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The toxic effects of monosodium glutamate (MSG) – The involvement of nitric oxide, prostanoids and potassium channels in the reactivity of thoracic arteries in MSG-obese rats

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ABSTRACT

We investigated the potential effects of monosodium glutamate (MSG)-induced obesity with regards to nitric oxide and prostanoid production, as well as potassium channel function, in rat thoracic arteries.

Newborn male Wistar rats were injected intraperitoneally with typically reported MSG (4.0 mg/g) once daily for 4 consecutive days. At 90 days postnatal, the rats were sacrificed and the thoracic aortas were evaluated for vascular responses and for prostanoid production.

Nitric oxide was studied with calcium ionophore (A23187), acetylcholine (ACh) and sodium nitroprusside (SNP). The release of prostanoids was measured under basal and ACh-stimulated conditions, and the vasomotor effect of exogenous thromboxane A_2 mimetic, U46619 was assessed. Potassium channel activities were analyzed using an NS1619 opener for BK_{Ca} channels and pinacidil for K_{ATP} channels.

Arteries from MSG-obese rats exhibited a reduced maximal contraction to potassium chloride and hyperresponsiveness to U46619, suggesting that MSG also alters the responsiveness of vascular smooth muscles. The endothelium-dependent relaxation to ACh and A23817 was attenuated, suggesting low nitric oxide bioavailability. The hypersensitivity of arteries to an exogenous nitric oxide donor, SNP, occurred. The secondary contraction to A23817 was augmented, suggesting increased activation of the prostanoid receptor. The prostanoid release was increased in both basal- and acetylcholine-stimulated rings. In addition, down-regulation of K_{ATP} and BK_{Ca} channels influenced hyperpolarizing mechanisms.

Our findings suggest that increased prostanoid production and hypersensitivity to thromboxane A_2 together with down-regulation of potassium channels and low nitric oxide bioavailability may contribute to the increase in blood pressure found in adult MSG-obese male rats.

1. Introduction

The prevalence of obesity is increasing worldwide and is considered to be at pandemic stage. Obesity is commonly associated with diabetes and is related to metabolic syndrome (MS). MS is defined as the co-occurrence of three out of five possible characteristics: abdominal obesity, insulin resistance, glucose intolerance, hypertriglyceridemia and reduced levels of HDL cholesterol. Obesity is a risk factor for coronary heart disease and cardiovascular disease (Ginsberg and Maccallum, 2009).

Monosodium glutamate (MSG) is commonly used in food preparation as a flavor enhancer. MSG intake results in the degeneration of ventromedial hypothalamic and arcuate nuclei (Dawson et al., 1997) that causes animal models such as rats to develop obesity, growth retardation (low growth hormone), as well as, sexual and behavioral dysfunction (Husarova and Ostatnikova, 2013). The MSG produces animals that mimic, at least in part, the clinical situation of metabolic syndrome with leptin resistance, hypophagia, hypoactivity, late puberty, ovarian weight reduction and high blood corticosteroids (Afifi and Abbas, 2011). The MSG animal models exhibit most features observed

Abbreviations: A23187, calcium ionophore; BK_{Ca}, Large Conductance Ca^{2+}-activated K^+ channels; cGMP, cyclic guanosine monophosphate; COX, cyclooxygenase; EDCF, endothelial derived contracting factors; EDHF, endothelial derived hyperpolarizing factor; E_{max}, maximal effect; K_{ATP}, ATP-dependent K^+ channels; L-NAME, Nω-nitro-L-arginine methyl ester; MS, metabolic syndrome; MSG, monosodium glutamate; pD_2, drug concentration exhibiting 50% of the E_{max} expressed as negative log molar; PG, prostaglandin; ROS, reactive oxygen species; SNP, sodium nitroprusside; TxA_2, thromboxane A_2; U46619, thromboxane A_2 mimetic

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in obese humans with insulin resistance, hyperinsulinemia and lipid profile abnormalities (Lobato et al., 2011).

Recent evidence using rat models indicates that MSG-induced obesity, to the typically reported 4.0 mg/g body weight, increases oxidative stress (Singh and Ahluwalia, 2012; Rosa et al., 2018) and enhances the formation of plasma prostaglandin E_2 (Cunha et al., 2010) and reduces sympathetic activity (Park et al., 2007). In addition, endothelial nitric oxide bioavailability is decreased (Cunha et al., 2014), which all together may contribute to the development of hypertension in adult obese MSG-treated rats (Cunha et al., 2010).

However, no studies have examined the participation of prostanoids from stimulated aortas, nor have they studied the contribution of the vasodilator calcium ionophore, A23187, in endothelium-dependent relaxation. Since permeability to potassium ions is one of the most important factors controlling the membrane potential, and controlling vascular tone (Brozovich et al., 2016), the role of potassium channels in aortas from the control and MSG-obese rats has also been examined using two types of potassium channel openers (*i.e.* NS1619 for BK_{Ca} channels and pinacidil for K_{ATP} channels).

In view of the above considerations, this study was undertaken to clarify the hypotheses that MSG-induced obesity exerts the following effects: i) increased production of prostanoids; ii) decreased nitric oxide dependent vasodilation; iii) decreased participation of hyperpolarizing mechanisms in the vasodilator responses, in which BK_{Ca} and K_{ATP} channels are engaged. To the best of our knowledge, these hypotheses have never been clarified.

2. Materials and methods

This study was approved by the Local Ethical Committee according to European guidelines (Directive 2010/63/EU for animal experiments) and conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publications No. 86–26, revised 2014).

2.1. Study protocol

Newborn male Wistar rats weighing between 7 and 8 g were divided into two groups of 12 animals each. The experimental group received typically reported intraperitoneal MSG injections at 4.0 mg/g of body weight, in a similar volume (1 μl/g), once a day for 4 consecutive single daily doses to induce obesity. The control group received injections of an equimolar saline solution (0.9%) at the same time, also starting at the first postnatal day.

All animals were housed until 90 days of life, under controlled conditions of a 12-h light-dark cycle, a temperature of 21–22 °C, a ventilation rate of 20 air changes per hour and a relative humidity 50 ± 10%. Water and standard rat chow was supplied *ad libitum*.

2.2. Vascular reactivity studies

Rats were anaesthetized and killed by decapitation. The thoracic aortas were then isolated and placed in ice-cold Krebs-Henseleit solution (KHS) of the following composition (mM): NaCl 115; $CaCl_2$ 2.5; KCl 4.6; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 25; and glucose 11.1; with pH 7.4. Thoracic aortas were cleaned of adherent tissue, were cut into 6–8 rings of 3- to 4-mm length, and were suspended horizontally under a resting tension of 1 g (determined during preliminary experiments) in 5-ml tissue baths containing KHS. The solution had been aerated with a mixture of 95% oxygen and 5% carbon dioxide, and maintained at +37 °C. Each ring was connected to a transducer to measure isometric force.

This was done in accordance with Villalpando et al. (2015) and Majewski et al. (2017). Briefly, after the initial equilibration period of 60 min, contractile responses elicited by either a increasing concentration of potassium chloride, KCl (1–60 mM) or a single maximum depolarizing concentration of KCl (60 mM) were assessed. The cumulative concentration-response curves of acetylcholine (0.1 nM–10 μM), calcium ionophore, A23187 (0.1 nM–1 μM) and sodium nitroprusside (0.1 nM–10 μM) were assessed to determine the relaxant responses on endothelium-intact rings that had been precontracted with submaximal concentrations of phenylephrine (1 μM). The rings were also assessed for contractile responses generated by a cumulative concentration of phenylephrine (1 nM–100 μM), thromboxane A_2 mimetic, and U46619 (1 nM–1 μM).

In another set of experiments, a 30 min preincubation with N(ω)-nitro-L-arginine methyl ester (L-NAME, 100 μM) was performed to determine nitric oxide availability. Also, COX inhibitor – indomethacin (10 μM) was used to analyze the involvement of arachidonic acid metabolites in vascular control. Thus contractile responses elicited by phenylephrine (1 μM) were analyzed in control conditions and after 30 min incubation with either L-NAME or indomethacin. In addition, relaxation to the cumulative concentrations of acetylcholine (0.1 nM–10 μM) was studied in the presence or absence of L-NAME and indomethacin.

2.3. Prostanoids quantification

This was done in accordance with Villalpando et al. (2015). Briefly, after a stabilization period in KHS at +37 °C for 30 min (pH 7.4), aortic rings from each group of rats were followed by 2 wash periods of 10 min using 200 μL of KHS. Once fresh KHS was replaced, arteries were exposed to phenylephrine (1 μM, 2 min) and then to cumulative acetylcholine concentrations (0.1 nM–10 μM) at 1-min intervals. The medium was collected and stored at −80 °C until further analysis. Production of thromboxane A_2, prostaglandin I_2 and prostaglandin E_2 were monitored by measuring their stable metabolites TxB_2, 6-keto-PGF1α and PGE_2, respectively. This was done using the appropriate enzyme immunoassay kit (Cayman Chemical). Results were expressed as pg prostanoid/mg tissue.

2.4. Drugs and reagents

The drugs used were: acetylcholine (ACh) chloride, phenylephrine (Phe) hydrochloride, N(ω)-nitro-L-arginine methyl ester (L-NAME) hydrochloride, indomethacin, potassium chloride (KCl), sodium nitroprusside (SNP), calcium ionophore A23187 (Sigma-Aldrich) and NS1619, U46619, pinacidil (Cayman chemical). Monosodium glutamate (purity ≥99%) was sourced from Biomus (Lublin, Poland). Stock solutions (10 mM) of drugs were prepared in distilled water, except for NS1619, indomethacin and pinacidil, which were dissolved in ethanol. Furthermore, U46619 and A23187 were dissolved in DMSO. These solutions were maintained at −20 °C and appropriate dilutions were made in fresh KHS on the day of the experiment.

2.5. Data analysis and statistics

The body weight and body length of rats were used to determine the Lee Index, calculated as the cube root of body weight (g) to nose-to-anus length (cm). Contraction was expressed in mg of tension for the KCl, phenylephrine, and U46619. Vasodilation was represented as a percentage of the maximal response to phenylephrine (1 μM). Values of pD_2 and the maximal response (E_{max}) were calculated by non-linear regression analysis. Data are expressed as means ± S.E.M (Standard Error of the Means) and were compared by either unpaired *t*-test with Welch's correction or ANOVA with *post-hoc* multiple comparisons test, when appropriate. Homogeneity of variance was tested for all data. A value of $P < .05$ was considered to be significant.