Vladmila V. BOJANIĆ[1]
Zoran Ž. BOJANIĆ[2]
Steva NAJMAN[3]
Jelena ŽIVANOV-ČURLIS[3]
Jasmina TOMIN[4]
Boris ĐINĐIĆ[1]
Todorka SAVIĆ[1]
Katarina KATIĆ[5]

[1]INSTITUTE OF PATHOPHYSIOLOGY, MEDICAL FACULTY NIŠ, SERBIA&MONTENEGRO
[2]INSTITUTE OF PHARMACOLOGY AND TOXICOLOGY, MEDICAL FACULTY NIŠ, SERBIA&MONTENEGRO
[3]INSTITUTE OF BIOLOGY, MEDICAL FACULTY NIŠ, SERBIA&MONTENEGRO
[4]INSTITUTE OF CHEMISTRY, MEDICAL FACULTY NIŠ, SERBIA&MONTENEGRO
[5]INSTITUTE OF PATHOLOGY, MEDICAL FACULTY NIŠ, SERBIA&MONTENEGRO

Diltiazem prevention of monosodium glutamate toxicity on hypothalamus in Wistar rats

KEYWORDS: Diltiazem; Sodium Glutamate; Hypothalamus; Drug Toxicity; Rats; Protective Agents

ABSTRACT

Administration of monosodium glutamate (MSG) to Wistar rats in neonatal period induced destructive lesions in some nuclei of hypothalamus and resulted in syndrome of obesity, stunting growth and hypogonadism. Sustained high concentration of MSG could induce to persistent depolarisation and alter ionic permeability of neural membranes. The overloading with intracellular Ca^{2+} and excessive activation of glutamate receptors may be cause of neural death. The aim of this study was to investigate if the pretreatment with diltiazem (L- calcium channel blocker) prevents the effects of MSG on hypothalamus in rats. The study was carried out on neonatal Wistar rats of both sexes. Wistar rats were injected subcutaneously with: 0.9% of sodium chloride (K group); 4 mg/g BW. MSG (MSG group); 5 mg/g BW diltiazem (D group) and 5 mg/g BW diltiazem and 60 minutes later with 4 mg/g BW MSG (D+ MSG group) at the second, the fourth, the sixth, the eight and the tenth day after birth. The rats were sacrificed at age of six months and histology examinations of hypothalamus were performed. The treatment with MSG resulted in focal hyperplasia of astrocytes with gliosis. Hypothalamic histology in K, D and D+MSG group of treated animals was normal. The pretreatment with diltiazem prevents the development of damages caused by MSG in hypothalamus in rats. Our results suggest the importance of calcium channel blockade in prevention of MSG toxicity.

INTRODUCTION

MSG is widely used as food additive. It has been demonstrated that subcutaneous administration of MSG to animals in neonatal period (mice, rats, rabbits) resulted in acute degeneration of the arcuate nuclei of hypothalamus and circumventricular organs. Adult rats treated neonatally exhibit a number of endocrine related disorders: syndrome of obesity, stunted growth, and hypogonadism.

The aim of the study was to determine if pretreatment with diltiazem (L- calcium channel blocker) prevents the effects of MSG on hypothalamus in rats.

Address correspondence to:
Doc. Dr. Vladmila Bojanić, Pariske komune 11/37, 18 000 Niš, Serbia and Montenegro, E-mail: bojanicv@eunet. yu
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MATERIALS AND METHODS

The present study was carried out on 24 male Wistar rats divided in four experimental groups with 6 animals in each. The animals were injected subcutaneously interscapulary with: 0.9% of sodium chloride (K group); 4 mg/g BW MSG (MSG group); 5 mg/g BW diltiazem (D group) and 5 mg/g BW diltiazem and 60 minutes later with 4 mg/g BW MSG (D+MSG group) at the second, the fourth, the sixth, the eight and the tenth day of life. Rats were housed under controlled conditions with room temperature of 23+/-2ºC and humidity of 55%-65% and 14 h light: 10 h dark cycle (lights on at 06.00). Animals were weaned at age of 29 days. They had free access to tap water and standard laboratory chow pellets ("Veterinarski zavod" Zemun). The animals were sacrificed at age of six months under pentobarbital anesthesia and morphometric and histological examinations were performed.

RESULTS AND DISCUSSION

Treatment with MSG resulted in hypothalamic lesions. The arcuate nuclei were uniformly destroyed along with neuronal constituents in the median eminence. Instead these tissues, gliosis was found, the most important indicator of central nervous system injury. The astrocytes were hypertrophic and hyperplastic, with small, dark nuclei located in a dense net of processus (glial fibrils). Multifocal, small microglial nodules, associated with neuronophagia were observed too.

Animals treated with MSG in neonatal period showed altered endocrine, metabolic and behavioral characteristics when adult. Animals treated with MSG were lethargic and they lacked sleekness of body coat seen in control and other groups of experimental animals. Our results documented statistically significant reduction of nasal- anal length ($p < 0.003$) in MSG treated rats (23.6+/-0.40) compared to rats of other experimental groups (26.3+/-0.2 K group; 26.0+/-0.3 D group; 26.5+/-0.5 D+MNG group). In rats treated with MSG tail length (19.6+/-0.3) was statistically significant shorter ($p<0.001$) then tail length of rats from K group (21.3+/-0.4), D group (21.2+/-0.2) and D+MSG group (22.2+/-0.2). Body weight of male rats treated with MSG (560.0+/-15.0 g) was not statistically significant changed ($p>0.05$) compared to other experimental groups (531.7+/-11.3 K group; 528.3+/-13.8 D group; 550.0+/-16.9 D+MNG group). Lee-index showed statistically significant increase ($p<0.01$) of quantity of adipose tissue in rats treated with MSG (0.342.+0.006) than in rats from K group (0.307+/-0.002), D group (0.310+/-0.002) and D+MNG group (0.309+/-0.005).

Neurotoxic properties of MSG result from its ability to induce depolarization of cell membrane (1). Glutamate functions physiologically as neurotransmitter (2) and it would be expected that postsynaptic region would be particularly sensitive to its increased extra cellular concentrations. Sustained high concentrations of MSG infiltrating the synaptic cleft region could lead to persistent depolarization, altered ionic permeability of neural membranes and to neural necrosis. Neuronal destruction in the brain is apparent in areas where blood-brain barrier is leaky (the circumventricular organs and contiguous structures) (3,4). The excessive activation of glutamate receptors and the overloading of intracellular Ca^{2+} lead to neuronal death (5,6). In this study is shown that pretreatment with diltiazem (L-calcium channel blocker) prevents the toxic effects of MSG on hypothalamic nuclei.

CONCLUSION

The pretreatment with diltiazem prevents the development of damages caused by MSG in hypothalamus of rats. Our results suggest the importance of calcium channel blockade in prevention of MSG toxicity.

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