Types I and III Parotid Collagen Variations and Serum Biochemical Parameters in Obese Rats Exposed to Monosodium Glutamate

Variaciones de Colágeno Parotídeo Tipos I y III y Parámetros Bioquímicos Séricos en Ratas Obesas Expuestas a Glutamato Monosódico

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SUMMARY: The objective of this study was to describe the effects of monosodium glutamate on the collagen of the parotid gland in an obesity model. 18 newborn male Sprague Dawley rats were used (first control group; second group of MSG1: 4 mg/g of monosodium glutamate weight, 5 doses, and third group of MSG2: 4 mg/g of monosodium glutamate, 5 doses, maintained for 8 and 16 weeks respectively). The content and type of collagen were analyzed, in addition to the levels of cholesterol, glucose, triglycerides and uric acid. Monosodium glutamate produced an increase in the obesity rates of the MSG2 group, in addition to an increase in blood cholesterol, glucose and uric acid levels compared to the control group. Type III collagen in the MSG2 group showed a statistically significant increase. Monosodium glutamate induced obesity, in addition to an increase in type III collagen fibers.

KEY WORDS: Collagen; Monosodium glutamate; Obesity, Salivary glands.

INTRODUCTION

Obesity is a chronic metabolic disorder characterized by the accumulation of excessive adipose tissue. It is currently considered a global epidemic and one of the main public health issues affecting both developed and developing countries (Eckel et al., 2002; Wong et al., 2004).

Monosodium glutamate (MSG) is the sodium salt of glutamate, an amino acid found naturally in many foods (Jinap & Hajeb, 2010; Behrens et al., 2011). MSG has long been used as a flavor enhancer in the food industry and is currently a widely consumed food additive (Shi et al., 2010), although its use is controversial due to its obesogenic potential (Lorden & Caudle, 1986; Von Diemen et al., 2006).

Several authors have reported a relationship between obesity and collagen synthesis, where modifications have been observed in both the amount and type of collagen fibers in obese individuals (Carrol & Tyagi, 2005; Toblli et al., 2005; Biondo-Simões et al., 2010). In addition to the obesogenic action of MSG, a possible relation that it has in collagen synthesis has also been reported (Tapiero et al., 2002). On the other hand, MSG has been related to morphofunctional changes in several organs (Henry-Unaeze, 2017), including the salivary glands (Hordienko et al., 2014). Our aim was to evaluate the influence of exposure time to MSG-induced obesity on the changes in the amount of types I and III collagen in the parotid gland of Sprague Dawley rats.

MATERIAL AND METHOD

Animals and Experimental Protocol. 18 newborn male Sprague Dawley rats were used (2 days old). At the beginning of the experimental period (day 1), the rats were divided into three groups: Control group (n=6): Group not exposed to MSG, exposed to saline solution (sodium chloride 0.9% (p/v) in distilled water) 8 ml/g administered subcutaneously. MSG1 group (n=6): exposed to subcutaneous MSG, 4 mg/g weight two doses, 2nd and 4th day and 2 mg/g weight, 6th,
8th, 10th day, kept for 8 weeks. MSG2 group (n=6): exposed to subcutaneous MSG, 4 mg/g weight two doses, 2nd and 4th day and 2 mg/g weight, 6th, 8th, 10th day (Baculikova et al., 2008).

The rats were housed in individual cages for 16 weeks in the vivarium of the Center for Excellence in Morphological and Surgical Studies (CEMyQ) at the Universidad de La Frontera, Temuco, Chile, kept at 22º C ± 2 ºC and 50 % – 70 % humidity and a 12 h light/dark cycle (8:00 a.m. – 8:00 p.m. / 8:00 p.m. – 8:00 a.m.). A standard laboratory diet (AIN-93M) and water ad libitum were administered. The study was approved by the Scientific Ethics Committee of the Universidad de La Frontera, Temuco, Chile, Nº051/2017.

Metabolic and biochemical profiles. The animals’ body weight was measured twice a week throughout the experiment on an analytical balance (Radwag, WTB2000, Poland). Obesity was evaluated using the Lee index, calculated by the cube root of body weight (g) divided between the snout-anus length (SAL) (cm). A value equal to or less than 0.3 was considered normal; greater than 0.3 the rats were classified as obese according to de Campos et al. (2007).

After euthanasia, the blood was extracted by cardiac puncture. For the biochemical analysis, the plasma was separated by centrifugation (3500 rpm) (Hettich, Mikro 200R, Tutlingen, Germany) and stored at -80 ºC until analysis. The physiological concentrations of total glucose (GLU-T), uric acid (URI), total cholesterol (COL-T) and high-density lipoprotein cholesterol (HDL-C) were analyzed with enzymatic kits and the triglycerides (TG) by colorimetric test in an automatic biochemistry analyzer (CM 250, Wiener Lab. Group).

Quantification of types I and III collagen of the parotid gland. After the parotid glands were extracted, they were fixed in buffered formalin 4 % (1.27 mol/l of formaldehyde in phosphate buffer 0.1 M pH 7.2) for 48 hours. Then they were dehydrated and included in Paraplast Plus (Sigma-Aldrich Co., St. Louis, MO, USA). Once the blocks were obtained, sections 5 mm thick were obtained (Leica® RM2255) and stained with Sirius Red F3BA 0.1 % p/v (Sigma-Aldrich Co., St. Louis, MO, USA) in an aqueous solution saturated with picric acid (Merck, Darmstadt, Germany).

For the evaluation of the collagen, one field per section was observed, in total 25 fields per group. The lower filter (Leica® Microsystems, Wetzlar, Germany) was placed on the iris diaphragm ring of the microscope field, while the upper filter was made of a combination of quarter-wave plates (Leica Microsystems, Wetzlar, Germany) placed underneath a linear polarizer, aligned with the transmission axis 45º from the fast axis of the wave plate. These two filters were aligned to maximize the darkness at the bottom (the filters were crossed). The histological images were obtained with a microscope (Leica® DM750, Wetzlar, Germany) and a digital camera (Leica® ICC50 HD, Wetzlar, Germany), and projected onto a LCD (View Sonic®, China). The total area (mm²) of types I and III collagen fibers (Junqueira et al., 1979) was analyzed with the Image-Pro Premier 9.1 software (Media Cybernetics, Warrendale, PA, USA).

Statistical analysis. The data were analyzed in the programs Microsoft® Excel Mac (v. 2011, CA, USA) and GraphPad Prism® (v. 5.0 San Diego, USA). For the distribution of the data the Kolmogorov-Smirnov test (analysis of data normality) or Shapiro-Wilks test was used. Depending on the normality of the data distribution, a one-way ANOVA or Kruskal-Wallis test was used, followed by Tukey’s post hoc test. A level of p<0.05 was considered significant.

RESULTS

The rats corresponding to the MSG1 and MSG2 groups increased their body weight compared to the control group. From the third month the MSG2 group presented a Lee Index of 0.4, results that were statistically significant (p= <0.0001). These changes in weight indicate obesity (Fig. 1).

With respect to the biochemical parameters, only cholesterol levels (COL-T) presented statistically significant variations (p=0.001). Glucose (GLU) and uric acid (URI) showed a statistically insignificant increase in both the MSG1 and MSG2 groups (Table I).

Type I collagen decreased slightly in both experimental groups compared to the control group; however, these differences were not statistically significant. Type III collagen increased in both the MSG1 and MSG2 groups (12066±4435 mm² and 8505±3782 mm², respectively) compared to the control group (p= <0.0001) (Fig. 2).

DISCUSSION

It has been described that the administration of MSG in newborn rats causes the destruction of the ventromedial and arcuate hypothalamic nuclei, subsequently developing into obesity due to the lack of control between energy absorption and output (Von Diemen et al.), affected mainly at the levels of leptin and insulin, increasing the appetite and the amount of food consumption (Matyskova et al., 2007).
The neonatal injection of MSG in rats caused an increase in body weight and obesity. According to what has been described in the literature, for MSG to cause obesity, it can be administered in rats during the neonatal period orally, intraperitoneally or subcutaneously (Bunyan et al., 1976; Shivshankar & Devi, 2005), in 4-10 doses, that vary from 2-4mg/g of weight in rats during the neonatal period, which was done and observed in this study. However, only the MSG2 group presented obesity, which was induced for a period of 16 weeks with a dose of 4 mg/g, similar to what has been reported in the literature (Miranda et al., 2017), demonstrating that the period of exposure to the drug is crucial in the induction (De Campos et al.) because an induction less than 16 weeks does not produce changes. Although the link between MSG and the increased risk of overweight has been widely reported, the data obtained from the studies on humans or on test animals is controversial and has not been fully verified (Ebert, 2009). Recent studies in healthy Chinese subjects have described MSG consumption as being positively related to a greater risk of overweight (He et al., 2008; 2011), similar to what was reported by Collison et al. (2010). By contrast, Shi et al. (2010) reported that MSG ingestion is not associated with a greater prevalence of obesity or with a clinically significant weight increase in Chinese adults. On the other hand, Insawang et al. (2012) indicated that the consumption of a larger amount of MSG is associated with the risk of suffering metabolic syndrome and overweight, regardless of other greater factors.

He et al. (2008) showed that MSG ingestion in humans may be associated with the increased risk of

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### Table I. Biochemical analysis of male Sprague Dawley rats subjected to MSG.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>MSG1</th>
<th>MSG2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL-T (mg/dL)</td>
<td>31.4 ± 20.0</td>
<td>81 ± 15.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71 ± 12.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>GLU (mg/dL)</td>
<td>138.2 ± 46.8</td>
<td>353.4 ± 115.4</td>
<td>313.2 ± 54.5</td>
<td>0.685</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>24.2 ± 5.5</td>
<td>31.6 ± 6.8</td>
<td>21.6 ± 8.8</td>
<td>0.116</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>78.2 ± 72.2</td>
<td>131.2 ± 35.9</td>
<td>100.2 ± 73.5</td>
<td>0.435</td>
</tr>
<tr>
<td>URI (mg/dL)</td>
<td>0.66 ± 0.38</td>
<td>0.54 ± 0.31</td>
<td>0.30 ± 0.29</td>
<td>0.257</td>
</tr>
</tbody>
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<sup>a</sup> Sig. Dif. Control/MSG1.  <sup>b</sup> Sig. Dif. Control/MSG2.
overweight, regardless of physical activity and total energy consumption (Beregova et al., 2014). On the other hand, it was observed that MSG-induced obese rats develop insulin resistance (Hirata et al., 1997) and MSG induces hyperinsulinemia in 3-month-old rats (Marmo et al., 1994).

Ahluwalia & Malik (1989) recounted a statistically significant increase in GLU-T and TG values in rats subjected to MSG, while Oida et al. (1984) only showed statistically significant changes in triglycerides. Our results revealed an increase in the plasma levels of URI, GLU-T, COLT-T and TG in mice in the MSG2 group compared to the mice in the control group, parameters associated with insulin resistance, where a moderate elevation in blood glucose concentration and high cholesterol and triglyceride concentrations are common (Muio & Newgard, 2008; Islam & Loots, 2009). The variability of results obtained in the biochemical parameters and the difficulty in comparing them to other studies may be related to the type of sample used, the variability of the models, nature, as well as the way and time obesity was induced, among others factors (Fuentes et al., 2008; Navarrete et al., 2015).

Regarding the action of obesity in terms of the amount of collagen fibers, changes at myocardial level have been reported, there being a remodeling of the extracellular matrix (ECM) (Galinier et al., 2005). Seo et al. (2015) suggest the existence of structural changes induced by obesity at the level of the ECM, particularly with respect to the amount and quality of interstitial collagen, whose fibers increase in thickness and aligned length. Also, there is evidence of changes in the collagen associated with unilocular adipose tissue, noting a remodeling of the ECM in db/db mice, in which overexpression of the mRNA level of types I, IV and VI collagen is observed (Khan et al., 2009). Moreover, an increase in types I, III and VI collagen has been reported in adipose tissue in obese individuals (Divoux et al., 2010).

Obesity induces modifications in the connective tissue of test animals, in obese rabbits induced by a high fat diet (Carroll & Tyagi) or in Zucker rats with a reported increase in types I and III collagen fibers in the myocardium (Toblli et al.). An increase in myocardial collagen has been reported in an obesity model induced by high fat consumption in Wistar rats (Leopoldo et al., 2010) and Wistar-Kyoto rats (Oliveira et al., 2009).

On the other hand, an increase in the amount of fibrous tissue has been reported in the stroma of the diaphragm of obese mice subjected to a diet rich in fat (Buras et al., 2019). Finally, in other studies no changes were found in the total collagen fraction in obese rats subjected to a high fat diet for 12 weeks (Carroll et al., 2006).

In the present study, Sprague Dawley rats, in which obesity was induced in periods of 8 and 16 weeks, underwent changes in the stroma of the parotid gland with respect to the amount of types I and III collagen. Type III collagen increased in both the MSG1 and MSG2 groups after the administration of MSG; previous studies have indicated increases in type III collagen related to abdominal and general obesity when the concentrations of serum aminoterminal propeptide of type III collagen (S-PIIINP) were measured. The latter decreases during weight loss in obese subjects (Rasmussen et al., 1995), which indicates that type III collagen is most affected during obesity, as was found in the stroma of the parotid gland of our experimental model. Type III collagen is composed of immature fibrils, likely due to fibrogenesis or fibrosis of the gland (Karsdal, 2016), which could explain the functional changes in the salivary glands during obesity (Roa & del Sol, 2018).

Although it has been reported that there are modifications at glandular level during obesity (Renzi et al., 1989 Bozzato et al., 2008; Moubarak et al., 2015), these are focused mainly at epithelial level, without noting the changes of the glandular connective tissue, as observed in the present study. The possible causes of the relationship between the amount and change of collagen types and obesity could be alterations in the adhesion of fibroblasts to the collagen matrix and the individual connections between the collagen chains (Schiro et al., 1991; Woodley et al., 1991); hence the increase in the endocinral action of the adipose tissue, where the leptin is, would also aid in the synthesis of collagen at odontoblastic level (Goldeladze et al., 2002).

CONCLUSION

Exposure to MSG determines the type and amount of collagen in the stroma of the parotid gland, which may possibly favor fibrosis of the gland, explaining in part the functional changes in obese patients.

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RESUMEN: El objetivo de este estudio fue describir los efectos del glutamato monosódico sobre el colágeno de la glándula parótida en un modelo de obesidad. Se utilizaron 18 ratas Sprague Dawley machos recién nacidas (primer grupo control; segundo grupo MSG1: 4 mg/g de peso de glutamato monosódico, 5 dosis, y tercer grupo MSG2: 4 mg/g de glutamato monosódico, 5 dosis, manteniéndolas durante 8 y 16 semanas respectivamente). Se analizó el contenido y el tipo de colágeno, además de los niveles de colesterol, glucosa, triglicéridos y ácido úrico. El glutamato monosódico produjo un aumento en las tasas de obesidad del grupo MSG2, además de un aumento en los niveles de colesterol en sangre, glucosa y ácido úrico en comparación con el grupo control. El colágeno tipo III en el grupo MSG2 mostró un aumento estadísticamente significativo. La obesidad inducida por glutamato monosódico, además de un aumento en las fibras de colágeno tipo III.

PALABRAS CLAVE: Colágeno; Glutamato monosódico; Obesidad; Glándulas salivales.

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