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

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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 \( \alpha \) - and \( \beta \)-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 epinephrine and \( \alpha \)- and \( \beta \)-adrenergic agonists. Epinephrine, Isoproterenol and phenylephrine increased glycogenolysis in the MSG-treated rats, compared to the controls (50 ± 2.8 Vs 17 ± 0.89 \( \mu \)mol 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.

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...

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 inferior vena cava and the flux was impulsioned by a perfusion pump to the oxygenator. Here, there occurs simultaneously oxygenation and impulsioned by 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), epinephrine (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 epinephrine, 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).
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Figure 1. Effect of epinephrine (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 phenylephrine (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 5 animals for both groups. *p<0.05.

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 α₁-adrenergic receptors in the liver of the adult male rat, where α₁-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 (García-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 phenylephrine 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.

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