

Effects of neonatal treatment with MSG (Monosodium glutamate) on hypothalamo- pituitary-thyroid axis in adult male rats

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Summary. Neonatal administration of MSG leads to a syndrome of endocrine dysfunction characterised by reduced growth, obesity and hypogonadism. The aim of the present investigation was to gain information on the structure and function of the pituitary-thyroid axis in MSG-treated rats. Neonatal Wistar rats received an s.c. MSG (4 mg/g body weight) or hyperosmotic saline (controls) on days 2, 4, 6, 8 and 10 of life. Histological and morphometrical studies were carried out on the thyroids of rats during the 4th month of life. Plasma TSH, T3, and T4 were measured by RIA kits. MSG-treated rats showed stunted growth, obesity and decreased pituitary weight. MSG administration resulted in increases in thyroid weight, absolute volumes of epithelium, colloid and stroma, and blood T3 level while T4 level remained unchanged. In enlarged thyroid gland, percentage fractions occupied by epithelium, colloid and stroma were similar to those observed in control rats.

The results obtained suggest that the rat hypothalamic centres involved in regulation of the pituitary-thyroid axis are slightly affected by neonatal MSG treatment.

Key words: Monosodium glutamate, Pituitary-thyroid axis, TSH, T3, T4

Introduction

Glutamate has been suggested as the major excitatory amino acid neurotransmitter in a number of neural loci including the hippocampus, cortex, cerebellum and hypothalamus (Van den Pol, 1991). Monosodium glutamate (MSG), an experimental neurotoxin, has been extensively used to investigate the role of the arcuate nucleus (AN) in endocrine regulation. In rodents between 80-90% of the arcuate neurons are destroyed by

neonatal administration of MSG (Olney, 1969; Holzworth-Mc Bride et al., 1976; Nemeroff et al., 1977; Rainbow et al., 1984). The chemical lesion is not entirely restricted to the AN since structures located near circumventricular areas are also damaged (Olney, 1969). In comparison with adult rats the central nervous system of the rats up to the 8th day of postnatal development is more sensitive to administration of such drugs as ibotenic acid and glutamic acid (Benawides et al., 1988). Adult rats that received MSG neonatally demonstrated a syndrome of endocrine dysfunction characterised by reduced growth, obesity, disturbances in the regulation of caloric balance, hypogonadism and behavioural deficiencies (Olney, 1969). The destruction of this hypothalamic area blocks GH secretion in adulthood due to the loss of GHRH produced by AN neurons (Corder et al., 1990). Much previous work was concentrated on the effects of MSG on pituitary-gonadal (Miśkowiak et al., 1993) and pituitary-adrenal (Magarinos et al., 1988) axis. On the contrary, data on MSG action on pituitary-thyroid axis are lacking. Therefore, the aim of the present study was to investigate the structure and function of the axis as affected by neonatal treatment with MSG. It is well known that thyroid hormones play a critical role in hypothalamo-pituitary GH-RH and GH synthesis and secretion (Valcavi et al., 1992).

Materials and methods

Neonatal male Wistar rats received an s.c. injection of MSG (4 mg/g body weight) or hyperosmotic saline (controls) on day 2, 4, 6, 8, and 10 of life. Animals were maintained under controlled conditions of light (14L:10D) and temperature (22 ± 2 °C) with free access to standard rat chow pellets and tap water. Body weight (BW) was recorded monthly throughout the experiment. MSG-treated and control rats were killed at the age of 4 months. Under Inactin anaesthesia blood from the heart was withdrawn.

Plasma was separated immediately by centrifugation

and stored at -20 °C until assay for TSH by an enzyme immunoassay (Abbott HTSH-EIA kit). In this method the intra-assay variance was 6% and the inter-assay variance, 8%. Serum T3 and T4 were quantified by RIA kits (ORIPi-Świerk, Poland)- intra assay and inter-assay variations: 4% and 6%.

Pituitary and thyroids were promptly removed, weighed, fixed in Bouin's solution and embedded in paraffin. Histological and morphometric studies were carried out on haematoxylin-eosin- (H+E) stained thyroid sections.

Stereometric studies were performed by the differential point-counting method of Weibel (1979). Under a magnification of x 3,000 the volume fractions of epithelium, colloid (follicular lumen) and stroma (connective tissue and blood vessels) were estimated directly on the screen. The H+E method does not allow recognition of thyroid C cells, in stereometric studies, therefore, these cells were included with the follicular cells. In each thyroid gland 3 sections were analyzed and in each section 50 fields were counted along the lines passing through the entire central part of the thyroid. The specific gravity of the thyroid glands (1.060 mg/mm³) was determined by their behaviour in a set of NaCl solutions of increasing concentration (Malendowicz and Bednarek, 1986). Knowing the weight of the gland and its specific gravity it was possible to calculate the volume in cubic millimeters of epithelium, colloid and stroma. These parameters are sensitive exponents of

function of thyroid gland (Uotila and Kanas, 1952; Palkovits, 1963; Malendowicz and Bednarek, 1986).

Results were expressed as mean \pm SD and statistical significance between the mean was evaluated by Student's t-test.

Results

If compared with control, MSG-treated rats had higher thyroid weight and lower pituitary gland weight (Table 1). MSG-administered rats were shorter (ca 15%) than control ones. There was no difference in body weight between control and MSG-treated rats in the course of the experiment. In MSG-treated rats much adipose tissue was located mainly within the greater omentum and behind the peritoneum. There was no difference in blood T4 and TSH level; however, blood T3 level was elevated in MSG-treated rats. Despite a significant change in thyroid gland weight the percentage fractions of epithelium, colloid and stroma and epithelium/colloid index were similar in thyroids of both control and MSG- treated rats. The volume fractions (mm³) of epithelium, colloid and stroma were higher in MSG-treated male rats than control ones -46%, 38% and 37% respectively (Table 2).

Discussion

Administration of MSG in this study took place at an age when the rat brain was rapidly developing. The present results confirm and extend earlier studies that neonatal MSG administration produced several abnormalities of the hypothalamo-pituitary axis (Dawson, 1986; Magarinos et al., 1988). Central nervous system glutamate receptors have been extensively studied since glutamate is thought to be a major excitatory neurotransmitter through the CNS. After binding to a cell membrane glutamate receptor can stimulate the nitric oxide (NO) synthesis. NO has little activity within the cell in which it is produced, but it rapidly leaks out of the cell and produces effects in neighbouring cells. It is suggested that apart from physiological effects NO may cause a damage to different nervous structures. Scher and Scher (1992) suggest that NO is a mediator in the Chinese restaurant syndrome, glutamate-induced asthma, «hot-dog headache», and pugilistic Alzheimer disease. Olney (1969), on the other hand, demonstrated that MSG

Table 1. Body weight, organ weights and plasma hormone levels in 4-month-old male control and MSG-treated rats. Results expressed as mean \pm SD, n= 6.

	CONTROL	MSG
Body weight (g)	306 \pm 12	337 \pm 21
Body length (cm) (naso-anal length)	23 \pm 0.4	20 \pm 0.3**
Pituitary weight (mg) mg/100g BW	10.6 \pm 0.97 3.5 \pm 0.21	7.5 \pm 1.1** 2.3 \pm 0.43**
Thyroid weight (mg) mg/100g BW	13.2 \pm 1.81 4.3 \pm 1.3	18.5 \pm 2.2** 5.5 \pm 0.5*
TSH (μ IU/ml)	0.1	0.1
T3 (ng/ml)	0.26 \pm 0.07	0.36 \pm 0.08*
T4 (ng/ml)	34 \pm 8.5	25 \pm 2.7

Significantly different from control: *, p < 0.05; **, p < 0.01.

Table 2. Percentage and volume representation of epithelium, colloid* and stroma of thyroid (control and MSG-treated rats). Results are expressed as mean \pm SD, n= 6.

	PERCENTAGE REPRESENTATION			VOLUME, mm ³			EPITHELIUM/COLLOID INDEX
	Epithelium	Colloid*	Stroma	Epithelium	Colloid*	Stroma	
Control	45.8 \pm 2.2	41.8 \pm 2.7	11.8 \pm 1.5	5.7 \pm 0.3	5.2 \pm 0.2	1.47 \pm 0.1	1.09
MSG	47.8 \pm 7.5	41.2 \pm 6.7	11.6 \pm 0.9	8.34 \pm 1.3**	7.19 \pm 1.1**	2.02 \pm 0.2**	1.16

*: surface of follicular interior. Significantly different from control: **, p < 0.001.

selectively destroys arcuate neurons when administered to newborn mice, and this approach is being frequently used in experimental works. The advantage of this model, as compared with electrothermocoagulation, is that it does not damage the axons that end in or pass through this region.

It is well known that MSG-treated rats are obese in adulthood and have reduced body length. In the present experiment we observed a 15% reduction of naso-anal length. Retardation of the growth may depend on impaired GH-RH and GH secretion (Corder et al., 1990).

To our knowledge there are no reports on the effect of neonatal MSG administration on thyroid structure and function. Present data indicate that basal TSH secretion is not impaired in MSG-treated rats and is not different from control ones. Our data indicate that the response of the thyroid MSG-treated rats are differentiated. We have demonstrated no significant differences in percentage representation of epithelium, colloid and stroma, epithelium/colloid index, and TSH and T4 plasma level between control and experimental animals. On the other hand significant differences were observed in thyroid weight, absolute volumes of epithelium, colloid and stroma and plasma T3 level between MSG- and saline-treated rats.

Since TRH is produced mainly in the paraventricular nuclei (Palkovits, 1979; Brownstein et al., 1982; De Greef et al., 1992), the results obtained suggest that this hypothalamic nuclei is not destroyed by MSG. This finding is in agreement with other authors who demonstrated that the cells in some regions of CNS are more resistant to neurotoxic glutamate than cells of AN (Hastings et al., 1985). This may be due to different types of receptors, differences in second messenger systems or different levels in ion channel activation (Van den Pol, 1991).

Recent work by Jessop et al. (1991) has shown that the CRF content of the paraventricular nucleus (PVN) and AN/median eminence was unchanged by MSG lesioning, indicating that these areas are not affected by MSG. However, MSG lesioning of the AN resulted in a significant decline in the PVN content of both substance P and substance K (Jessop et al., 1991) and a decrease in NPY concentration in PVN and AN neurons (Abe et al., 1990).

The results obtained suggest that the rat hypothalamic centres involved in regulation of the pituitary-thyroid axis are slightly affected by neonatal MSG treatment.

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