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

Alterações neuroendócrinas interferem com a mineralização do 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 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 in males and females, and 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.

DESCRIPTIONS: Sodium glutamate; Hypothalamic diseases; Dental physiology; Saliva; Rats.

RESUMO: Administração neonatal de glutamato monosódico (MSG) em ratos provoca distúrbios neuroendocrinos que acarretam alterações em vários sistemas orgânicos. Nestes trabalhos, avaliaram-se as repercussões desse tratamento sobre os dentes e glândulas salivares. Nos ratos machos e fêmeas, 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; grupo controle recebeu solução salina no mesmo esquema de injetação. O momento da erupção de iniciais dos ratos foi determinado entre os 4º e 10º dias de vida. Os animais tratados com MSG mostraram redução do fluxo salivar (males: –27%; fêmeas: –40%) e do peso das glândulas submaxilares (cerca de –12%). O peso corporal reduziu-se apenas em 7% para os machos, e não houve alterações no peso das fêmeas. A saliva dos ratos tratados com MSG apresentou aumento nas concentrações de proteínas totais e de amilase. A erupção dos iniciais ocorreu mais cedo em ratos dos grupos tratados com MSG, mas a taxa de erupção foi significativamente reduzida. A microdureza dos incisivos foi menor nos grupos tratados com MSG, comparados aos grupos controle. Nossos resultados mostram que o tratamento neonatal com MSG causa quadros bem definidos de alterações bucal e dentária em adultos de ratos, incluindo redução do fluxo salivar, que coexistiu com sinergia da síntese de proteínas e distúrbios de mineralização e erupção dentária. Esses dados apoiam a ideia de que certos núcleos hipotalâmicos sensíveis ao MSG exercem um efeito modulatório sobre os fatores que determinam a susceptibilidade à cárie.

DESCRIPTORES: Glutamato de sódio; Doenças hipotalâmicas; Fisiologia dentária; Saiva; Ratios.

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INTRODUCTION

Monosodium glutamate (MSG) is a widely used foodstuff flavouring compound, especially in oriental food. In rats and mice, the newborn ad ministration of MSG leads to extensive damage of certain hypothalamic nuclei, thus causing severe neuroendocrine disturbances in adulthood. The abnor malities are first observed by Olney 19 (1969), included growth impairment, marked obesity (which can develop with out hyperphagia) and reduction of organ weights, among others 1,3. Marked repression was observed in the development of tooth enamel and of cartilaginous elements and chondrocytes 6, reduced ratio of mineral to position, and slower bone maturation 6.

The formation, eruption and growth of teeth are processes under the concerted and timely influences of several hormones, such as growth hormone (GH), and thyroid 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 8, and that the salivary secretion in rats is under strong hypothalamic influences 41.

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

MATERIAL AND METHODS

Animals and treatments

Neonatal 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 main tained on routine laboratory care conditions (12 h dark-light cycle, lights on 08:00 a.m. to 20:00 h). The experiment was conducted under local anesthesia with sodium pentobarbital (Hypnodol®, Cristalia, 40 mg/kg BW, IP). Salivary secretion was stimulated by pilocarpine nitrate (Sigma, 5 mg/kg BW, IP). Whole saliva was then collected 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 19 (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 37°C.

Microhardness

The upper and lower incisors were removed and dissected free from any foreign adherent tissue. The right teeth were embedded longitudinally placed into acrylic resin; the left teeth were dissected free from any adherent tissue. Two in denations were made, one on the crown and the other on the root, at the median portion of enamel thickness. A microhardness tester Shimadzu HVM-2000® coupled to a Knoop-like penetrator set was used with a 50 g load. Microhardness results are given in terms of kgf/mm² x 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 neo na tal MSG treat ment caused a sig- nificant impairment of body weight gain of male rats, but this effect was not so clearly ev i dent in fe -males. The weights of salivary glands relative to the body weight were differently influenced by MSG treat ment, as it caused an about 12% re duc- tion of both male and fe male submandibular gland weights, a 21% reduction of male (but not of fe -male) parotid glands, and did not in ter fere with the weights of sublingual glands of ei ther sex.

Salivary function studies are summarized in Table 1. MSG caused re mark able re duc tions in the pilocarpine-stim u lated sali vary flow, both in male (~27%) and in female rats (~40%). On the other hand, salivary con tents of total protein and amylase ac tiv ity rose strongly, both in males (mean rise 31%) and in females (mean rise 49%).

The effects of neo na tal MSG ad min is tra tion on the erup tion of rat in ci sors are seen in Table 2. The total and daily rates of male rat tooth eruption were faster than those of females, and this differ -ence was maintained or even somewhat accen tu ated as an ef fect of MSG. On the other hand, erup tion it self oc curred signifi cantly earlier in male or female rats treated with MSG than in their sex-matched con tro ls.

Table 3 shows the microhardness analysis of rat teeth. Overall, micro hard ness was signifi cantly higher for male than for fe male teeth, what ever the section or the anatomic al region considered. Micro hard ness values ob tained in tooth lon gi tu di -nal sections were higher than those in transverse sections, and also higher for the crowns when compared to the roots. We ob served that neo na tal MSG treatment caused, in adulthood, an evenly lowered tooth microhardness, thus maintaining not only that sex dimorphism but also the differ -ences pre vi ously seen re garding the tooth sections and anatomic al regions.

**GRAPH 1** - Body and sali vary gland we ights of con trols or rats ne o na tally tre a ted with mo no so di um glu ta ma te (MSG). The re la ti ve we ights of sub man di bu lar (SM), pa ro tid (P) and su blin gual glands (SL) are given as mean ± SEM of 10 ani -mals for every group. The in sert shows the body we ights at sa cri fi ce (mean ± stan dard er ror of the mean (SEM), n = 10). *p < 0.01 in re la ti on to the cor re spon ding con trol.
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. Being so, in this paper we examined the presumed 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, which plays a decisive role on its sexual dimorphism. Since 70% of total saliva are from the SMG, 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) |
| Males                           | 17.60 ± 0.50ᵃ  | 8.39 ± 0.28ᵃ    | 14.70 ± 0.47ᵃ  |
| Females                         | 23.40 ± 0.60ᵃ  | 8.13 ± 0.34ᵃ    | 15.03 ± 0.38ᵃ  |
| MSG-treated                     | 12.80 ± 0.50ᵇ  | 10.32 ± 0.4ᵃᵇ   | 20.39 ± 0.60ᵇ   |
| Females                         | 14.10 ± 0.60ᵇ  | 12.18 ± 0.2ᵃᵇ    | 22.29 ± 0.2ᵇ    |

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 |
| Males                           | 11.859 ± 0.10⁰ | 0.566 ± 0.005⁰ | 25             | 75             |
| Females                         | 11.488 ± 0.14¹ | 0.549 ± 0.006⁸  | 44             | 56             |
| MSG-treated                     | 11.416 ± 0.08⁵  | 0.542 ± 0.004⁵  | 53             | 47             |
| Females                         | 10.841 ± 0.07¹  | 0.517 ± 0.003⁶  | 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/mm² × 10⁻³) |
|                                | Longitudinal section | Transverse section | Crown | Root |
| Males                           | 271.84 ± 2.26ᵃ | 259.98 ± 3.39ᵃ | 273.98 ± 2.07ᵃ | 257.84 ± 3.4³ᵃ |
| Females                         | 262.62 ± 2.02ᵃ | 253.85 ± 2.75  | 263.44 ± 2.07ᵃ | 253.03 ± 2.6⁹ᵃ |
| MSG-treated                     | 262.78 ± 2.12ᵇ | 253.79 ± 2.4⁹  | 265.24 ± 1.7⁶ᵇ | 251.33 ± 2.5⁰ᵇ |
| Females                         | 257.52 ± 1.12ᶜ | 245.08 ± 2.2³ᶜ | 256.01 ± 1.9⁹ᶜ | 246.59 ± 2.5¹ᶜ |

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 response sensitivity 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 mammalian species, the number of muscarinic and β-adrenergic receptors in the SMG can be 25-51% higher in males than in male rats, and this could explain the higher salivary flow of our control females (Table 1). On the other hand, the sex difference disappeared in our MSG-treated animals (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 a result of MSG treatment was largely amylase.

Regarding enamel mineralization, as observed in other tissues and functions, a reasonable degree of sexual 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 in salivary dysfunction, the hormonal imbalance due to MSG treatment can certainly 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. 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 at 45-day rat in incisors and molars. In addition, GH deficiency reduces RNA expression in preameloblasts and pre-odontoblasts and the synthesis of two proteoglycans, decorin and biglycan, thus impairing the correct tooth formation and mineralization.

Over all, 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, 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.

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CONCLUSION

In conclusion, neonatal MSG treatment causes a series of oral disturbances in adult hood in rats, including salivary flow and eruption and salivary gland function. 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.

ACKNOWLEDGEMENT

Otoniel An tonio Macedo dos Santos has been recipient of a FAPESP scholarship (Grant no. 00/11959-7).
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