

STUDY ON ADRENAL GLAND MORPHOLOGY IN MICE TREATED WITH MONOSODIUM GLUTAMATE

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Summary. *Monosodium glutamate is neurotoxin, potentially toxic to everyone - even to those people who do not respond with adverse reactions such as migraine headache, asthma, nausea and vomiting, fatigue, disorientation, and depression. However, it is used worldwide to improve the food preference. Hypothalamic lesions induced by neonatal treatment with MSG are primary in the preoptic and arcuate nuclei and in the median eminence, resulting in Cushing's obesity, stunted growth, lethargy and sterility. Having in the mind the contradictory data on the role of the adrenal glands in this type of obesity, the objective of the present experiment was to study MSG-effect on the adrenal gland morphology. Seven newborn C57BL/6J mice, 9 days after the birth, were injected daily with MSG (4.4mg/g body WT) sc. Controls received no treatment. Mice were killed at 4 months of age, and the adrenal glands were removed. For histological studies, specimens were fixed in 10% formalin, routinely processed and embedded in paraffin. Each section (5nm in thickness) was stained with HE, Van Gieson and PAS techniques, and observed microscopically. Besides Cushing's obesity, the most important morphological finding was hypertrophy of both adrenal glands, compared with controls. Microscopically, the cortex was widened and composed predominantly of fasciculate cells with large cells and abundant intracytoplasmic lipid droplets showing microvesicular pattern. Thus, the authors confirm the suggestion that mice neonatal treatment with MSG causes a complex disruption of the hypothalamic-pituitary-adrenal axis.*

Key words: *Adrenal gland, hypothalamic obesity, monosodium glutamate, mice*

Introduction

The etiology of obesity is multifactorial and is becoming a problem of public health, due to its increased prevalence in health and the consequent repercussion on the health of the population (1- 4). The great similarity and homology between the genomes of rodents and humans make these animal models a major tool for studying conditions affecting humans which can be simulated in rats (1). The most widely used model to induce obesity in mice and rats are the lesions of the arcuate nucleus (AN) and, in part, of the ventromedial hypothalamus (VMH), induced by administering monosodium glutamate (MSG) (5-8). MSG is used worldwide to improve both food preference and intake for general population (9-13). The addition of MSG to meat, vegetables, soups, and food products has been implicated in the cause of the "Chinese syndrome" (13), or even in a neurological reaction in humans (11, 14-15). The real

mechanism by which this hypothalamic injury leads to obesity is not known, but what is known is that it does not happen because of the increased food intake (5, 7-8).

Aim

Having in mind the contradictory data on adrenal gland role in the pathogenesis of MSG-obese rats and mice, the aim of this work is to study the MSG-effect on adrenal gland morphology in mice.

Material and Methods

A total number of 14 mice (Jcl: ICR strain) was used in this study. Seven mice were rendered obese by a subcutaneous injection of 10% MSG, 4.4 mg/g body wt, every 24 h for the first nine postnatal days (at the neonatal period). Another group of 7 mice, which were in-

jected with saline solution, 0.02 ml/g body wt, every 24h for the first nine postnatal days, served as a control group. Mice were killed when they were 4 months old and their adrenal glands were removed. For histological studies, specimens were fixed in 10% formaldehyde, over night, processed to paraffin wax and cut at 4 μ m. The sections were stained routinely with haematoxylin and eosin (HE) and histochemically with Periodic Acid Schiff (PAS) and Van Gieson techniques and then observed microscopically.

Results

Treated animals were quite lethargic as adults and, they lacked the sleekness of body coat, which was seen in controls. Obesity of the face, neck, trunk and abdomen and thin extremities (Cushing's type of obesity-buffalo type), evidenced macroscopically (Fig 1), was associated with liver steatosis (Fig 2), pancreas lipomatosis and spleen atrophy. Macroscopically, the adrenal glands of the treated mice were enlarged and had rounded edges. Microscopically, the adrenal glands had diffuse cortical hypertrophy: the adrenal cortical zona fasciculata was widened and composed predominantly of clear large cells with abundant intracytoplasmic lipid droplets showing microvesicular cytoplasm, compared to untreated animals (Fig. 3, 4, 5, 6).



Fig.1. Control (left) and buffalo type obesity (right)

Discussion

Obesity is a serious metabolic disorder whose prevalence has increased in epidemic proportions around the world (4). The glutamate industry would like us to believe that MSG is not a problem for humans be-

cause brain is protected from MSG by the blood-brain barrier (14-16). However, that is not true. Furthermore, throughout life, certain regions of the brain, known as the circumventricular organs, lack a blood brain barrier (17) and the blood brain barrier can be damaged from, among other things, high fever, stroke, head traumas, seizures, repeated ingestion of MSG. Elevated levels of glucagon, induced with MNG, then increase insulin levels, leading to a feeling of hunger and, therefore, over-eating (6). Previous studies have demonstrated that mice neonatal MSG treatment destroys growth-hormone releasing-hormone (GHRH) neurons within the hypothalamic arcuate nucleus, decreases serum GH and insulin-like growth-factor (IGF-1) concentrations, and retards linear growth (5, 18-19). However, the prevention of the Cushing's type of obesity in GSM-treated mice by adrenalectomy has induced a suggestion that high levels of corticosterone in mice blood are related to these abnormalities (20).

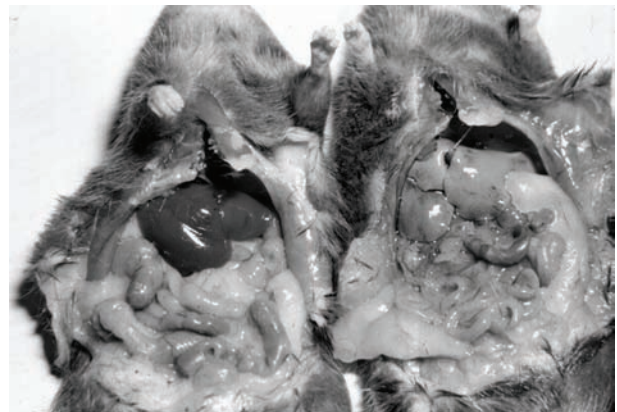


Fig. 2. Dark-brown liver (left) and Fat liver (right)

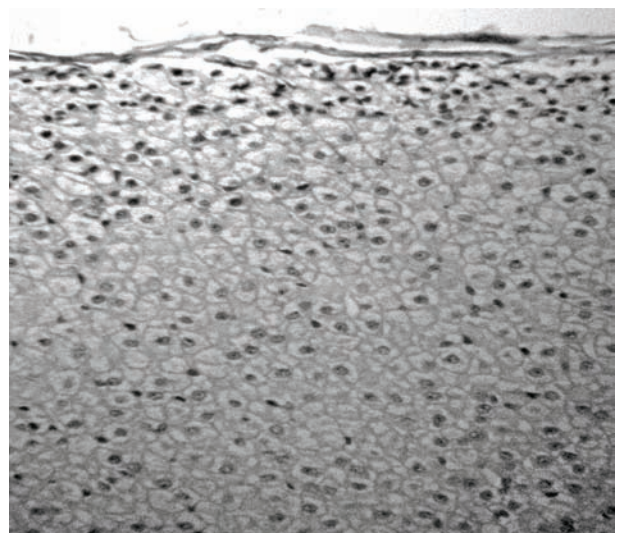


Fig. 3. Experimental mouse: wide adrenal cortex (without medulla). H&E x 200

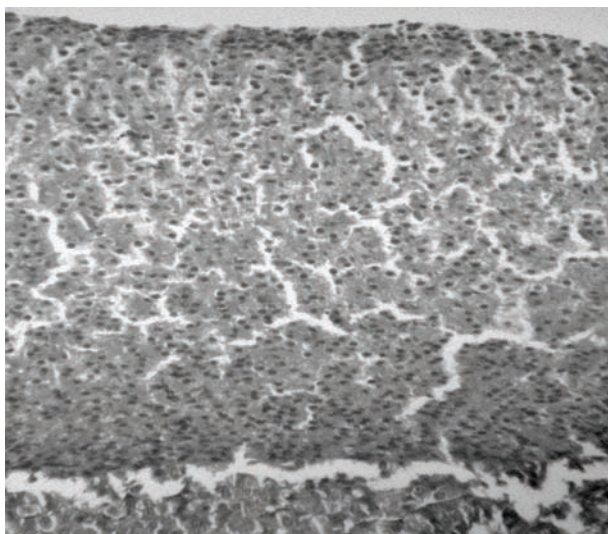


Fig. 4. Control mouse: normal cortex depth (with medulla). H&E x 200

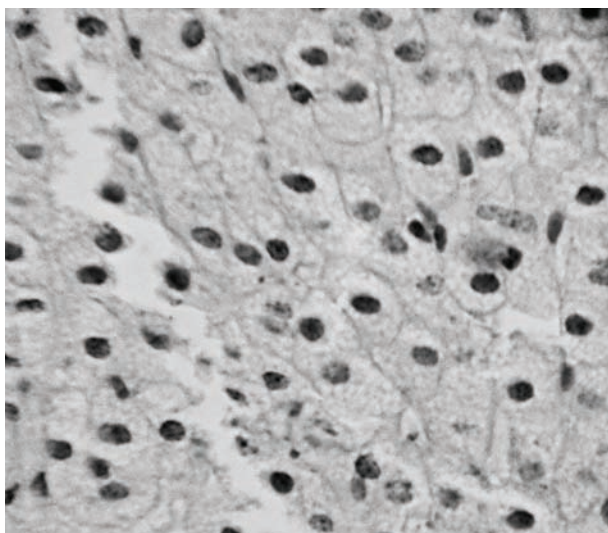


Fig. 5. Experimental mouse: large cells with microvesicular cytoplasm. H&E x 400

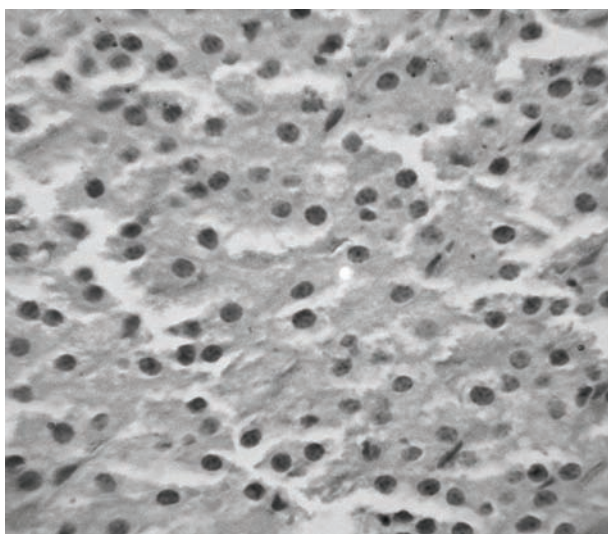


Fig. 6. Control mouse: small cells with eosinophilic cytoplasm. H&E x 400

Hypothalamus is thought to contain the "biological clock" that regulates certain body functions that vary in different times of the day (e.g. body temperature, hormone secretion, hunger) or those that vary over a period of many days (e.g. menstrual cycle) (21-22). The hypothalamus, with its nuclei that have been referred to as satiety and feeding centers, participates in body weight control (23). In addition to regulating eating behavior, a number of CNS neuropeptides regulate the neuroendocrine pathways (21-25). Thus, hypothalamic arcuatus nucleus, contains many of neurons that control endocrine function of adenohypophysis. (6, 25-26). They include Growth hormone (GH), Adenocorticotropin hormone (ACTH), Thyrotropin (TH), Gonadotropins and Prolactin. Synthetic material of vasopressin and a substance completely distinct from octapeptide vasopressin, a 41-amino acid peptide, isolated from pituitary extracts and named corticotrophin-releasing factor (CRF), stimulates the release of ACTH by pituitary (25, 27). The hypothalamic nucleus that causes secretion of growth hormone releasing hormone (GHRH) is the ventromedial nucleus causing hunger in hypoglycemic states (27-29). In contrast, the secretion of growth hormone inhibitory hormone (GHIH), named somatostatin, is controlled by other nearby areas of the hypothalamus (16). Ghrelin, a novel peptide discovered 1999 in the nucleus arcuatus of the hypothalamus also stimulates the release of GH in the pituitary and induces a rise in the serum concentration of ACTH and cortisol, involved in energy homeostasis and participating in the genesis of hypothalamic obesity (23).

Hypersecretion of ACTH, associated with hyperplasia of basophilic pituitary cells in the rats, treated with MSG, reported in our earlier studies (7-8), could be explained by neurotoxic effect of MSG not only on GHRH but also on GHIH (6, 23) that suppresses anterior pituitary gland GH secretion and, activates ACTH secretion. The possible existence of both stimulatory and inhibitory factors involved in the regulation of ACTH release was reported by some authors (23, 25-30).

Glutamate is one of the most commonly known excitotoxins (substances that react with specialized receptors in the brain in such a way which leads to the destruction of certain types neurons). Recent studies have indicated that the damage by monosodium glutamate was much more widespread and that it includes such areas as the hippocampus, circumventricular organs, locus ceruleus, amygdala-limbic system, subthalamus and striatum (29-31).

In many disorders, the source of glutamate is when endogenous-injured brain cells release large amounts of glutamate from surrounding astrocytes. This glutamate can further damage surrounding normal neuronal cells (esp. brain trauma, strokes). But, food borne excitotoxins contribute significantly to this accumulation of toxins (10).

Furthermore, it has been shown that plasma levels of leptin, the adipose tissue-derived peptide important for the regulation of food intake, energy metabolism and

reproductive function, represent a metabolic key regulator of the reproductive function and a signal between the nutritional status and reproduction. The biologically active variant of the leptin receptor is localized in the arcuate and ventromedial nuclei, also in gonadotropes, Leiding and male germ cells. Both corticosterone and leptin plasma levels are significantly increased in adult MSG rats, indicating a permanent hypothalamic nuclei lesions in these animals (32).

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In conclusion, the authors have pointed out that the buffalo type obesity in the mice treated with MSG represents the main factor of hypothalamus-pituitary-adrenal axis dysfunction in MSG rats.

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PROUČAVANJE MORFOLOGIJE NADBUBREŽNE ŽLEZDE U MIŠEVA TRETIRANIH MONONATRIJUM GLUTAMATOM

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Kratak sadržaj: Mononatrijum glutamat je potencijalno toksičan za svakog - potencijalno toksičan čak i za one ljude koji ne reaguju na njegovo unošenje neprijatnim reakcijama kao što je migrena, astma, gađenje i povraćanje, zamor, dezorijentacija i depresija. Uprkos tome, mononatrijum glutamat se koristi širom sveta u cilju poboljšanja kvaliteta hrane. Hipotalamične lezije, izazvane tretiranjem neonatusa mononatrijum glutamatom su primarno lokalizovane u preoptičkom i arkuatnom jedru i u eminenciji mediani, dovodeći do Kušingovog tipa gojaznosti, zakržljalog rasta, pospanosti i steriliteta. Imajući na umu kontradiktorne podatke o ulozi nadbubrežnih žlezda u ovom tipu gojaznosti, predmet proučavanja u ovom eksperimentu je efekat mononatrijum glutamata na morfoloiju nadbubrežne žlezde. Sedam novorođenih C57BL/6J miševa je tretirano potkožnim ubrizgavanjem mononatrijum glutamata (4,4mg/g telesne težine), jedanput dnevno, u toku prvih 9 dana života. Kontrolne životinje nisu tretirane. Miševi su žrtvovani u 4 meseca starosti, a zatim su nadbubrežne žlezde izvađene. Za histološko proučavanje, uzorci su fiksirani u 10% rastvoru formalina, rutinski obrađivani i ukalupljeni u parafin. Svaki presek (5nm debljine) je bojen HA, Van Gieson i PAS metodama i analiziran mikroskopski. Pored Kušingovog tipa gojaznosti, najvažniji morfološki nalaz je hipertrofija nadbubrežnih žlezda, u odnosu na kontrole. Mikroskopski, kora je proširena i sastavljena pretežno od fascikulatne zone, sa velikim ćelijama koje su obilovale lipidnim kapljicama u citoplazmi, pokazujući mikrovezikularni izgled. Prema tome, autori su potvrdili sumnju da tretiranje novorođenih miševa izaziva složeno razaranje hipotalamo-hipofizno-adrenalne ose.

Ključne reči: Nadbubrežna žlezda, hipotalamusna gojaznost, mononatrijum glutamat, miševi