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THE INFLUENCE OF GLUTAMINE AND ETHANOLAMINE-O-SULPHATE ON NEUROACTIVE AMINO ACIDS CONTENT IN THE RAT ORGANS IN NORM AND WITH EXPERIMENTAL STREPTOZOTOCINE DIABETES

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The research studies the separate and combined action of inhibitor of GABA-T ethanolamine-O-sulfate (EOS) and glutamate and GABA precursor glutamine on glutamine family amino acid content in the brain and liver of rats. The study demonstrates a higher efficiency of combined administration of those compounds at GABA level in brain and liver. The combined administration of those compounds produces a protective effect on the amino acids in the organs of rats with experimental streptozotocine diabetes, more specifically, in the brain and the pancreas. The pancreas of rats maintains concentrations of neuroactive amino acids the compared with brain. Given the role of GABA in the regulation and secretion of insulin, the combined use of glutamine and EOS can be substantial in the first type diabetes.

Brain – pancreas – diabetes – neuroactive amino acids

Ուսումնասիրվել է ԳԱԿԹ-տրանսամինազի արգելակիչ էթանոլամին-O-սուլֆատի (ԷOU) և ԳԱԿԹ-ի նախորդ գլուտամինի անջատ և միասնական ազդեցությունը գլուտամինի ընտանիքի ամինաթթուների պարունակության վրա առնետի ուղեղում և լյարդում: Յույց է տրվել միասնական ազդեցության ավելի բարձր արդյունավետությունը ԳԱԿԹ-ի մակարդակի վրա ուղեղում և լյարդում: Բացահայտվել է նյութերի միասնական տրման պաշտպանիչ ազդեցությունը առնետի օրգաններում ամինաթթուների պարունակության վրա, մասնավորապես ուղեղում և ենթաստամոքսային գեղձում փորձարարական ստրեպտոզոտոցինային դիաբետի ժամանակ: Յույց է տրվել նյարդաակտիվ ամինաթթուների ուղեղի հետ համեմատման կոնցենտրացիաների առկայությունը ենթաստամոքսային գեղձում: Հաշվի առնելով ԳԱԿԹ-ի դերը ինսուլինի սինթեզի և սեկրեցիայի կարգավորման մեջ գլուտամինի և ԷOU-ի միասնական օգտագործումը կարող է էական լինել առաջին տիպի դիաբետի ժամանակ:

Ուղեղ – ենթաստամոքսային գեղձ – դիաբետ – նյարդաակտիվ ամինաթթուներ

Исследовано раздельное и совместное действие ингибитора ГАМК-трансаминазы этаноламин-О-сульфата (ЭОС) и предшественника глутамата и ГАМК глутамина на содержание аминокислот семейства глутамина в мозге и печени крыс. Показана большая эффективность совместного введения препаратов на уровень ГАМК в мозге и печени. Выявлен протективный эффект совместного введения препаратов на уровень аминокислот в органах

крыс, в частности в мозге и поджелудочной железе при экспериментальном стрептозотоциновом диабете. Показано наличие сравнимых с мозгом концентраций нейроактивных аминокислот в поджелудочной железе крыс. С учетом роли ГАМК в регуляции синтеза и секреции инсулина совместное применение глутамина и ЭОС может быть существенно при диабете первого типа.

Мозг – панкреас – диабет – нейроактивные аминокислоты

The amino acids of glutamine family play a key role in nitrogen metabolism. They provide jointing processes of protein α-amino nitrogen release and its resynthesis. Possibly this role determines the use of these amino acids not only as a source of energy and plasticity but also as potent mediator of metabolism, particularly as neurotransmitter. It should be mentioned that glutamate- and GABAergic systems are the principal neurotransmitter systems, involved in nearly all functions (energy, plasticity, information, cognition) of brain in norm and pathology [3, 13, 19, 22].

Hence, the specified amino acids and their agonists and antagonists are used in the treatment of a number of neurodegenerative and neuropsychical processes of the central nervous system [4, 27]. It is known that the main source of glutamate and GABA in brain is glutamine wich, in contrast to glutamate, is not neurotoxic and may preferably be used as GABA source. The latter, as per our data, can be formed in a roundabout way [15, 16]. In this connection, it is more reasonable to use glutamine together with non toxic or low toxic inhibitors of GABA-T for the purpose of GABA generation. The presence of GABA and the enzymes of its synthesis and breaking up in pancreas has long been known [11, 12, 24]. The participation of glutamic acid decarboxylase localized in β-cells in the launching of autoimmune processes, inducing their death is also well known (2). The determination of antibody to glutamic acid decarboxylase is used as a screening test on the diabetes type I risk of development [2, 18]. In addition, the GABA participation to autoimmunity inhibition on the CD-3, CD-4 and CD-8 levels of T-cells [30, 32] as well as ability to stimulate insulin synthesis and secretion by β-cells of Lanherhance islets have been shown [6, 29].

In the present study, we examined the influence of glutamine as a GABA source and EOS as an inhibitor of GABA-T on the content of the glutamine family amino acids in the brain, liver and pancreas in norm and experimental diabetes, induced by streptozotocine.

Materials and methods. Mail white rats with weights of 180-200 g were used for the experiments. The animals were housed in a vivarium of the Institute of Biochemistry of the National Academy of Sciences of Armenia and had access to standard foods. The animals were split into 4 groups, with 5 rats in each group: I-control group received intraperitoneal 0.5 ml physiological solution a day, II – 40mg/kg glutamine, III – 500 mg/kg etanolamine-O-sulfate, IV – both preparations together. In 3 days the animals were decapitated with light ether anesthesia, brain and liver were removed, carefully cleaned from covers and blood in the cold, and samples of tissues were homogenated in 6% $HClO_4$. After proteins precipitation, the extracts were neutralized with 2 M KOH to pH 5.0, centrifuged at 6000 revolutions per minute and 0^0C for 10 min. The supernatants remained at 0^0C for 24 hours, and then were centrifuged again for 20 min at 16000 revolutions per minute. The separation of perchlorate extracts amino acids was performed by high voltage paper (FN11, Germany) electrophoresis in pyridine-acetate buffer, pH 3.9. The electrforesis duration was 1.5 hour at current power 0.5 mA/cm and 1300 V. The amino acids were revealed by 0.2 % solution of ninhydrin in 95 %acetone with some drops of ice acetic acid. The electroforegrams were dried in thermostat at 60^0C for 20 min, then the amino acids were eluted by 5ml of 0.005 % $CuSO_4$ in 75% ethanol and after exposition during 1 hour the colour intensity was recorded at 540 nm on the Specol-11. Amino acids contents were calculated using standard curves, constructed with amino acids of Sigma Chemical Company (USA) [17]. Blood glucose was determined using Accu-Chek glucometer (Germany). In a separate series of experiments, the influence of preliminary intraperitoneal administration of glutamine and EOS on the amino acids content in rat organs (brain, liver, and pancreas), subjected to the toxic action of diabetogen streptozotocine (60 mg/kg), was studied.

The rats were separated into 2 groups: the first group received physiological solution the duration 3 days, second group received EOS mixture with glutamine. On the forth day streptozotocine was administrated intraperitoneally to all animals and in 5 days the rats were decapitated with light ether anesthesia. Amino acids contents were determined in brain, liver and pancreas.

In separate experiments, the content of amino acids in the pancreas of sheep was determined.

Statistical analysis was done by GraphPad Prism software using unpaired Student's t-test. The differences were considered as significant at the level of $p<0.05$ and marked with asterisk.

Results and Discussion. The literature data testify to the presence of appreciable quantity of GABA in animal pancreas. With this regard, we determined the content of neuroactive amino acids of the sheep pancreas in the initial series of our experiments. The data obtained testify to the presence of high level of neuroactive amino acids such as aspartate, glutamate, GABA and glutamine in the rat pancreas. The concentrations of the specified amino acids can compare with those present in brain. Given that GABA is basically present in β-cells of Lanherhanse islets, for the latter the GABA level in pancreas is most likely to be higher than in brain.

Table 1. The content of amino acids in the rat pancreas

Amino acids	μM/g
Glu	4.1±0.03
GABA	0.98±0.06
Gln	4.6±0.2
Asp	1.3±0.15
Ethanolamine	0.68±0.08

There are also remarkable ethanolamine quantities observed in the pancreas, which like GABA, favour the glucose carrier via biological membrane [23]. Oleil-ethanolamine also promoted glucose transport on the streptozotocine induced model of rat diabetes [5]. At the same time, the glucose inhibits the insulin secretion by β-cells through the intensification of GABA-shunt [31]. In addition both ethanolamine acylation [1] and acetylation [17] as well as anti-inflammatory and anti-allergy properties of ethanolamine acyl derivatives provide possible still long ago [7, 28]. It should also be noted that EOS is an inhibitor widely presented in pancreas GABA-T [9]. The synthesis of that inhibitor is quite possible in liver, where the enzymes of monoamines sulfatation are quite active.

Tab. 2 shows the data of the content of amino acids in the brain and liver after a 3 day intraperitoneal administration of EOS, glutamine and its mixture to rats.

The content of amino acids in rat brain increases more in the case of GABA-T inhibitor EOS administration (7.8 against 4.5 μM/g) than in the case of GABA precursor glutamine (6.2 against 4.5 μM/g). Interestingly, in case of combined administration of EOS and glutamine the effects on the GABA cumulate. Thus, the combined application of non toxic inhibitors of GABA-T and GABA precursor is reasonable in pathologic conditions related to brain GABA-ergic mechanisms.

It is characteristic that the level of neurotoxic dicarboxylic amino acids in the brain doesn't change, while the glutamine concentration increases both when administered separately and in combination with EOS. In the liver compared the brain, although small but statistically significant increase of GABA level is observed more, with pronounced in combined administration of preparations. At the same time, a decrease of aspartate and ethanolamine concentration is observed.

Table 2. The influence of intra-peritoneal administration of glutamine and EOS on the amino acid and ethanolamine content in rat brain and liver

Amino acids μM/g	Control		EOS		Glutamine		EOS + glutamine	
	brain	liver	brain	liver	brain	liver	brain	liver
	8.5±0.9	2.0±0.3	10.2±0.7	1.8±0.2	9.3±0.7	1.7±0.3	7.9±0.4	1.7±0.2
GABA	4.5±0.4	0.13±0.02	7.8±0.6*	0.16±0.01*	6.2±0.3*	0.14±0.01	11±0.5*	0.19±0.02*
Gln	4.5±0.4	3.2±0.26	3.9±0.2	3.0±0.38	6.2±0.3*	3.6±0.5	6.3±0.5*	3.1±0.3
Asp	3.9±0.4	2.9±0.4	3.9±0.4	2.3±0.25*	4.2±0.3	1.4±0.2*	3.6±0.5	1.5±0.1*
EA		2.2±0.3		2.1±0.17		2.1±0.18		1.3±0.3*

** - p<0.05, n=5, EA-ethanolamine*

In a separate part of experiments we investigated the change in the content of amino acids in rat brain, liver and pancreas. After a combined intra-peritoneal administration of EOS and glutamine to rats during 3 days, streptozotocine has been injected on the 4th day. Rats of an other group were injected with physiological solution during 3 days, and then streptozotocine was injected on the 4th day. 5 days later the rats were decapitated with a light ether anesthesia, and the content of amino acids in specified organs was determined.

The results presented in tab. 3 show that the preliminary administration of EOS and glutamine to rats promote the conservation of amino acid concentrations in organs, particularly of GABA, in brain and pancreas.

Many authors, back in 70-80s, showed the increase of GABA level in brain and peripheral organs in various modes of EOS administration [8, 9, 1, 29].

We revealed brain an alternative way of GABA formation from glutamine via GABA-amid, which is activated in neurointoxication [15, 16]. The presence of this process in the β-cells of pancreas is quite possible. Some GABA-T inhibitors are used in the treatment of brain activity disturbances, related to GABA ergic mechanisms [4, 27]. Also the EOS influence on the content of neuroactive amino acids in the liver and kidney [26], but not in pancreas is shown, where GABA affects both the endocrine [11, 12, 24] and the exocrine function [6, 25].

Table 3. The influence of intraperitoneal glutamine and EOS administration on the content of amino acids and ethanolamine in rat with experimental diabetes

Amino acids	CT			Gln+ EOS +CT		
	brain	liver	pancreas	brain	liver	pancreas
Asp	2.2±0.065	2.0±0.18	1.2±0.1	2.6±0.3	2.9±0.2*	1.4±0.2
Glu	6.5±0.7	1.4±0.3	2.1±0.2	8.0±0.6*	1.6±0.25	4.6±0.7*
Gln	4.0±1.0	2.4±0.36	3.1±0.4	6.5±0.4	3.9±0.3*	7.1±0.9*
GABA	1.7±0.1	0.4±0.07	0.7±0.1	3.2±0.5*	0.65±0.1*	1.2±0.2*
EA	1.45±0.25	1.05±0.06	0.63±0.1	2.3±0.3*	1.2±0.2	1.1±0.2*

n=5, CT- streptozotocine, EA-ethanolamine

The data obtained (tab. 3) with regard to maintaining the level of GABA and its precursors in pancreas in case of induced damage of β-cells by streptozotocine, where GABA, according to most studies, assists the insulin synthesis and secretion [2, 11]. Of course, there are data on the inhibition, by GABA and baclofen, insulin synthesis and secretion by β-cells of perfused pancreas with safe microcirculation [10]. The authors associated this with the activation of GABAB receptors. Along with it, there is dependency of this effect on both the concentration of amino acids and on the glucose in the perfused solution.

The participation of GABAergic mechanisms in the regulation of blood glucose homeostasis is widely known. The inhibition of central GABAA receptors leads to an increase of blood plasma glucose level [20]. Consequently in the pathogenesis of diabetes, not only the disturbances of the pancreas GABA ergic mechanisms are important, but also those of brain.

Glutamine and EOS administration also provide a higher level of amino acids in such organs as brain and liver. Moreover, the blood glucose concentration, compared to streptozotocine group, is 1.4 times less (16.5±1.2 against 23.7±1.9 mM/l). Possibly, the preparations influence not only the synthesis and secretion of insulin but the glucose utilization as well.

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