

Effect of neonatal treatment with MSG (Monosodium glutamate) on thyroid of the adult male rats

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Summary. Monosodium glutamate (MSG), administered to newborn rats produces extensive lesions in neurons of the arcuate nuclei of the hypothalamus. The cells represent the site of neurohormone production, including the production of both growth-hormone releasing hormone (GHRH) and somatostatin.

Studies were performed on male Wistar strain rats, subcutaneously injected with MSG, at 4 mg/g body weight, on days 2, 4, 6, 8 and 10 of life. When the rats reached the age of 18 months, they were additionally stimulated with a single dose of TSH (Ambinon). When the rats reached the age of 6, 12, or 18 months, their thyroids were isolated and fixed in Bouin's solution. In HE-stained preparations, planimetric and volumetric proportions occupied by the epithelial fraction, colloid and stroma, the number of thyroid follicles per mm² and the thickness of epithelium were determined. Serum T3 and T4 levels were quantified by RIA. Significance of differences was tested using Student's t test.

The weight of experimental rat thyroids showed no significant variations as compared to the controls but were greatest in the group of 12-month-old rats. The same was noted for the volumetric fractions of epithelium, colloid and stroma. The planimetric fractions occupied by epithelium, colloid and stroma in the thyroid remained unchanged and amounted to 60%, 31% and 9%, respectively. The number of follicles per mm² thyroid cross-section in the MSG-treated 6-, 12- or 18-month-old rats was 131.3, 116.2 and 130.4, respectively, and did not differ from control values. Thickness of follicular epithelium showed no significant variations.

Serum T3 levels in the rats examined after 6, 12 or 18 months were increased by 67%, 89% and 33%, respectively, as compared to serum T4 levels. When compared to the controls, the serum T4 level was lower only in the 12-month-old MSG-treated rats and the serum T3 level was higher in 18-month-old MSG-treated rats. The ability of the thyroid to respond to Ambinon stimulation was preserved.

The results of our investigations suggest that the rat

hypothalamic centers involved in regulation of the pituitary-thyroid axis are slightly affected by neonatal administration of MSG.

Key words: MSG, thyroid, T3, T4, Morphometry

Introduction

Glutamic acid is the commonest neurotransmitter in the central nervous system and its concentration is approximately a million-times greater than that of neuropeptides (Pilc, 1992). Monosodium glutamate (MSG), administered to rodents in the perinatal period, penetrates those cerebral regions which lack the blood/brain barrier. MSG induces lesions in arcuate nuclei (AN) and in 2/3 of the neurocytes in ventromedial nuclei (Yamamoto et al., 1993). Administration of MSG destroys dopaminergic, cholinergic and other neurons in AN which terminate in the median eminence (Belluardo et al., 1990). The region of the ventral hypothalamus, and AN neurons, in particular, are known to participate in the secretion of numerous hormones, including also somatoliberin, which stimulates the synthesis and release of growth hormone in the pituitary, and somatostatin, which inhibits these processes (Maiter et al., 1991; Thompson et al., 1994). Damage to arcuate nuclei of the hypothalamus induced by perinatal administration of MSG, evokes a number of behavioural changes in the organism, including changes in the endocrine system which are evident in individuals reaching sexual maturity. The lesions are manifested by obesity, retarded growth, lower carcass protein, higher carcass lipid content (Ribeiro et al., 1997), and disturbances in the pituitary-adrenal axis (Magarinos et al., 1988) and the pituitary-gonadal axis (Miskowiak et al., 1993a,b).

In previous studies on the effects of perinatal administration of MSG on the reproductive system in male rats (Miskowiak et al., 1993a,b) we demonstrated decreased weight of testes, increased weight of ventral prostate, decreased serum testosterone, but unchanged serum LH and FSH levels. With the metabolic-hormonal disturbances which follow perinatal administration of MSG (Miskowiak, 1995) we also expected a response

from the pituitary-thyroid system. In a preceding report (Miskowiak and Partyka, 1993) we detected no changes in thyroid histology in 4-month-old rats, postnatally treated with MSG, nor alterations in the so-called planimetric presentation of epithelium, colloid and stroma in the gland (Miskowiak and Partyka, 1993).

The present report is aimed at defining the long-term effects of MSG on the pituitary-thyroid system in 6-, 12-, and 18-month-old rats, perinatally treated with MSG, and at appropriate morphometric and biohormonal (T3 and T4) parameters.

Material and methods

The studies were performed on male Wistar strain rats, subcutaneously injected with MSG (Sigma), at 4 mg/g body weight, on days 2, 4, 6, 8, and 10 of life. Control rats received at the same time injections of hypertonic, 2% NaCl solution. The animals were housed in standard light (14L:10D) and temperature (20 ± 2 °C) conditions with free access to water and a granulated chow. When the animals reached the age of 6, 12 or 18 months, their blood was sampled from the heart under general anaesthesia, for estimation of serum T3 and T4 levels by RIA (ORIP, Swierk). The eighteen-month-old rats were subjected to a single dose TSH stimulation (Ambinon, 2IU, Organon Oss, Holland) 24 h before sacrifice. Body weight and naso-anal length were measured in each rat. For morphometric and stereometric studies the thyroids were isolated and weighed, fixed in Bouin's solution, embedded in paraffin, and sectioned at 3 to 5 μ m.

Morphometric studies of the gland were performed on H+E-stained histological preparations of the thyroid gland using Weibel's differential point counting method (Weibel, 1979). At x400 magnification, shown directly on the screen the numbers of cross-sections of type M 48 grid lines were established, positioned over epithelium, colloid and the connective tissue-vascular stroma of the thyroid. The proportions occupied by each of these structures in the gland could thus be calculated. Thyroid C cells were included in the follicular epithelium fraction. 30 measurements were made on each preparation along the line passing through the centre of

the section. The volume of the fractions was calculated from both the proportions of thyroid volume which they occupied and the specific density of the thyroid (1.060 mg/mm³), as determined by Malendowicz and Bednarek (1986).

The thickness of follicular epithelium was established at x1200 magnification using the Multiscan computer program. All measurements were made along the line passing through the section's centre. Between 98 to 130 measurements were made in each preparation.

In addition, the number of thyroid follicles present was determined from examination at x300 magnification of each section using the Multiscan program. The preparation was shifted repeatedly in such a way that adjacent optical fields were analysed. In order to avoid double registration of objects, only the objects positioned within the counting frame and overlapped by its left and upper limit were scored (Wojnar and Majorek, 1994).

The results were subjected to statistical analysis using Student's t test.

Results

These experiments demonstrated that rats perinatally treated with MSG showed marked obesity and a decreased naso-anal length at the ages of 6, 12, or 18 months.

In the 6-, 12- or 18-month-old rats, perinatally treated with MSG, the absolute weights of thyroid glands did not differ from those of the controls (Table 1). However, in the group of 12-month-old experimental rats, the relative thyroid weights proved higher by 27%. In the 18-month-old rats, perinatally treated with MSG, serum T3 levels were higher by 57% than those in the corresponding controls (Table 1). By contrast, serum T4 levels were lower, by approximately 19% in the group of 12-month-old experimental rats.

In each of the three groups of experimental animals the T3 level, as compared to the T4 level, was elevated, by 67%, 89% and 33%, respectively compared to their controls (Table 1). Morphometric studies showed that thyroid follicle numbers per mm² area of the gland cross-section were similar in all groups whether treated

Table 1. Body weight and length, thyroid weight and T3, T4 and T3/T4 ratio of the controls of rats treated neonatally with MSG, investigated after 6, 12 and 18 months.

	6 MONTHS		12 MONTHS		18 MONTHS	
	Control	MSG	Control	MSG	Control	MSG
Body weight (g)	334.0 \pm 58.6	337.0 \pm 52.1	472.2 \pm 24.8	484.8 \pm 21.0	521.3 \pm 16.4	517.5 \pm 4.4
Body length (cm)	23.2 \pm 0.9	21.6 \pm 0.6*	25.2 \pm 0.7	22.6 \pm 0.4 *	26.2 \pm 0.5	23.0 \pm 0.7*
Thyroid weight (mg)	14.8 \pm 3.4	15.5 \pm 2.2	17.6 \pm 0.9	23.0 \pm 5.3	22.4 \pm 3.1	19.2 \pm 3.6
Relative thyroid weight (mg /100 g)	4.5 \pm 0.9	4.6 \pm 0.5	3.7 \pm 0.3	4.7 \pm 0.9*	4.3 \pm 0.2	3.7 \pm 0.4
T3 (ng /ml)	0.26 \pm 0.07	0.36 \pm 0.08	0.33 \pm 0.07	0.49 \pm 0.21	0.37 \pm 0.06	0.58 \pm 0.14*
T4 (ng /ml)	31.0 \pm 10.3	25.0 \pm 2.8	36.1 \pm 2.6	29.2 \pm 5.3*	31.3 \pm 7.9	35.0 \pm 4.9
T3/T4 ratio	0.9 \pm 0.4	1.5 \pm 0.4*	0.9 \pm 0.2	1.7 \pm 0.7*	1.2 \pm 0.3	1.6 \pm 0.4*

Results expressed as means \pm SD; n = 6. *: significantly different from control; p < 0.05.

MSG and thyroid

Table 2. Amount of the thyroid follicle per mm² thyroid area of control rats and rats treated neonatally with MSG, investigated after 6, 12 and 18 months.

AGE MONTHS	CONTROL		MSG	
	Number of thyroid follicles per section of thyroid	Number of thyroid follicles per mm ² thyroid area	Number of thyroid follicles per section of thyroid	Number of thyroid follicles per mm ² thyroid area
6	686.0±130.9	133.3±25.4	459.5±180.5	131.3±30.0
12	522.7±127.3	117.0±17.8	502.3±222.0	116.2±13.0
18	592.2±124.7	114.6±14.1	346.8±89.9	130.4±17.9

Results expressed as means±SD; n = 6.

Table 3. The thickness of the thyroid follicle epithelium and percentage and volume fractions occupied by epithelium, colloid and stroma in the thyroids of control rats and rats treated neonatally with MSG, investigated after 6, 12 and 18 months.

	6 MONTHS		12 MONTHS		18 MONTHS	
	Control	MSG	Control	MSG	Control	MSG
<i>The thickness of epithelium (μm)</i>	10.6±0.8	10.3±0.8	10.4±0.6	10.2±0.6	10.2±0.9	9.2±0.4*
<i>Percentage fractions in the thyroids</i>						
Epithelium	59.2±4.7	60.3±4.0	62.5±4.9	58.9±5.7	58.0±4.3	56.4±3.9
Colloid	31.0±3.4	32.8±4.0	30.0±3.8	32.3±5.4	31.5±5.6	31.6±3.3
Stroma	9.8±2.0	6.9±1.0	7.5±2.3	8.8±1.6	10.4±3.9	9.0±1.8
<i>Volume fractions in thyroids (mm³)</i>						
Epithelium	8.3±0.7	8.8±0.6	10.4±0.8	12.8±2*	11.6±0.9	10.8±0.7
Colloid	4.3±0.5	4.8±0.6	5.0±0.6	7.0±2*	6.7±1.2	5.7±0.6
Stroma	1.4±0.3	1.0±0.1*	1.2±0.4	1.9±0.4*	2.2±0.8	1.6±0.3
<i>Volume fractions/100g body weight</i>						
Epithelium	2.5±0.2	2.6±0.2	2.2±0.2	2.6±0.2*	2.4±0.2	2.1±0.1*
Colloid	1.3±0.2	1.4±0.2	1.1±0.1	1.5±0.2*	1.3±0.2	1.1±0.1
Stroma	0.4±0.1	0.3±0.04*	0.3±0.1	0.4±0.1*	0.4±0.2	0.3±0.1

Results expressed as means±SD; n = 6. *: significantly different from control; p<0.05.

or not treated with MSG, and ranged from 114.6 to 133.3 (Table 2).

The thickness of thyroid follicle epithelium did not differ between the 6- and 12-month-old rats treated with MSG, on the one hand and the respective controls on the other, and amounted to approximately, 10.4±0.6 μm (Table 3). Ambinon stimulation resulted in a decrease in the thickness of the epithelium to 9.2 μm in the 18-month-old experimental rats.

The volume fraction occupied by epithelial cells, colloid and stroma in the thyroids did not vary in any of the groups of rats and averaged approximately 60%, 31% and 9%, respectively (Table 3). In 12-month-old MSG treated rats increased volume of the fractions was noted, as compared to the controls, which reflected the increased thyroid weight in the age group (Tables 1 and 3). In thyroids of the comparable groups of 6-month-old rats no differences in volumes of the epithelium and colloid were disclosed but stroma volume underwent a slight reduction. Thyroids of 18-month-old rats, following MSG treatment, showed lowered volumes of all fractions, as compared to the controls, but the difference was significant only in the case of the relative

volume of the epithelial fraction.

Discussion

The hypothalamo-pituitary-thyroid axis represents an integral part of the body endocrine system, closely associated with metabolic processes. The individual elements of the system: hypothalamus, pituitary and thyroid gland, are strictly linked to each other since their functions are controlled by a set of feedbacks between TRH, TSH and thyroid hormones. The principal effects of thyroid hormones involve the transcription control of target genes in the body, control of neurotransmitter activity and of the number of their receptors as well as their effects on neuron functions (Puymirat, 1992; Yen and Chin, 1994). In the axis, thyroid hormones represent the principal regulators of TSH synthesis and release in the pituitary (Jackson, 1994). Neurodegenerative lesions induced by MSG probably do not include paraventricular nuclei (NPV); the only site of TRH production in the brain involved in the system of feedbacks with thyroid hormones (Jackson, 1994). This is suggested by the presence of nitrogen oxide synthetase

(NOS) in microcellular neurons of the nucleus (Torres et al., 1993) and by its protective effect in MSG-induced neurotoxicity (Brünning, 1992; Turski and Turski, 1993). Torres et al. (1993) have reported that already on the first day after birth NOS can be noted in NPV of the rat, although maturation of the nuclei, i.e. increase in perikaryon number and in dendrite branching, continues until the 21st day of life. A favourable T3 effect has also been observed from the 17th day of intrauterine life, on expression of the NOS gene (Puymirat, 1992; Ueta et al., 1995). In experimental studies on the hypothalamus the presence of functional links between arcuate and paraventricular nuclei have been demonstrated. In earlier experiments we showed that the perinatal introduction of MSG is not neutral to T3 level, thyroid weight and its effect on lipid metabolism (Miskowiak and Partyka, 1993; Miskowiak, 1995).

In the present experiments the thyroid weight of experimental rats has shown no significant changes, as compared to the control, while the absolute weights were elevated in only the 12-month-old rats. In contrast, Kubota et al. (1994) reported reduced rat thyroid weights following perinatal treatment with MSG.

The level of T3, elevated as related to T4 by 67%, 89% and 33% in individual MSG-treated groups, respectively, may have indicated augmented requirements for the hormone. However, serum T3 levels elevated as compared to the controls were only noted in the 18-month-old-experimental rats. The T4 level, in turn, was lowered only in the 12-month-old animals. In rats perinatally treated with MSG, Kubota et al. (1994) noted T4 levels within normal limits and similar results were obtained by Yamamoto et al. (1993) in 1-, 2-, 3-, and 5-month-old mice subjected to MSG action.

Ambinon stimulation in 18-month-old rats, perinatally treated with MSG, had a positive effect on both T3 and T4 levels. The T4 level in 6- and 12-month-old rats, decreased by around 19% as compared to the control, after Ambinon stimulation manifested 12% tendency for an increase.

The T3 level in the animals also increased by 57% as compared to the control: the proportions of the thyroid gland occupied by epithelium, colloid and stroma did not change in any of the MSG-treated groups of rats i.e., epithelium accounted for 60%, colloid for 31% and stroma for 9% of thyroid volume. Conde et al. (1991) have reported a lower volume fraction occupied by epithelium (42%), a similar colloid fraction (32%) and a greater stroma representation (26%) in thyroids of 4-month-old control rats.

As compared to the data observed in control animals, significant differences were noted in the volumes of epithelium, colloid and stroma in the group of 12-month-old MSG treated rats. The differences corresponded to an increased weight of thyroids in 12-month-old MSG treated rats. The values of relative volumes were lower for individual thyroid fractions in the 6-month-old rats treated with MSG (where epithelium accounted for 2.6 mm³, colloid for 1.4 mm³

and stroma for 0.3 mm³) than in the study of Malendowicz and Bednarek (1986) in 3-month-old controls. Their figures were 4.4 mm³, 1.8 mm³ and 1.2 mm³ for the epithelium, colloid and stroma, respectively. These differences can be related to higher body weight of our older i.e. 6-month-old, rats.

The thickness of thyroid follicle epithelium amounted to 10 µm and exceeded by approximately 40% the value obtained by Delverdier et al. (1991). The latter authors fixed their material in formalin while Bouin's solution was used in our study. Thyroid functional condition is related to, amongst other factors, the number and size of thyroid follicles. The larger follicles, located at the periphery of the gland, are metabolically less active (Delverdier et al. 1991).

Previous studies on the effect of the perinatal injection of MSG on thyroid gland have documented no obvious changes in structure or function of the gland, in conditions of GHRH deficiency or upon stimulation of the gland with Ambinon. In our studies the number of follicles per mm² thyroid section remained unchanged in the three groups of rats, perinatally injected with MSG. The morphological and functional parameters of thyroid gland, including gland volume fractions occupied by epithelium, colloid and stroma, height of follicular cells and number of follicles per mm², have demonstrated no significant differences in 6-, 12- and 18-month-old experimental rats as compared to the respective control groups. However, the volumes of epithelium, colloid and stroma in the thyroid glands of 12-month-old rats, perinatally treated with MSG, were higher than those in control animals. Furthermore we observed an increase in the serum T3 level as related to the serum T4 level.

Our results suggest that the rat hypothalamic centers involved in regulation of the pituitary-thyroid axis are slightly affected by neonatal administration of MSG.

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MSG and thyroid

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