HISTOPATHOLOGIC CHANGES AT THE HYPOTHALAMIC, ADRENAL AND THYMIC NUCLEUS ARCUA TUS IN RATS TREATED WITH MONOSODIUM GLUTAMATE

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The aim of this paper was to investigate the impact of neurotoxic monosodium glutamate (MSG) on a rat’s hypothalamus, adrenal gland and thymus nucleus arcuatus (ARCN). In newborn animals subcutaneous MSG injections induce lesions at the hypothalamic nucleus arcuatus level and induce hypothalamic-hypophyseal-adrenal-thymic axis disorders.

Experimental and control group of animals included 10 Wistar rats each. Experimental group animals were treated with monosodium glutamate (4 mg MSG/g BW) on the 2nd, 4th, 6th, 8th and 10th day of their postnatal life. Five animals from experimental and control group were sacrificed 7 days after MSG treatment for the purpose of studying histopathological changes on the thymus. The remaining animals were sacrificed 6 months after MSG treatment in order to study histopathological changes in ARCN level and adrenal gland. Paraflin sections of the hypothalamic tissue, adrenal gland and thymus were hematoxylin-eosin (HE) stained.

Macroscopically, the treated animals demonstrate skeletal development arrest and cushingoid type of obesity (‘buffalo type’). Histopathological analysis of nucleus arcuatus in experimental animals demonstrates significantly reduced number of neurons. Other cells express degenerative changes in the form of pyknotic nuclei. Adrenal glands in experimental rats demonstrate cortex hyperplasia, with clearly enlarged reticular and fasciculate areas and signs of hemorrhagic necrosis in the medulla. At thymus level, depletion of thymocytes in the cortex is observed with hemorrhage in the medulla, coupled with degeneration of tissues cytoarchitecture. These findings suggest the impaired hypothalamic-hypophyseal adrenal-thymic (HPTT) axis function in newborn rats treated with MSG. Acta Medica Medianae 2005; 44 (3): 35 – 42.

Key words: monosodium glutamate, nucleus arcuatus, adrenal glands, thymus

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used experimental model in the early postnatal period in rodents involves the study of ARCN-ME region (7,8,9). While the tanycyte (modified astroglial cells forming tight junctions and making up the inner BBB surface) network is already established in neonatal mice, arcuate and other neural axons grow into the ME during the first 25 days of neonatal life (10). That is why even large doses of MSG administered to immature animals do not cause evident tanycyte or ME terminal axon lesions, but the ARCN neurons are significantly damaged (11,12). This nucleus is the production site of numerous stimulatory and inhibitory hormones (13,14,15); that is why the disturbance of its function in neonatal period (by MSG treatment) leads to numerous endocrine and metabolic disorders. Neurotoxic effects of MSG are reflected in growth retardation, obesity, sterility, reduction of growth hormone, gonadal steroid and thyroid hormone levels, but the serum levels of gonadotropin and TSH are not necessarily reduced (16,17,18).

To a lesser extent, treatment of neonatal animals with MSG also damages other hypothalamic regions, including paraventricular nucleus (PVN) whose neurocytes are the site of corticotrophin releasing hormone (CRH) (19). The hypothalamic-hypophyseal-adrenal (HHA) axis response to stress induced stimuli starts in the neurons of this nucleus whose terminals are situated in the median eminence where they excrete ACTH (adrenocorticotrophic hormone) secretagogues, such as CRH and arginine vasopressin (AVP) (19). The secreted ACTH from the adenohypophysis stimulates secretion of glucocorticoids from the adrenal cortex. CRH neurons participate in the regulation of food intake and energetic homeostasis, reproduction, inflammatory responses, endocrine and cardiovascular functions. These effects are achieved by CRH through its synapsis to CRH R1 and CRH R2 receptors (20) belonging to the family of G protein-connected receptors. While CRH R2 is of limited spreading, the CRH R1 is largely abundant throughout the cortex, cerebellum, mesencephalon and pons. The phenotype of the cells expressing CRH receptors within the hypothalamus, remains unclear.

The neuroanatomic and electrophysiological studies indicate that PVN is closely connected with ARCN via two-way monosynaptic nerve projections (21). This arcuate paraventricular circuit is involved in neuroendocrine and autonomous control of numerous bodily functions. A population of neurons in ARCN excretes neuropeptide Y (NPY), a potent orexigenic peptide, involved in numerous hypothalamic functions. The NPY neurons of ARCN are projected and come into close contact with CRH neurons in PVN. The CRH neurons in PVN make synapsis with nerve terminals which express NPY Y1 receptors, while the subset of CRH neurons of PVN expresses NPY Y5 receptor subtype (22). That is the reason why the NPY function in stimulating food intake is partly performed through regulation of CRH neurons in PVN. Although the acute NPY treatment stimulates expression of CRH, the conditions in which the NPY is chronically increased (during starvation and chronic hyperphagia) are associated with CRH suppression. Inversely, it has been demonstrated that CRH modulates NPY signalization. The infusion of CRH in PVN stimulates releasing of NPY within this same region. However, the infusion of CRH antagonist in PVN, and not in VMH (ventromedial hypothalamus) intensifies NPY response (in the same region) to nutrition. These findings suggest the possibility of reciprocal feedback regulation between NPY and CRH neurons, which could be of relevance in the regulation of nutrition. High degree of functional internuclear connection is the cause of indirect functional disorder at PVN level in MSG treated animals.

The effect of MSG treatment in newborn animals on the hypothalamic-hypophyseal-adrenocortical axis in adult animals has been the subject of contradictory data (23, 24, 25, 26). Some authors (26) are reporting an increase in basal and stress induced corticosterone levels in MSG treated rats. Other studies (25) are showing drop in plasma level of ACTH and drop of mRNA for CRH in hypothalamic PVN. This finding is contradictory to studies evidencing that mRNA levels for CRH in hypothalamus are unchanged, compared to the increased levels of mRNA for pro-opiomelanocortin in the adenohypophysis (26). According to the authors of these studies, there is a connection between the increased plasma level of corticosterone and its prolonged response to stress induced stimulation in MSG treated neonatal rats and the reduced degree of corticosterone clearance (27).

Bearing in mind these facts, we have investigated in our study the effects of MSG at hypothalamus and adrenal gland ARCN level; because of high influence of this gland hormone and numerous neuropeptides of arcuate-paraventricular axis on the thymus, this organ has also been the subject of histopathological study.

**Material and methods**

Albino Wistar rats of both sexes were used in the study. The experimental group of animals, comprising 10 rats, was subcutaneously treated with monosodium glutamate (4 mg/g BW) on 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> postnatal day. The control group also comprised 10 animals and was treated with equivalent volumes of physiological saline. Five animals from both groups were sacrificed 7 days after MSG treatment for the purpose of studying histopathological changes in the thymus. The remaining animals of both groups were sacrificed 6 months after MSG treatment with a view to studying histopathological changes in ARCN and adrenal gland. Paraffin sections of hypothalamic, adrenal and thymic tissue were HE stained.

**Results**

Macroscopically, experimental animals demonstrated a significant degree of obesity (Fig. 1). In controls, ARCN demonstrated normal cyto-architecture (Fig. 2), while in experimental animals reduction in the number of neurons was evident (Fig. 3). Degenerative changes were also visible in the nerve cell perikaryons and the surrounding neuropil. Some nerve cells demonstrated scanty but denser cytoplasm, karyolysis or the complete loss of nuclei. A severe
edema could be observed in the neuropil, as well as the reduced number of dendritic projections (Fig. 4). The adrenal gland in control animals appeared to have normal constitution with glomerulus area, fasciculate area, and reticulate area in cortex and medulla (Fig. 5), while in experimental animals adrenal cortex hyperplasia in the fasciculate and reticulate areas could be observed (Fig. 6-PAS method). The medulla showed the presence of fatty degeneration (Fig. 7) and hemorrhagic necrosis (capillary diffuse hemorrhage) (Fig. 8). The thymus in control group of animals demonstrated normal constitution (Fig. 9) while in the experimental group depletion of thymocytes in cortex was observed with the increased presence of macrophages (Fig. 10). The thymus medulla showed the presence of extravasation of blood elements, followed by destruction of normal tissue cytoarchitecture (Fig. 11).
Increased plasma level of glutamate causes the selective loss of neurons in the brain of newborn rats. *Nucleus arcuatus* and median eminence regions demonstrate the highest sensitivity to glutamate during developmental maturation in the early postnatal life (1,2). Increased plasma level of glutamate induces glutamate receptor expression in the selective lesion of ventromedial *nucleus arcuatus* (ARCN) neurons (3). This nucleus has the main role in neuroendocrine (5) and autonomous integration and its lesions induce, among other phenomena, disturbed function of hypothalamic-hypophyseal-adrenal axis. The extremely complex function of this system is reflected in the presence of intrahypothalamic internuclear connections which modulate the functions of numerous hypothalamic neuropeptides and hormones (21).

ARCN is a part of the periventricular hypothalamus region. Anatomically, the nucleus is organized so as to communicate with different brain parts including hypophysis, limbic system, some thalamic nuclei, medium brain, brain stem autonomous nuclei and hypothalamus. The nucleus uses about 15 neurotransmitters and neuropeptides as well as neurons of various phenotypes (21). ARCN receives powerful neural signals from other parts of the periventricular region, and in particular from PVN. Interaction between ARCN and PVN has been confirmed.
neuroanatomically, considering the fact that ARCN is the main source of NPY-ergic PVN innervation and NPY release into this brain nucleus. The hypothalamic arcuate paraventricular nervous system is included in the centralized control of bodily functions through neuroendocrine and autonomous regulatory mechanisms. An important physiological role of this hypothalamic circuit is reflected in the regulation of food intake. Through extrahypothalamic projections up to the vagus preganglial neurons and thoracic par of spinal cord, the ARCN affects the autonomous mother brain nuclei and sympathetic activity.

Neuroanatomic and physiological findings suggest that neural activation of ARCN neurons exerts influence on CRH-immunoreactive PVN neurons, indirectly, through the activation of interpolated NPY receptors and NPY releasing neurons. It is a proof that the reciprocal interaction between CRH and NPY mechanisms is of particular importance in arcuate paraventricular-hypothalamic nervous system. On the other hand, based on anatomic studies Li and his associates have come to conclusion that NPY neurons of ARCN directly regulate the function of CRH neurons in PVN (28). Most probably, an important role is assumed by Y1-receptor-positive neurons in PVN parvocellular part. Besides, the results of pharmacological experiments indicate that NPY shows stimulatory effect on hypothalamic CRH neurons (29).

Further results suggest that stimulation of arcuate GABA-ergic POMC neurons induce inhibition of NPY nervous activity in ARCN and afferent terminal sites, i.e. in PVN and LHA (lateral hypothalamic area). Microinjection of excitatory amino-acid in ARCN may affect PVN nervous activity with activation of GABA-ergic interneurons in ARCN-PVN circuit (30).

From the functional point of view, it is important to know that CRH upregulates mRNA for its own PVN receptor (31), which proves the existence of positive CRH feedback on CRH neurosecretory cells in PVN. In that way, the CRH itself can modulate positive secretory activity of CRH neurosecretory cells in PVN even in the state of stress. Besides, exogenous CRH selectively activate transcription of the gene coding CRH type R1 receptor (R1) inside PVN. The effects on CRH R1-mRNA and on CRH gene transcription may be completely prevented by pre-treatment with CRH antagonists. This observation suggests the existence of CRH-CRH synaptic connections at PVN level.

An important influence on HHA axis is exerted by hypothalamic noradrenergic and serotonergic nerve terminals that regulate the activity of CRH neurons in PVN (acting interactively) and stimulate secretion of ACTH and corticosterone (32). Both noradrenaline (NA) and 5-hydroxy-tryptamine (5-HT) have an important role in mediating stress stimuli effects on the activity of HHA. Weidenfield has proved by experiments that there is interaction between noradrenergic and serotonergic agonists in ACTH and corticosterone secretion simulation in rats (32). Noradrenergic input directly stimulates postsynaptic α1-adrenoceptors in PVN, but also regulates serotonergic input into these CRH cells of PVN. Similarly, serotonergic input, directly stimulating postsynaptic 5-HT receptors in PVN regulates noradrenergic input into PVN (32). Possible basis for reciprocal effects of noradrenergic and serotonergic systems on CRH neurons may include, partly at least, mediation of these effects by excitatory (glutamatic) and inhibitory (GABA-ergic) hypothalamic interneurons (32). Glutamate is a potent factor of CRH release: glutamic interneurons stimulating NA are present in PVN and mediate NA excitatory effect on magnocellular PVN neurons. On the other hand, 5-HT may inhibit release by GABA activation of 5-HT1A receptors. The 5-HT1A receptor mediated inhibition of GABA-ergic neurotransmission is demonstrated in amygdala, and it is known that GABA-ergic neurons encircle PVN in the form of a ring. These interneurons may have a role in their mutual interaction between 5-HT and NA. Thus, elimination of 5-HT input in PVN may lead to increased level of GABA, which may inhibit NA excitatory effect on CRH neurons. Elimination of NA input in PVN leads to decreases of glutamic activation, thus intensifying GABA inhibitory effect (32). It may be concluded that there is an interaction between NA and 5-HT innervation of CRH cells in PVN which are responsible for the activation of HHA axis. This interaction may explain, at least partially, finding evidence equivalent reductions of HHA axis response on nervous stimulation after neurotoxic lesions, whether on NA or 5-HT system.

The hypothalamic nuclei, in particular PVN, are important brain structures through which central nervous system responds to immune challenges (33). The catecholamine cells of the brain stem in nucleus tractus solitarius (NTS) and ventrolateral medulla (VLM) have a critical role in transmission of system immune signals to PVN. Through treatment with proinflammatory cytokine interleukine-1β, in combination with Fos marking, Buller was the first to investigate the effect of PVN lesions on NTS and VLM catecholamine and non-catecholamine cell response (33). The results obtained have demonstrated an important reduction of the number of Fos-positive non-catecholamine, noradrenergic and adrenergic cells in NTS and VLM after administering interleukine 1β. This study shows that PVN has the capability of NTS and VLM response modulation on the immune signal and that this can be the result of decreasing projections from PVN (33). In that way, the nervous system responds to the immune signal by including complex reciprocal connections between PVN and brain stem as well as between the brain stem nuclei themselves.

The thymus is an immune system organ in powerful interaction with neuroendocrine system, in particular during ontogenetic and early postnatal period (34). The proper development and function of the thymus require activity of a number of endocrine glands, but this organ is also included, by the feedback mechanism, in the regulation of integral neuroendocrine homeostasis. The complex effect of hypophysis on the thymus is demonstrated when the hypophysis is removed, or by administering antihypophyseal serum, which causes thymus atrophy. Hypoplasia of the thymus and other lymphoid organs, observed in dwarf-mice, disappears after substitutional therapy with growth hormone (35). The growth hormone accelerates the proliferation of
thymus cells and this effect is partly achieved directly (as on other bodily cells), and partly indirectly, by mediation of thymosin synthesis induction in thymus epithelial cells. Numerous data are available on the effect of thymus on the hypophysis (36). Neonatal thymectomy leads to degranulation of growth hormone excreting cells, while the administration of small doses of thymosin leads to increased secretion of somatotropin hormone and prolactin. Higher doses of thymosin produce contrary effects. Out of all hormones, those secreted by adrenal gland, in particular glucocorticoids, are of particular interest and they provoke acute thymus involution. Similar effect is demonstrated by ACTH, in an indirect way of course, through mediation of adrenal gland. Moreover, some lesions of the central nervous system, in particular of the hypothalamus, result in thymus atrophy, which can be explained by activation of HHA axis. Thymus involution within the general reaction to stress is interpreted similarly. Thymus involution caused by glucocorticoids is the result of destruction of thymocytes (primarily the cortical ones), sensible to cortisol effect. The origin of this process of thymocyte destruction may be found in their apoptosis induction. The process starts with changes in the nucleus, and the key event inducing cell death occurs due to activation of endogenous endonuclease which then effectuates fragmentation of DNA onto oligonucleotides. These changes are morphologically characterized by condensation of chromatin, which is marked as apoptosis. All subsequent disorders of glucose metabolism, as well as protein synthesis in thymocytes, that were formerly designated as primary effects of glucocorticoids, are of secondary nature. A strong connection between the adrenal gland and thymus is observed after bilateral adenalecetomy that induces thymus hypertrophy. The enlarged thymuses are encountered in patients suffering from Addison’s disease. In AKR mice with innate hypofunction of adrenal glands, the thymuses are increased in size compared to other mouse strains. There are proofs of a reverse influence of thymus on adrenal gland cortex ontogenesis. Thymectomy reduces ACTH and corticosterone concentration in blood.

The results of this experiment indicate that the ARCN region in rats is very sensitive to monosodium glutamate treatment in the early postnatal period. Histopathologic analysis of the examined nucleus is reflected in the reduced number of nervous cells and marked degenerative changes in the remaining cells. Many neurons manifest the loss of nucleus and reduction in the number of dendritic projections, which morphologically proves a long-time existence of degenerative changes. Damaged ARCN structure is the origin of many functional insults, as this nucleus is the site of synthesis of many neuropeptides and hormones. The growth hormone releasing hormone (GHRH) is synthesized in the neurons of this nucleus. That is why the experimental animals show linear-type growth retardation. Their obesity is of hypo-phagic, Cushingoid type, conditioned by the increased level of cortisol. Hypophagia is the result of NPY loss (NPY being a powerful food intake stimulant) in ARCN secreting neurons. The intrahypothalamic arcuate-paraventricular nuclear axis is the principal factor in food-intake regulation by neuropeptide-Y (20).

The extremely complex function of this system leads to contradictory results presented in the available studies (25, 26) on MSG effect on adrenal gland. Some authors (25, 26) have described the increase in plasma level of corticosterone in basal and stress situations, with or without simultaneous changes in the secretion of ACTH. The increased plasma level of corticosterone without increased secretion of ACTH in MSG treated rats has been explained by changed sensitivity of adrenal cortex to the circulating ACTH or by the existence of adrenocortical secretagogues, different from ACTH, found in these animals (25). Possible direct influence of MSG treatment on adrenal function and corticosterone production, has not yet been studied. Some other authors (26) have explained the increased plasma level of corticosterone through changes in the peripheral corticosterone metabolism. Findings presented in this paper do not comply with findings of those authors stating that the weight of the adrenal gland has been reduced and plasma concentration of corticosterone increased (26). Unlike these findings, this experiment has demonstrated hyperplastic adrenal glands with clearly enlarged zona reticularis and zona fasciculata compared to the control group. The processes within and between the transcriptional, translational and posttranslational mechanisms inside each level of this axis shall clear up the problem at molecular level.

The results of this experiment suggest interaction between the thymus and HHA axis. Damage of constitution and function of ARCN which is the source of growth hormone releasing hormone (GHRH) leads to reduced secretion of this hormone which has a stimulating effect on the thymus (accelerates proliferation of thymus cells). In addition to the reduced level of growth hormone, the increased levels of glucocorticoids in experimental group animals, contribute to depletion of thymocytes in the thymus cortex.

**Conclusion**

In newborn animals treated with MSG, we can observe clearly evident reduction of nucleus arcuatus neurons, while the remaining neurons demonstrate degenerative changes and karyolysis, together with the severe edema of the neuropil. The adrenal cortex in these animals manifests clear hyperplasia of fasciculate region and reticulate region, while the medulla shows the signs of hemorrhagic necrosis. The thymus cortex demonstrates depletion of cortical thymocytes and hemorrhage in the medulla. These findings suggest the significance of hypothalamic-hypophysial-adrenal axis for the adrenal gland function, since the lesions of the hypothalamic structures are reflected on the structure and function of this gland. Functional disorder of this axis is therefore reflected on the structure and function of the thymus.
References


Histopatološke promene
u nivou nukleus arkuatusa hipotalamusa, nadbubrežne žlezde i timusa pacova tretiranih monosodijum glutamatom

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Cilj ovog rada bio je ispitivanje uticaja neurotoksičnog monosodijum-glutamata (MSG) na nukleus arkuatus (ARCN) hipotalamusa, nadbubrežne žlezde i timusa pacova. Kod novorodjenih životinja supkutane injekcije MSG-a indukuju lezije u nivou nucleus arkuatus-hipotalamusa i dovode do poremećaja hipotalamo-hipofizno-adrenalno-timusne osovine.

Eksperimentalna i kontrolna grupa životinja obuhvatila su po 10 pacova Wistar soja. Životinje eksperimentalne grupe tretirane su monosodijum-glutamatom (4 mg MSG/g/TT), 2, 4, 6, 8 i 10-og dana postnatalnog života. Po pet životinja iz eksperimentalne i kontrolne grupe žrtvovano je posle 7 dana od tretmana MSG-om u cilju ispitivanja histopatoloških promena na timusu. Ostale životinje žrtvovane su 6 meseci od tretmana MSG-om radi analize histopatoloških promena u nivou ARCN-a i nadbubrežne žlezde. Parafinskii preseci tkiva hipotalamusa, nadbubrežne žlezde i timusa bojeni su hematoksilin-eozinskom (HE) metodom.


Ključne reči: monosodijsul glutamat, nukleus arkuatus, nadbubrežne žlezde, timus