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Neonatal treatment with monosodium glutamate (MSG): structure of the TSH-immunoreactive pituitary cells

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Summary. Glutamic acid represents the most abundant stimulatory neurotransmitter in the central nervous system. Monosodium glutamate (MSG), subcutaneously administered to newborn rats in the perinatal period, induces lesions in 80 to 90% of the neurocytes of arcuate nuclei in the hypothalamus. These nuclei are the site of production of numerous stimulatory and inhibitory hormones including growth hormone releasing hormone (GHRH).

The present studies were performed on male Wistar strain rats, subcutaneously injected on days 2, 4, 6, 8, and 10 of postnatal life with MSG at a dose of 4 mg/g body weight. Eighteen-month-old rats were additionally treated with Ambinon. When the animals reached the ages of 6 or 12 months, their body weight, body length and weight of pituitary were determined. On paraffin sections, using imrnunohistochemical techniques, TSH-immunoreactive cells were detected and characterised by computerised image analysis. The results were subjected to statistical analysis using Student's *t* test.

The rats which were perinatally treated with MSG and examined after 6 or 12 months of life were obese and shorter than control rats by 7% and 10% respectively. They also exhibited a reduction in the weight of the pituitary of 30% and 40% respectively in the two age groups.

The proportion of TSH-immunoreactive cells in the pituitary remained unchanged and amounted to 4.5% in the 6-month-old and 5.4% in the 12-month-old rats, respectively. The number of TSH-positive cells per mm^2 area remained unchanged. The area and circumference of the cells in the 12-month-old rats were reduced by 22% and 18%, respectively.

Perinatal injury to hypophyseal arcuate nuclei induced by monosodium glutamate injection, was not associated with any significant alterations in pituitary structure, as defined by the proportion of pituitary volume occupied by TSH-immunoreactive cells.

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Introduction

Glutamic acid is one of the most abundant stimulatory neurotransmitters in the central nervous system and is also one of the amino acids most widely present in food. Monosodium glutamate (MSG), introduced to rodents at a time when the blood brain barrier (BBB) has not yet sufficiently developed, i.e. in the perinatal period, easily penetrates the brain, damaging as many as 80% to 90% of the neurocytes of hypothalamic arcuate nuclei (Olney, 1969; Waxman, 1990). MSC administration in neonatal rats caused reversible changes in BBB permeability. BBB vulnerability in MSC-treated rats during immobilization stress exposure was increased in the hypothalamus and decreased in the brain stem (Skultetyova et al., 1998). MSG did not alter neurohypophyseal vasopressin (VP) profiles: VP content of the posterior pituitary and microdissected regions of the hypothalarnus, serum VP concentration were similar in MSC-treated and control rats (Clough et al., 1986). However, Johnston and Negro-Vilar (1986) examined the effect of neonatal MSG treatment on oxytocin, vasopressin and somatostatin concentration in the suprachiasmatic, paraventricular, supraoptic and arcuate nuclei, median eminence and neurointermediate pituitary lobe. The data demonstrate that changes in neuropeptide content are not limited to the arcuate nucleus. However, MSG neonatal treatment resulted in a substantial loss of the arcuate nucleus (AN) neurons. The AN neurons produce a number of neuropeptides, including somatoliberin, somatostatin, gonadoliberin, substance P, substance K, NPY and 13-endorphins (Dawson and Bierkamper, 1987; Magarinos et al., 1988; Mitsushi et al., 1990; Jessop et al., 1991; Sasaki et al., 1994).

Perinatal injection of MSG results in a number of endocrine-behavioural alterations. These alterations may be classified as either stimulatory, e.g. those related to lipid metabolism, or inhibitory, e.g. those affecting the pituitary-gonadal axis (Miśkowiak et al., 1993a,b;

Miśkowiak, 1995), and the pituitary-adrenal axis (Magarinos et al., 1988).

Recently, Sasaki et al. (1994) performed immunocytochemical studies on GH-, LH-, FSH-, and prolactin-immunoreactive cells in the pituitaries of mice injected in the perinatal period with MSG.

Data on MSG action on pituitary-thyroid axis are lacking. Therefore, the aim of the present study was to investigate the structure of the TSH-immunoreactive (TSH-i) cells as affected by neonatal treatment with MSG. Utilising this immunocytochemical method for localising the cells, we decided to determine the number of cells per mm^2, their area and circumference and their % share in the volume of the pituitary.

Materials and methods

The studies were performed on male Wistar strain rats, subcutaneously injected on days 2, 4, 6, 8, and 10 of life with 4 mg of monosodium glutamate (MSG, Sigma)/g body weight. Control rats received 2% NaCl solution at the same time. The animals were housed in standard conditions of illumination (14L:10D) and temperature (20±2 °C), with free access to water and pelleted chow. When the animals reached the age of 6 or 12 months, their weight and naso-anal length were measured. After sacrifice their pituitaries were isolated, weighed and fixed in Bouin's solution, embedded in paraffin and serially cut in 3-5 μm-thick sections.

TSH-immunoreactive cells in the pituitary were detected by using both specific anti-rat TSH antibodies (Bioproducts) at 1:1000 and the unlabelled antibody technique using peroxidase-antiperoxidase complexes (PAP).

In order to quantitate the % share of TSH-immunoreactive cells in the pituitary volume, the numbers of type A grid line cross-sections were defined, which overlapped the cells or the remaining elements of pituitary gland on the screen (Weibel, 1979). In parallel, the number of TSH-immunoreactive cells per total section surface was determined.

The area and circumference of the TSH-immunoreactive cells were determined using computerised image analysis, applying the Multiscan program with corrections based on data from Wojnar and Majorek (1994). The measurements were performed at x1200 magnification, analysing the entire pituitary cross-section of area. Appropriate selection of TSH-immunoreactive cells by the program was ensured by the use of RGB filter and by setting the detection threshold at objects with dimensions ranging from 4.0 to 16.0 x 10^{-5} mm^2.

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

Results

The experiments showed that rats treated perinatally with MSG at the age of 6 and 12 months exhibited different degrees of obesity and their body length decreased by 7% and 10% respectively.

The observed reduction in pituitary mass in 6- and 12-month-old MSG-treated rats amounted to 30% and 40% compared to their corresponding controls (Table 1).

Immunocytochemical testing of TSH-secreting cells demonstrated that the cells formed islands in the pituitary, mostly in peripheral portions of the gland.

The share formed by TSH-secreting cells in the entire volume of the anterior lobe of the pituitary approximated 5% both in rats pretreated with MSG and in control, 6- or 12-month-old rats.

The number of TSH-immunoreactive cells per mm^2 cross-section area showed a decreasing tendency in MSG-treated rats (by 30% in the 6-month-old rats and by 23% in the 12-month-old rats, respectively). However, in none of the groups did the differences reach the level of statistical significance.

The mean areas and circumference of the TSH-immunoreactive cells were lower only in the 12-month-old rats, by 22% and 18%, respectively (Fig. 1, Table 2).

Discussion

In these experimental studies we noted the development of obesity, decreased body length and reduced body weight in the first months of life in rats which received perinatal doses of MSG. These alterations in the appearance of the animals are typical

Table 1. Body weight and length, pituitary weight and % participation of TSH-immunoreactive cells in the volume of the anterior pituitary of control rats and rats treated neonatally with MSG, investigated after 6 and 12 months. Results expressed as means±SD; n=6 in each group.

	6 MONTHS		12 MONTHS	
	Control	MSG	Control	MSG
Body weight (g)	334.0±58.6	337.0±52.1	472.2±24.8	484.8±21.0
Body length (cm)	23.2±0.9	21.6±0.6*	25.2±0.7	22.6±0,4*
Pituitary weight (mg)	11.1±0.7	7.6±1,1*	11.8±1.2	7.2±0.9*
Relative pituitary weight (mg/100 g)	3.4±0.4	2.3±0.4*	2.5±0.1	1.5±0.2*
Pituitary area (mm^2)	3.2±0.5	2.1±0.3	3.4±0.7	2.4±0.6
The number of TSH cells per total section surface of the pituitary/(mm^2)	23.9±4.4	16.8±8.8	30.8±12.2	23.6±12.3
Participation of TSH-cells in the anterior pituitary (%)	5.2±1.0	4.5±2.3	5.8±3.4	5.4±2.7

Significantly different from control: *, p<0,05

and have been noted by previous authors (Magarinos et al., 1988; Maiter et al., 1991; Badger et al., 1992). Brain and pituitary weight deficits following neonatal MSG treatment have been well documented (Dawson and Bierkamper, 1987; Miśkowiak and Partyka 1993).

Our analysis of pituitary weight in experimental rats of two different age groups demonstrated decreases of 30% and 40% respectively, as compared to the controls. Dawson (1986) has indicated that even two doses of MSG administered in the perinatal period (at 4 mg/g body weight, on the 2nd and 4th days of life) decrease pituitary weight by 23% in the first month of life and by as much as 52% in the 3rd month of life. The reduction of pituitary weight in MSG-treated rats has been linked to GHRH deficiency due to damage to neurons of the arcuate nuclei which are thought to exert a trophic effect on pituitary cells (Maiter et al., 1991). Even if pituitaries of rats perinatally-treated with MSG exhibit a decrease in protein content of 60%, the concentration of GH per protein unit has shown no significant differences (Maiter et al., 1991). Bhat et al. (1995) are of the opinion that MSG perinatally introduced to the body, may also directly affect pituitary cells since, according to them, NMDA receptors have been detected on at least a few percent of some anterior lobe cells in the pituitary.

Receptor autoradiography in the rat hypothalamus and pituitary confirmed the widespread presence of all major glutamate receptor subtypes with roughly the following relative densities: ventromedial, paraventricular, supraoptic, arcuate, pituitary neural lobe (small), anterior pituitary lobe (negligible) - (Meeker et al., 1991). Recently Bains and Ferguson (1997) tested resistance of subpopulations of paraventricular nucleus neurons using electrophysiological method to excitotoxicity neurotoxins. These results indicated the selective resistance of magnocellular subdivision of PVN neurons to excitotoxic cell damage.

In mature animals maintenance of a low extracellular glutamate level in the brain is warranted by the blood brain barrier and the very effective system of

Table 2. Area and circumference of the TSH-immunoreactive anterior pituitary of the control rats and rats treated neonatally with MSG and investigated after 6 and 12 months. Results expressed as means±SD; n=6 in each group.

AGE (months)	CONTROL			MSG		
	number of TSH-cells analyzed	area of the TSH-cell (mm^2x10^{-5})	Circumference of the TSH-cell ($mmx10^{-2}$)	number of TSH-cells analyzed	area of the TSH-cell (mm^2x10^{-5})	Circumference of the TSH-cell ($mmx10^{-2}$)
6	276±45	11.0±2.2	5.0±0.8	382±75	9.3±1.5	5.4±0.5
12	228±58	10.5±1.0	5.7±0.4	338±115	8.2±2.0*	4.7±0.2*

Significantly different from control: *, p<0,05

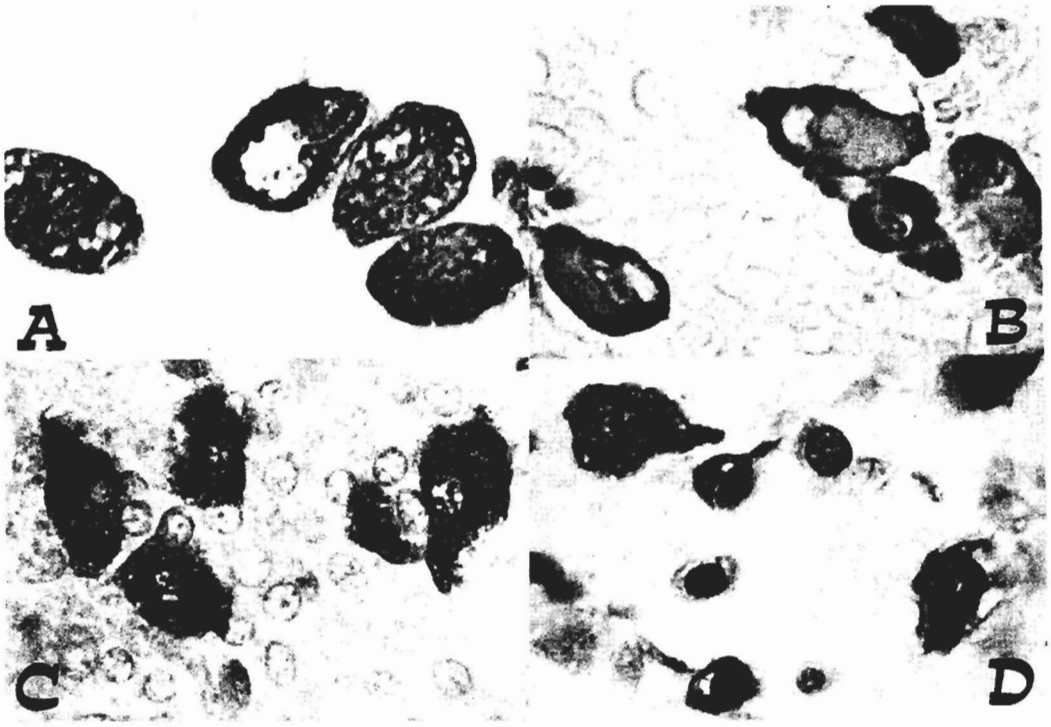

Fig. 1. TSH-immunoreactive cells in the anterior pituitary gland of the 6- and 12-month-old rats. **A.** 6-month-old controls. **B.** MSG-treated-6-month-old rats. **C.** 12-month-old controls. **D.** MSG-treated-12 month-old rats. x 800

glutamate trapping by glia and neurons. Penetration of perinatally-administered glutamate to the brain of rodents may take place due to immaturity of the barrier at the time and may also be due to immaturity of glutamate trapping and metabolism (Laake et al., 1992; Fairman et al., 1995).

Thyreotropin (TSH) is a glycoprotein released by thyreotropic cells of the pituitary and affects both the structure and function of the thyroid gland (Hisao, 1988). The hormone is released under the influence of hypothalamic TRH but its stable basic secretion is also observed in the pituitary. TRH gene expression in parvocellular neurons of PVN remains under regulation by thyroid hormone circulating in the bloodstream. Thyroid hormone receptors were found in the greatest percentage of TRH neurons in the PVN (Lechan et al., 1994).

TSH stimulates growth of the thyroid gland, due to thyreocyte proliferation on the one hand and to functional alterations, ie. a stimulated release of the colloid from the vesicles, on the other. TSH is produced and released to the bloodstream as a mixture of multiple isoforms, differing in bioactivity but exhibiting the same immunoreactivity (Beck-Peccoz and Persani, 1994).

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).

The share of TSH-immunoreactive cells as a percentage of the total volume of the pituitary anterior lobe in 6- and 12-month-old rats, namely 4.5% and 5.4% respectively, was the same in the MSG-pretreated rats as in the control animals. Similarly, no differences in the input of GH-, FSH-, LH-, PRL-positive cells in the volume of the pituitary have been demonstrated by Sasaki et al. (1994) between 2- and 2.5-month-old control mice and similar mice perinatally treated with MSG (2 mg/g body weight, 7 injections).

Sasaki et al. (1994) determined total numbers of GH-, FSH-, LH-, and PRL-immunoreactive cells in pituitaries and found that MSG administration was followed by a decrease in numbers, as compared to the controls, of 46%, 40%, 37% and 35%, respectively. Counting the number of TSH-immunoreactive cells per mm^2 pituitary area in MSG-pretreated 6- and 12-month-old rats revealed a decrease in the density of 30% and 23%, respectively. Torres et al. (1995) suggest that thyreotrope stimulation augments their secretory activity by mobilisation of preformed secretory granules. This stimulation also promotes hypertrophy of cytoplasmic organelles taking part in thyreotropin synthesis and, as a result, augments the immunocytochemically identifiable population of thyreotropes.

In the present study MSG pretreatment resulted in a reduction of both area and circumference of TSH-immunoreactive cells in 12-month-old rats, by 22% and 18%, respectively. Sasaki et al. (1994) demonstrated a similar decrease in the size of FSH-, LH-, and GH-immunoreactive cells by around 20% and of PRL-immunoreactive cells by 13%.

Our experimental studies in rats on the effect of perinatal injection of MSG on thyroid gland including gland, volume fractions occupied by epithelium, colloid and stroma, height of follicular cells, number of follicles per mm^2, have demonstrated no extensive differences in 6- and 12-month-old experimental rats as compared to the respective controls (Miśkowiak and Partyka, 1999). We have also observed also an increase in serum T3 level as related to serum T4 level. Bogdanov et al. (1996) examined the effects of consumption of a high oral dose of MSG or a high systemic administration of MSG on glutamate levels in plasma, and on glutamate release estimated using brain microdialysis, from the arcuate nucleus of hypothalamus of adult rats. In feeding experiments glutamate levels in arcuate nucleus dialysates were unchanged, while i.p. administration of MSG caused dose-dependent increases in glutamate levels within arcuate nucleus dialysates.

The model of MSG-induced neurotoxic injury in animal hypothalamus also allows the explanation of certain neuroendocrinological syndromes in humans. The majority of hypothalamic syndromes are due to destructive lesions of the hypothalamus. These may include primary or metastatic tumors, structural abnormalities, infiltrative diseases, trauma, vascular abnormalities or iatrogenic causes. Hypopituitarism can be manifested as either selective deficiences of individual hypothalamic hormones or panhypo-pituitarism, where all pituitary hormones are involved. Isolated deficiencies of anterior pituitary hormones are usually genetic or developmental disorders in which there is an absence of a specific hypothalamic-releasing hormone.

The primates appears to be particulary resistant to glutamate. No evidence of central nervous system damage was found following MSG force-feeding. Fasting male subjects each received a large oral dose of MSG (12.7 g) or MSG vehicle as control. Plasma glutamate levels rose 11-fold within 1h of MSG ingestion, but PRL, LH, FSH, GH, testosterone and cortisol were unchanged. Plasma levels of TSH, T4 and T3 showed minimal changes after MSG treatment (Fernstrom et al., 1996). These results suggest that acute pharmacological elevations of plasma glutamate levels in adult men produced minimal, if any, effects on hypothalamic or pituitary function. However, Scher and Scher (1992) suggest that nitric oxide is a mediator in the Chinese restaurant syndrome, glutamate-induced asthma, "hot dog headache", and pugilistic Alzheimer's disease.

These experimental studies on the effect of perinatal introduction of MSG on the TSH-immunoreactive cells of the pituitary have revealed no dramatic alterations as compared to the appropriate controls. The results obtained suggest that the rat hypothalamo-pituitary axis is slighty affected by neonatal MSG treatment.

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