Glycogenolysis response to adrenergic agonists in the liver of rats treated with monosodium glutamate (MSG)

Rosana Torrezan[1*], Sabrina Grassiolli[2], Nilton de Almeida Brito[1] e Paulo Cezar de Freitas Mathias[3]

*[1]Departamento de Ciências Morfofisiológicas, Centro de Ciências Biológicas, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brasil. [2]Programa de Pós-graduação em Ciências Biológicas, Centro de Ciências Biológicas, Universidade Estadual de Maringá, Maringá, Paraná, Brasil. [3]Departamento de Biologia Celular e Genética, Centro de Ciências Biológicas, Universidade Estadual de Maringá, Maringá, Paraná, Brasil. *Autor para correspondência. e-mail: rtorrezan@uem.br*

ABSTRACT. Administration of MSG to neonate rats causes lesions in the arcuate nucleus (AN), followed by a syndrome of neuroendocrine dysfunction characterized by obesity and decreased sympathetic activity. The aim of the present investigation was to examine the responses of hepatic glycogenolysis to α- and β-adrenergic agonists in rats' treatment with MSG. Male Wistar rats received subcutaneous injections of MSG (4 mg g^{-1} body weight) or hyperosmotic saline (controls) during five days after birth. Ninety days after treatment, the livers of the MSG or controls rats were perfused *in situ* with epinephryne and α- and β-adrenergic agonists. Epinephryne, Isoproterenol and phenylephrine increased glycogenolysis in the MSG-treated rats, compared to the controls (50 ± 2.8 Vs 17 ± 0.89 μmol min^{-1} g^{-1} of liver, $p<0.0001$; 64 ± 0.15 Vs 37 ± 0.39, $p<0.0001$; 35 ± 2.48 Vs 27 ± 0.98, $p<0.05$, respectively). Results indicated that the lesion in the AN increased glycogen catabolism to adrenergic agonists, possibly, due to the reduced activity of the sympathetic-adrenal axis.

Key words: adrenergic agonists, monosodium glutamate, arcuate nucleus, obesity, glycogenolysis.

RESUMO. Resposta glicogenolítica à agonistas adrenérgicos no fígado de ratos tratados com glutamato monossódico (MSG). Administração de glutamato monossódico (MSG) em ratos neonatos causa lesão no núcleo arqueado (NA), seguido por uma síndrome de disfunção neuroendócrina caracterizada por obesidade e reduzida atividade simpática. O objetivo da presente investigação foi examinar a resposta da glicogenólise hepática a agonistas adrenérgico em ratos tratados com MSG. Ratos Wistar machos receberam injeções subcutâneas de MSG (4 mg g^{-1} de peso corporal) ou salina equimolar (controles) durante cinco dias após o nascimento. Noventa dias após o tratamento, os fígados de ratos-MSG ou controles foram perfundidos *in situ* com epinefrina e agonistas α- e β-adrenérgico. Isoproterenol, fenilefrina e epinefrina aumentaram a glicogenólise em ratos-MSG, comparados aos controles (50 ± 2,8 Vs 17 ± 0,89 μmol min^{-1} g^{-1} de fígado, $p<0,0001$; 64 ± 0,15 Vs 37 ± 0,39, $p<0,0001$; 35 ± 2,48 Vs 27 ± 0,98, $p<0,05$, respectivamente). Concluiu-se que a lesão do NA aumentou o catabolismo do glicogênio aos agonistas adrenérgicos, possivelmente devido à reduzida atividade do eixo simpático - medula adrenal.

Palavras-chave: agonistas adrenérgicos, glutamato monossódico, núcleo arqueado, obesidade, glicogenólise.

Introduction

Glutamate has been suggested as the major excitatory amino acid neurotransmitter in a number of neural loci, including the hippocampus, cortex, cerebellum, and hypothalamus (Van Del Pol, 1991). In rodents, approximately 80-90% of the arcuate nucleus neurons are destroyed by neonatal administration of MSG (Olney, 1969; Lem Key-Johnston and Reynolds, 1974; Nemeroff *et al*., 1982). These anatomical changes are associated with metabolic and endocrine disturbances in the adult, which lead to growth stunting, sexual dysfunction and obesity (Miskouwak and Partyka, 1993; Perelló *et al*., 2003; Martins *et al*., 2004). Lesions in the AN are also related to disturbances in the activity of the Autonomic Nervous System, followed by increases in the parasympathetic activity (Seress, 1982; Balbo *et al*., 2000) and decreases in the sympathetic activity (Van Del Pol, 1991; Yoshida *et al*., 1998). In this way, monosodium glutamate (MSG), an experimental neurotoxin, has been extensively used to investigate the role of the AN in metabolic regulation.

The mechanisms that mediate the brain's glucoregulation of glycogenolysis in the liver may include not only the autonomic nervous pathways for

secreting such glucoregulatory hormones such as epinephrine, glucagon and insulin (Iguchi *et al*., 1986), but also direct innervation of the liver (Lautl, 1983).

Cholinergic stimulation of any nuclei of the medial hypothalamus causes hyperglycemia because of the action of the catecholamines secreted by the adrenal medullas on the hepatocytes, stimulating glyconeogenesis (Migliorini *et al*., 1989; Brito *et al*., 1993).

The electrical stimulation of sympathetic nerves or the infusion of norepinephrine activates the glucose output of perfused livers, an effect mediated via both α and β-adrenergic receptors on the plasma membranes of the liver cells (Jungerman *et al*., 1987; Lopes *et al*., 1998). Although much is known about the importance of the sympathetic nervous system for the regulation of peripheral metabolism, the influence of the AN on hepatic glycogenolysis by catecholamines remains to be investigated.

In the present study, the hepatic responses to adrenergic agonists in AN-lesioned rats using perfused livers was investigated in order to understand the role of the AN in the dominance of either α or β adrenergic responses in the liver.

Material and methods

Neonatal male Wistar rats received subcutaneous injection of MSG (4 mg g^{-1} body weight) or hyperosmotic saline (controls) daily for five days after birth. The animals were maintained under controlled conditions of light (12L:12D) and temperature (22 ± 2°C) and fed with commercial diet and water *ad libitum*. Investigations of hepatic perfusion *in situ* were performed at 9:00 a.m. 90 days after the treatment with MSG or saline. The MSG-treated rats and the controls were anesthetized with sodium pentobarbital (49 mg kg^{-1} body weight, i.p). After laparotomy, the portal vein and the inferior vena cava were cannulated. The cannule was introduced in the portal vein under a perfusion flux of about 10 mL min^{-1}. Soon after, the abdominal vessels below the liver were sectioned so as to free the organ of any blood. A second cannule was introduced in the inferior vena cava and the flux was increased to values large enough to allow adequate oxygenation (4 mL min^{-1} g^{-1} of liver). After cannulation, the liver was perfused with Krebs/Henseleit-bicarbonate (KH). This fluid was impulsioned by a perfusion pump to the oxygenator. Here, there occurs simultaneously oxygenation and warming to 37°C. Thus, perfusion was carried out in an open (nonrecirculating) system, the direction of flux being from the periportal to the perivenous hepatocytes. Metabolic rates were measured using the following experimental protocol. After a preperfusion period (10min), epinephryne (0,1 μM), phenylephrine (2 μM) or isoproterenol (20 μM), dissolved in the perfusion fluid, were infused during the 10-30min interval. Samples of the effluent perfusion fluid were collected at 2min intervals and analyzed for D-glucose. All metabolic rates were referred to the wet weight of the liver.

At the end of perfusions periepididymal fat pads were removed from rats. After washing with saline solution the tissues were weighted. All data are presented as mean ±SEM.

When not indicated, all reagents were obtained from Sigma Chemical Company, St. Louis, MO, USA. The Animal Ethical Committee from the State University of Maringá, Paraná State, approved the experimental protocols.

The effects of epinephryne, phenylephrine and isoproterenol on glycogen catabolism were evaluated by means of the area under curve (AUC), expressed as micromoles per gram, employing a computer program (GraphPad Prism). In addition, statistical analyses were performed by Student's t test.

Results

MSG treated rats showed less mass body (268 ± 5.2 g) than untreated animals (367 ± 4.5 g) ($p<0.05$). AN neurons produce and release the GH releasing factor. MSG-rats were shorter (19.5 ± 0.13 cm) than controls (23 ± 0.14 cm) ($p<0.05$). MSG-rats also accumulated more fat in periepididymal pads, 2.12 ± 0.08 g compared to untreated rats, 1.07 ± 0.04 g ($p<0.05$). The obesity induced by the MSG injections can also be shown by the increase in the Lee index, 330 ± 2 compared to control animals, 311 ± 1 ($p<0.05$). Both biometric and the mass of fat in periepididymal pads presented in rats treated with MSG suggested that there was lesion on AN.

The infusion of epinephrine 0.1 μM, 2 μM phenylephrine or 20 μM isoproterenol, both concentrations employed as a maximal dose, promoted a rapid and transient increase in glycogenolysis in both groups (Figures 1, 2 and 3). However, this increase was significantly greater and lasted longer in the MSG-treated rats, compared to the controls ($p<0.0001$). Peak values were attained less than five min after the beginning of the infusion of either epinephrine, phenylephrine or isoproterenol. The action of the α-agonist (Figure 2) was more prolonged than that of the β-agonist (Figure 3).

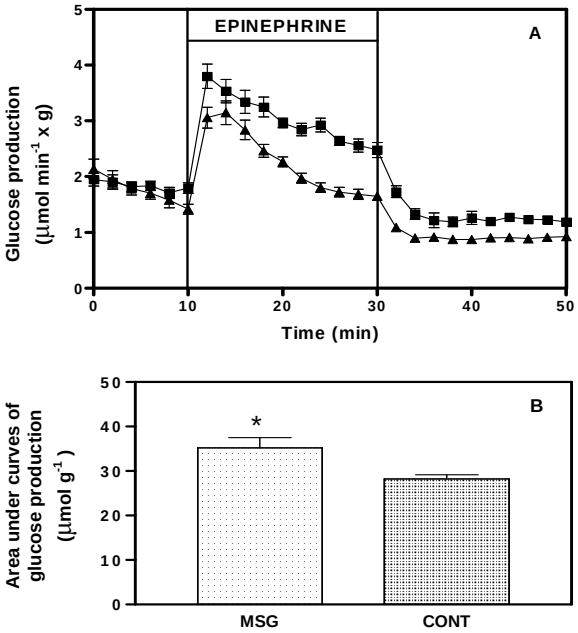

Figure 1. Effect of ephinephrine (0.1 µM) on hepatic glucose production in fed rats, which received monosodium glutamate (MSG group, ■) or hyperosmotic saline (CONT group, ▲) daily for five days after birth. Liver experiments were performed ninety days after the treatment. **(A)** Each point of the curve represents the mean ± SEM of 8 animals for both groups. **(B)** The valves represent the mean area under curve ± SEM of 5 animals for both groups. ⋆p<0.05.

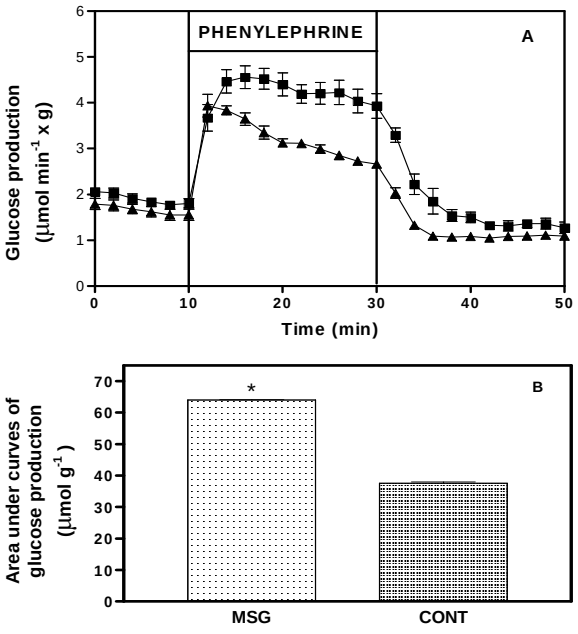

Figure 2. Effect of phenylefrine (2 µM) on hepatic glucose production in fed rats, which received monosodium glutamate (MSG group, ■) or hyperosmotic saline (CONT group, ▲) daily for five days after birth. Liver experiments were performed ninety days after the treatment. **(A)** Each point of the curve represents the mean ± SEM of 8 animals for both groups. **(B)** The valves represent the mean area under curve ± SEM of 8 animals for both groups. ⋆p<0.0001.

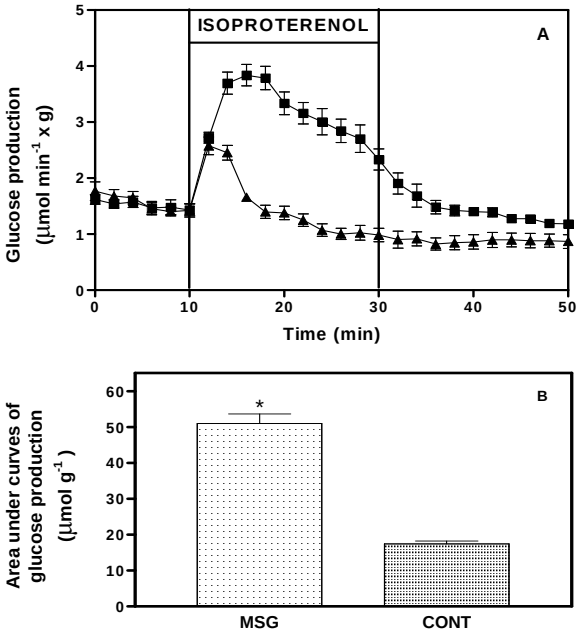

Figure 3. Effect of isoproterenol (20 µM) on hepatic glucose production in fed rats, which received monosodium glutamate (MSG group, ■) or hyperosmotic saline (CONT group, ▲) daily for five days after birth. Liver experiments were performed ninety days after the treatment. **(A)** Each point of the curve represents the mean ± SEM of 7 animals for both groups. **(B)** The valves represent the mean area under curve ± SEM of 7 animals for both groups. ⋆p<0.0001.

The degree of activation of the glycogenolysis by epinephrine (Figure 1A), phenylephrine (Figure 2A) and isoproterenol (Figure 3A) was clearly influenced by the treatment with MSG. The ablation of the AN by MSG caused a marked and significant increase in the liver responses to the α and β-adrenergic agonists, revealed by the greater glycogen catabolism in the MSG-treated rats during the infusion of these agonists.

Discussion

A wide variety of factors regulate hepatic sensitivity and/or responsiveness to adrenergic agonists, among these, their exposure to catecholamines (Bergmeyer and Bernt, 1974; Tokin and Matsubara 1987; Bazotte *et al*., 1989).

With the use of the perfusion system of rat livers, it was possible to follow the events occurring during the infusion of the adrenergic agonists.

After establishing the steady state of glucose production, infusion of phenylephrine and isoproterenol caused an increase in glycogenolysis. The changes in glucose production reflects the rate of glycogenolysis, and the relative activities of α and β-adrenergic receptors are evaluated by measuring the changes of glucose production by α and β

adrenergic agonists.

Catecholamine-induced glycogenolysis is thought to be mediated primarily by β-adrenergic receptors in fetal rat livers (Lopes *et al*., 1998) whereas it is currently known to be mediated by α_1-adrenergic receptors in the liver of the adult male rat, where α_1-adrenergic receptors comprise approximately 80% of the total α-adrenergic receptor population (Sherline and Glinsmann, 1974). Our results showed that the glycogenolytic response of the α-adrenergic agonist (Figure 2) was longer-lasting than that of the β-adrenergic agonist (Figure 3) in both groups. This result was expected since the α-adrenergic receptors predominate and the β-adrenergic receptors are more susceptible to desensitization when exposed to their agonists (Garcia-Sáinz *et al*., 1989). Lopes *et al*. (1998) also showed that during hypoglycemia the secretion of counter-regulatory hormones exposes the α and β-adrenergic receptors to elevated plasma concentrations of catecholamines, causing decreased responsiveness of the hepatic glycogen metabolism to phenilephrine and isoproterenol, the response of the β-adrenergic receptor being smaller. However, our results showed that the activation of glycogenolysis by maximally effective doses of isoproterenol (20 μM) and phenylephrine (2 μM) was greater in MSG-treated rats than in controls. Therefore, it is reasonable to suggest that the lesion in the hypothalamic arcuate nucleus promoted a greater responsiveness of the α and β-adrenergic receptors. According to previous studies, the models of obesity induced by lesions in the arcuate or ventromedial hypothalamic (VMH) nuclei are accompanied by an increase in parasympathetic activity and a decrease in the sympathetic tonus (Yoshida *et al*., 1998; Balbo *et al*., 2002; Bray and Champagne, 2005), suggesting that possibly the basal secretion of catecholamines by the adrenal medulla is smaller in these animals.

Inoue and Bray (1980) have suggested that VMH lesions increased the sensitivity of the β-adrenergic receptors on the β-cells of the pancreatic islets, leading to hyperinsulinemia.

Matsui *et al*. (1993) showed that the lesion in the VMH causes changes in the levels of hepatic adrenergic receptors and that the increase in the β-adrenergic responses is caused mostly by the reduction of plasma epinephrine in virtue of the decreased sympathetic tonus.

Studies of liver perfusion of adrenomedullated animals showed increases only in the responses to β-adrenergic agonists, without changes in the responses to the α-adrenergic agonists, suggesting that the deficiency of catecholamines released from the adrenal gland results in an increase in β-adrenergic receptor function (Wolfe *et al*., 1976; Matsui *et al*., 1993). Nevertheless, our results demonstrated that AN lesions induced by MSG treatment promoted increased responses not only of the β-adrenergic receptor, but also the α-adrenergic receptor as well. It is reasonable to conclude that this change in the responsiveness of the α- and β-adrenergic receptors of the liver may be attributed to the smaller activity of the sympathetic-adrenal axis in the MSG-treated animals once, as mentioned above, this neurotoxin causes ablation of the AN, followed by obesity and reduced sympathetic tonus.

Conclusão

Results indicated that the lesion in the AN increased glycogen catabolism to adrenergic agonists, possibly, due to the reduced activity of the sympathetic-adrenal axis.

References

BALBO, S.L. *et al*. Vagatomy reduces obesity in SG-trated rats. *Res. Commum. Mol. Pathol. Pharmacol*., Westbury, v. 6, p. 291-296, 2000.

BALBO, S.L. *et al*. Parasympathetic activity changes insulin response to glucose and neurotransmitters. *Diabetes Metab*., New York, v. 28, n. 6, p. 3S13-3S17, 2002.

BAZOTTE, R.B. *et al*. The sensitivity of glycogenolisys to glucagons, epinephrine and cyanidein lives from rats in different metabolic conditions. *Res. Commum. Chem. Pharmacol*., Westbury, v. 64, p. 793-205, 1989.

BERGMEYER, H.U.; BERNT, E. *Methods of enzymatic analysis*. Washington, D.C.: Academic Press, 1974. v. 2, p. 1205-1215,.

BRAY, G.A.; CHAMPAGNE, C.M. Beyond energy balance: there is more to obesity than kilocalories. J. *Am. Diet Assoc*., Washington, D.C., v. 105, n. 5, p. 17-23, 2005.

BRITO, N.A. *et al.* Intra-ventromedial o hypothalamic injection of cholinergic agents induces rapid hyperglycemia, hyperlactactemia and glyconeogenesis activation in fed, conscious rats. *Brain Res*., Amsterdan, v. 626, p. 339-342, 1993.

GARCIA-SÁINZ, J.A. *et al.* Hepatocyte β-adrenergic responsiveness and guanine nucleotide-binding regulatory proteins. *Am. J. Physiol*., Baltimore, v. 256, p. 384-389, 1989.

IGUCHI, A.M. *et al.* Mechanism of central hypoglycemia effect of cholinergic agonists in fasted rats. *Am. J. Physiol*., Bethesda, v. 251, p. 431-437, 1986.

INOUE, S.; BRAY, G.A. Role the autonomic nervous system in the development of ventromedial hypothalamic obesity. *Brain Res. Bull*., New York, v. 5, p. 119-125, 1980.

JUNGERMAN, K. *et al*. Regulation of liver metabolism by the hepatic nerves. *Adv. Enzyme Regul*., Elmsford, v. 26,

p. 63-88, 1987.

LAUTL, W.W. Afferent and efferent neural roles in liver function. *Prog. Neurobiol*., Oxford, v. 21, p. 323-348, 1983.

LEM KEY-JOHNSTON, N.; REYNOLDS, W.A. Nature and extent of brain lesion in mice related to ingestion of monosodium glutamate. *J. Neuropathol. Exp. Neurol*., Lawrence, v. 74-78, 1974.

LOPES, G. *et al.* Responsiveness of glycogen catabolism to adrenergic agonists during insulin – induced hypoglycemia in rat livers. *Gen. Pharmacol*., New York, v. 4, p. 593-599, 1998.

MARTINS, A.C. *et al.* Adrenal medullary function and expression of catecholamines-synthesizing enzymes in mice with hypothalamic obesity. *Life Sci*., New York, v. 74, n. 26, p. 3211-3222, 2004.

MIGLIORINI, R. *et al.* Rapid activation of gluconeogenesis after intracerebroventricular carbacol. *Am. J. Phisiol*., Bethesda, v. 257, p. 486-490, 1989.

MISKOUWAK, B.; PARTYKA, M. Effects of neonatal treatment with MSG (monosodium glutamate) on hypothalamo-pituitary-thyroid axis in adult male rats. *Histol. Histopath*., Múrcia, v. 8, p. 731-734, 1993.

MATSUI, Y. *et al.* Responses to catecholamines in perfused livers hypothalamic-lesioned rats. *Am. J. Physiol*., Bethesda, v. 265, p. 117-123, 1993.

NEMEROFF, C.B. *et al.* Analysis of the disruption of hypothalamic – pituitary regulation in rats treated neon atally with monosodium glutamate (MSG): Evidence for the involvement of tuberoinfundibular cholinergic and dopaminergic systems in neuroendocrine regulation. *Endocrinology*, Baltimore, v. 101, p. 613-622, 1982.

OLNEY, J.W. Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science,* Washington, D.C., v. 164, p. 719-721, 1969.

PERELLÓ, M. *et al*. Adrenal enucleation in MSG-damaged hyperleptinemic male rats transiently restores adrenal sensitivity to leptin. *Neuroendocrinology,* Basel, v. 78, n. 3, p. 176-84, 2003.

SERESS, L. Divergent effects of acute and chronic monosodium L-glutamate treatment on the anterior and posterior parts of the arcuate nucleus. *Neuroscience,* Oxford, v. 7, p. 2207-2216, 1982.

SHERLINE, P.; GLINSAMANN, R.T. Acute hormonal regulation of cyclic AMP content and glycogen phosphorylase activity in fetal liver in organ culture. *Endocrinology,* Baltimore, v. 94, p. 935-939, 1974.

TOKIN, M.; MATSUBARA, T. Effects of adrenergic agonists and antagonists on glycogenolysis in isolated perfused liver. *J. Pharmacol*., London, v. 45, p. 236-242, 1987.

VAN DEL POL, N.A. Glutamate and aspartate immunoreactivity in hypothalamic presynaptic axons. *J. Neurosci*., Baltimore, v. 11, p. 2087-2101, 1991.

WOLFE, B.B. *et al.* β-Adrenergic receptors in rat liver: effects of adrenalectomy. *Proc. Natl. Acad. Sci*., Washington, D.C., v. 73, p. 1343-1347, 1976.

YOSHIDA, T. *et al.* Beta 3 adrenergic agonist induces a functionally active uncoupling protein in fat and slow-twitch muscle fibers. *Am. J. Physiol*., Bethesda. v. 274, p. 469-475, 1998.

Received on April 04, 2006.
Accepted on November 20, 2006.