EARLY POSTNATAL GLUTAMATE TREATMENT RESULTS IN ALTERED VASCULAR RESPONSIVENESS TO SEROTONIN AND NORADRENALINE IN ADULT RATS

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Objectives. To evaluate possible alterations of vascular responsiveness to vasoactive hormones in the vessel preparations from adult rats treated neonatally with high doses of glutamate.

Methods. The responses to noradrenaline and serotonin in perfused hindlimb vascular bed and isolated renal artery were measured in MSG-treated (2 and 4 mg/g BW) and control groups of adult rats at the age of 10 weeks. Acetylcholine test was used to assess the endothelium-dependent relaxation of the hindlimb vascular preparation. The vessel specimens from this vascular bed were evaluated histologically.

Results. Vasoconstrictory responses to noradrenaline and serotonin were significantly reduced in the hindlimb vascular bed in MSG-treated rats. In the renal artery, a significant decrease of the responses to noradrenaline was found without significant changes in the responses to serotonin. The observed changes were more pronounced in groups treated with a high dose of MSG. Comparison of relaxing responses to acetylcholine in the hindlimb preparation did not show any statistically significant differences in control and MSG treated groups. Histological evaluation of this preparations did not reveal any endothelial damage or morphological changes of vessel wall.

Conclusions. The obtained results showed reduced vascular responsiveness to vasoconstrictory agents in adult rats neonatally treated with MSG suggesting that early postnatal administration of glutamate may result in irreversible changes in cardiovascular function.

Key words: Noradrenaline - Serotonin - Glutamate (MSG) - Neurotoxic damage - Blood vessels - Vascular responsiveness - Maturation processes

Neurotoxic effects of glutamate are well known. Thus, after the administration of monosodium glutamate (MSG) to rodents in the early postnatal period Olney (1969) found neurotoxic damage to the arcuate nucleus, circumventricular organs, retina and some other neural structures. As a consequence, a series of symptoms indicating endocrine, behavioral and metabolic disturbances may be observed in adult animals treated neonatally with MSG (Klingberg et al. 1987; Jezova et al. 1995).

As to the endocrine system, MSG treated rats or mice exhibit a clear dysfunction of the gonadal axis as well as the growth retardation which has been shown to result from neurotoxic damage to hypothalamic regulatory centers (Bakke et al. 1978). Less serious alterations of other neuroendocrine functions such as hypothalamic-pituitary adrenocortical axis were also demonstrated (Škultetyova et al. 1998). The most evident metabolic consequence of glutamate treatment in the neonatal period is the development of obesity which might be accompanied with the signs of insulin resistance (Zorad et al. 1997).

The cardiovascular function of MSG pretreated rats is less understood, but several alterations were described. Under basal conditions, slightly reduced values of blood pressure (Tokarev et al. 1996) and some morphological changes in the heart (Hamao-

KA and SAWADA 1987) were observed in MSG lesioned rats. A detailed study using continuous monitoring of blood pressure in conscious rats revealed that the pressor responses to vasoactive agents were reduced in MSG-treated animals (TOKAREV et al. 1997). Moreover, acute blockade of nitric oxide synthase in rats pretreated with MSG was associated with diminished blood pressure response (TOKAREV 1998).

The present studies were aimed to evaluate possible alterations of vascular responsiveness to vasoactive hormones in blood vessel preparations from adult rats treated with high doses of glutamate in the neonatal period. For this purpose, constrictive responses to noradrenaline and serotonin in perfused hindlimb vascular bed and isolated segment of a renal artery were measured. Administration of acetylcholine was used to assess the endothelium-dependent relaxation of the hindlimb vascular bed.

Materials and Methods

Animals: Female SPF Sprague-Dawley rats (Charles River Laboratories, WIGA, Silzfeld, Germany) were mated and housed individually until delivery of pups. Constant temperature (23-25 °C) and a 12 L:12 D cycle (lights on 6.00 -18.00 h) were maintained in the animal room. Standard pelleted diet and tap water were provided *ad libitum*. After weaning on postnatal day 21, the offspring were separated according to treatment and sex.

MSG treatment: The pups of both sexes were used in this study. MSG (Merck, Darmstadt, Germany) dissolved in 0.9 % NaCl was injected intraperitoneally (i.p.) in doses of 2 or 4 mg/g body weight on 2nd, 4th, 6th, 8th and 10th postnatal day. Controls received an equivalent volume of hypertonic saline (10 % NaCl) on the same time schedule as MSG treated rats.

The experiments were performed in MSG-treated and control groups (6-8 rats per group) of adult rats at the age of 10 weeks. The animals were anesthetized with pentobarbital sodium (65 mg/kg i.p.) and sacrified.

Hidlimb vascular preparation: After laparotomy all visceral organs were completely removed after the ligations of appropriate blood vessels. The abdominal aorta and inferior vena cava were carefully isolated. Then a polyethylene cannula was inserted into abdominal aorta distal to the renal arteries and fixed by the ligature. Heparin (0.1 %) with Tyrode's solution (the composition in mM: NaCl 137, KCl 2.7, $MgCl_2$ 1.1, NaH_2PO_4 0.32, $CaCl_2$ 0.9, $NaHCO_3$ 11.9, glucose 5.5) was administered very slowly into the vascular bed. The hindlimb vascular preparation was connected to the perfusion system and perfused at constant flow rate of 3 ml/min with Tyrode's solution, kept at 37 °C and pH 7.25-7.35. The outflow from inferior vena cava was directed into a reservoir without recirculation. The perfusion fluid was continuously gassed with a 95 % O_2 and 5% CO_2 mixture.

Isolated renal artery: In another group of animals anesthetized with pentobarbital sodium the renal artery was carefully excised together with the kidney and placed in Tyrode's solution. After isolation and cannulation the vessel segment was transfered into a vessel chamber and perfused at similar conditions as described above (flow rate 2.5 ml/min). Both vascular preparations were allowed to equilibrate with the perfusion fluids for 30 minutes.

Treatments: Noradrenaline hydrogentartarate (Leciva, Czech Republic) and serotonin (Sigma, St.Louis, Mo) were injected directly into the cannula in a volume of 0.1 ml in doses of 0.01, 0.1, 1.0 and 10.0 μg.

In the hindlimb vascular preparation the endothelial function was evaluated using acetylcholine test. The relaxation response to acetylcholine chloride (Dispersa AG) administered in a dose of 10 μg was expressed as the percentage of noradrenaline-induced contraction (conc. $2x10^{-6}$mol/l). The substances were dissolved in physiological saline solution. For dilution of drugs Tyrode's solution was used. All solutions were prepared fresh before each experiment.

The specimens of blood vessels from hindlimb preparation were evaluated histologically using the routine formaline paraffine technique.

Statistical evaluation: Means±S.E. were calculated and compared for statistical significance with the aid of analysis of variance (ANOVA) and non-parametric test by Kruskal-Wallis. P values less than 0.05 were considered significant.

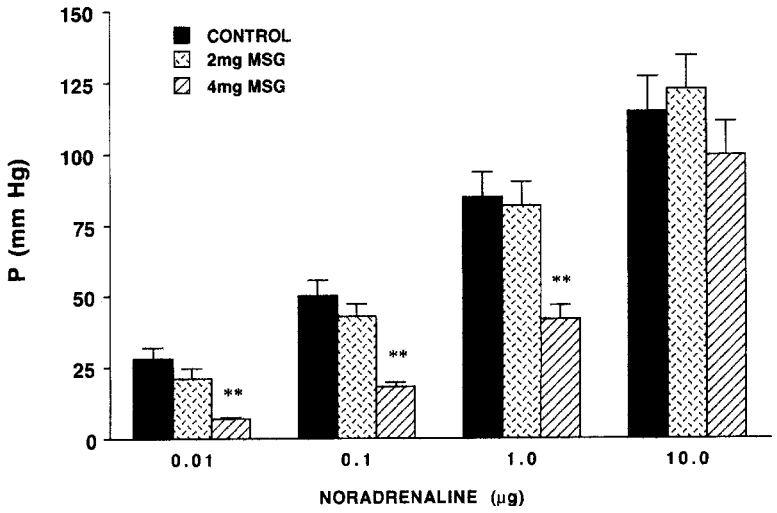

Fig.1
Effect of noradrenaline on hindlimb vascular bed reactivity in rats treated neonatally with saline (CONTROL) or MSG. P - perfusion pressure. Means of 8 values ± SEM. Statistical significance vs. appropriate control group: ** - P<0.01.

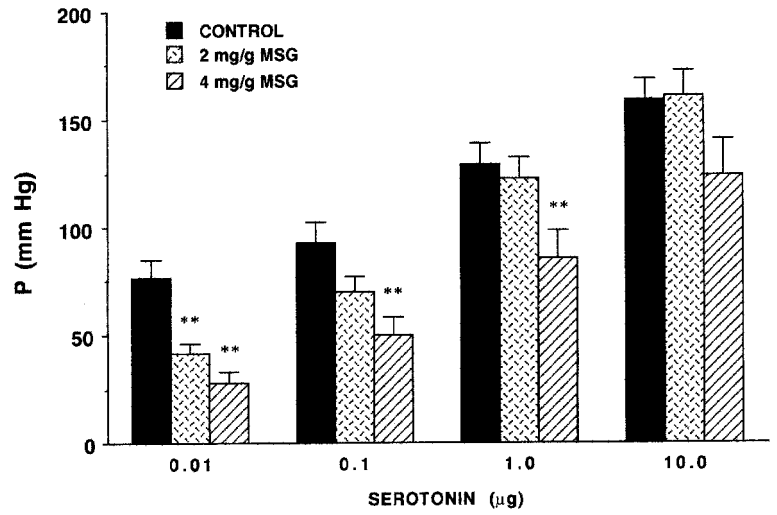

Fig.2
Effect of serotonin on hindlimb vascular bed reactivity in rats treated neonatally with saline (CONTROL) or MSG. P - perfusion pressure. Means of 8 values ± SEM. Statistical significance vs. appropriate control group: ** - P<0.01.

Results

In both types of vessel preparations, a gradual dose-related increase of vasoconstrictory responses to noradrenaline and serotonin was found in all investigated groups.

Glutamate treatment with 4 mg resulted in significantly decreased vasoconstrictory responses of hindlimb vascular bed to noradrenaline and serotonin (Fig.1 and 2) at doses 0.01, 0.1 and 1.0 μg ($P< 0.01$). In the group treated with 2 mg MSG no significant differences in responses to noradrenaline and serotonin were found except of the lowest dose of serotonin (Fig.2).

The comparison of relaxing responses to acetylcholine in hindlimb preparations of control and both MSG treated groups did not show any statistically significant differences (Tab.1).

Tab.1.
Endothelium-dependent relaxation to acetylcholine in perfused hindlimb vascular bed from control and MSG treated rats (% of noradrenaline-induced contraction). Means of 8 values ± SEM.

Groups	Responses [%]
Control	54.29 ± 19.4
2 mg/g MSG	44.9. ± 9.7
4 mg/g MSG	44.01 ± 11.5

In renal artery preparations, a significant decrease of vasoconstrictory responses to noradrenaline (0.01, 0.1 and 1.0 μg) was found in the group treated with 4 mg MSG (Fig.3). However, no significant differences in the responsiveness to serotonin in renal arteries of the same group were observed (Fig.4).

Histological evaluation of vascular wall of both control and MSG treated hindlimb vascular bed preparations did not show any considerable changes, the endothelial lining being well preserved (Fig.5).

Discussion

This study demonstrated reduced vascular responsiveness to alpha-adrenergic stimulation in two different vessel preparations of rats treated with glutamate in the neonatal period. Moreover, the vasoconstrictory responses to serotonin were reduced in hindlimb vascular bed, but not in isolated renal artery. In the hindlimb vascular preparation, the observed changes were more pronounced in groups treated with a high dose of MSG (4 mg/g BW).

Attenuation of the vascular responses to noradrenaline observed in present experiments may be related to the interference of MSG effects with blood vessel maturation during early postnatal development. Thus, the hormones and drugs may interact at one or more sites of the vessel wall including adrenergic innervation, vascular smooth muscle receptors, second messenger system and contractile proteins. Drug-vascular smooth muscle interactions are considered to be age related (Fleisch 1980). For example, it was found that the responses of rat aorta to noradrenaline and serotonin were higher in the younger (2 months) than in older (1 year) animals (Cohen and Berkowitz 1976). It may be suggested that some components of receptor-effector system

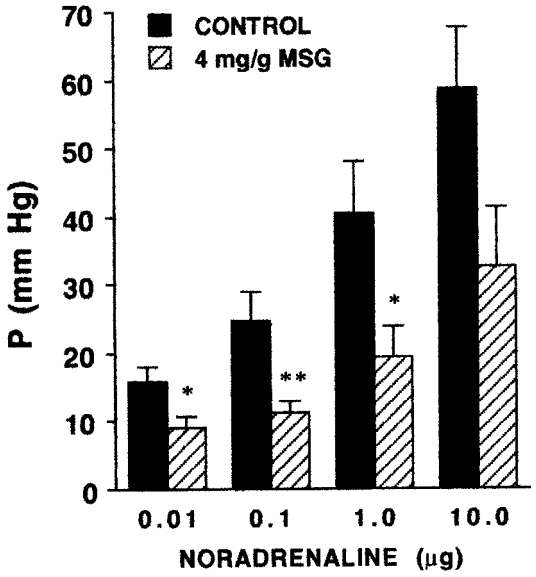

Fig.3
Constrictory effect of noradrenaline in the renal artery in rats treated neonatally with saline (CONTROL) or MSG. P - perfusion pressure. Means of 6-7 values ± SEM. Statistical significance vs. appropriate control group: * - P<0.05; ** - P<0.01.

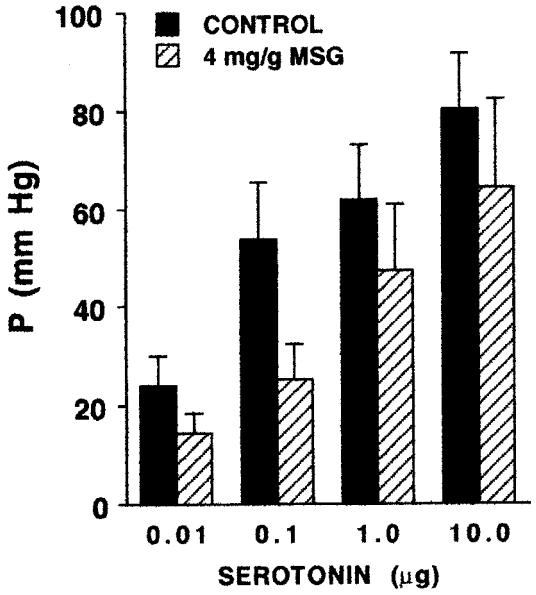

Fig.4
Constrictory effect of serotonin in the renal artery in rats treated neonatally with saline (CONTROL) or MSG. P - perfusion pressure. Means of 6-7 values ± SEM.

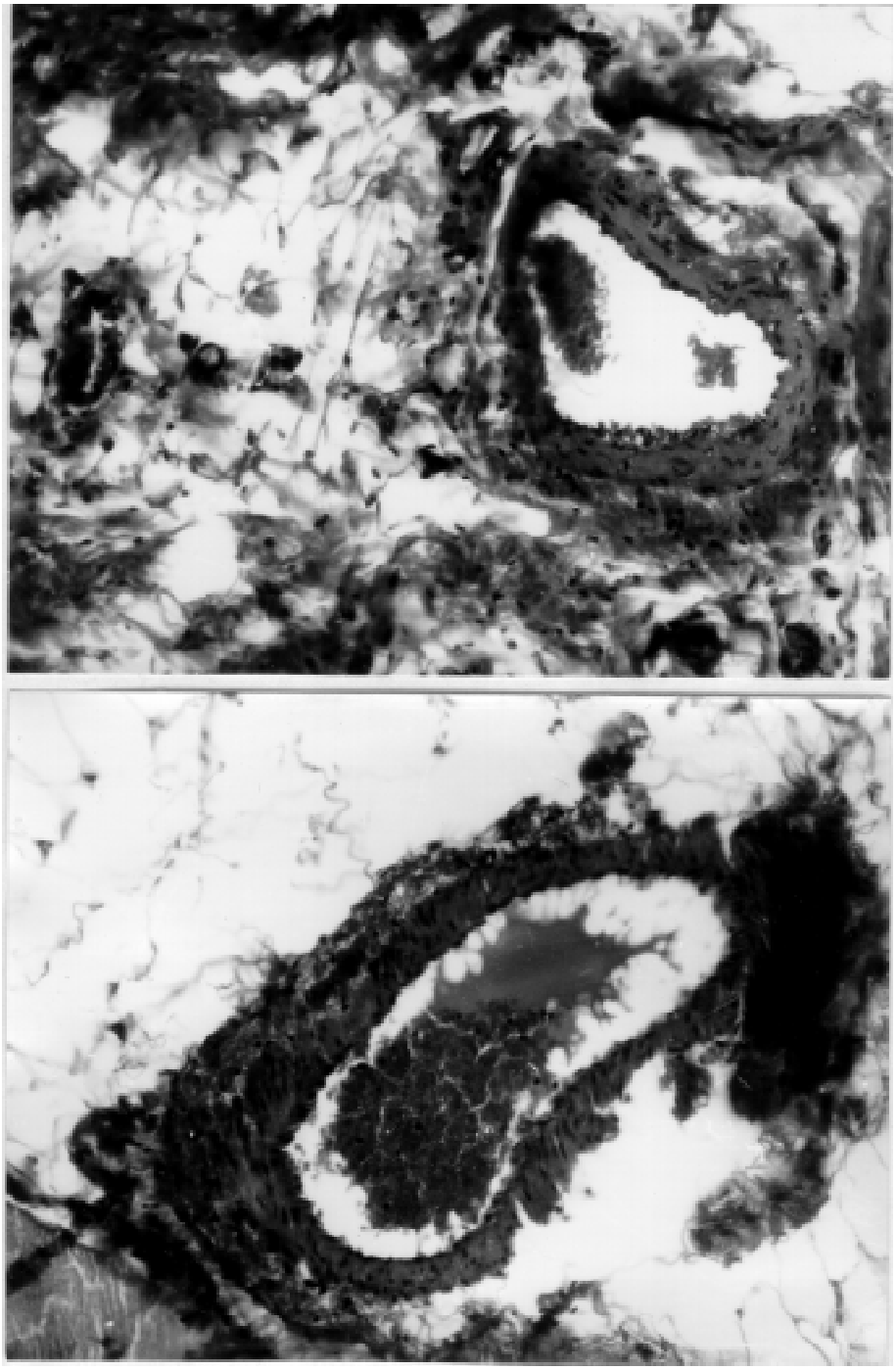

Fig.5
Rat hindlimb vascular preparation from control (upper part) and 4 mg MSG treated animals (lower part). Endothelium intact. Hematoxylline and eosin (HE), x200

may undergoe a change with increasing age and that early pharmacological manipulation may modify the vascular responses in later life period.

Vasoconstrictory effect of noradrenaline on vascular smooth muscle is known to be mediated by postsynaptic alfa-1 adrenergic receptors which appear to be completely developed to the end of gestation (Van Petten et al. 1978). One explanation of altered responsiveness to noradrenaline may be a decreased sensitivity of adrenergic receptors in vascular smooth muscle in MSG-lesioned rats. This suggestion is supported by recent in vivo findings of reduced blood pressure response to alfa-adrenergic agonist phenylephrine as demonstrated in MSG-treated rats (Tokarev et al. 1997).

Serotonin action on vascular smooth muscle was found to be variable not only among the species but also between the animals of the same species. However, most studies have shown prevailing constrictory effect of serotonin on isolated blood vessel (Fleisch 1980) which was confirmed also in our experiments. The variability was exhibited by different responses to serotonin between hindlimb vascular bed and renal artery in MSG-treated rats. In this respect, serotonin effects were found to vary considerably among blood vessels of the same species also in other studies (Duckles and Banner 1984), though the consequences of MSG treatment have not been studied previously.

Constrictory responses to noradrenaline and serotonin in the hindlimb were significantly diminished in animals treated with the high dose of MSG (4 mg/g). However, with the exception of the response to the lowest dose of serotonin, the rats treated with 2 mg/g of MSG showed no significant differences. Both doses of MSG used were reported to be neurotoxic inducing the damage predominantly in the hypothalamus (Olney 1969; Meister et al. 1989; Caputo et al. 1996). The degree of neurotoxic lesions in mouse brain was found to be related to the dose of MSG injected (Abraham et al. 1971). Moreover, glutamate receptors occur not only in the central nervous system but also in some peripheral tissues, e.g. in the pancreas (Bertrand et al. 1992), adrenals (Yoneda and Ogita 1986) and sympathetic ganglia (Erdo 1991). Thus, potential damage to peripheral tissues including vascular wall produced by high doses of MSG might contribute to altered responsiveness of cardiovascular system. It is not likely that morphological changes in the vessel wall play a role in the mechanisms of glutamate-induced alterations. Histological evaluation of the hindlimb vascular bed failed to show any structural differences between control and MSG treated animals.

In vivo studies revealed a diminished cardiovascular response to acute blockade of nitric oxide (NO) synthase in rats pretreated with MSG (Tokarev 1998). Deleterious effect of MSG observed in isolated vascular preparations does not seem to be related to altered NO production in endothelial cells. This suggestion is supported by present morphological and functional examination. In MSG-treated rats, no serious morphological changes in endothelial lining occured and the ability of vessels to relax in response to acetylcholine remained undisturbed.

In summary, our results indicate that the early postnatal administration of MSG may result in irreversibile changes in cardiovascular function in adult rats which may be the consequence of central and possibly even peripheral impairment of maturation processes during the development.

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