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HISTOPATHOLOGIC CHANGES AT THE HYPOTHALAMIC NUCLEUS ARCUATUS AND THYROID LEVEL IN RATS TREATED WITH MONOSODIUM GLUTAMATE

SUMMARY

The aim of this paper was to investigate the impact of neurotoxic monosodium glutamate (MSG) on rat hypothalamic nucleus arcuatus and thyroid gland. In newborn animals subcutaneous MSG injections induce lesions at the hypothalamic nucleus arcuatus level and induce hypothalamic-hypophyseal-thyroid (HPT) axis disturbances as well.

Experimental and control group of animals included 10 Wistar rats each. In the experimental group animals were treated with monosodium glutamate (4 mg MSG/g BW) on their 2, 4, 6, 8 and 10 day of postnatal life and they were sacrificed 6 months after MSG treatment. Paraffin sections of the hypothalamic tissue and thyroid gland were stained with hematoxylin-eosin (HE).

Macroscopically, the treated animals demonstrate skeletal development arrest and Cushingoid type of obesity ("buffalo type"). Histopathologic analysis of nucleus arcuatus in experimental animals demonstrate significantly reduced number of neurons. Other cells express degenerative changes in the form of pyknotic nuclei. Thyroid gland in experimental rats demonstrate thyreocyte atrophy with colloid hypersecretion. These finding suggest the impaired hypothalamic-hypophyseal-thyroid axis function in newborn rats treated with monosodium glutamate.

Key words: **monosodium glutamate, nucleus arcuatus, thyroid gland**

INTRODUCTION

Glutamate is an amino-acid widely available in the central nervous system (CNS) where it functions as an excitant neurotransmitter. It is highly concentrated in those regions of the brain that are essential in cognitive processes mediation, eg. in the cerebral cortex, hippocampal gyrus dentatus and striatum (1), indicating an important role of this amino acid in higher cognitive functions (including memory). On the other hand, it acts as a neurotoxin *(excitotoxin)* which can destroy central neurons (2). Glutamate ability to destroy neurons is mediated by the interaction with N-methyl-D-aspartate (NMDA) receptors which induces intracellular calcium increase, free radical generation, activation of proteases, phosoholipases and endonucleases, and the transcriptional activity of apoptotic programmes (3). Excitotoxic pathways probably contribute to neuronal death in ischaemic insults, head traumas, epilepsy, viral encephalopathy and chronic neurodegenerative diseases such as amyotrophic lateral sclerosis, Huntington and Alzheimer disease (4,5).

Cellular bodies of hypothalamic secretory neurons are situated in the areas protected by the blood-brain barrier (BBB), while their terminal axons are localized in eminentia mediana (ME) which lacks BBB (6). That is the reason why the ME region (which accepts the axonal terminals from the nearby arcuate nucleus (ARCN) and other hypothalamic se-

cretory neurons) is the most sensitive to excitotoxic glutamate exposure. Moreover, the fenestrated capillary endothelium of the ME makes it available to plasma amino acids, so that the initial gluta-mate-induced neuronal damage could be the result of circulating level of these acids rather than the cerebro-ventricular pool. The ARCN-ME region in the early postnatal period in rodents was an often used model in the studies of monosodium glutamate (MSG) induced neurotoxicity (7-9) due to its marked sensitivity, consistent cytoarchitecture and prominent anatomic site. Though the tanycyte (modified astroglial cells forming tight connections and making up the inner BBB surface) network is already established in neonatal mice, arcuate and other neural axons ingrown into the ME during the first 25 days of neonatal life (10). Large doses of MSG administered to immature animals do not cause evident tanycyte or ME terminal axon damage, but the ARCN neurons are significantly damaged (11,12). This nucleus is the production site of numerous stimulatory and inhibitory hormones; that is why the disturbance of its function in neonatal period (by MSG treatment) leads to numerous endocrine and metabolic disorders and altered behaviour in the adult age (13-15). Neurotoxic effects of MSG induce 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 (16-18).

Neonatal MSG treatment in a lesser degree damages other hypothalamic regions as well, including paraventricular nucleus (PVN) the neurocytes of which are the secretion site of thyreotropin-releasing hormone (TRH) (19). Hypophysiotropic TRH neurons of the PVN effectuate a dense network of axosomatic and axodendritic synapses with the fibers of certain ARCN neurons. Immunohistochemical analysis demonstrated that these fibers belong to neuropeptide-Y (NPY) secreting ARCN neurons (20). Gene expression studies revealed the presence of agouti-related protein (AGRP), endogenous antagonist of melanocortic receptors, co-localised in the neurons with NPY (21). It was also established that AGPR--immunoreactive nerve fibres terminate in the parvocellular PVN portion (22,23). Within the hypothalamus there are internuclear connections, such as arcuate-paraventricular neuronal connection, due to which ARCN damage influences the function of PVN.

Bearing in mind these facts, with this study we investigated the MSG influence at the hypothalamic and thyroid ARCN level.

MATERIAL AND METHODS

White Wistar rats of both sexes were used in the study. The experimental group of animals, comprising 10 rats, was subcutaneously treated with MSG (4 mg/g BW) on 2,4,6,8 and 10 postnatal day. The control group comprised also 10 animals and was treated with equivalent volumes of saline. Animals from both groups were sacrificed 6 months after treatment. Paraffin sections of hypothalamic and thyroid tissue were HE stained.

RESULTS

Macroscopically, experimental animals demonstrate a significant degree of obesity (fig. 1).

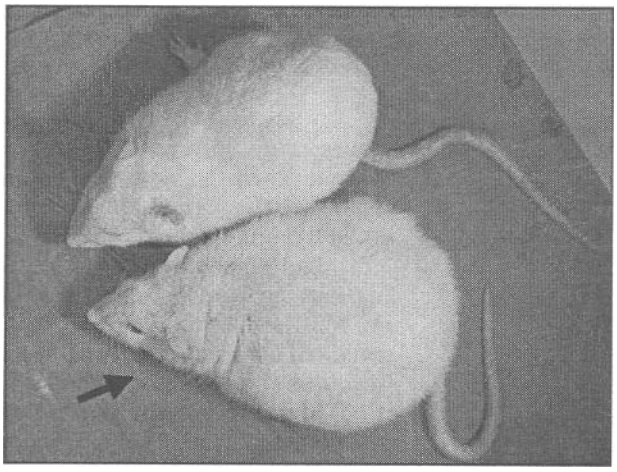

Figure L Experimental animal demonstrating a significant degree of obesity

Degenerative changes were also documented in the nerve cell perikaryons and the surrounding neuropil. Some nerve cells demonstrate scanty but denser cytoplasm, caryolysis or the complete loss of nuclei (fig. 2). A severe edema could be observed in the neuropil, as well as the reduced number of dendritic extensions (fig. 3). The thyroid gland in control animals appeared to have normal constitution (fig. 4), while in experimental animals thyreocytic atrophy and follicular fields without colloid could be observed (fig. 5). In some of the follicles hyposecretion (in the costate colloid form) (fig. 6) was evident, while some of the thyroid portions expressed nodular hyperplasia in the form of microadenoma.

DISCUSSION

Increased plasma level of glutamate causes the selective loss of neurons in the brain of newborn rats. Nucleus arcuatus and median eminence regions

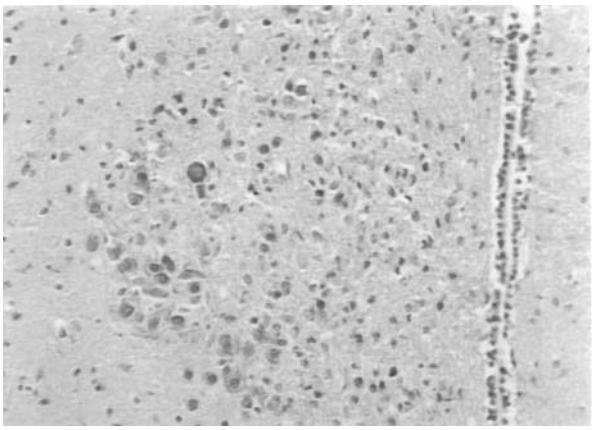

Figure 2. Scanty, but more dense cytoplasm, caryolysis or the complete loss of nuclei in ARCN in experimental animals

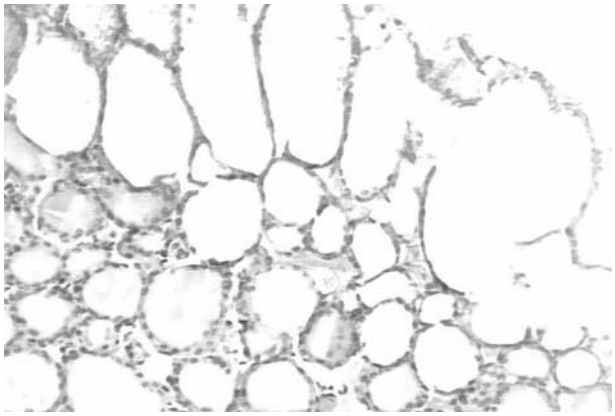

Figure 5. Thyreocytic atrophy and follicular fields without colloid in experimental animals

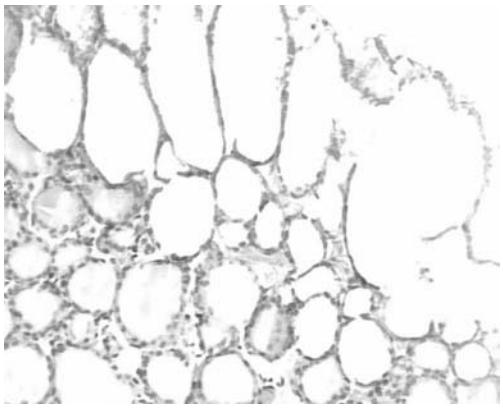

Figure 3. Severe edema in the neuropil in ARCN in experimental animals

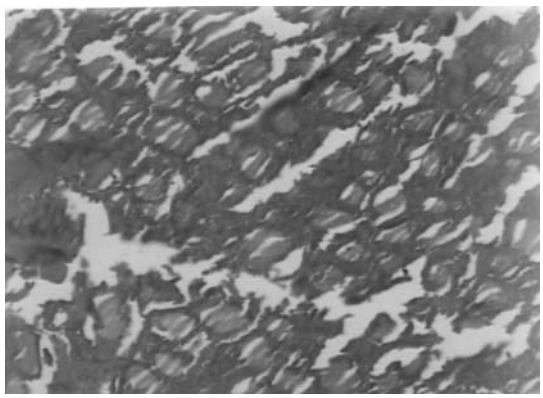

Figure 6. Follicular hyposecretion in the costate colloid form in some follicles

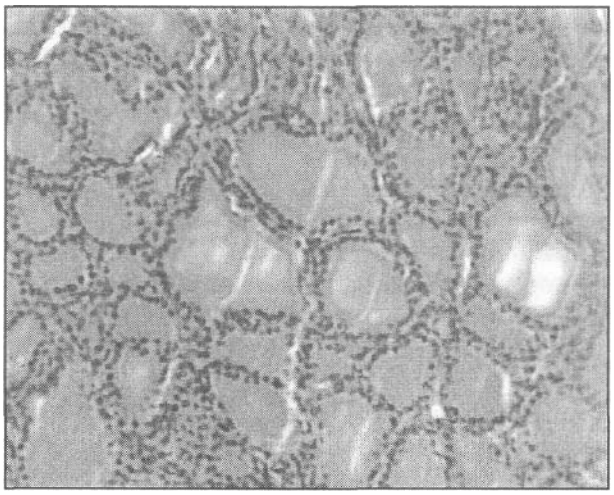

Figure 4. Normal thyroid structure in control animals

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 during selective damage 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-thyroid axis. The extremely complex function of this system is reflected by the presence of intra-hypothalamic internuclear connections which modulate the functions of numerous hypothalamic neuropeptides and hormones (21).

Stable energetic homeostasis is established through the balance between the two adverse components: food intake/energy conservation and energy consumption (24). Thyroid hormone, the pe-

ripheral end-product of the hypothalamic-pitui-tary-thyroid axis (HPT), is an important stimulator of energy consumption, mainly in the form of increased thermogenesis (25). In the HPT axis, regulation of thyroid-stimulating hormone (TSH) secretion primarily depends on TRH release from the hypophyseotropic TRH neurons of the peri ventricular parvocellular PVN portion and negative feedback regulation by the circulating thyroid hormone levels (19). With reduced thyroid hormone level in the plasma, TRH biosynthesis and secretion from these neurons increase, raising the threshold for thyroid hormone feedback inhibition of thyrotrophs of the anterior pituitary lobe, which increases TSH secretion. On the other hand, the increase of thyroid hormone in the plasma suppresses the biosynthesis and secretion of TRH, leading to the lower threshold for feedback regulation by thyroid hormone on the thyrotrophs, suppressing the TSH secretion (19). This regulatory system changes during fasting, when reduced circulating thyroid hormone levels are associated with seemingly paradoxical reduction of TRH synthesis and TSH secretion (26). Such a condition of transient central hypothyroidism reduces thyroid thermogenesis, serving as an important mechanism of energy preservation up to the moment when food intake takes place again.

Numerous neuropeptides and hormones may modulate the function of hypophysiotropic TRH secreting neurons, thus influencing the HPT axis. It was demonstrated that the reduction of plasma level of leptin (anorexic hormone secreted by fatty tissue) acts as the critical signal on the hypophysiotropic TRH neurons in the way that they become sensitive again to the inhibitory feedback effects of thyroid hormone. In fasting animals exogenous leptin establishes again the normal level of pro-TRH gene expression and normal levels of both total and free thyroid hormone (27). Leptin effects on the hypophysiotropic TRH neurons are not direct but indirect, through the neural projections from ARCN into PVN. This postulate is based on the abundant expression of mRNA for leptin receptor in ARCN and its low expression at the PVN level (28). Supplementing this postulate is also the fact that the arcuate nucleus ablation by monosodium glutamate prevents the ability of leptin to restore the HPT axis to its normal state in fasting animals (29).

One of the mediators modulating the leptin effects on the HPT axis is a powerful orexigenic peptide, NPY. Axonal neurons of this peptide effectuate numerous synaptic contacts with TRH neurons in the PVN (30). Gene expression for NPY is dramatically increased during fasting in ARCN, while in the PVN, NPY concentration is simultaneously increased. Leptin administration prevents this expression in the ARCN. Since most of the NPY neurons of the ARCN co-express AGRP (22), it follows that the NPY and AGRP axonal terminals of the same neurons innervate hypophysiotropic TRH neurons (31). The increase of gene expression for AGRP during fasting and the possibility of its suppression by leptin (22) point to the fact that this peptide is a strong modulator of the HPT axis.

AGRP innervation of the TRH neuron in the medial and periventricular parvocellular PVN portion suggests the importance of this peptide in hypophysiotropic neuronal regulation. TRH neurons in the frontal, dorsal and ventral parvocellular portions of this nucleus are also innervated by the AGRP neuron nerve terminals, but they do not have direct hypophysiotropic function since they are not projected into the median eminence (19). Since numerous neurons of the dorsal and ventral parvocellular portion send their projections into the parasympathetic and sympathetic centers of the brain stem and spinal cord, AGRP may be included into the autonomous regulation. In fasting, when AGPR gene expression increases, AGPR may simultaneously influence hypophysiotropic neurons, as well as the autonomous nerve system through the projection into the TRH neurons in the PVN.

The results of this experiment indicate that the ARCN region in rats is very sensitive to the treatment with MSG in the early postnatal period. Histopathologic analysis of the investigated nucleus is reflected in the reduced number of cells and marked degenerative changes in the remaining cells. Many neurons demonstrate the loss of nucleus and reduction of number of dendritic projections, which morphologically proves long-lasting degenerative changes. In this way disarranged ARCN structure is the basis of various functional insults since this neuron is the site of synthesis of numerous neuropeptides and hormones. In its neurons growth hormone releasing hormone (GHRH) is synthesized; that is the reason why experimental animals demonstrate linear-type growth retardation. Their obesity is of a hypophagic type, Cushingoid, conditioned by increased cortisol level. Hypophagia results from the loss of NPY (powerful food-intake stimulator) secreting ARCN neurons. Intrahypothalamic arcuate-paraventricular nuclear axis is the principal factor of food-intake regulation by neuropeptide-Y (20). This axis is of importance for TRH neurons in the PVN, in which thyreotro-pin-releasing hormone is synthesized, since these neurons effectuate numerous axiomatic synapses with ARCN neuron axonal terminals. That is the reason why the ablation of ARCN with MSG, in addition to the PVN damage, induces TRH synthesis reduction and HPT-axis disorder. In this experiment, the confirmation of the disturbed axis function is the histopathologic finding of the thyroid, reflect-

ing tyreocyte atrophy and colloid hypersecretion. Nodular hyperplasia, all the way to microadenoma, can be interpreted as a compensatory reaction to the lack of TSH due to lower TRH synthesis.

CONCLUSION

In newborn animals treated with MSG clearly evident is the reduction of nucleus arcuatus neurons, while in the remainder of neurons degenerative changes and caryolysis can be observed, together with the severe edema of the neuropil. The thyroid of these animals contains atrophic tyreocytes, colloid hyposecretion and fields of nodular hyperplasia, all the way to the picture of microadenoma. These findings suggest the significance of hypothalamic-hypophyseal-thyroid axis for the thyroid function, since the damage of hypothalamic structures is reflected in thyroid hypofunction.

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HISTOPATOLOSKE PROMENE U NIVOU NUCLEUS ARCUATUS I TIROIDNE ZLEZDE KOD PACOVA TRETIRANIH MONOSODIJUM - GLUTAMATOM

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SAŽETAK

Cilj ovog rada bio je ispitivanje uticaja neurotoksičnog monosodijum glutamata (MSG) na nukleus arcuatus hipotalamusa i tireoidnu žlezdu pacova. Kod novorođenih životinja subkutane injekcije MSG indukuju lezije u nivou nucleus arcuatus-a hipotalamusa i dovode do poremećaja hipotalamo-hipofizno-tiroidne osovine.

Eksperimentalna i kontrolna grupa životinja obuhvatale su po 10 pacova Wistar soja. životinje eksperimentalne grupe tretirane su monosodijum-glutamatom (4mg MSG/g/TT), 2,4, 6, 8 i 10-og dana postnatalnog života, a žrtvovane su posle 6 meseci od tretmana MSG-om. Parafinski preseci tkiva hipotalamusa i tireoidne žlezde bojeni su hematoksilin-eozinskom (HE) metodom.

Tretirane životinje makroskopski pokazuju: zastoj skeletnog razvoja i Kušingoidni tip gojaznosti (buffalo tip). Histopatološka analiza nukleus arkuatusa eksperimentalnih životinja ogleda se u značajnom smanjenju broja neurona. Preostale ćelije ispoljavaju degenerativne promene predstavljene piknotičnim jedrima. Tireoidna žlezda kod eksperimentalnih životinja pokazuje atrofiju tireocita sa hiposekrecijom koloida. Ovi nalazi ukazuju da je funkcija hipotalamo-hipofizno-tireoidne (HPT) osovine poremećena kod životinja neonatalno tretiranih monosodijum glutamatom.

Ključne reči: **monosodijum-glutamat, nukleus arkuatus, tireoidna žlezda**