POSTNATAL MONOSODIUM GLUTAMATE TREATMENT RESULTS IN ATTENUATION OF CORTICOSTERONE METABOLIC RATE IN ADULT RATS

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Objective. To study the basal and ACTH stimulated production of corticosterone by adrenal cortex on one hand and the binding and degradation of corticosterone in the liver of adult rats which were treated with monosodium glutamate (MSG) during the neonatal period.

Methods. Male offsprings of Sprague-Dawley rats were injected i.p. with MSG (4 mg/g of b.w. in saline) on alternated days for the first 10 days of life, their littersmates being used as controls. On the 21st postnatal day they were weaned and used for the observation at the age of 65-75 days. After sacrifice the level of corticosterone in serum and the release of corticosterone from incubated adrenals under basal and ACTH stimulated conditions (ACTH in 6 concentrations from 1.25 to 80 mU/ml medium) were estimated. In addition, glucocorticoid binding to cytosol receptors in the liver and muscle tissues was determined. Corticosterone degradation rate was measured by decrease of corticosterone concentration added to the medium after the incubation with liver slices.

Results. Adult rats neonatally treated with MSG had reduced weight of adrenal glands, while plasma corticosterone levels and its basal production by adrenals in vitro were significantly higher than in controls. In MSG treated rats the stimulation of corticosterone production by ACTH was diminished. Glucocorticoid binding to liver cytosolic receptors was significantly decreased, while that in muscle tissue was only slightly elevated. Moreover, a decreased corticosterone degradation rate in liver slices was observed in rats treated neonatally with MSG.

Conclusions. These results are in agreement with previously observed decrease of corticosterone clearance rate in MSG treated animals and suggest that elevated corticosterone levels in plasma and its prolonged response to stressogenic stimulation are due to elevated corticosterone production in adrenals and lower degradation rate in the liver.

Key words: Monosodium glutamate – Rat – Corticosterone metabolism – Adrenals – Liver – Muscle tissue – Receptors

Numerous reports have shown that administration of monosodium glutamate (MSG) during the sensitive early postnatal period results in neurotoxic lesions of the hypothalamus and circumventricular organs (Olney 1969; Burde et al. 1971; Lemke-Johnson and Reynolds 1974) with manifestation of endocrine and metabolic abnormalities at adult age. These neurotoxic effects of MSG treatment result in stunted growth, obesity, infertility, decrease of growth hormone, gonadal steroid and thyroid hormone levels, although gonadotropin and TSH serum levels were not always reduced (Redding et al. 1971; Bakke et al. 1978; Nemeroff et al. 1977a,b; Rodriguez-Sierra et al. 1980; Nemeroff et al. 1981).

With respect to the hypothalamic-pituitary-adrenocortical axis activity, conflicting results were reported in adult rats neonatally treated with MSG. Despite the reduction in the weight of the pituitary and adrenal glands, normal values of plasma and pituitary ACTH content and plasma corticosterone concentrations were reported under both resting and stress conditions (Krieger et al. 1979; Conte-Devolx
et al. 1981; ACS et al. 1982). However, Dolnikoff et al. (1988); Spinedi et al. (1984); Magaranos et al. (1988) and, recently, Skultetyova et al. (1998) found increased basal or stress-induced corticosterone levels in MSG treated rats. One study (Larsen et al. 1994) reported decreased level of ACTH in plasma and that of corticotropin releasing hormone (CRH) mRNA in the paraventricular nucleus, while Skultetyova et al. (1998) clearly demonstrated unchanged CRH mRNA levels in the hypothalamus and higher levels of proopiomelanocortin mRNA in the anterior pituitary. According to the observation by Skultetyova et al. (1998), elevated basal corticosterone in plasma and prolonged corticosterone responses to stressogenic stimulation in neonatally MSG treated rats seem to be due to decreased clearance rate of corticosterone.

Previous studies in MSG treated rats mentioned above were related mainly to the evaluation of possible changes in hypothalamic and pituitary control of adrenocortical function. The aim of the present study was to investigate: 1. the changes of adrenocortical function at the level of adrenal glands by estimating the basal and ACTH stimulated production of corticosterone by adrenal cortex, 2. the binding and degradation of corticosterone in the liver of adult rats treated with MSG during the neonatal period.

Materials and Methods

Animals. Male offsprings of Sprague-Dawley rats (Charles-River Wiga, Silzfeld, Germany) were injected intraperitoneally on alternated days for the first 10 days of life with MSG (4 mg/g of body mass, Merck, Darmstadt, Germany) dissolved in 0.9 % NaCl. Littermate controls (a half from the same litter) were injected with equivalent volumes of 10 % NaCl (isosmotic with MSG solution). The animals were weaned at the 21st postnatal day and housed under controlled conditions (23±2 °C, 12:12 h light-dark regime, in standard cages, 6 animals/cage), with free access to rat chow pellets and tap water. The rats were used for the observation at the age of 65-75 days.

Corticosterone production. Animals were sacrificed by decapitation, blood samples were collected for corticosterone determination. The adrenals were removed, cleaned from surrounding tissues, quartered and weighed. Each quarter of adrenal was preincubated in 1 ml of Krebs-Ringer-bicarbonate (KRBG, pH 7.4) with glucose (1 mg/ml) for 30 min in O2- CO2 (95 %, 5 %) atmosphere. The preincubation medium was discarded and adrenal tissues were incubated in fresh KRBG for 1 hour period. The corticosterone concentration was determined in incubation medium using a radioimmunoassay (Sigma Bio-science, Corticosterone RIA protocol, St. Louis, USA).

ACTH stimulatory effect. The adrenal glands were removed and cleaned and preincubated as described above. ACTH (Sigma, St. Louis, USA, sp. act. 91.5 IU/mg) was added to fresh KRBG incubation medium (at doses 1.25, 2.5, 5, 10, 40 and 80 mU/ml) and incubation continued for 60 min. Concentration of corticosterone in incubation medium was estimated by protein displacement method according to Beittins et al. (1971) or by specific radioimmunoassay.

Glucocorticoid binding. Specific glucocorticoid binding to cytosol receptors in liver and muscle tissues was assessed by the modification of the method described by Alexandrova (1978). Briefly, liver and m. quadriceps from hind limbs were removed and quickly frozen in liquid nitrogen. The tissues were pulverized in a porcelain mortar, the powder was then transferred to centrifuge tubes and ice cold Tris-HCl buffer (0.01M) with EDTA (0.001 M), sodium molybdate (0.01 M), glycerol 10 % and dithiothreitol (0.002 M), pH 7.4, (6 ml/g of tissue) was added. After centrifugation at 100,000 g for 60 min, the supernatant was removed and used as a cytosol for the binding assay. An aliquot of cytosol was used for protein determination according to Lowry et al. (1951). Receptor determinations were performed by dextran-coated charcoal adsorption procedure using 3H-dexamethasone (DEX, spec. act. 42.5 Ci/mmol, Amersham, Little Chalfont, England) as binding ligand during 22 h incubation period. The 600-fold excess of cold DEX was used for determination of non-specific binding. Scatchard plots of data were employed for calculation of glucocorticoid receptor binding capacity.

Corticosterone metabolism in liver. Corticosterone degradation by liver tissue was determined from the decrease of corticosterone concentration added to incubation medium after the incubation with liver slic-
es. Livers from control and neonatally MSG treated animals were removed immediately after decapitation, weighed, and tissues slices (thickness of 0.2 mm) were prepared by using Stadie-Riggs microtome. Pieces of 100-150 mg were incubated in 2 ml of Krebs-Ringer phosphate buffer (KRP, pH 7.4) with 30 μg/ml of corticosterone at 37 °C for 1 hour. The samples of tissue from the same livers were incubated in KRP medium but at the temperature 0 °C and serve as blank controls. Aliquots of incubation medium were extracted into dichloromethane and this extract was evaporated. The dry rest was dissolved in 0.1 ml of KRP and corticosterone concentration was determined using radioimmunoassay (Sigma, Bioscience, Corticosterone RIA protocol). The amount of corticosterone metabolized by liver tissues during the incubation period was calculated as a difference between the values of corticosterone in the samples incubated at 0 °C (controls) and samples incubated at 37 °C. The degradation of corticosterone was expressed per unit of fresh weight of liver and also total amount of metabolized corticosterone per whole liver was calculated. The preliminary experiments showed that there was no interference of dihydro- and tetrahydrocorticosterone metabolites with the radioimmunological determination of corticosterone.

**Statistical evaluation.** The data were processed by one way analysis of variance and the unpaired Student t-test was used when comparisons were performed for two groups only.

### Results

In agreement with previous observations the rats neonatally treated with MSG showed decreased body and liver weights, and lower absolute and relative weights of adrenal glands (Tab. 1). In spite of decreased adrenal weight, an elevation of basal corticosterone levels in plasma was observed (Tab. 1). The determination of basal production of corticosterone by adrenal glands as expressed per unit of adrenal or body weight, was increased in adult rats neonatally treated with MSG as compared to the values in controls (Fig. 1).

The stimulation of corticosterone production by ACTH added to adrenal tissues in vitro (expressed as increases over basal production of the hormone) was lower in MSG treated animals (Fig. 2).

To explain the decreased corticosterone clearance rate observed previously in vivo, the binding and metabolism of corticosterone was studied. Lower corticosterone binding was found in the liver (Fig. 3), while a slight elevation of binding parameters was observed in muscle tissues of MSG treated rats (Fig. 3).

A decrease of the rate of corticosterone metabolism in liver tissue was noted in MSG treated animals which was highly significant when total amount of corticosterone per whole liver was calculated (Fig. 4).

### Discussion

Neurotoxic lesions in the arcuate nucleus and other parts of the brain induced by MSG treatment during
Fig. 2 Stimulation of corticosterone production by adrenal glands after the addition of ACTH expressed as increase over the basal production. C – control rats, MSG – rats neonataly treated with monosodium glutamate (MSG). Comparison of C vs. MSG: * = C vs. MSG P<0.05

Fig. 3 Binding of corticosterone to cytosol receptors in the liver (L) and skeletal muscle (SM) from control rats (C) and rats neonataly treated with monosodium glutamate (MSG). Comparison of C vs. MSG: * = P<0.05, ** = P<0.01

Fig. 4 Mean values of corticosterone metabolized by liver tissues (expressed as differences of corticosterone levels in samples incubated at 0 °C and 37 °C) from control (C) or monosodium treated rats (MSG). The amount of metabolized corticosterone is calculated per unit of liver weight (A) or per total liver (B).

early postnatal period are accompanied by changes in the content of neurotransmitters and neuropeptides produced in the hypothalamus, such as β-endorphin, ACTH, somatoliberin, neurotensin and met-enkephaline (Ferland et al. 1977; Clemens et al. 1978; Walas and Fonnun 1978; Krieger et al. 1979; Hong et al. 1981). No effects on LHRH, TRH and somatostatine content were noted (Nemeroff et al. 1977 a,b). Changes in hypothalamic function may result in altered content and release of anterior pituitary hormones and consequently in changes in the weight and function of endocrine glands.

A significant decrease of absolute weights of pituitary, adrenal, thyroid glands and gonads was observed in rats treated neonataly with MSG at the age of 40 and 100 days (Redding et al. 1971). The content of ACTH, TSH, PRL in the pituitary and plasma did not show any significant changes compared
to the values in controls, however, a marked decrease in growth hormone and luteinizing hormone levels was observed after MSG treatment (Redding et al. 1971; Nemeroff et al. 1977b; Krieger et al. 1979; ACS et al. 1982; Antoni et al. 1982).

It has been proposed that neuroendocrine disturbances induced by neonatal MSG-treatment include also changes in the function of the hypothalamic-pituitary-adrenocortical axis. In spite of repeatedly observed decreased weights of the pituitary and adrenals, contradictory results were reported on ACTH and corticosterone concentrations. The normal values of plasma and pituitary ACTH content and plasma corticosterone concentrations under resting and stress conditions were observed (Krieger et al. 1979; Conte-Devolx et al. 1981; ACS et al. 1982; Liu et al. 1986). Other authors described increased basal and stress induced plasma corticosterone levels, with or without concomitant changes in ACTH release (Spinelli et al. 1984; Magarinos et al. 1988; Dolnikoff et al. 1988; Larsen et al. 1994; Skultetyova et al. 1998). Larsen et al. (1994) reported that plasma ACTH levels were decreased in MSG treated rats, while corticosterone levels were elevated. In agreement with lower ACTH release, CRH mRNA levels in hypothalamic paraventricular nucleus were significantly lower in MSG-treated rats, but these data were not confirmed in other experiments (Skultetyova et al. 1998). After exposure to restraint stress, responses of plasma corticosterone levels were elevated in MSG lesioned animals, but plasma ACTH levels were similar to those in controls. Larsen et al. (1994) suggested that the adrenal cortex of MSG treated rats exhibits an altered sensitivity to circulating ACTH or that adenocortical secretagogues other than ACTH operate in these animals. However, the possible direct influences of MSG treatment on adrenal function, on corticosterone production, and on the response of adrenal tissue to ACTH has not been studied as yet. Further, higher levels of plasma corticosterone could be due to the changes in peripheral metabolism of corticosterone (Skultetyova et al. 1998).

The results of our experiments showed that, in spite of lower adrenal weight, the resting plasma corticosterone concentrations were higher in MSG treated rats as compared to the control group. This could be due to elevated production of corticosterone by smaller adrenals or by slower rate of disappearance and degradation of corticosterone in tissues. The determination of corticosterone production showed that in MSG treated rats the basal production is elevated suggesting that activation of adrenal cortex function plays a role in keeping the increased plasma corticosterone levels even at resting conditions.

The response of adrenal tissue to ACTH stimulation under in vitro conditions did not support the hypothesis of higher sensitivity of adrenal cortex to ACTH in MSG treated animals (Larsen et al. 1994). We have observed a lower response of adrenals to various doses of ACTH added in vitro in MSG-treated rats.

Skultetyova et al. (1998) observed a decreased corticosterone clearance rate in 10-14 week old rats treated neonatally with MSG. The slower clearance rate might be due, at least partially, to increased serum levels of corticosterone binding globulin found in MSG treated rats (Magarinos et al. 1988). However, the changes of processes of the degradation of corticosterone in the liver can also be involved. The results of our experiments show that there is decreased binding of corticosterone suggesting a lower uptake of hormone in the liver. Further, a diminished degradation of corticosterone was found in liver tissues in animals treated with MSG. This decreased rate of metabolism of corticosterone in the liver is in agreement with slower clearance rate observed in vivo by Skultetyova et al. (1998). The slower rate of corticosterone degradation can also explain the higher (Larsen et al. 1994) or prolonged (Skultetyova et al. 1998) increase of corticosterone concentration in MSG treated rats after the exposure to stress stimuli.

The results of our measurement of corticosterone production and metabolism are in agreement with decreased clearance rate of this hormone observed by Skultetyova et al. (1998) and demonstrated that higher production of corticosterone in adrenals and lower rate of hormone metabolism in the liver are responsible for increased corticosterone concentrations in plasma of rats treated neonatally with MSG.

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