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EFFECT OF GLUTAMIC ACID *IN VITRO* ON THE SECRETION OF GLUCOCORTICOIDS BY THE ADRENAL GLANDS OF RABBITS (*ORYCTOLAGUS CUNICULUS*) – PRELIMINARY STUDIES

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Abstract

Glutamic acid is the main neurotransmitter responsible for stimulating the central nervous system. Released by the postganglionic cells of the nervous system, it reaches all organs it can interact with through specific receptors. The presence of glutamic acid receptors has also been demonstrated in the adrenal glands, which indicates that it may modulate their hormonal activity. The aim of the study was to determine the in vitro effects of glutamic acid in relation to adrenocortical secretion of cortisol and corticosterone of rabbits. The results of the experiment confirmed the direct influence of glutamic acid on the endocrine activity of the adrenal cortex of rabbits in the secretion of cortisol and, to a large extent, corticosterone. The use of ketamine, a nonspecific ionotropic receptor antagonist for glutamic acid for the incubation of adrenal tissue, indirectly indicates the presence of this type of receptors in the adrenal glands of rabbits and their participation in

the modulation of hormonal activity in the secretion of glucocorticoids. Varied reaction and the proportions of the release of cortisol and corticosterone implicate the role of glutamic acid in various pathways of adrenal steroidogenesis. The results prove the validity of the use of ketamine for anaesthesia in the species, the adrenal glands of which synthesise cortisol.

Key words: glutamic acid, cortisol, corticosterone, adrenal glands, rabbit

Introduction

Glutamic acid (Glu) is the main neurotransmitter responsible for stimulating over 50% of neurons in the central nervous system (CNS). Glu acts on target cells through two main types receptors. The first ones include ionotropic receptors: NMDA (*N*-methyl-D-aspartate receptors), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) and kainate receptors, which are related to the flow of calcium, sodium and potassium ions. The second group consists of metabotropic receptors, which are divided into three subgroups characterized on the basis of the mutual similarity of the amino acid sequences and the method of signal transduction. Glu, by affecting target cells in the CNS and in the periphery, plays an important functions: it is responsible for binding and removing excess ammonia through the blood-brain barrier, has an antidepressant effect, removes symptoms of general fatigue, and indirectly contributes to the improvement of overall activity digestive. Research shows that both the deficiency and the excess of glutamate can contribute to the development of diseases such as bipolar disorder, schizophrenia, depression, Alzheimer's disease (Hyde et al., 2011; Jin et al., 2018).

It has been clearly shown that Glu is released from the cells of the adrenal medulla (Romero et al., 2003) and can also directly affect the adrenal glands. This is confirmed by the presence of many of its receptors, including the endocrine gland in rodents as well as in humans. Controlling their activity results in an increased release of catecholamines from the adrenal medulla when NMDA receptors are activated in mice (Watanabe et al., 1994), but also affects the adrenal cortex, where AMPA receptors are located (Kristensen, 1993). For many years, research has been carried out on stress and the complex reactions of shaping the body's adaptive processes. It has been established that the adrenal glands are important in the context of the body's endocrine response to stress. They condition the relevant metabolic reaction, and also provide the body with the energy necessary to counteract stress. In turn, the control of the

functions of all organs is conditioned by the activity of the nervous system, whose is responsible for integrate the functioning of the body, register stimuli and process the information contained in them. The body has two systems that regulate its responses to stressors. The first is the autonomic nervous system, along with the sympathetic adrenal system (SAS). It is triggered at the very first moment of a stress reaction, and its purpose is an immediate reaction to the stress factor (Sapolsky et al., 2000). The results of the Llewellyn-Smith team (1992, 1995) showed the presence of glutamatergic innervation in sympathetic-adrenal neurons.

The second and also a key regulatory system regulating the body's responses to stressors is the hypothalamic-pituitary-adrenal axis (HPA) responsible for the control of the amount of released glucocorticoids. In addition to the preparation of the functioning of the organism in the case of disturbed homeostasis of this system called the stress axis, controls the intensity of the stress reaction by regulating its activity, and also sends a signal to its end. The degree of stimulation of both of these systems depends on the type of stress factor acting on the body, its frequency, intensity and duration (Hardy and Pollard, 2006). Each adrenal gland has two distinct structures, the outer - adrenal cortex and the inner –adrenal medulla. The adrenal medulla is made of chromaffin cells, responsible for the synthesis of catecholamines, mainly adrenaline, but also norepinephrine. The cortex is an essential part of the gland and consists of three layers: zona glomerulosa synthesizing mineralocorticoids (aldosterone), zona fasciculata synthesizing glucocorticoids (cortisol, corticosterone and cortisone), zona reticularis directly adjacent to the adrenal medulla and synthesizing and estrogens (Challis et al., 2000). In most mammals, cortisol is the main glucocorticosteroid, which has a significant impact on the rate and direction of metabolism, especially protein and fat metabolism, while corticosterone is predominant in birds and rodents. The synthesis and increased release of cortisols and corticosterone into the circulatory system in stressful situations facilitates metabolic adaptation to changed environmental conditions, and is directly conditioned by the influence of stress-generating factors on the body. Stressors include numerous physical and mental stimuli that trigger emotions i.e. fear, anxiety, anxiety or forced contact with humans (Rosol et al., 2001).

In the light of the background data presented concerning the properties of glutamic acid, it seems interesting to determine its role in the regulation of adrenal glands function in the secretion of glucocorticoids. Ketamine has been used in the clinic for many years, as a non-specific antagonist of ionotropic glutamatergic receptors with analgesic and anesthetic

applications. This drug enhances the effects of dopamine and stimulates the brain's reward system, but also acts as an analgesic, antidepressant in humans and is used in addiction treatment therapy (Wolff and Winstock, 2006).

The aim of the study was to determine in vitro the amount of glucocorticoid steroids secreted into the incubation medium - cortisol and corticosterone from rabbit adrenal tissue after three doses of glutamic acid, and also after inhibition of the activity of ionotropic receptors for this neurotransmitter.

Material and methods

The experiment was carried out on 7 immature female rabbits (Oryctolagus cuniculus) at the age of 12 weeks. The animals were maintained in individual cages with dimensions consistent with the recommendations for the battery system, standing in a hall equipped with lighting (14 L:10 D), with forced and controlled ventilation and free access to water and feed (DeHeus). The material for analyzes (adrenal glands) was obtained thanks to the Department of Genetics Animal Breeding and Ethology of the University of Agriculture in Krakow. The rabbits were decapitated and the recovered adrenal glands were placed in Petri plate on ice in a physiological saline solution. Then, the adrenal glands from each rabbit were cut into smaller pieces of similar weight (approx. 50 mg), covering the cortex and medulla of this gland. Adrenal tissue sections were placed in incubation wells in a multi-well plate (cell culture; Sigma-Aldrich, St. Louis, USA) containing 1 ml of incubation Krebs medium (Krebs phosphate buffer with 0.3% glucose and 0.1% bovine albumin BSA). Tissue incubation was carried out in an atmosphere of carbogen - 95% O_2 and 5% CO_2 at 38 ° C in incubator (Sanyo, Tokyo, Japan). The dose was previously experimentally determined; these are unpublished data and data from a previous experiment (Szpręgiel et al., 2020). After 10 minutes of incubation in Krebs medium, the adrenal glands sections were transferred to subsequent wells containing pure Krebs medium and 3 different doses of glutamic acid (L-glutamic acid monosodium salt hydrate; Sigma-Aldrich, St. Louis, USA): I - 5 μM, II - 50 μM, III - 200 μM in a volume of 1 ml of Krebs medium. Every 30 minutes, each section of adrenal glands was transferred to consecutive incubation wells with Krebs medium containing glutamic acid in the above-mentioned doses. The medium collected from the 30, 60, and 90 minute incubation wells of rabbit adrenal glands was frozen until analyzed. Subsequent sections of adrenal glands obtained from each rabbit were placed in 1 ml of Krebs buffer in incubation wells with three doses of ketamine at a concentration: I - 1 μM, II - 10 μM and III - 20 μM (Bioketan, Lure, France) for a period of 60

minutes (pre-incubation), then, as before, a section of adrenal glands was transferred to subsequent wells every 30 min, which contained only pure Krebs buffer. The medium collected from the 30, 60 and 90 minutes experimental wells was frozen until analyzed. Cortisol concentration in the incubation medium was determined by radioimmunoassay (RIA) using Cortisol-RIA-CT kits (DIAsource, Louvain-la-Neuve, Belgium). The radioactivity of the samples was measured in a gamma counter "Wizard" (LKB, Vienna, Austria). The sensitivity of this hormone determination method was 0.9 μg / L, the intra-run error was 11.5%, and the extra-run error was 6.2%. The concentration of corticosterone in the incubation medium was determined by the immunoenzymatic method (ELISA) using Corticosterone-EIA-4164 kit (DRG, Marburg, Germany). The results were reported by spectrophotometric analysis at a wavelength of 450 nm using an Epoch 2 (BioTek, Winooski, USA). The sensitivity of this hormone determination was 0.56 ng / ml, the intra-run error was 6.1%, and extra-run error was 3.6%. The results were converted for 1 mg of adrenal glands.

Data analyses

The results were statistically analyzed using two-way analysis of variance in completely randomized blocks. The significance of differences between the mean values was determined by Duncan's test. The calculations were carried out using SigmaStat 2.03 software (SPSS Science Software GmbH, Erkrath, Germany). A probability of $p < 0.05$ or $p < 0.01$ indicated statistically significant or highly statistically significant differences, respectively, between the mean values. Figures were prepared using Grapher 12 (Golden Software Inc., Colorado, USA).

Results

In the control group, during a 90-minute experiment, a gradual decrease in cortisol secretion was demonstrated from 1.5 ± 0.25 ng / mg of tissue after the first 30 minutes to 0.54 ± 0.22 ng / mg of tissue after 90 minutes, only this value turned out to be significantly lower than the initial one (P <0.01; Fig. 1). In all three experimental groups, no significant differences were found in each of the measurements (30 min, 60 min, 90 min) to the same extent after the use of glutamic acid at the concentration of 5, 50 and 200 μM (P> 0.05).

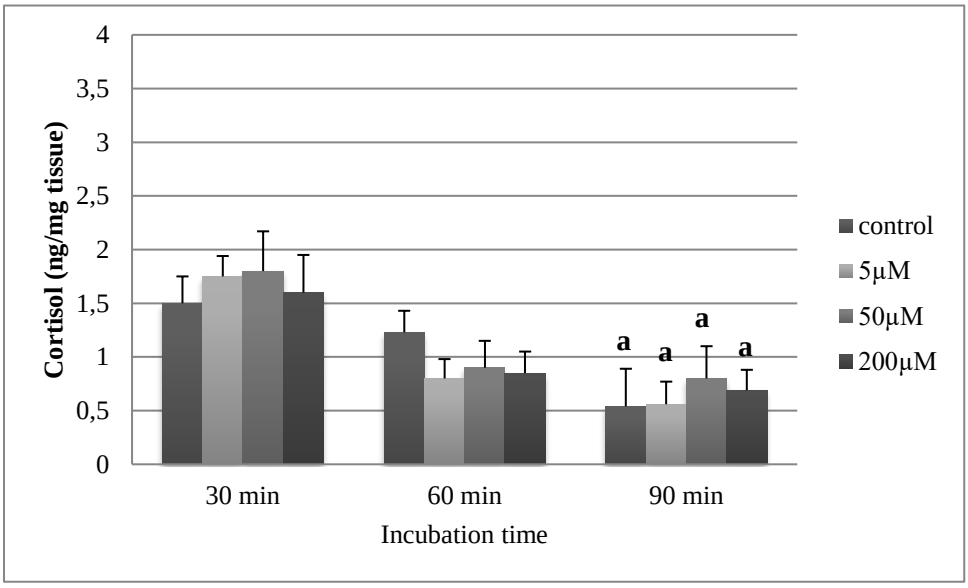

Fig. 1. Effect of glutamic acid on cortisol secretion from rabbit adrenal tissue.

a - significantly different values (P <0.01) compared to the values after 30 min of incubation

b - significantly different values (P <0.01) compared to the control group value

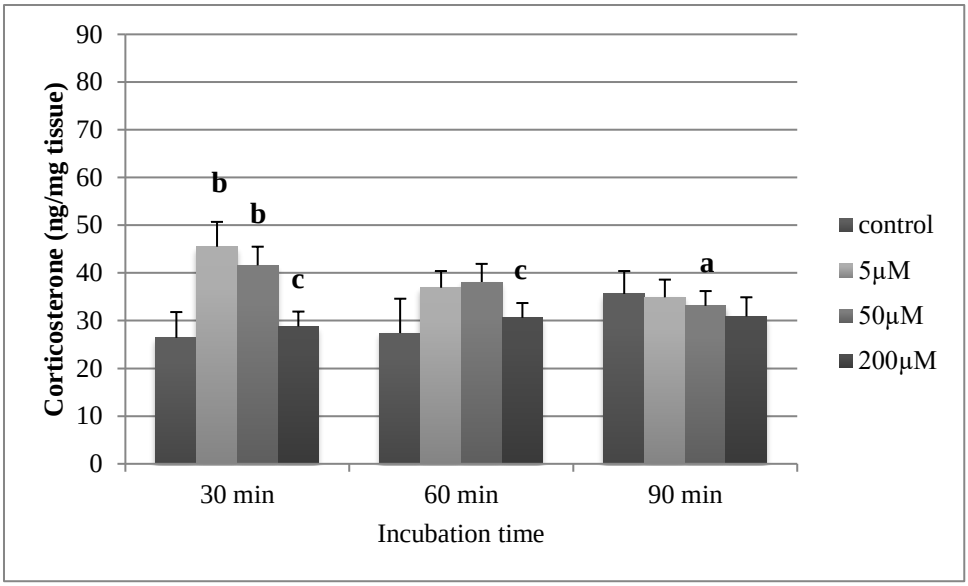

Fig. 2. Effect of glutamic acid on corticosterone secretion from rabbit adrenal tissue.

a – significantly different values (P<0.01) compared to the values after 30 min of incubation

b – significantly different values (P<0.01) compared to the control group value

c – significantly different values (P<0.01) between experimental groups in a given time

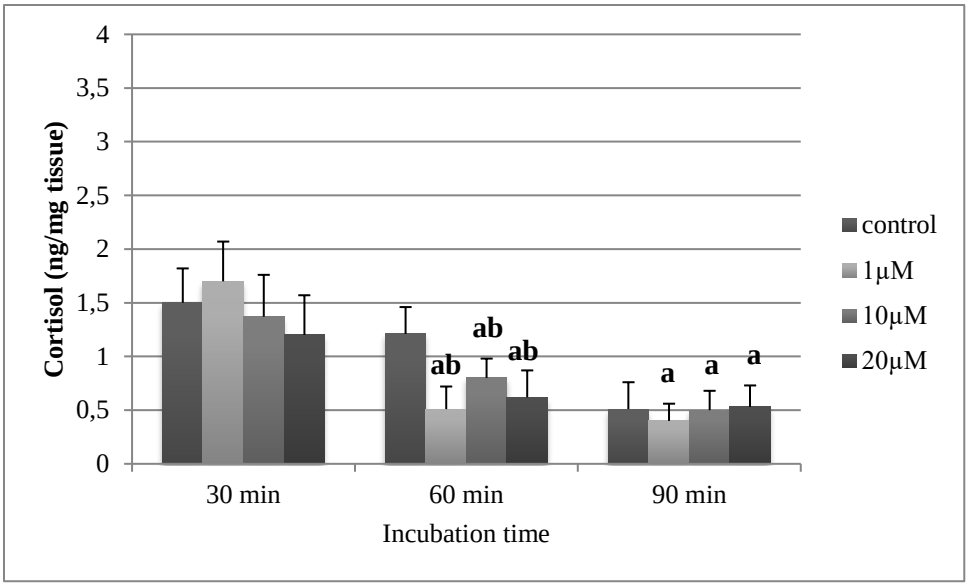

Fig. 3. Effect of ketamine on cortisol secretion from rabbit adrenal tissue.

a – significantly different values (P<0.01) compared to the values after 30 min of incubation

b – significantly different values (P<0.01) compared to the control group value

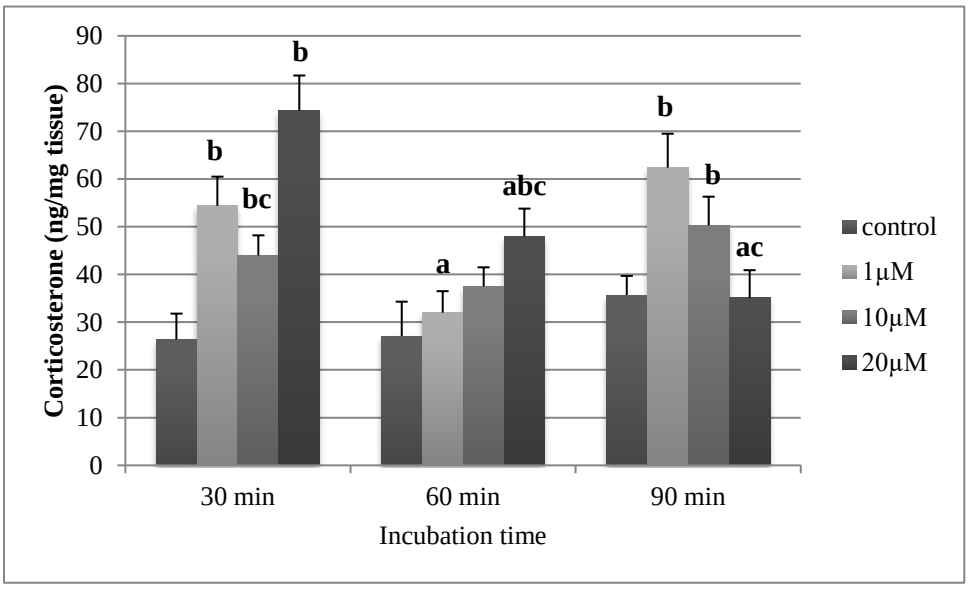

Fig. 4. Effect of ketamine on corticosterone secretion from rabbit adrenal tissue

a – significantly different values (P<0.01) compared to the values after 30 min of incubation b – significantly different values (P<0.01) compared to the control group value

c – significantly different values (P<0.01) between experimental groups in a given time

Discussion

The present studies have shown that in vitro Glu can act directly on the adrenal glands in terms of the amount of both glucocorticoids secreted from the adrenal glands of rabbits. Glu is the most excitatory neurotransmitter in the nervous system found naturally in mammals, and its concentration in peripheral blood is stable between 20 and 50 μM (Hediger and Welbourne, 1999). The numerous results clearly demonstrate the key role glutamic acid plays in the conduction of nerve impulses by nerve cells. However, the full and objective characterization of this neurotransmitter requires attention to the negative effects associated with its excessive occurrence in the body. The consequence of too high concentration of Glu, caused by the excessive influx of calcium and sodium ions into the nerve cells, may be edema, structural changes, and even death of neurons (Waelsch, 1951). The excessive amount of glutamic acid released into the extracellular space in the CNS causes much faster transmission of information between cells. This process can also be accompanied by apoptosis, which results in the formation of a "wave" in which many more neurons die than in the case of normal concentrations of glutamic acid (Koyama et al., 1997).

In mammals, the concentration of glucocorticoids in the blood plasma is the result of the degree of stimulation of the neuroendocrine activity of the hypothalamic-pituitary-adrenal axis. The hypothalamus reacts to nagative physical or emotional factors and activates this neuroendocrine system, the final effect of which is to increase the synthesis and release into the circulation of glucocorticosteroids: cortisol and / or corticosterone (Buttgereit et al., 2004). Rabbits are able to synthesize both of these hormones, and their physiological properties are similar (Szeto et al., 2004). The own experiment described in the presented study showed that the adrenal glands of rabbits secrete less cortisol, confirming that the steroidogenesis pathway in these animals is much more focused on the synthesis of corticosterone (Buckingham, 2006). The redirection of glucocorticoid synthesis towards corticosterone is associated with a greater activity of the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) (Rasmussen et al., 2013). The redirection of glucocorticoid synthesis towards corticosterone is associated with a greater activity of the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) (Rasmussen et al., 2013). At the same time, a differentiated reaction of the adrenal glands to glutamic acid was found in relation to both discussed glucocorticosteroids. In the case of cortisol, no statistically significant differences were found between the values determined after the first 30 minutes of the

experiment for all three doses of glutamic acid compared to the control group. Based on the analysis of the release of cortisol into the incubation medium, only a trend of higher values was observed in all three experimental groups. In the further minutes of the experiment, a decrease in cortisol secreted into the incubation medium was found, in all groups the trend of greater inhibition of this hormone secretion was more noticeable in the experimental groups, although the differences were still not significantly different. The profile of corticosterone secretion from the adrenal glands of rabbits showed that after the first 30 minutes of the experiment, a significant increase in the amount of corticosterone secreted into the incubation medium was found, as compared to the values of the control group. After 30 and 60 minutes of the experiment, a dose-dependency effect was observed, although in the 60 minute of the experiment the values were significantly lower compared to the values obtained after the first 30 minutes. The unexpected effect of glutamic acid on the release of this hormone after 90 minutes of incubation of adrenal glands has been demonstrated. The cortisol values determined at this time unexpectedly turned out to be inversely proportional to increasing the dose of glutamic acid.

The reaction of the adrenal glands of rabbits to the action of glutamic acid requires the presence of specific receptors for this neurotransmitter in this organ. Glutamic acid acts on target cells through two types of receptors - metabotropic and ionotropic. Both types of receptors are responsible for signal transmission in the central nervous system and peripheral tissues (Julio-Pieper et al., 2011). It has been shown that these receptors are present both in the hypothalamus, pituitary gland and in the adrenal glands, which may suggest their role in the neuroendocrine regulation of the HPA axis activity (Mahesh et al., 1999; Coutinho and Knopfel, 2002; Pokusa et al., 2014). The adrenal glands response to glutamic acid administration found in our studies was not statistically significant changes in the amount of cortisol excreted into the medium, which may be related to a weak stimulation or the lack of specific Glu receptors in the adrenal glands. In our research, the activity of NMDA receptors in the adrenal glands of rabbits was blocked by the use of their non-specific antagonist, ketamine. It is a multifunctional, organic chemical compound, classified as a psychoactive, non-competitive antagonist of NMDA receptors, which interacts with voltage-dependent $Ca2^{+}$ ion channels (Hirota and Lambert, 1996). Ketamine is widely used in medicine and veterinary medicine as a pre-operative anesthetic drug; in addition to inducing anesthesia, it also has a strong analgesic effect. It is also used in complex anesthesia. Ketamine causes a specific type of anesthesia, its action is to selectively inhibit certain structures of the CNS, which leads to loss of

consciousness. The clinical results show that appropriate dosing of ketamine alleviates symptoms of acute depression (Zarate et al., 2006).

The data of the current study have shown that ketamine changes the endocrine activity of rabbit adrenal glands in vitro. The administration of this chemical at all three doses used (1, 10, and 20 μM) generally resulted in a decrease in the amount of cortisol secreted from adrenal glands in rabbits. This effect was clearly demonstrate after 60 minutes of experiment. After 90 minutes of the experiment, the amount of secreted cortisol continued to decrease, however, due to similar values found in the control group during this incubation time, the results turned out to be insignificant. On the other hand, the analysis of the results illustrating the effect of ketamine on the secretion of corticosterone from the adrenal glands of rabbits showed that the use of this chemical compound caused a significant increase in the secretion of corticosterone to the incubation medium in all experimental groups. This effect clearly demonstrate when the highest dose of ketamine (20 μM) was applied after the first 30 minutes of incubation. In the following minutes of the experiment (60 and 90 minutes) the trend was similar, but compared to a 30 minute of incubation, the values were lower. Therefore, it can be assumed that ketamine may affect the final stage of the steroidogenesis process as a factor inhibiting the activity of cytochrome P450c17 responsible for the conversion of pregnenolone into 17-OH-pregnenolone and progesterone to 17-OH-progesterone. Furthermore, it may be presumed that ketamine causes an increase in the activity of the enzyme 3β-HSD or other cytochrome P450 enzymes responsible for the conversion of 11-deoxycorticosterone into corticosterone.

Our own studies have shown a variable effect of glutamic acid on the secretion of cortisol and corticosterone from adrenal glands, while the parallel studies also showed a variable effect of glutamic acid on the secretion of catecholamines from the adrenal glands. It has been shown that different doses of glutamic acid inhibit the release of adrenaline from the adrenal glands of rabbits, in contrast to noradrenaline, where these changes were not observed (Szpręgiel et al., 2020). The adrenal medulla is made of chromaffin cells responsible for the synthesis of catecholamines - mainly adrenaline, but also noradrenaline. Both of these hormones are involved in mobilizing the body to escape or fight in stressful situations. Apart from the above-mentioned functions, after stimulation of the visceral nerve, they directly influence the release of adrenal cortex hormones, including cortisol and corticosterone (Rosol, 2001).

The above considerations lead to a significant suggestion, in relation to the obtained results of our own experiment, that the adrenal activity of rabbits exposed to the stress factor should be additionally examined, which would certainly cause changes in the degree of stimulation of the HPA axis. It is also worth emphasizing the daily cycle of glucocorticoid secretion, because they are characterized by a clear time dependence - circadian and rapid pulsation. The circadian rhythm of glucocorticoid secretion is closely related to the activity of animals. In daytime animals, it occurs in the early morning hours, while the pulsatile secretion of glucocorticoids occurs on average 1-2 times per hour (Dickmeis, 2009). Such pulsatile secretion of glucocorticoids, most likely due to individual characteristics, also occurs in rabbits. It was found that male rabbits secrete corticosterone and cortisol in a circadian rhythm, reaching the peak of secretion in the afternoon, while the lowest daily concentration of this hormone was observed after 06:00. This means that rabbits differ in phase with the human rhythm of glucocorticoid secretion (Szeto et al., 2004).

In conclusion, the conducted experiment showed that the adrenal glands are also under the control of the nervous system and the mediators released. This makes it possible to additionally regulate the activity of this endocrine gland. Paradoxically, it is the steroid hormones synthesized by this gland that are released in any significant disturbance of the organism's homeostasis. The results of these studies induce to undertake further experiments within the discussed topic, e.g. during the action of stress factors on the body. As there are numerous receptors for this neurotransmitter, further research should focus on elucidating the role of other types of glutamic acid receptors, also at the molecular level.

References

Buckingham J.C. (2006). Glucocorticoids: exemplars of multi-tasking. Br. J. Pharmacol., 147(1): 258–268.

Buttgereit F., Straub R.H., Wehling M., Burmester G.R. (2004). Glucocorticoids in the treatment of rheumatic diseases: an update on the mechanisms of action. Arthritis and Rheumatism, 50 (11): 3408–3417.

Challis J.R.G., Matthews S.G., Gibb W., Lye S.J. (2000). Endocrine and paracrine regulation on birth at term and preterm. Endocr. Rev., 21 (5): 514–550.

Coutinho V., Knopfel T. (2002). Metabotropic glutamate receptors: electrical and chemical signal- ing properties. Neuroscientist, 8 (6): 551–561.

Dickmeis T. (2009). Glucocorticoids and the circadian clock. J. Endocrinol., 200 (1): 3–22.

Hardy K., Pollard H. (2006). The organization of the stress response, and its relevance to chiroprac- tors: a commentary. Chiropractic & Osteopathy, 14 (25): 1–13.

Hediger M.A., Welbourne T.C. (1999). Introduction: glutamate transport, metabolism, and physi- ological responses. Am. J. Physiol., 277 (4): F477–F480.

Hirota K., Lambert D.J. (1996). Ketamine: its mechanisms of action and unusual clinical uses. Br.

J. Anaesth., 77 (4): 441–444.

Hyde T.M., Lipska B.K., Ali T., Mathew S.V., Law A.J., Metitiri O.E., Straub R.E., Ye T., Colantuoni C., Herman M.M., Bigelow L.B., Weinber ger D.R., Klein- m a n J.E. (2011). Expression of GABA signaling molecules KCC2, NKCC1, and GAD1 in cortical development and schizophrenia. J. Neurosci., 31 (30): 11088–11095.

Jin P., Pan Y., Pan Z., Xu J., Lin M., Sun Z., Chen M., Xu M. (2018). Alzheimer-like brain metabolic and structural features in cholesterol-fed rabbit detected by magnetic resonance imaging. Lipids Health Dis., 17 (1): 61.

Julio - Pieper M., Flor P.J., Dinan T.G., Cryan J.F. (2011). Exciting times beyond the brain: metabotropic glutamate receptors in peripheral and non-neural tissues. Pharmacol. Rev., 63 (1): 35–58.

Koyama Y., Kimura Y., Baba A. (1997). Induction of glutamine synthetase by L-alpha-aminoadi- pate in developmental stages of cultured astrocytes. Neurosci. Lett., 223 (1): 65–68.

Kristensen P. (1993). Differential expression of AMPA glutamate receptor mRNAs in the rat adrenal gland. FEBS Letters, 332 (1-2): 14–18.

Llewellyn-Smith I.J., Phend K.D., Minson J.B., Pilowsky P.M., Chalmers J.P. (1992). Glutamate-immunoreactive synapses on retrogradely-labelled sympathetic preganglionic neurons in rat thoracic spinal cord. Brain Res., 581 (1): 67–80.

Llewellyn-Smith I.J., Minson J.B., Pilowsky P.M., Arnolda L.F., Chalmers J.P. (1995). The one hundred percent hypothesis: glutamate or GABA in synapses on sympathetic preganglionic neurons. Clin. Exp. Hypertens., 17 (1-2): 323–333.

Mahesh V.B., Zamorano P., De Sevilla P., Lewis D., Brann D.W. (1999). Characteriza- tion of ionotropic glutamate receptors in rat hypothalamus, pituitary and immortalized gonadotropin-releasing hormone (GnRH) neurons (GT1-7 cells). Neuroendocrinology, 69: 397–407.

Pokusa M., Prokopova B., Hlavacova N., Makatsori A., Jezova D. (2014). Effect of blockade of mGluR5 on stress hormone release and its gene expression in the adrenal gland. Can.

J. Physiol. Pharmacol., 92 (8): 686–692.

Rasmussen M.K., Ekstrand B., Zamaratskaia G. (2013). Regulation of 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase: a review. Int. J. Mol. Sci., 14 (9): 17926–17942.

Romero O., Figueroa S., V icente S., González M.P., Oset-Gasque M.J. (2003). Mo- lecular mechanisms of glutamate release by bovine chromaffin cells in primary culture. Neuroscience, 116 (3): 817–829.

Rosol T.J., D e Lellis R.A., Harvey P.W., Sutcliffe C. (2001). Endocrine System. In: Haschek and Rousseaux's Handbook of Toxicologic Pathology (Third Edition), Haschek W.M., Rousseaux C.G., Wallig M.A. (eds). Academic Press, Cambridge, USA, pp. 2391–2492.

Sapolsky R.M., Romero L.M., Munck A.U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev., 21: 55–89.

Szeto A., Gonzales J.A., Spitzer S.B., Levine J.E., Zaias J., Saab P.G., Schneider - m a n N., M c C a b e P.M. (2004). Circulating levels of glucocorticoid hormones in WHHL and NZW rabbits: circadian cycle and response to repeated social encounter. Psychoneuroendocrinology, 29 (7): 861–866.

Szpręgiel I., Wrońska D., Pałka S., Kmiecik Ł. Kania B.F. (2020). Udział kwasu glutaminowego w regulacji aktywności nadnerczy królików w wydzielaniu amin katecholowych – badania wstępne. Rocz. Nauk. Zoot., 47 (1): 99–110.

Waelsch H. (1951). Glutamic acid and cerebral function. Advances in Protein Chemistry, 6: 299–341. Wa t a n a b e M., M i s h i n a M., I n o u e Y. (1994). Distinct gene expression of the N-methyl-D-aspar- tate receptor channel subunit in peripheral neurons of the mouse sensory ganglia and adrenal gland.

Neurosci. Lett., 165 (1-2): 183–186.

Wolff K., Winstoc k A.R. (2006). Ketamine: from medicine to misuse. CNS Drugs, 20 (3): 199–218. Zarate Jr C.A., Singh J.B., Carson P.J., Brutshe N.E., Ameli R., Luckenbaugh D.A. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch. Gen. Psychiatry, 63 (8): 856–864.