which is described in the literature [73,74], is not a true denervation of the uterus, and it is the conversion of the mediator in the adrenergic terminals of the uterus - instead of noradrenaline, has a pronounced fluorescence, mediator becomes a precursor of catecholamines - tyrosine having a less pronounced fluorescence, which creates the illusion of sympathectomy. It is possible that this phenomenon is due to the suppression in the adrenergic synapses (under the influence of progesterone) of the expression of enzymes genes involved in

the synthesis of dihydroxyphenylalanine (DOPA), dopamine and noradrenaline from tyrosine. Therefore neurotransmitter in the synapse becomes tyrosine, wich have high ESBAR- activity. Thus, tyrosine enhances the effectiveness of beta-AR activation on postsynaptic and extratsynaptic sites of the plasma membrane of muscle cells of the uterus under the influence of catecholamines of blood and amniotic fluid that prevents desensitization of these receptors. This provides braking CAU, ie implementation beta-ARIM. On the eve of the labor is likely to be gradual restoration of synthesis of classical adrenergic mediator - in the beginning it becomes dopamine and then noradrenaline. This reduces the degree of the effect of beta-ARIM on the myometrium and promotes the induction of labor, especially in this period and increased expression of alpha-AP gene.

Physiological properties and mechanisms of action of ESBAR and its analogs

The study of this issue mainly conducted experiments on LS NPRUH showing [61,68], that the blood serum of pregnant women and umbilical cord blood serum (as a source of ESBAR) and analogs of ESBAR –histidine and tyrosine, and in some cases tryptophan increase the inhibitory effect of above-threshold (but not maximum) concentrations of adrenaline, noradrenaline, and dopamine, but not enhance the inhibitory effects of synthetic beta$_2$-agonists (ginipral and partusisten). This suggested that ESBAR and its analogs bind to the so-called amino acid site of beta$_2$-AR and thus allosterically enhance its affinity to catecholamines, but not to synthetic agonists of adrenoceptor having different structure [61,68].

It has also been demonstrated [61,68], that the ability of ESBAR and its analogs (histidine, tryptophan, tyrosine) to improve the inhibitory effect of adrenaline is manifested not only on intact LS NPRUH having spontaneous CA, but also on LS NPRUH whose activity is enhanced artificially, e.g., high potassium depolarization (KCl 60 mM) Krebs solution (рис. 3) or oxytocin (5x10-3 IU/mL). It speaks of the universality action of ESBAR and its analogs, capable in any environment to improve the efficiency of beta$_2$-AR activation In experiments with LS NPRUH was found [61,68], that the effect of ESBAR and its analogs observed relatively quickly - within the first 1-3 minutes from the start of exposure. After a 20-minute application of ESBAR its effect lasts 10 min (after exposure to 500- and 103-fold dilutions of serum) or 80 min (after exposure to 100-fold dilutions serum). ESBAR and its analogs exert their activity on the background of blockade of alpha-AR by nicergoline (10-6 g/mL) [68]. All this has allowed to argue [61,68], that ESBAR and its analogs are positive modulators of direct action, the effect of which is associated with increased affinity of receptor to agonist, to increase the efficiency of the transmission signal from the beta$_2$-AR inside the cell effectors and to counter the development of desensitization. Indeed, in experiments with LS NPRUH it is shown that histidine (3 x10-8, 3x10-7 and 3x10-6 g/mL, but not 3x10-11 g/ mL) inhibits the development of desensitization observed during the 30-minute continuous exposure at high concentration of adrenaline (10-6 g/mL) [68,75], and histidine (10-6 g /mL) counteracts desensitization, developed at 5-fold the short-term (10 min) effects of adrenaline (10-7 g/mL) as an inhibitor of spontaneous contractions [58]. Accordingly, the histidine may have antidesenstization effect in different types of effects of adrenaline to the test object. We suggested [58,68,75], that the basis of histidine antidesenstization effect (histidine as analog of ESBAR) is its ability to inhibit enzymes that cause phosphorylation of the beta$_2$-AR (kinase of beta$_2$-AR or protein kinase A or protein kinase C) and / or activate of phosphatase and thereby reduce the degree of phosphorylation of the beta$_2$-AR, which increases the efficiency of their activation. Unfortunately, we have not studied the effect of serum (as a source of ESBAR), histidine and other analogs of ESBAR on activity of enzymes involved are known [11-13] to desensitization and on phosphatase activity, which is known [76], prevents desensitization. However, regardless of the mechanism of histidine antidesenstization effect, we can talk about the possibility of using ESBAR and its analogs (tryptophan, tyrosine, mildronat, preductal) in clinical

practice in order to improve the efficiency of activation of beta$_2$-AR of cells [58]. In this aspect is important data on the interaction of blood serum (as a source of ESBAR) with analog of ESBAR [59]. They were obtained in experiments with LS NPRUH, which evaluated the effect of a unique ESBAR (histidine, tryptophan, tyrosine, mildronat and preductal) on the inhibitory effect of adrenaline (10-8 g /mL), including the presence in the environment 100-fold dilution of serum of nonpregnant women study was conducted on the background tone, caused by high potassium (60 mM KCl) Krebs solution. (Figure 3). Thus the inhibitory effect was evaluated adrenaline source (1), its effect when combined action with one of the analogues of ESBAR at concentration of 10-4 g / mL (2), the effect of adrenaline together with 100-fold dilution of the serum of nonpregnant women (3) and the effect of adrenaline together with the 100-fold dilution of serum and with one of the analogues ESBAR (4). It was found that the 100-fold dilution of serum of nonpregnant women as a source ESBAR not prevent the expression of beta-adrenosensibilizatory activity of histidine and other analogues of ESBAR though potentiating effect analogues of ESBAR not observed. These data support our proposal about possibility of ESBAR analogs in clinical practice to increase the efficiency of activation of beta-AR of myometrium and other entities, for example, in the treatment of brachial asthma or for inhibiting preterm labor.

Ability of ESBAR and its analogs to restore the effectiveness of the activation of beta-AR, wich decline by the ozone, lysophosphatidylcholine or blocker of beta-AR

As part of the study of the mechanism of action of ESBAR and his counterparts in the experiments with LS NPRUH it was studied the role of these factors in the recovery efficiency of the activation of beta$_2$-AR artificially reduced ozonated Krebs solution [51,53,77], or lysophosphatidylcholine [58,71], or blocker of beta-AR propranolol [56,58].

In particular, it has been established [51,53,77], that perfusion with ozonized Krebs solution (at a concentration of ozone in the environment 5x10-8 g /mL) reduces beta- adrenoreactivity of strips, i.e. reduces of the ability of adrenaline to inhibit their spontaneous CA or tonus induced by high potassium (60 mM KCl) Krebs solution. It was found that a 100-fold dilution of the serum of nonpregnant women as a source of ESBAR and L-histidine (3x10-6 g /mL), L-tryptophan (10-6 g /mL), D, L-tyrosine (2x10-6 g /mL), preductal (10-6 g/mL) and mildronat (10-5 g / mL), even on the background of the ozonized Krebs reduced adrenoreactivity of the strips to the initial level, i.e. remove beta-adrenoblocking effect of ozone. It is set for strips having spontaneous CA, and strips, which initially increased the tone of high potassium (60 mM KCl) Krebs solution. Moreover, in these experiments we have shown that a 100-fold dilution of serum and L-histidine (3x10-6 g/mL) even increased adrenoreactivity of myometrium i.e. increased the inhibitory effect of adrenaline (10-8 g/mL). Although the nature of beta-adrenoceptor blocking action of ozone is unclear, it can be assumed that the ozone is due to the accumulation of reactive species of oxygen destroys the native structure of proteins involved in signal transduction from the beta$_2$-AR into the uterine muscle cells. Therefore, we identified the fact indicates the ability of ESBAR and its analogs (as original chaperones) to restore the native structure of proteins involved in signaling induced by activation of the beta$_2$-AR.

We have also been shown [58,71], that beta-adrenoreactivity of LS NPRUH reduces by lysophosphatidylcholine (LPC) - at a concentration 10-4 g / mL it reduces the ability of adrenaline (10-8 g /mL) to inhibit spontaneous CA of strips or lower tone of strips, wich induce by high potassium (60 mM KCl) Krebs solution. Thus histidine (10-4 g / mL) restores the effectiveness of beta$_2$-AR activation. Similar results were obtained using a chicken egg yolk as a source of well-known [78] LPC. It been shown, that a chicken egg yolk in dilution 1:50 reduces beta- adrenoreactivity of LS NPRUH but histidine (10-5 g/mL) even in the presence of egg yolk restore it [58]. We explain the effect of beta-adrenoceptor blocking of LPC his ability as shown by several authors [78], to activate protein kinase C, which is known [13], together with the protein

kinase A [12] and kinase of $beta_2$-AR [11] phosphorylation of $beta_2$-AR and thus speeds up the process of desensitization. From this perspective, it is believed, that the ability of histidine to restore the effectiveness of $beta_2$-AR activation is probably due to his influence increased of $beta_2$-AR dephosphorylation, which is likely to occur due to activation of phosphatase and inhibition of protein kinase C.

In another series of our experiments with LS NPRUH nonselective blocker of $beta_1$- and $beta_2$-AR propranolol (obzidan) at concentrations of 10-9 -10-6 g/mL dose-dependently partially or completely block the ability of adrenaline (10-7 g / mL) to lower the tone, increased with high potassium (60 mM KCl) Krebs solution [56,58]. Unlike propranolol selective $beta_1$-AR blockers metoprolol and atenolol (10-9-10-6 g / mL) did not show this effect, indicating the absence of $beta_1$-AR in myocytes of longitudinal layer of the uterine horn of nonpregnant rats [56,58]. On the background of propranolol 100-fold dilution of the serum of nonpregnant women (as a source of ESBAR) and any of its analogs - histidine, tryptophan and tyrosine (all -10-5 and 10-4 g / mL), and mildronat and preductal (both - 10-6 g / mL) restores the ability of adrenaline to show an inhibitory effect or hinder manifestation of the blocking action of propranolol. Thus, a series of control experiments indicated that partial blockade of inhibitory effect of adrenaline used in a concentration of 10-7 g / mL, propranolol should be applied at a concentration of 10-9 g / mL, and for full blockade - at a concentration of 10-7 g /mL. On a 100-fold dilution of the blood serum, these values for propranolol were respectively 10-8 g / mL (partial blockade) and 10-6 g /mL (complete block), i.e. in 10 times higher than in controls. On the background of histidine (10-5 and 10-4 g /mL), propranolol could partially or totally block the effect of adrenaline at a concentration of only 10-7 / mL, against tryptophan - at a concentration of 10-6 g/mL, against tyrosine - in concentrations 10-7 g / mL, against mildronat - in concentrations of 10-7 and 10-6 g / mL, respectively, against preductal - 10-9 and 10-6 g /mL. Thus, blood serum as the source of ESBAR and all analogues of ESBAR increased concentration of propranolol necessary for blockade inhibitory effect of adrenaline. Given that the propranolol is blocker of competitive type [79], i.e. it competes with catecholamines for binding site to the active site of beta-AR, the results suggest that ESBAR joining away from the center binding allosterically increases the affinity of agonist to the receptor and thus prevents the action of the blocker. A similar effect ESBAR and its analogs observed in experiments with rat myocardium [39,42,80].

Thus, in general, the results of our experiments with the LS NPRUH suggest that the basis for action of ESBAR and its analogs (histidine, tryptophan, tyrosine, preductal and mildronat) is their ability to increase the affinity of the active site relative to the catecholamines, the ability to counter the desensitization of beta-AR (by reducing the activity of enzymes involved in phosphorylation beta-AP, i.e. kinase of beta -AR, protein kinases A and C), by increasing the activity of phosphatase, and the ability to restore the native structure of proteins involved in beta-AR-signaling. Results of experiments with other objects, listed below, support this idea about the mechanism of action of ESBAR and its analogues.

Influence of ESBAR and its analogs on efficiency of activation of beta-AP of other smooth muscle, myocardium and blood cells

To study the mechanism of action of ESBAR and its analogs and physiological role of ESBAR we examined the ability of blood serum as a source of ESBAR and analogues of ESBAR (histidine, tryptophan, tyrosine, preductal, mildronat) for the manifestation of the effects of adrenaline on smooth muscle of the uterus of women, cow trachea, pig coronary artery, rat stomach, and myocardium of frog, rats and humans and also human blood cells – erythrocytes and platelets.

Myometrium of pregnant women: We have previously found [1,5,15] that physiological properties of isolated myometrium of nonpregnant and pregnant

women differ significantly from the myometrium nonpregnant and pregnant rats [14,15], rabbits [3,15] and pigs [2,15]. It has been shown [1,15], that adrenaline increases the contractile activity (CA) of isolated myometrium nonpregnant women, due to the activation of alpha-AR, but inhibits CA of myometrium of pregnant women (due to activation of $beta_2$-AR) and again increases CA of myometrium from partutient (due to activation of alpha-AR).In the clinical setting we have identified [7,15], that the 10-minute intravenous infusion of adrenaline (0,6 micrograms / min) to women before the onset of labor significantly reduces the numbers of uterine contractions (from 3,2 to 2.5 for 10 minutes, or up to 76% of baseline), and its introduction into an active phase I of stage of labor is the reduction in the contraction frequency (from 3.3 to 2.9 for 10 minutes, i.e. up to 86%) was not statistically significant. Consequently, in labor's ability of adrenaline reduced of contractile activity of uterus (CAU) decreases. At the same time, in both cases adrenaline caused a statistically significant increase in maternal heart rate (corresponding up to to 124% and 113% of baseline), systolic pressure (up to 110% and 108%) and diastolic pressure (up to 110% and 112%), i.e. reaction of the cardiovascular system to the introduction of adrenaline during labor has not changed. In another study, when using outdoor hysterography set [8,15], that 5-minute intravenous infusion of $beta_2$-adrenomimetic partusisten (0.00125 mg per minute) in pregnant women at 28-36 weeks gestation (in the absence of symptoms of threat of premature labor (TPL) significantly reducing amplitude of large waves (on 52% of the original level), frequency of contractions generation (on 47%) and the total activity (on 74%). In women in late pregnancy i.e. at time of 38-42 weeks, the same dose of partusisten causes less decrease CAU - including amplitude (on 27%) and the total activity (on 42%), while women in the active phase of the first stage of labor, the introduction of this dose is much less pronounced reduction of large amplitude waves (only on 16%) and the total activity (only on 25%). These observations suggest that before labor and during labor agonist of beta -AR partusisten even in a small dose can inhibit of the CAU. However, this ability in labor is clearly reduced, that indicating a decrease of beta-adrenoreactivity of myometrium of women in vivo. In similar studies, we have shown [15] that in pregnant women (28-36 weeks) with no signs of threat of premature labor (TPL) partusisten test, wich performed as described previously [8],inhibits background of CAU, while in pregnant women with signs of threat of premature labor (TPL) this test in some cases was negative, ie, not accompanied with inhibition of CAU. This is evidenced about the decreasing of beta- adrenoreactivity of myometrium at TPL. All these data support the notion that activation of the myometrium $beta_2$-AR in pregnant women results in inhibition of CAU before labor and this inhibitory effect before labor is significantly reduced.

Investigation isolated myometrium of pregnant women showed [15-18,68], that the blood serum of pregnant women (in dilutions 1:50, 1: 100, 1: 500, 1: 103) and amniotic fluid (at dilutions 1:10, 1: 100, 1:500, 1: 103) did not affect the expression of the stimulating effect of adrenaline (10-7 and 10-6 g/mL), but reduce it after removal of serum or after prolonged (up to 90 min) exposure to amniotic fluid (Figure 5). Thus,

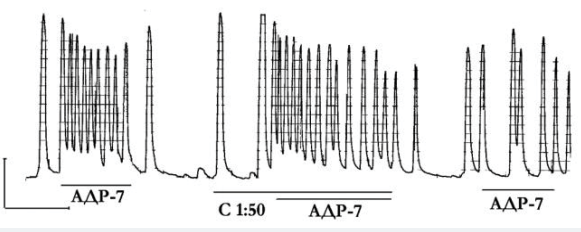

Figure 5: Mechanogram of myometrium strip of pregnant woman showing β- adrenosensitizing effect of 50-fold dilution of blood serum of pregnant women (C 1:50) but not at the time of perfusion and after it. Horizontal liness under the mechanogram reflect the time of exposure to substances, including adrenaline, 10-7 g/mL (АДР-7) and blood serum in dilution 1:50 (C 1:50). Calibration — 10 mN, 10 min. From [11].

these experiments no clearly demonstrate the ability of ESBAR to enhance of beta-adrenoreactivity of pregnant women myometrium of failed. At the same time histidine (3x10-7 and 3x10-6 g/mL) and trimetazidine (preductal, 10–7 g / ml) prevented the manifestation of the stimulating effect of adrenaline, used at a concentration of 10-8 g / mL and 10-7 g /mL, as well as reversial uterostimulatory effect of adrenaline in uteroinhibitory effect (Figure 4). In other words, histidine and trimetazidine (preductal) showed (beta- adrenosensibilizatory activity. Unfortunately, such studies have not been conducted using other analogs of ESBAR - tryptophan, tyrosine and mildronat. Thus, experiments with isolated myometrium of pregnant women is not given conclusive evidence of the applicability of the hypothesis of beta-ARIM against the CAU and the participation of ESBAR in its implementation at women. At the same time, the results of our studies are consistent with the above view that pregnancy at women is accompanied by the conversion of an adrenergic mediator (replacing norepinephrine with tyrosine), which helps maintain high beta-AR activation efficiency, and thereby inhibit CAU.

The content of ESBAR in obstetric and somatic pathologies

Judging by the effective titer of blood serum dilution, it was found that the content of ESBAR at human depends on gender (in women, especially in pregnant women is higher than in men), at pregnant women it depends from the presence of obstetric complications So, the content of ESBAR increased in preeclampsia [15,67] and the weakness of labor activity [15,67] while reducing at placental insufficiency [15,67], but not changed at threat of premature labor [15,67]. As well as in pregnant women with hypertension or vegeto-vascular dystonia. The content of ESBAR varies with somatic pathology - it was reduced at myocardial ischemia or coronary heart disease [62-64], essential hypertension [25,26,65,66], and bronchial asthma [30,31]. This indicates the involvement of ESBAR in the pathogenesis of obstetric complications and a number of somatic diseases.

Conclusion

So, summarizing of the results of 20 year studies about presence in the blood (as well as urine, cerebrospinal fluid, saliva, and amniotic fluid) of endogenous modulators of adrenergic and M-cholinergic reactivity, allow us to consider them as humoral components of the autonomic nervous system (ANS). The focus of this article is given to the endogenous sensitizer of beta-adrenergic receptor (ESBAR). Most likely, that ESBAR is a water-soluble low molecular weight compound, and its analogs are histidine, tryptophan, tyrosine, mildronat and preductal. Several dilutions of human blood serum and the analogs of ESBAR capable to increase the efficacy of activation of beta-AP of smooth muscle of the rat uterus, women uterus, pig coronary artery, cow renal artery, cow trachea, rat stomach and also beta-AP of myocardium from frog, rat and human, as well as beta-AP human erythrocytes (the action of histidine) and human platelets (under the action of tryptophan). The content of ESBAR at human depends on gender (in women, especially in pregnant women is higher than in men), at pregnant women it depends from the presence of obstetric complications and its and it varies with somatic pathology. Although the nature of ESBAR hitherto unknown and ESBAR not isolated in pure form, that it is an obstacle to recognition of its existence, but the physiological effects of ESBAR and its analogs indicate, that ESBAR playing an important role in the regulation of the activities of visceral organs and probably brain structures. In general, ESBAR and its analogs are considered to be direct modulators (urgent action) of $beta_1$-AR and $beta_2$-AR. In the various operating conditions of cells ESBAR and its analogs with short latency increase initial effectiveness of the activation of beta-AR or restore it if it was lowered during prolonged agonist interaction with beta-AR (i.e. at desensitization of beta-AR), or exposure adrenoblockers or when exposed to damaging factors such as ozone or LPC. It is assumed that the basis of this action of ESBAR is the ability of ESBAR or its analogs bind with amino acid site of beta-AR and thus allosterically enhance the affinity of the beta-AR to catecholamines. Simultaneously ESBAR and its analogs are

likely to inhibit the phosphorylation of beta-AR (possibly due to inhibition of kinase of beta-AP, proteinkinase A and proteinkinase C) and accelerate the dephosphorylation of beta-AR by activating phosphatase, and (like chaperones) restore the native protein structure involved in the beta-AR-induced signaling. This is obvious evidence of the need for a more thorough study of these provisions and the selectivity with respect to different populations of beta-AR ($beta_1$-AR, $beta_2$-AR and $beta_3$-AR). Substantiates the notion that prevents of desensitization of beta-AR, ESBAR promotes the functioning of the beta-adrenergic inhibitory mechanism (beta-ARIM) in pregnant women, necessary for inhibition of contractile activity of uterus (CAU). This function of ESBAR is realized by involving adrenergic terminals which are supposed to occur conversion mediator at during pregnancy, i.e. instead of adrenaline or noradrenaline tyrosine temporaril becomes of mediator. Therefore ESBAR and its analogs can play an important role in the prevention of preterm labor. ESBAR involved in the regulation of activity of the heart, circulatory system and respiratory tracts. Therefore deficiency of ESBAR- content may be a cause of myocardial ischemia (coronary heart disease), essential hypertension or bronchial asthma, and the use of ESBAR and its analogs may be a promising method for the prevention and treatment of these pathologies, as well as such a formidable status as heart failure. It is alleged that the heart rate variability (HRV) reflects not only the state of the autonomic nervous system (ANS), as is commonly believed, but also reflects of the blood levels of endogenous modulators, including ESBAR. We believe that the selection ESBAR in its pure form, the development of reliable and affordable method for determining the content of ESBAR, creating unique arsenal analogs of ESBAR and study of the possibility and feasibility of clinical application - all this is an important tasks for future research.

Core tip

In humans and animals there are factors that significantly modulate the efficiency of activation of beta- and alpha- adrenergic receptors, as well as the M-cholinergic receptors. Although these endogenous modulators (sensitizers and blockers) has not yet been isolated in pure form, but their physiological effects are very pronounced. The main purpose of our review - to draw attention to these factors, and, above all, to the endogenous sensitizer of beta-adrenergic receptor (ESBAR). This factor, as well as its analogs (histidine, tryptophan, tyrosine, preductal, mildronat) is capable s able to restore the effectiveness of the activation of beta-adrenergic receptors, reduced under the influence of various effects, including at heart failure.

Tsirkin VI, Sizova EN, Polezhaeva TV, Khlybova SV, Morozova MA, Truhin AN, Korotaeva YV, Khodyrev GN. Endogenous sensitizer of beta-adrenergic receptors (ESBAR) and its analogs (review).

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