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Research Article

HYPOTHALAMIC OBESE AND NON-OBESE RATS EXPRESS A SIMILAR FUNCTIONAL MUSCARINIC M_3 SUBTYPE IN THE CONDUCTANCE ARTERY

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ABSTRACT

The substantial homology between the genomes of rodents and humans has made hypothalamic obese rats, obtained by neonatal treatment with monosodium L-glutamate (MSG), a good tool for studying the effects of obesity on the functional characteristics of muscarinic receptors. The affinity of muscarinic receptors for quinuclynidyl benzylate is increased in cells in the central nervous system in MSG-obese rats, and the population of M_3 receptors expressed in pancreatic islets isolated from non-obese rat transitions toward the M_2 subtype in MSG-obese animals. We investigated whether neonatal treatment with MSG could also modify the M_3 muscarinic receptor subtype that is expressed in vascular tissues of rats. The $EC_{50\%}$ values for acetylcholine-induced relaxation in obese and non-obese rats were similar in the intact aortic ring preparations precontracted with norepinephrine. Atropine, 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP), and pirenzepine induced similar parallel rightward shifts of the log [concentration]-response curve of acetylcholine-induced relaxation in preparations isolated from both types of animals. The pA_2 values for atropine, 4-DAMP, and pirenzepine in obese animals were not different from controls. Methoctramine did not produce any effect on the log [concentration]-response curve for acetylcholine in obese and non-obese rats. MSG does not modify the muscarinic receptor subtype expressed in the conductance artery of rats.

Keywords: Muscarinic receptors, aorta, obesity, monosodium L-glutamate, arterial blood pressure

INTRODUCTION

Obesity is associated with a spectrum of metabolic and cardiovascular disorders [1] and may be induced in animals by neuroendocrine, dietary, or genetic changes [2]. Although the studies performed with such animal models have shown that the central nervous system regulates energy expenditure and food intake, the mechanisms by which hypothalamic injury leads to obesity are not fully understood [2]. However, the obesity induced by hypothalamic injury is known to not be caused by an increase in food intake [2]. The substantial homology between the genomes of rodents and humans have made these animal models a good tool for studying the effects of obesity on different biological parameters [2] , such as the arterial blood pressure and the responsiveness of vascular tissues to activation by different drugs. Thus, it has been verified that the neonatal treatment of rat with monosodium L-glutamate (MSG), a condition that produces obese animal (hypothalamic obese rat or MSG-obese rat), lowers the responsiveness of both α-adrenergic receptors and angiotensin II receptors in vascular tissues in these animals [3]. Moreover, it has been proposed that the neonatal treatment of rats with MSG could alter the expression of muscarinic receptors in different tissues in these animals, as it was verified that the population of M_3 muscarinic receptor subtypes transitions toward the M_2 subtype in pancreatic islets isolated from MSG-obese rats [4], and the number of muscarinic receptors, as well as the affinity of such receptors for quinuclynidyl benzylate, are increased in cells of the central nervous system in MSG-obese animals [5] .

Although vascular muscarinic receptors are well established to not be activated under physiological conditions (i.e., the absence of cholinergic innervation and the absence of acetylcholine molecules in the blood), the activation of such receptors by different cholinomimetic drugs significantly reduces arterial blood pressure under therapeutic conditions [6]. The endothelial M_3 receptor is well known to be the main subtype of muscarinic receptor involved in acetylcholine-induced endothelium-dependent relaxation in aortic ring preparations isolated from spontaneously hypertensive rats [7] and non-hypertensive animals [8]. However, unknown is whether the obesity produced by neonatal treatment of rats with MSG modifies the specific muscarinic receptor subtypes that are expressed in vascular tissue in such animals, or whether the muscarinic receptors in the vessels of MSG-obese rats are less or more responsive to activation by acetylcholine than in non-obese animals. Since information about vascular muscarinic receptors expressed in MSG-obese rats could be useful for managing therapeutic procedures in obese patients, the responsiveness of vascular muscarinic receptors to activation by acetylcholine, and the subtype of vascular muscarinic receptors expressed in aortic ring preparations isolated from MSG-obese rats were evaluated in the current study.

METHODS

Drugs

Monosodium L-glutamate, acetylcholine, pirenzepine, methoctramine, 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP), and norepinephrine were obtained from Sigma (St. Louis, MO, USA).

Animals and obesity

The Ethical Committee for Animal Experiments of the State University of Maringa approved the animal protocols. Neonate male Wistar rats were subcutaneously injected during the first 5 days of life with MSG (4 g/kg body weight). Control animals received equimolar saline solution. Both animal groups were weaned on postnatal day 21. All animals were housed under a 12 h/12 h light/dark cycle (07:00 to 19:00 h) and controlled temperature (21 ± 2°C). Water and standard rodent chow (Nuvital, Curitiba, Brazil) were available *ad libitum*. Retroperitoneal, perigonadal, and subcutaneous fat pads were removed, washed, and weighed to estimate obesity induced by MSG treatment. The Lee index (body weight [g] 1/3/nasal-anal length [cm] × 1000) was calculated as a predictor of obesity in MSG rats.

Arterial blood pressure

When 90 days old, rats from both groups were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and urethane (600 mg/kg, i.p.). A polyethylene catheter (PE 10) containing heparinized saline (50 IU heparin/ml 0.9%) was introduced into the femoral artery to permit the recording of mean arterial blood pressure (MLT0380/D transducer) in each animal on a computer equipped with Powerlab chart software (AD Instruments, Pty, Ltd., Castle Hill, NSW, Australia).

In vitro preparations

After obtaining the arterial pressure values for each rat, the animals were subjected to medial laparotomy, and the thoracic aorta was

immediately excised. The vessel was cut into 3 mm rings and gently dissected free of fat and connective tissue. Rings were then mounted into 30 ml organ baths filled with oxygenated (95% O_2, 5% CO_2) Krebs buffer (118 mmol/L NaCl, 4.75 mmol/L KCl, 2.5 mmol/L CaCl2, 1.2 mmol/L MgSO4, 25 mmol/L NaHCO3, 1.2 mmol/L KH2PO4, 0.03 mmol/L EDTA, and 11 mmol/L glucose) at 37°C, and the pH of the solution was maintained at 7.4. The aortic ring preparation was connected to strain gauges (Grass, FT 03), and isometric tension was recorded on a computer equipped with Powerlab chart software (AD Instruments, Pty, Ltd., Castle Hill, NSW, Australia). After the rings were allowed to equilibrate for 1 h, the preparations were gradually stretched to an optimal resting tension (1 g).

The endothelium was considered to be functionally intact when the addition of acetylcholine (1.0 μM) produced 100% relaxation (return of tension to values recorded before any previous contraction of rings) of the precontraction induced by norepinephrine (10 nM). After the resting tension stabilized, norepinephrine (10 nM) was administered to induce a rapid increase in vascular tone, which was followed by stable vasoconstriction (tonic contraction). Acetylcholine (0.01-1.0 μM) was then added to the bath. Norepinephrine was the drug chosen to produce vessel precontraction because it is an endogenous agonist and when compared with epinephrine has a higher affinity for α-adrenergic receptors [9]. The norepinephrine concentration we used had produced about 50% of the maximal tension in isolated rat aortic ring preparations devoid of endothelium [10].

In the experiments with atropine, pirenzepine, methoctramine, and 4-DAMP, each antimuscarinic agent was administered 15 sec after norepinephrine. The lowest concentration of each antimuscarinic drug able to produce a small rightward shift in the dose-effect curve produced by acetylcholine was determined, and the multiple dose values were used to obtain the others dose-effect curves necessary to infer the pA_2 values (GraphPad Prism version 3.02 for Windows, GraphPad Software®) for the antimuscarinic agents [11; 12] .

Data analysis

Data were analyzed using Student's *t*-test or analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Obesity and arterial blood pressure

The MSG-obese animals had a Lee index and retroperitoneal, perigonadal, and subcutaneous fat values that were significantly ($p< 0.05$) higher than those in non-obese rats (Table 1). Mean arterial blood pressure in obese animals was not significantly (*p> 0.05)* different from that found in non-obese animals (Table 1).

EC_{50} values for acetylcholine

The EC_{50} values for acetylcholine in the intact aortic ring preparations precontracted with norepinephrine (10 nM) in obese (2.27 ± 0.26 nM, $n = 35$) and non-obese (2.37 ± 0.32 nM, $n = 35$) animals were not significantly different ($p > 0.05$) (Fig. 1).

pA_2 values for muscarinic antagonist

Atropine (12.5, 50.0, and 200.0 nM, $n = 4$), 4-DAMP (25.0, 50.0, and 100.0 nM, $n = 4$), and pirenzepine (1.0, 10.0, and 100.0 μM, $n = 4$) caused a parallel rightward displacement of the log [concentration]-response curve for acetylcholine in MSG-obese and non-obese rats (Fig. 2). The pA_2 values (-log M) for atropine, 4-DAMP, and pirenzepine for the relaxing effect induced by acetylcholine were similar ($p > 0.05$) in obese and non-obese rats (Table 2). Methoctramine (until 10 μM) did not produce any effect on the log [concentration]-response curve for acetylcholine in MSG-obese and non-obese rats (data not shown).

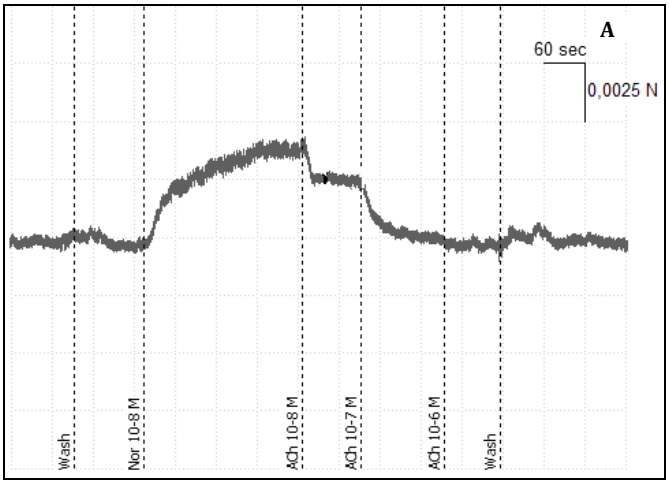
Responsiveness of α-adrenergic receptors

A decreased responsiveness of α-adrenergic receptors to norepinephrine-induced effect was detected in the vascular tissue of MSG-obese rats (Table 3).

Table 1: Effects of MSG-neonatal treatment in adult rats

	Control ($n = 22$)	MSG ($n = 30$)
Mean arterial blood pressure (mmHg)	94.4 ± 1.06	90.0 ± 3.25
Lee index	303.2 ± 1.20	315.0 ± 1.84*
Retroperitoneal fat (g/100 g)	1.3 ± 0.07	2.2 ± 0.07*
Perigonadal fat (g/100 g)	0.9 ± 0.05	1.7 ± 0.06*
Subcutaneous fat (g/100 g)	1.3 ± 0.07	3.6 ± 0.15*

Data are expressed as mean ± SEM of 22 to 30 experiments obtained from non-obese (control) and MSG-obese (MSG) rats. The Lee index and the various fats are described in the Methods. *p < 0.05, significant difference from control (Student's t-test).



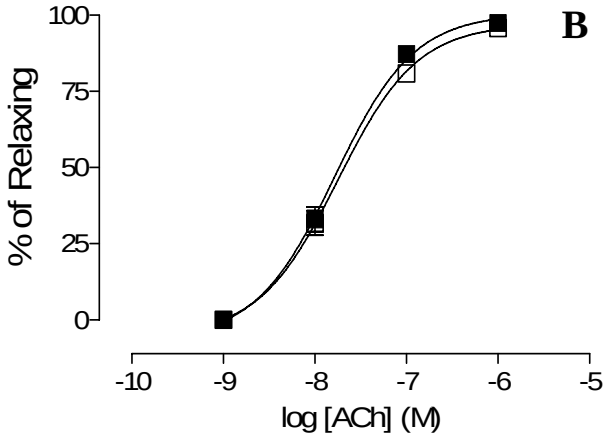
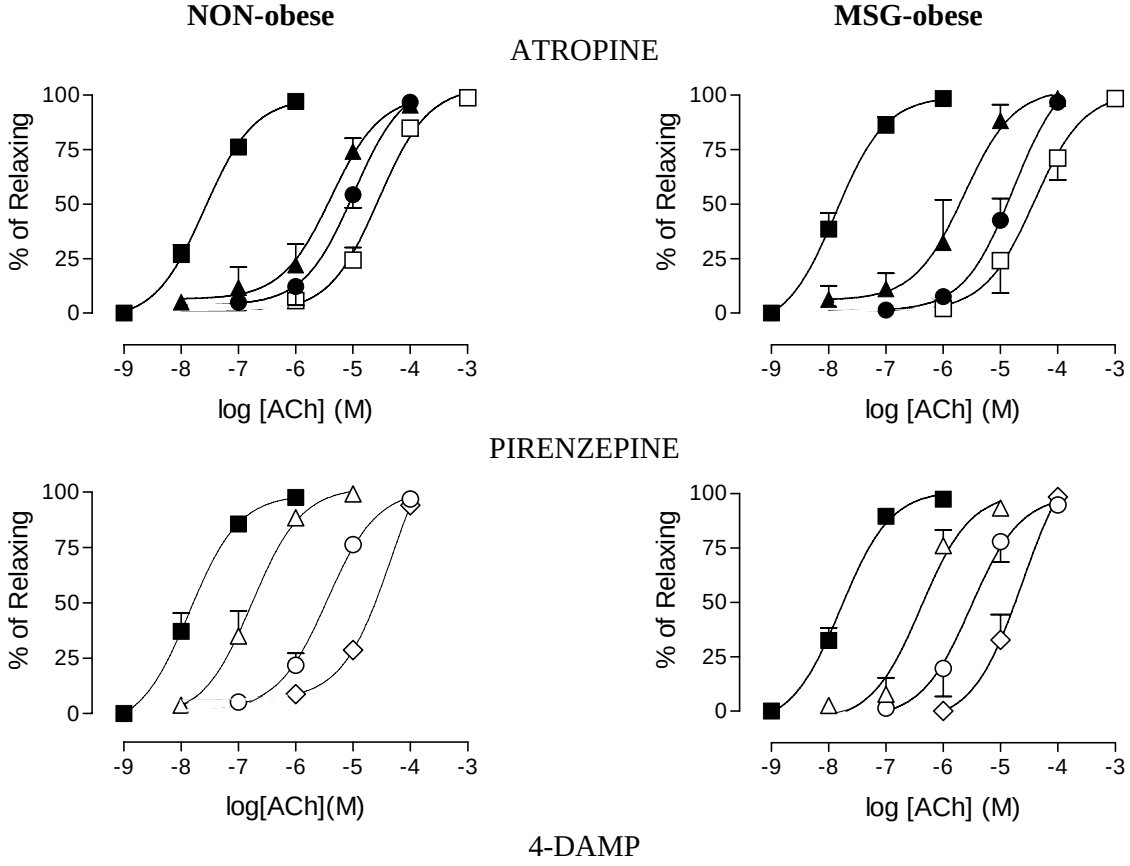


Fig. 1: (A) Relaxing effect produced by acetylcholine (ACh, 10^{-8} to 10^{-6} M) in intact rat aortic ring preparations isolated from MSG-obese rats and precontracted with 10 nM norepinephrine (Nor). The wash indicates an exchange of nutrient solution containing Nor and ACh by drug-free Krebs buffer. (B) The percentage (%) of relaxing effect produced by acetylcholine (ACh) in intact rat aortic ring preparations in MSG-obese (, *n* = 35) and non-obese (, *n* = 35) rats precontracted with 10^{-8} M norepinephrine. Symbols indicate mean ± SEM of 35 experiments. The EC_{50} values (mean ± SEM) for ACh were similar ($p > 0.05$) in MSG-obese rats ($2.27 \pm 0.3 \times 10^{-8}$ M) and non-obese rats ($2.37 \pm 0.3 \times 10^{-8}$ M).



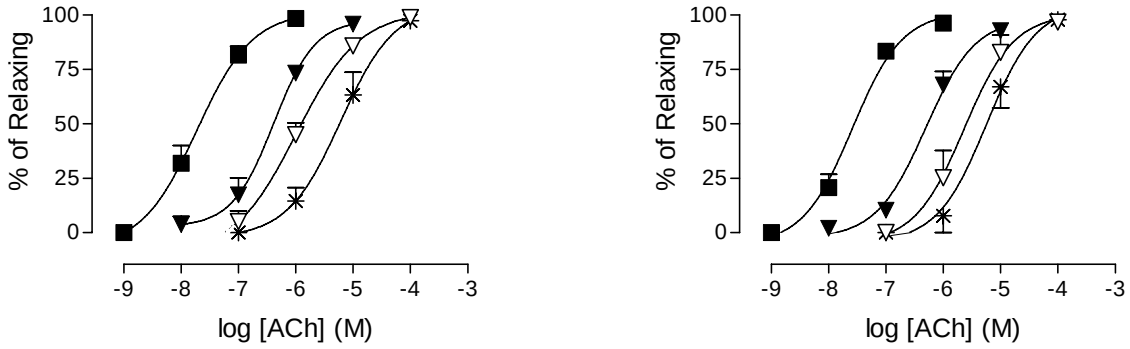


Fig. 2: Competitive antagonism by atropine (▲ 12.5 nM, ● 50 nM, □ 200 nM), pirenzepine (△ 1μM, ○ 10 μM, ◇ 100 μM), and 4-DAMP (▼ 25 nM, ▽ 50 nM, ✳ 100 nM) of the relaxing effect produced by acetylcholine (ACh) in the aortic ring preparations in rats precontracted with 10 nM norepinephrine. The preparations were isolated from non-obese and MSG-obese rats. Symbols indicate mean ± SEM of 4 to 12 experiments.

Table 2: pA_2 and slope values for atropine, pirenzepine, and 4-DAMP in intact aortic ring preparations in MSG-obese and non-obese rats precontracted with norepinephrine (10 nM).

	Non-obese		MSG-obese	
	Slope	*pA_2 (-log M)*	*Slope*	*pA_2 (-log M)*
Atropine	0.9 ± 0.2	10.0 ± 0.1	1.2 ± 0.2	10.1 ± 0.1
Pirenzepine	1.2 ± 0.2	7.4 ± 0.2	1.1 ± 0.1	7.3 ± 0.1
4-DAMP	0.9 ± 0.5	9.2 ± 0.1	1.2 ± 0.6	9.2 ± 0.1

Values are expressed as mean ± SEM of 11 to 12 observations. pA_2 and slope were obtained using Arunlakshana and Schild methods for slope equal to unity. Data were subjected to ANOVA.

Table 3: Maximal tension produced by norepinephrine (10 nM) and interval (ΔT) of time for norepinephrine to produce its maximal effect in aortic ring preparations in MSG-obese and non-obese rats.

	NON-obese	MSG-Obese
Maximal tension (N)	0,00284 ± 0,0001	0,00224 ± 0,0002*
ΔT (min)	2.4 ± 0.10	2.7 ± 0.2

Data are expressed as mean ± SEM of 15 to 20 observations. *$p < 0.05$, significant difference from control (non-obese rats; Student's *t*-test).

DISCUSSION

MSG-treated neonatal rats developed obesity in adulthood. The parameters that were used to infer obesity in the present study produced data similar to those previously reported [4]. However, it was recorded in the current study that the muscarinic receptor subtype inducing vascular relaxation did not transition toward another muscarinic receptor subtype in MSG-obese animals. Although such a conclusion conflicts with previous results observed in pancreatic cells [4], the results are consistent with data showing that the pA_2 values found for atropine, pirenzepine, and 4-DAMP in the aortic ring preparations isolated from MSG-obese rats were not different from those found in the preparations isolated from non-obese animals. The muscarinic receptor subtype in vascular tissue in MSG-obese and non-obese animal was determined to be the M_3 subtype. The order of affinity found for both groups of animals was atropine > 4-DAMP >> pirenzepine. Methoctramine did not produce any effect on the concentration-effect curve induced by acetylcholine in either group of animals.

The order of affinity found in the present study is similar to the orders of affinity reported for other tissues expressing M_3 muscarinic receptors [7; 13; 14; 15], and the pA_2 value for 4-DAMP was consistent with activation of a homologous population of M_3 muscarinic receptors [7]. Therefore, MSG treatment appears to induce changes in the population of muscarinic receptors in a specific tissue, if such tissue already naturally expresses more than one muscarinic receptor subtype. This might be the case for pancreatic cells in rats, in which both M_1 and M_3 muscarinic receptors are abundant [16], and MSG treatment induces changes that determine the functional expression of another muscarinic receptor subtype (M_2) in such tissues [4]. The present study also showed that MSG treatment did not produce any change in the potency of acetylcholine in inducing endothelium-dependent relaxation. The EC_{50} values for acetylcholine and the maximal reduction in tension produced by the cholinomimetic were similar in aortic ring preparations isolated from MSG-obese rats and non-obese animals. Furthermore, the present data indicate that M_3 muscarinic receptors exhibit preserved functional characteristics in MSG-obese rats. In contrast, neonatal MSG treatment in rats has been hypothesized to decrease the responsiveness of the cardiovascular system, particularly in response to α-adrenergic stimulation when phenylephrine is intravenously administered in awake MSG-obese rats [3], or when the functional activity of vascular tissue is evaluated with the use of perfusion techniques and direct norepinephrine administration in the catheterized vascular tissue of such animals [17]. Although the results of the present study were obtained with the use of a different type of bioassay, the decreased responsiveness of α-adrenergic receptors was also detected in the vascular tissue of MSG-obese animals. The maximum constrictor effect induced by norepinephrine in the aortic ring preparations from MSG-obese rats was significantly ($p < 0.05$) lower than that found in preparations isolated from non-obese animals. The reduced responsiveness of vascular tissues to α-adrenergic activation recorded in the aortic ring preparation isolated from MSG-obese animals was unlikely to have influenced the data obtained with the use of acetylcholine in such preparations. Under such conditions, one would expect that the

vasodilator effect and EC_{50} value for acetylcholine would be altered in preparations isolated from obese animals. Additionally, the mean arterial blood pressure in anesthetized MSG-obese rats was slightly lower than, but not significantly ($p > 0.05$) different from, that found in non-obese animals. Although these results were obtained with the use of anesthetized animals, the data are consistent with a previous study in which arterial pressure was measured in awaked MSG-obese animals [3].

Altogether, the present data show that the vascular responsiveness of α-adrenergic receptors is reduced in aortic rings preparations isolated from MSG-obese rats, but neonatal MSG treatment does not alter the functional characteristics and muscarinic receptor subtype that is naturally expressed in vascular tissues in rats. The present findings may have clinical significance and may help understand and control the effects of drugs on arterial blood pressure in obese patients.

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