Glycomics and Lipidopics

Extended Abstract

Early Glycine Supplementation Re-Establishes Adrenal Catecholamine Secretion in Hypothalamic Obesity Model in Rats but does not Affect Visceral Adiposity

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Abstract:

Obesity is a worldwide epidemic that features a multifactorial syndrome characterized by a chronic positive energetic unbalance. Neonatal administration of monosodium L-glutamate (MSG) causes lesion on the arcuate nucleus of hypothalamus that led to development of obesity in the adult life in rodents characterized by a notorious accumulation of catecholamine in the adrenal medulla. The amino acid glycine induces catecholamine secretion of adrenal medulla. Thus, the objective of our work was to evaluate the possible effects of glycine administration in the MSG-obesity model in rats and investigate its impact on adrenal catecholamine medulla homeostasis. Male Wistar rats received MSG solution (4mg/g body weight) subcutaneously in the cervical area for 5 days after delivery, controls received saline solution. Animals were also divided in two groups, in which one received tap water added with glycine (0.1g/Kg) after weaning on 21st day until 90 days of life.Biometrical variables, visceral fat pads weight, total content and basal secretion of adrenal cathecolamine were evaluated. Glycine increased Lee index of all tested groups and had no effect on visceral adiposity. However, glycine treatment completely reestablished catecholamine total content and basal secretion of MSG-obese group. In conclusion, although glycine treatment apparently completely reestablishes catecholamine secretion homeostasis it is not sufficient to significant directly reduce visceral adiposity in MSG obesity model in rats.Obesity is a multifactorial syndrome characterized by a chronic positive energetic unbalance. Its incidence is increasing in alarming proportion; currently, approximately 500 million people are obese world-wide representing a risk factor for type 2 diabetes and cardiovascular illnesses. Extensive academic effort has been put on medical research to better understand the complexity of this disorder. The experimental obesity model induced by monosodium L-glutamate (MSG) has been widely used for being representative of the metabolic disturbance observed in human obesity. The neonatal administration of MSG causes lesion on the arcuate nucleus of hypothalamus.

Neonatal administration of MSG led to development of obesity in the adult life in rodents. Moreover, the animals develop an abnormal deposition of fat. Also, MSG-obese animals present hyperinsulinemia, insulin resistance and a notorious accumulation of catecholamines in the adrenal medulla due to impaired secretion process, among other features. Catecholamine from adrenal medulla has an important role in the regulation of glucose, lipids and protein metabolism. The sympathoadrenal system includes the sympathetic nervous system and the chromaffin cells from the adrenal medulla, which secretes catecholamine (primarily epinephrine) into the bloodstream. Chromaffin cells are cholinergically innervated by the splanchnic nerve; acetylcholine released upon stimulation of this nerve activates neuronal cholinergic receptors in chromaffin cells, thereby inducing membrane depolarisation and triggering catecholamine secretion.The metabolic effect of the epinephrine results in the increase of serum glucose, lipolysis, oxygen consumption and thermogenesis. Therefore, abnormalities in the release mechanisms and/or production of catecholamine can contribute for the development of obesity. Some articles have shown that the amino acid glycine can induce catecholamine secretion of adrenal medulla, although its mechanism of action in chromaffin cells is still not clear. Yadid and collaborators had demonstrated preferential release of epinephrine from the chromaffin cells of the adrenal medulla in response to glycine. Thus, the objective of our work was to evaluate the possible effects of glycine administration in the MSG-obesity model in rats and investigate its impact on adrenal catecholamine medulla homeostasis. All animal protocols were performed according to Brazilian College of Animal Experimentation (COBEA) [23] and Brazilian Federal Law and the procedure protocols were approved by the Ethical Committee for Animal Handling (UFJF – Juiz de Fora, Minas Gerais State, Brazil) (Permit Number: 002/2012). Male Wistar rats were used in this study (n=60 for all experimental procedures). The animals were obtained from the Center of Reproductive

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Biology (CRB) of the Federal University of Juiz de Fora. During the first 5 days after delivery, animals were injected subcutaneously in the cervical area with MSG solution (4mg/g body weight) [7]. Control animals received equimolar saline solution. The weaning of the animals was performed at the 21st day. The animals were divided in four groups: Control and MSG which received tap water; and, Control-Glycine and MSG-Glycine which were treated with tap water added with glycine (0.1g/Kg) according to Alarcon-Aguilar et al [24]. After weaning, all animals groups were weighted weekly. Animals received water and commercial chow (Nuvital, Curitiba, Brazil) ad libitum and placed in an environmentally controlled room (23 ± 3 °C and 12h light / darkness photocycle (07:00-19:00 h)) during the whole protocol period. To evaluate obesity onset, all 90 day old rats were euthanized by exsanguination through cardiac puncture under sodium pentobarbital (45mg/100g body weight i.p.) anesthesia. Epidydimal and retroperitoneal fat pads were removed and weighted to estimate obesity induced by MSG treatment. The Lee index was calculated from the ratio [body weight (bw)$^{1/3}$ (g)/nasoanal length (NAL) (cm)]×1000 and used as a predictor of obesity in MSG-rodents. Adrenal glands were removed and weighted. During handling, glands were maintained in standard Krebs–Hepes solution composed of (in mM): Cl- 154.2; Na+ 144.0; Ca2+ 2.5; Mg2+ 1.18; SO42- 1.2; K+ 3.5; glucose 11.1; Hepes (acid N-(2-hydroximethylpiperazine)-N-(2-ethanosulfonic)) 25.0; bovine serum albumin (BSA) 0.5%, on an ice bath. Right glands of all experimental groups were used to evaluate the total catecholamine content – epinephrine and norepinephrine – quantified by using the trihydroxyindole fluorescence method [26]. Results were obtained by plotting the values on a linear regression of the standard epinephrine curve. On the other hand, left adrenal glands were used in secretion experiments. Dissection of adrenal medulla was undertaken with stereoscopic lens and ophthalmologic surgical instruments. Isolated medullae were, moreover, impaled on steel sticks for better manipulation, and left in a rest of 40 minutes in standard Krebs-Hepes solution. Costar 96-well cell culture plates were used. Each well contained 200 µL of standard Krebs-HEPES or modified Krebs-HEPES solution containing5 µM of glycine [27]. Catecholamine secretion experiments were realized according to previous described method. All results are presented as mean ± SEM. $p < 0.05$ was considered statistically significant. One-way ANOVA with Bonferroni post-test was performed using GraphPad Prism version 5 for Windows (GraphPad Software, San Diego California USA). The biometrical analysis of the studied groups is shown in the Table 1. Control animals has a 3.5% increased on NAL after glycine treatment ($p<0.05$). However, the NAL did not differ between the MSG groups. Furthermore, glycine treatment enhanced the Lee Index in both groups ($p<0.001$). The MSG animals have shown diminished weight of the adrenal glands compared to Control animals, but no differences were seen after the glycine treatment when we compared to their respective pairs.

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