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Fisiologia

Neuroendocrine alterations impair enamel mineralization, tooth eruption and saliva in rats

Alterações neuroendócrinas interferem com a mineralizaçãodo esmalte, a erupção dentária e a saliva em ratos

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ABSTRACT: Neonatal administration of monosodium glutamate (MSG) in rats causes definite neuroendocrine disturbances which lead to alterations in many organ systems. The possibility that MSG could affect tooth and salivary gland physiology was examined in this paper. Male and female pups were injected subcutaneously with MSG (4 mg/g BW) once a day at the 2nd, 4th, 6th, 8th and 10th day after birth. Control animals were injected with saline, following the same schedule. Lower incisor eruption was determined between the 4th and the 10th postnatal days, and the eruption rate was measured between the 43rd and the 67th days of age. Pilocarpine-stimulated salivary flow was measured at 3 months of age; protein and amylase contents were thereby determined. The animals treated with MSG showed significant reductions in the salivary flow (males, –27%; females, –40%) and in the weight of submandibular glands (about –12%). Body weight reduction was only about 7% for males, and did not vary in females. Saliva of MSG-treated rats had increased concentrations of total proteins and amylase activity. The eruption of lower incisors occurred earlier in MSG-treated rats than in the control group, but on the other hand the eruption rate was significantly slowed down. The incisor microhardness was found to be lower than that of control rats. Our results show that neonatal MSG treatment causes well-defined oral disturbances in adulthood in rats, including salivary flow reduction, which coexisted with unaltered protein synthesis, and disturbances of dental mineralization and eruption. These data support the view that some MSG-sensitive hypothalamic nuclei have an important modulatory effect on the factors which determine caries susceptibility.
DESCRIPTORS: Sodium glutamate; Hypothalamic diseases; Dental physiology; Saliva; Rats.

RESUMO: A administração neonatal de glutamato monossódico (MSG) em ratos provoca distúrbios neuroendócrinos que acarretam alterações em vários sistemas orgânicos. Neste trabalho, avaliamos as repercussões desse tratamento sobre dentes e glândulas salivares. Ratos machos e fêmeas recém-nascidos foram injetados com MSG (4 mg/g peso corporal, s.c.) uma vez ao dia nos 2º, 4º, 6º, 8º e 10º dias após o nascimento; o grupo controle recebeu solução salina no mesmo esquema. O momento da erupção do incisivo inferior foi determinado entre o 4º e o 10º dia de vida, e o ritmo de erupção foi medido entre o 43º e o 67º dia. O fluxo de saliva e o conteúdo salivar de proteína e amilase foram determinados sob estimulação com pilocarpina aos 3 meses de idade. Os animais tratados com MSG mostraram reduções significativas do fluxo salivar (machos: –27%; fêmeas: –40%) e do peso das glândulas submandibulares (cerca de –12%). Apenas em machos houve discreta redução do peso corporal (7%). A saliva dos animais tratados com MSG apresentou aumento na concentração de proteínas totais e na atividade de amilásica. A erupção dos incisivos inferiores ocorreu mais precocemente nos ratos tratados do que nos controles, porém a taxa de erupção apresentou-se significativamente reduzida. A microdureza também foi menor nos animais tratados. Nos sos resultados mostram que o tratamento de ratos recém-nascidos com MSG causa um quadro definido de alterações buco-dentais no animal adulto, traduzidas por redução do fluxo de saliva (sem redução da síntese proteica) e distúrbios de mineralização e erupção dentárias. Esses dados apontam para o importante papel modulador que certos núcleos hipotalâmicos sensíveis ao MSG exercem sobre os fatores que regulam a susceptibilidade à cárie.
DESCRITORES: Glutamato de sódio; Doenças hipotalâmicas; Fisiologia dentária; Saliva; Ratos.

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INTRODUCTION

Monosodium glutamate (MSG) is a widely used foodstuff flavouring compound, especially in oriental food. In rats and mice, the neonatal administration of MSG leads to extensive damage of certain hypothalamic nuclei, thus causing severe neuroendocrine disturbances in adulthood. The abnormalities, first observed by Olney[19] (1969), included growth impairment, marked obesity (which can develop without hyperphagia) and reduction of organ weights, among others[1,3]. Marked repression was observed in the ossification of developing endochondral bone, with the persistence of cartilagenous elements and chondrocytes[9], reduced ratio of mineral deposition, and slower bone maturation[26].

The formation, eruption and growth of teeth are processes under the concerted and timely influences of several hormones, such as growth hormone (GH)[2,28,29] and thyroid[14], sex[24], and adrenal hormones[25]. On the other hand, it is known that the development and the function of rodent salivary glands depend upon neurohormonal factors[18], and that the salivary secretion in rats is under strong hypothalamic influences[21].

Since the process of tooth formation and the dental microenvironment are important factors influencing caries susceptibility, the putative modulatory role of hypothalamus in these processes was evaluated by studying the tooth microhardness, the salivary flow and the concentration of protein and amylase in saliva of control or neonatally MSG-injected rats.

MATERIAL AND METHODS

Animals and treatments

Neonate male and female Wistar rats were treated with 5 subcutaneous injections of monosodium glutamate (MSG, Sigma Co., 4 mg/g body weight) dissolved in physiological saline. Injections were done once a day at the 2nd, 4th, 6th, 8th and 10th day of life. Control animals were treated with the drug vehicle. Volume injected was always 0.02 ml/g BW.

Rats were weaned at 21 days and put thereafter on regular Purina rat chow and water *ad libitum*. The animals were maintained on routine laboratory care conditions (12 h dark/light cycle, lights on 08:00 a.m., 24±2ºC). The experimental protocol was approved by the Ethics Committee on Animal Experimentation, UNESP School of Dentistry, Araçatuba.

Tooth eruption

Inferior incisors eruption day was determined upon daily examination from the 4th up to the 10th day of age. The rate of normofunctional tooth eruption was determined by examination every 2 days of the superior incisors in the period from 43 to 67 days of age. A starting mark at the gingival limit level was made under light ether anesthesia in the tooth enamel with a cylindrical bur and mensurations were then carried out following the method described by Gerlach *et al.*[10] (2000).

Salivary flow

At 90 days of age, after 12 h fasting, the rats were anesthetized with sodium pentobarbital (Hypnol®, Cristalia, 40 mg/kg BW, IP). Salivary secretion was stimulated by pilocarpine nitrate (Sigma, 5 mg/kg BW, IP). Whole saliva was then collected[4] into preweighed vessels and maintained on crushed ice during 20 min after the first drop had fallen. Volumes were estimated by weight, assuming the specific gravity of saliva to be 1.0 g/ml. After collection, the animals were killed by excess pentobarbital anesthesia, and the parotid, submandibular and sublingual salivary glands were carefully dissected out and weighed.

Protein and amylase determinations in saliva

Total protein in saliva was determined by the method of Lowry *et al.*[15] (1951) and the salivary amylase activity by that of Caraway[7] (1959). One unit of amylase activity is referred to as the amount of enzyme needed to hydrolyze 10 mg of starch in 30 min at 37ºC.

Microhardness

The upper and lower incisors were removed and dissected free from any foreign adherent tissue. The right teeth were embedded and longitudinally placed into acrylic resin; the left teeth were sectioned and placed transversely into the resin[23]. In both sections (longitudinal and transversal), two indentations were made, one on the crown and the other on the root, at the medium portion of enamel thickness. A microhardness tester Shimadzu HMV-2000® coupled to a Knoop-like penetrator set was used with a 50 g load. Microhardness results are given in terms of $kgf/mm^2 \times 10^{-3}$.

Statistical analysis

Weight and microhardness data were studied by ANOVA and followed, whenever appropriate, by Kruskal-Wallis or non-parametric multiple com-

parisons tests. A 2.01 version of the GraphPad InStat® software was used for this purpose.

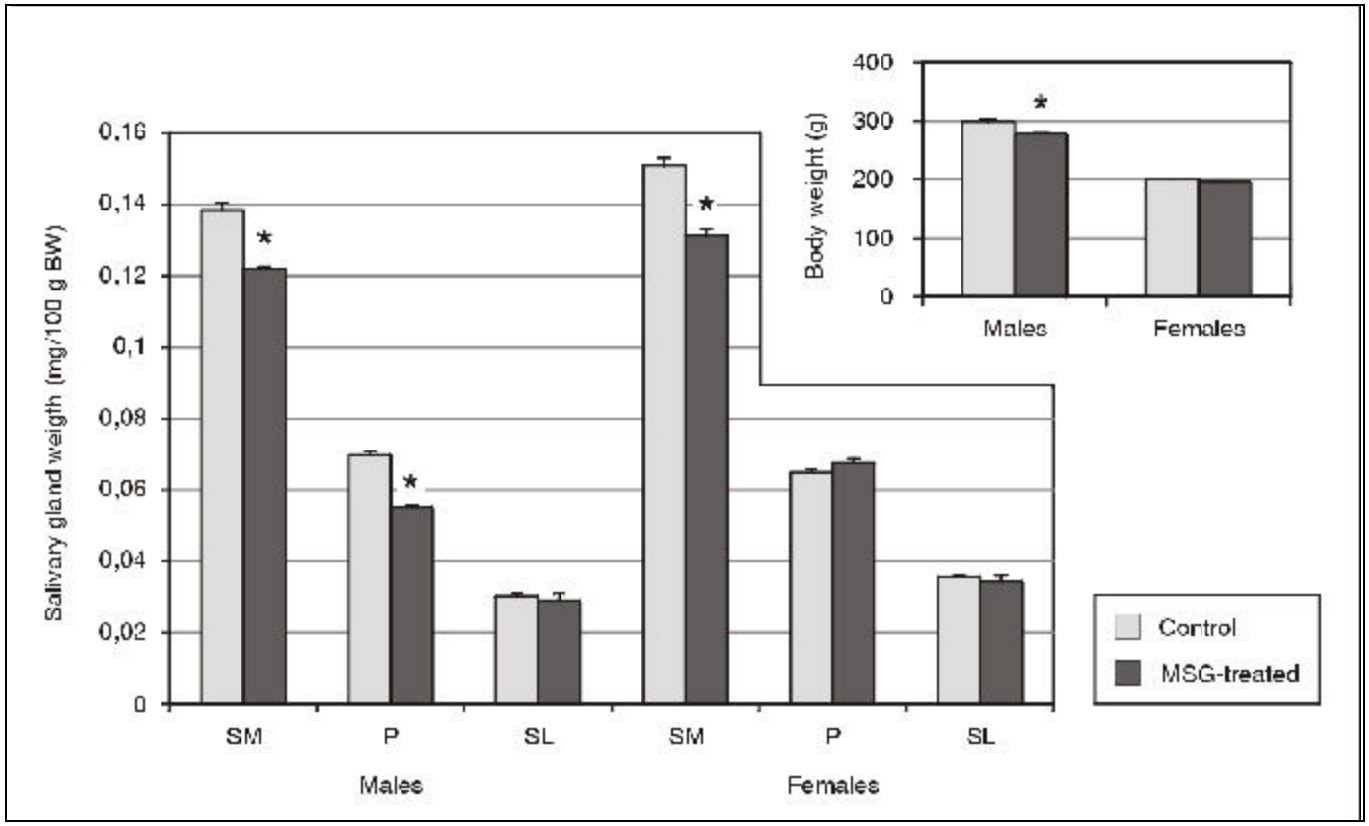
RESULTS

Ponderal data are seen in Graph 1. The insert shows that neonatal MSG treatment caused a significant impairment of body weight gain of male rats, but this effect was not so clearly evident in females. The weights of salivary glands relative to the body weight were differently influenced by MSG treatment, as it caused an about 12% reduction of both male and female submandibular gland weights, a 21% reduction of male (but not of female) parotid glands, and did not interfere with the weights of sublingual glands of either sex.

Salivary function studies are summarized in Table 1. MSG caused remarkable reductions in the pilocarpine-stimulated salivary flow, both in male (–27%) and in female rats (–40%). On the other hand, salivary contents of total protein and amylase activity rose strongly, both in males (mean rise 31%) and in females (mean rise 49%).

The effects of neonatal MSG administration on the eruption of rat incisors are seen in Table 2. The total and daily rates of male rat tooth eruption were faster than those of females, and this difference was maintained or even somewhat accentuated as an effect of MSG. On the other hand, eruption itself occurred significantly earlier in male or female rats treated with MSG than in their sex-matched controls.

Table 3 shows the microhardness analysis of rat teeth. Overall, microhardness was significantly higher for male than for female teeth, whatever the section or the anatomical region considered. Microhardness values obtained in tooth longitudinal sections were higher than those in transverse sections, and also higher for the crowns when compared to the roots. We observed that neonatal MSG treatment caused, in adulthood, an evenly lowered tooth microhardness, thus maintaining not only that sex dimorphism but also the differences previously seen regarding the tooth sections and anatomical regions.



GRAPH 1 - Body and salivary gland weights of controls or rats neonatally treated with monosodium glutamate (MSG). The relative weights of submandibular (SM), parotid (P) and sublingual glands (SL) are given as mean ± SEM of 10 animals for every group. The insert shows the body weights at sacrifice (mean ± standard error of the mean (SEM), n = 10). *$p < 0.01$ in relation to the corresponding control.

DISCUSSION

The early postnatal administration of monosodium glutamate (MSG) to rats is known to permanently damage neurons in the hypothalamic arcuate nucleus. The ensuing inappropriate brain-neuroendocrine-immune regulation was recently demonstrated to influence periodontal disease susceptibility and progression[5]. Being so, in this paper we examined the presumable consequences of MSG treatment on salivary functional characteristics and on dental mineralization, which could contribute to dental decay.

Our results showed that male rats neonatally treated with MSG not only had body weight gain reduction but also lower submandibular gland (SMG) weights (Graph 1). Especially in rodents, it is well established that SMG development and differentiation are under the influence of a multihormonal control[18], which plays a decisive role on its sexual dimorphism. Since 70% of total saliva are from the SMG[11], the hormonal imbalance triggered by MSG could explain the reduction of SMG weight (Graph 1) and the impaired salivary response to pilocarpine stimulation (Table 1). In addition, it is conceivable

TABLE 1 - Salivary functions of male and female rats. Results for controls and rats neonatally treated with monosodium glutamate (MSG). Results are mean ± standard error of the mean (SEM) of 16 observations throughout.

Groups		Salivary flow (µl/min per 100 g BW)	Total protein content (mg/ml)	Amylase activity (U/ml × 10^{-2})
Control	Males	17.60 ± 0.50^{a}	8.39± 0.28^{a}	14.70 ± 0.47^{a}
	Females	23.40 ± 0.60^{c}	8.13± 0.34^{a}	15.03 ± 0.38^{a}
MSG-treated	Males	12.80 ± 0.50^{b}	10.32 ± 0.41^{b}	20.39 ± 0.60^{b}
	Females	14.10 ± 0.60^{b}	12.18± 0.24^{c}	22.29 ± 0.26^{c}

Means followed by distinct superscript letters are significantly different from each other (ANOVA, $p < 0.05$).

TABLE 2 - Eruption of the incisors of male and female rats. Results for controls and rats neonatally treated with monosodium glutamate (MSG). Results are mean ± standard error of the mean (SEM).

Groups		Eruption rate (mm)		Eruption observed (% of the litter)	
		Total	Per day	At the 8th day	At the 9th day
Control	Males	11.859 ± 0.100^{a}	0.566 ±0.0050^{a}	25	75
	Females	11.488 ± 0.141^{b}	0.549 ±0.0068^{b}	44	56
MSG-treated	Males	11.416 ± 0.085^{b}	0.542 ±0.0045^{b}	53	47
	Females	10.841 ± 0.071^{c}	0.517 ±0.0036^{c}	67	33

Means followed by distinct superscript letters are significantly different from each other (ANOVA, $p < 0.05$). For the eruption rate and eruption day, data are from 16 observations for every group.

TABLE 3 - Microhardness of incisor enamel of male and female rats. Results for controls and rats neonatally treated with monosodium glutamate (MSG). Results are mean ± standard error of the mean (SEM) of determinations carried out in 56 teeth for every group.

Groups		Microhardness (kgf/mm^{2} × 10^{-3})			
		Longitudinal section	Transverse section	Crown	Root
Control	Males	271.84 ± 2.26^{a}	259.98 ±3.39^{d}	273.98 ± 2.07^{a}	257.84 ± 3.43^{d}
	Females	262.62 ± 2.02^{b}	253.85 ± 2.75^{e}	263.44 ± 2.07^{b}	253.03 ± 2.69^{e}
MSG-treated	Males	262.78 ± 2.12^{b}	253.79 ± 2.49^{e}	265.24 ± 1.76^{b}	251.33 ± 2.50^{e}
	Females	257.52 ± 1.12^{c}	245.08 ± 2.23^{f}	256.01 ± 1.99^{c}	246.59 ± 2.51^{f}

Means followed by distinct superscript letters are significantly different from each other (Kruskal-Wallis, $p < 0.05$).

that some reduction in the density and/or responsiveness of autonomic muscarinic receptors of the gland could contribute to the altered response.

As a part of the histomorphological and biochemical sexual dimorphism of the salivary glands in various mammal species[22], the number of muscarinic and β-adrenergic receptors in the SMG can be 25-51% higher in female than in male rats[6], and this could explain the higher salivary flow of our control females (Table 1). On the other hand, this sex difference disappeared in our MSG-treated animals, presumably as a function of the drug-induced alterations of circulating levels of gonadal steroids[17].

Concentrations of total proteins and of amylase activity in saliva of MSG-treated rats were significantly higher than those found in controls (Table 1). However, if these values are taken as a function of the salivary flow, such differences are blunted (for example, control males = 0.148 ± 0.004 and MSG-treated males = 0.132 ± 0.005 mg protein secreted/min per 100 g BW; $p > 0.1$). These results suggest that the net synthesis and/or release of salivary proteins were not affected by the drug, and only the fluid production was in fact reduced. On the other hand, the magnitude of protein increase in saliva was undistinguishable from that showed by amylase, thus suggesting that the specific protein which increased as an effect of MSG treatment was largely amylase.

Regarding enamel mineralization, as observed in other tissues and functions, a reasonable degree of sex dimorphism exists in the microhardness of the tooth enamel, that of males being higher than that of females. Though MSG treatment was able to significantly diminish both crown and root microhardness, those sex differences were not abolished (Table 3).

Among other factors possibly involved, the hormonal imbalances due to MSG treatment conceivably play a very important role. Pre- or postnatal hypothyroidism slows down dental development, leading to defects in the enamel which are observed later in life[14]. Interference with the growth hormone (GH) could also impair the formation and mineralization of the teeth, since GH receptors are present in dividing cells, preameloblasts, differentiating preodontoblasts, and in secreting ameloblasts and odontoblasts of 45-day rat incisors and molars[28]. In addition, GH deficiency reduces rRNA expression in preameloblasts and pre-odontoblasts[29] and the synthesis of two proteoglycans, decorin and biglycan, thus impairing the correct tooth formation and mineralization[30].

Overall, the hormonal alterations caused by neonatal treatment of rats with MSG most presumably interfered with several steps of tooth formation, including the intestinal absorption of Ca^{2+}, and the synthesis of proteins and proteoglycans which built up the extracellular matrix and play a further role in the process of dental mineralization. Also, the enzymes involved in amelogenesis could also be affected.

Inferior incisors eruption both in male and female rats occurred at the 9th postnatal day in controls and at the 8th day in MSG-treated animals (Table 2). The limiting factor to dental eruption is the resorption of alveolar bone, which forms a path for eruption and depends upon the formation and activity of osteoclast cells[27]. The process as a whole, mediated through the expression of osteoprotegerin, is regulated by a delicate hormonal balance between synergistic (parathyroid hormone, glucocorticoids)[13,27] and antagonistic influences (estrogens, GH)[2]. Although the exact mechanism by which neonatal MSG caused an accelerated rat incisors eruption is at present an unresolved question, this might be the result of multifactorial-dependent, increased osteoclastic activity.

The rates of incisor eruption were found to be slower in female than in male rats; neonatal MSG treatment caused a global reduction in these rates but did not interfere with the observed sex difference (Table 2). It is known that eruption regulation in rats depends upon the balanced activity of cementoblasts and of periodontal ligament cells, which in turn are stimulated by GH and inhibited by parathyroid hormone (PTH)[20]. Even though in this experimental model the serum levels of PTH have not been studied yet, and the participation of corticoid hormones cannot be discarded[16], our results can be partially explained by the reduction of GH circulating levels caused by MSG[8].

CONCLUSION

In conclusion, neonatal MSG treatment causes a series of oral disturbances in adulthood in rats, including salivary flow reduction and incisors mineralization and eruption disturbances. Our data support the view that the cohort of hormonal imbalances caused by hypothalamic malfunctioning can be accounted for by many (if not all) of the alterations reported herein, and may culminate in higher caries susceptibility.

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