Diltiazem prevention of toxic effects of monosodium glutamate on ovaries in rats

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Abstract. The female reproductive system is very sensitive to different harmful environmental factors. A great danger is hidden in an increased use of food additives like monosodium glutamate (MSG). Numerous studies have shown that application of high doses of MSG to different kinds of animals during the neonatal period may cause lesions of neural structures and the retina. Later in adulthood animals exhibit a series of neuroendocrine disorders: a stunted growth, obesity and infertility. The mechanism of MSG action is not well explained yet. We hypothesized that high concentration of MSG could alter permeability of neural membrane for calcium. We studied whether pretreatment with diltiazem prevented the effects of MSG on ovaries in rats. Female rat pups were treated with: 0.9% NaCl, MSG, diltiazem or diltiazem with MSG. MSG treatment resulted in a cystic degeneration of ovaries and irregular and prolonged estrus phase of estrus cycle. The other treated groups of rats had normal ovarian histology and estrus cycle. The pretreatment with diltiazem prevented development of morphological and functional disorders of ovaries. Our results suggest that calcium overloading play an important role in mechanisms of MSG toxicity.

Key words: Monosodium glutamate — Rat — Ovary — Estrus cycle — Diltiazem

Introduction

Female infertility is a very real medical problem in developed countries. The female reproductive system is very sensitive to different harmful environmental factors. Modern lifestyle with an increased number of women who decide to have babies later in life and the negative influence of alcohol, smoking habit, and excessive overweight or underweight and contraception are factors which should not be underestimated. A great advance in technology is followed by an increased use of chemicals which can seriously harm female fertility. Some chemicals are counteractive with estrogen. A great danger is hidden in an increased use of different food additives like monosodium glutamate (MSG). MSG is the salt of nonessential glutamic acid. It has a property to enhance the perception that flavours are well blended and full-bodied. MSG also compensates for the absence of superior ingredients in food and disguise unwelcome tastes. This popular taste enhancer is widely used not only in the food industry, but also in homes and restaurants.

This taste enhancer is present in: flavoured chips and snacks, soups or sauces (canned, packed), prepared meals, frozen foods and meals, fresh sausages, marinated meats, and stuffed or seasoned chicken, bottled soy or oriental sauces, manufactured meats, some hams, luncheon chicken and turkey, flavoured tuna, vegetarian burgers and sausages.

Numerous studies have shown that parenteral application of high doses of MSG (1–4 mg/g b.w.) to animals dur-

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ing the neonatal period may cause lesions of the preoptic nuclei, arcuate nuclei, the circumventricular organs and the retina (Lukas and Newhouse 1957; Potts et al. 1960; Cohen 1967; Olney 1969, 1971; Olney and Ho 1970; Lemkey-Johnston and Reynolds 1972, 1974; Olney et al. 1972; Sasaki and Sano 1986; Gao et al. 1994). Animals treated with injections of MSG during the first 10 neonatal days, exhibit a series of neuroendocrine disorders during their adult lives. The most prominent disorders are: stunted growth, obesity and decreased fertility. Studies were carried out in different kinds of animals: mice, rats, rabbits, hamsters, dogs, chickens and monkeys. Many studies have shown that MSG can harm when given orally, too (Olney and Ho 1970; Burde et al. 1971, 1972; Olney 1971; Lemkey-Johnston and Reynolds 1972; Olney et al. 1972; Takasaki 1979, 1980).

The results of some studies indicate that MSG could exhibit harmful effect on human body including: obesity, urticaria (Botey et al. 1988; Roberts 1996), asthma (Schaumburg et al. 1969; Rosenblum et al. 1971; Kenney and Tidball 1972; Reif-Lehrer and Stemmermann 1975; Andermann et al. 1976; Diamond et al. 1986; Allen et al. 1987; Sands et al. 1991; Scopp 1991; Ehlers et al. 1998). The way of MSG action is not well explained yet. It is well known that MSG has high excitotoxic potential, which is mediated at the level of second messenger by calcium anions (Gao et al. 1994; Lin et al. 1995). We therefore hypothesised that high concentration of MSG could alter permeability of neural membrane for calcium which could be involved in the mechanisms of MSG toxicity. The purpose of this investigation was to study if pretreatment with diltiazem (L-calcium channel blocker) could alter the effects of MSG on ovaries and estrus cycle in rats.

Materials and Methods

The study was carried out in neonatal female Wistar rats. The animals were injected subcutaneously interscapularly with the same volume of: 0.9% NaCl solution (C group), 4 mg/g b.w. of MSG (M group), 5 mg/g b.w. of diltiazem (D group) and 5 mg/g b.w. of diltiazem and 60 min later with 4 mg/g b.w. of MSG (DM group). The injections were administered at 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} day of life. The animals were housed under controlled conditions. The room temperature was 23 ± 2°C and air humidity 50 ± 5%.The rhythm of light and darkness was established (light phase from 6:00 a.m. to 6:00 p.m.). The rats were weaned at age of 28 days. Females were housed in standard cages. They had free access to tap water and to standard laboratory chow pellets ("Veterinarski zavod" Zemun). Females were marked with a non-toxic pencil.

Vaginal smears were collected from three months old females during 25 days. There were six females in each group. The cages with rats were carried to the experimental room by the same order at 8:00 a.m. every morning. Vaginal secretion was taken with a clean plastic pipette filled with 0.1 ml of normal saline (0.9% NaCl) by inserting into rat vagina not too deep. Vagina was washed out and one drop of vaginal fluid was placed on a clean glass microscope slide. A different slide was used for each animal. Vaginal fluid drop was pushed with the clean spreader to the end of slide with smooth movement. The slide was left to dry in the air.

Vaginal smears were fixed with methanol for 5 min. Methanol was rinsed with distilled water for 5 min and after that slides were gently totally covered with 10% Giemsa stain for 10 min. The stain was rinsed with clean distilled water and slides were dried. Stained slides were observed under light microscope. Three types of cells could be recognized: epithelial cells, cornified cells and leukocytes. The proportion among these cells was used for determination of the estrous cycle phase.

The animals were sacrificed at age of four months under pentobarbital sodium anesthesia (40 mg/kg b.w. intraperitoneally). The ovaries were carefully removed and prepared according to appropriate procedures with HE methods for micromorphological examinations. All procedures on animals followed Guideline for work on experimental animals approved by Ethic Committee of Faculty of Medicine in Niš.

Results of statistical analysis are expressed as means ± standard deviation. Statistical significance was determined with analysis of variance (ANOVA) test. The differences were considered significant at $p < 0.05$ level. All statistical analyses were performed using the SPSS statistical software (version 15).

Results

Determination of estrus cycle length and duration of its phases is performed. Average estrus cycle in rats neonatally treated with MSG was 5.2 days and it was significantly prolonged, compared to duration of average estrus cycle in rats in C group (4.1 days) (Fig. 1). There were no significant differences between control and the other groups – D (4.3 days) and DM (4.6 days).

In females of M group estrus phase was more often registered than in females from the other groups (Fig. 2). The difference was statistically significant between M and C groups (9.67 ± 2.42 vs. 6.67 ± 1.21; $p < 0.05$), as well as between M and D groups (9.67 ± 2.42 vs. 6.67 ± 0.82; $p < 0.01$). Metaestrus was recorded more often in animals of groups C and D, than in females from DM and M groups. In females of DM group and M group diestrus phase was more often recorded than in rats of C and D groups, but this was

not statistically significant. In rats of M group proestrus was registered statistically significant more rarely than in C group (4.00 ± 0.63 vs. 6.00 ± 0.00; $p < 0.001$), D group (4.00 ± 0.63 vs. 6.17 ± 0.98; $p < 0.001$), as well as in DM group (4.00 ± 0.63 vs. 5.50 ± 0.55; $p < 0.01$).

The ovaries in rats from C, D, and DM groups showed normal morphology (Fig. 3). Their ovaries had wide cortex with numerous primary, secondary and Graafian follicles, as well as newly formed and degenerated *corpora lutea*. Histology of ovaries of MSG-treated rats showed cystic degeneration. Fibrotically changed stroma and arteriolar hyalinosis were found. Ovaries contained many atretic follicles and no *corpora lutea*.

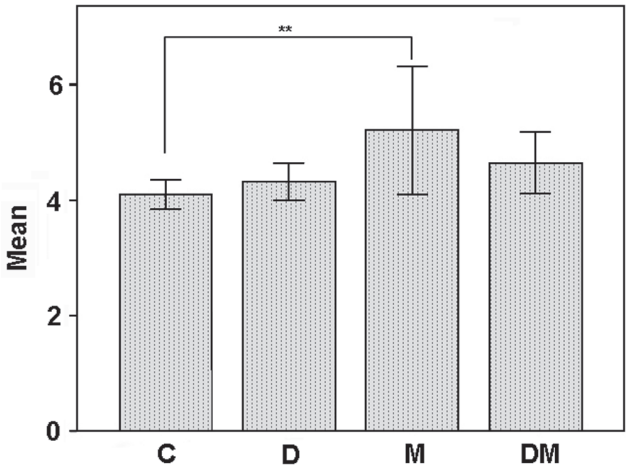

Figure 1. The average duration of estrus cycle of Wistar rats from different groups. C, control; D, diltiazem-treated; M, MSG-treated; DM, diltiazem- and MSG-treated; ** $p < 0.01$, error bars: 95% CI (ANOVA).

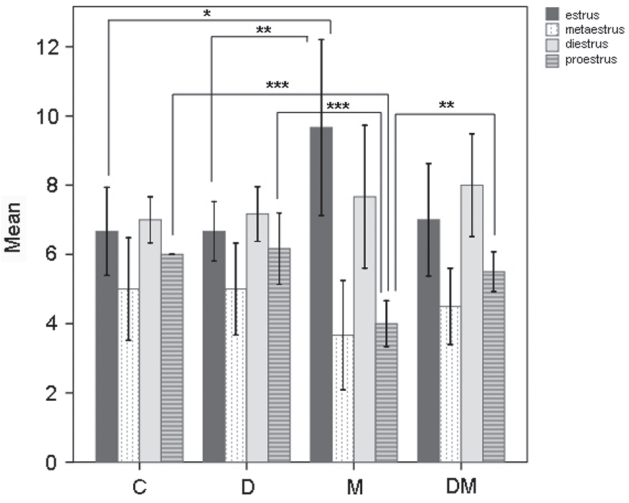

Figure 2. The summ of days with registered estrus phasis of estrus cycle within period of cycle determination and statistical significance of diferences between groups. C, control; D, diltiazem-treated; M, MSG-treated; DM, diltiazem- and MSG-treated; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, error bars: 95% CI (ANOVA).

Discussion

There are conflicting reports concerning the effect of neonatal treatment with MSG on female neuroendocrine system. Olney (1969) registered sterility in female mice, which were treated with MSG during first ten days of their neonatal life. There were twice as more atretic follicules in their ovaries than in animals of the control group. The wall of their uterus was very tiny. Adamo and Ratner (1970) reported that female Wistar rats after administration of a single dose of MSG (4 mg/g b.w.) in neonatal period, later cycled normally and were capable of mating and producing normal litters. Although their relative ovarian weights were significantly less then in control animals, ovarian histological findings were normal.

In this paper the histology of ovaries of MSG-treated rats showed cystic degeneration. Ovaries contained many atretic follicles with no *corpora lutea* and fibrotically changed stroma. Arteriolar hyalinosis was found too. Ovaries of rats from C, D and DM groups showed normal morphology. They had primary, secondary and Graafian follicles, as well as newly formed and the degenerated *corpora lutea* in ovary cortex depending on phase of cycle.

Nagasawa et al. (1974) found that estrus cycle of the MSG-treated mice had longer periods of estrus and shorter periods of diestrus than in controls. Clemens et al. (1978) reported the weight reduction of ovaries and uteri and irregular prolonged estrus cycle in MSG-treated Sprague-Dawley rats. Lorden and Caudle (1986) found decreased ovary weight and delayed puberty in mice neonatally treated with MSG. MacDonald and Wilkinson (1990) had quite opposite findings. They found that neonatal treatment with MSG accelerated sexual maturation of rats. Sasaki and Sano (1986) reported that ovaries in MSG-treated mice contained many atretic follicles but no *corpora lutea*. Shapiro et al. (1986) recorded prolongation of estrus in rats neonatally treated with MSG.

The results of this study showed that estrus cycles of rats neonatally treated with MSG were prolonged significantly compared to the duration of estrus cycles in rats neonatally treated with normal saline, diltiazem and diltiazem with MSG.

Estrus cycle is prolonged due to extension of estrus phase and diestrus phase in MSG-treated rats compared to the other experimental groups. Changed permeability of neural membrane for calcium due to effect of MSG is involved in the mechanisms of MSG toxicity.

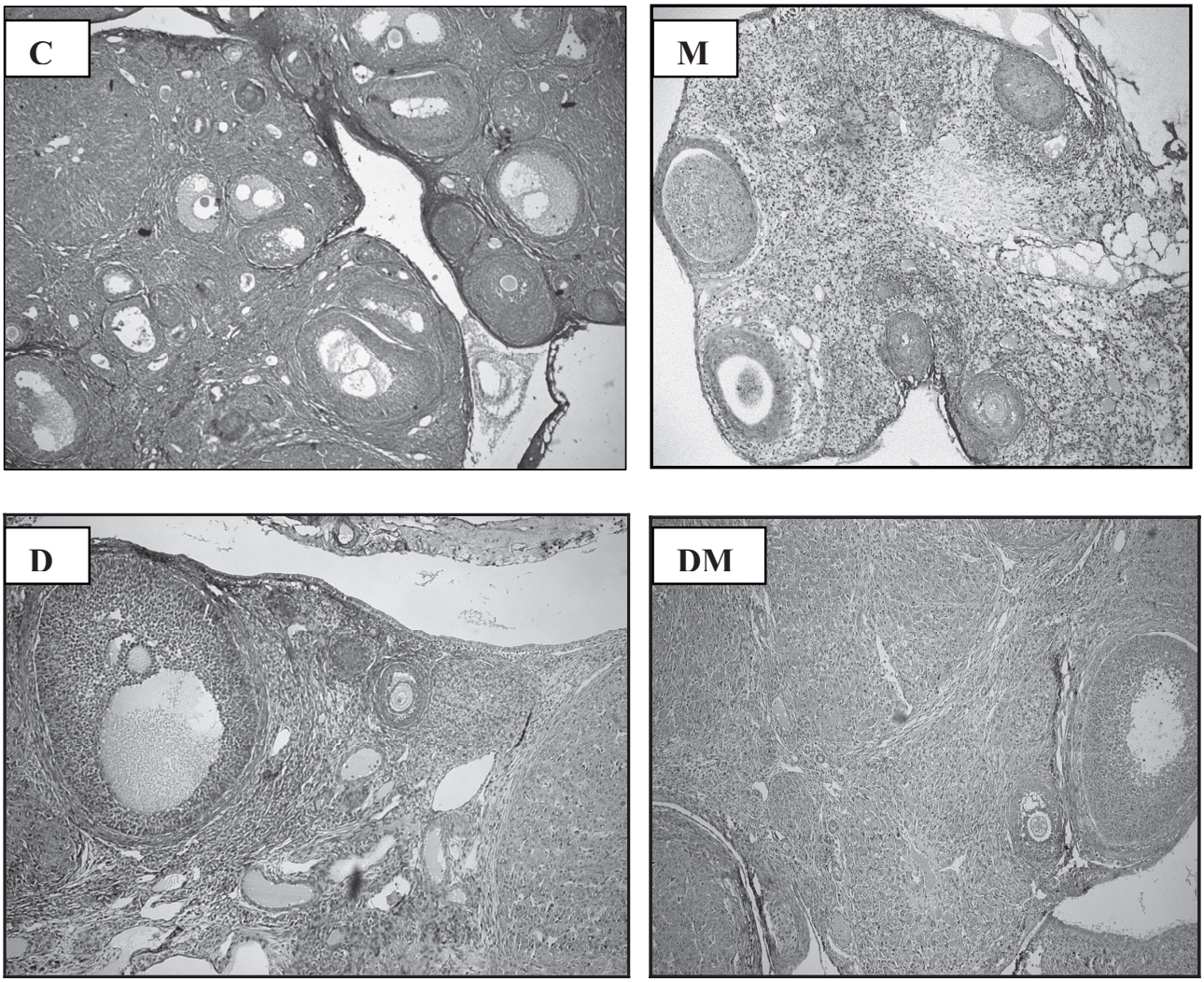

Figure 3. Sections of ovaries of female rats from: group C (control), group D (neonatally treated with diltiazem), group M (neonatally treated with MSG) and group DM (neonatally treated with diltiazem and MSG); HE stained. Magnification ×100.

The toxic effect of MSG on female reproductive system has been atributed to its direct effect on nuclei of hypothalamus for years. Central effect of MSG is proven (Lamperti and Blaha 1979).

Recent studies have shown that glutamate receptors play very important role in pathogenesis of disorders induced by MSG. Glutamate is predominant excitatory neurotransmitter in the mammalians central nervous system (Robinson 2006; Schlett 2006; Greenwood and Connolly 2007; Liguz-Lecznar and Skangiel-Kramska 2007). There are two basic types of glutamate receptors: ionotropic (NMDA, kainate and AMPA) and metabotropic (mGluR) (Smith et al. 2001; Weston et al. 2006; Gerber et al. 2007). Neurotoxicity of MSG is related with glutamate receptors activation (Gao et al. 1994; Beas-Zarate et al. 2001). Sustained high concentration of MSG could alter ionic permeability of neural membrane and induce persistent depolarisation. Such excessive activation of glutamate receptors and overloading with intracellular calcium can induce neural death (Gil-Loyzaga et al. 1993; Bojanic 1997). Glutamate receptors are present in different tissues: hypothalamus, heart, lungs, liver, kidneys, endocrine system, ovaries, uterus, etc. (Gill et al. 2008). Eweka and Om'Iniabohs (2007) referred that prolonged administration of high doses of MSG induced degenerative and atrophic changes in rat ovaries.

The results of our study showed that the pretreatment with diltiazem was efficient in prevention of MSG toxicity on ovaries histology and estrus cycle in Wistar rats.

We consider that excessive activation of glutamate receptors could be responsible for neurotoxic potencial of

MSG. Our subsequent studies should be done to elucidate if chronic low MSG doses activation of glutamate receptors in peripheral tissues could induce damage of ovaries in adult animals.

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