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The Dual Effect of Isoproterenol on Insulin Release Is Suppressed in Pancreatic Islets from Hypothalamic Obese Rats

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Hyperinsulinemia in obesity has been attributed to insulin oversecretion by pancreatic beta-cells. Beta-cells are equipped with cholinergic and adrenergic receptors; whereas overall acetylcholine action is to potentiate, catecholamines' effect is to inhibit glucose-induced insulin release (GIIR) via α_2-adrenoceptor. However, it has been shown that β-adrenergic agonists potentiate glucose response. GIIR was studied in pancreatic islets from hyperinsulinemic adult obese rats, obtained by L-glutamate monosodium (MSG) neonatal treatment. Islets from MSG-rats were more glucose responsive than control ones. Isoproterenol, a β-adrenergic agonist, inhibited the GIIR in islets from MSG-obese rats. Results indicate that MSG treatment causes alteration on function of beta-cell adrenoceptors.

Key Words: β-Adrenergic receptors; insulin secretion; MSG-obesity; pancreatic islets.

Introduction

Pancreatic beta-cells from humans and animals secrete insulin, largely induced by blood glucose oscillations *(1,2)*. Glucose stimulates the beta-cell metabolism that provokes the insulin granule exocytosis. However, other factors, such as neurotransmitters, that play an important role on blood insulin concentration control may interfere in glucose-stimulated insulin secretion. While acetylcholine released by parasympathetic ends into beta-cells and binds muscarinic receptors that enhance insulin *(3–5)*, noradrenaline released by sympathetic neural terminals or adrenaline and noradrenaline secreted by adrenal medulla chromaffin cells stimulate α-adrenoceptors, which inhibit insulin secretion *(6)*. Because beta-cells are also equipped with β-adrenergic receptors, it has been established that their stimulation by catecholamines potentiate glucose-induced insulin secretion *(7)*. It has been shown that isoproterenol, a nonspecific β-adrenoceptor agonist, potentiates insulin secretion stimulated by glucose in human and rodent pancreatic islets *(8,9)*.

Fasting hyperinsulinemia revals pancreas endocrine malfunction in obesity *(10,11)*. While pancreatic beta-cells from obese humans and rodents are very sensitive to glucose, high vagal activity causes acetylcholine release to beta-cell, which, in turn, activates beta-cell muscarinic receptors. Furthermore, the cholinergic pancreatic response is to potentiate glucose-induced insulin secretion, which contributes to blood insulin concentration rise *(12)*. Although several studies shed some light on the role of parasympathetic activity on hyperinsulinemia in obese animals *(10,11,13,14)*, little has been exploited to study the activity of pancreatic beta-cell β-adrenoceptor on blood insulin level rise.

The current study was undertaken to examine the effects of a β-adrenergic agonist isoproterenol on glucose-induced insulin secretion from isolated pancreatic islets of hyperinsulinemic hypothalamic obese rats obtained in neonatal treatment with L-glutamate monosodium (MSG).

Results

Rats submitted to treatments with MSG showed body weight decrease (43.8%, $p < 0.001$) and short length (21.2%, $p < 0.001$), when compared to controls. However, MSG-rats showed an increase in the Lee index by 7.3% ($p < 0.001$) compared to untreated rats. MSG-rats fat weight on the epididymal pad was increased 2.5-fold compared with untreated rats ($p < 0.001$). While MSG-rats showed no changes in their plasma glucose concentration compared with untreated animals, they presented a threefold higher plasma insulin concentration than controls, $p < 0.001$, as shown in Table 1.

Pancreatic islets from control rats released 3.2 ± 0.2 ng/islet of insulin after being stimulated by 16.7 m*M* glucose, whereas MSG-rats' pancreatic islets released 4.7 ± 0.4 ng/islet ($p < 0.001$). Islets from MSG-rats, incubated with 5.6 m*M* glucose, released insulin twofold higher than control rat islets, 0.54 ± 0.04 and 0.26 ± 0.04 ng/islet, respectively ($p < 0.05$). Insulin release was obtained from incubations

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Table 1
Effect of Neonatal Treatment of MSG in 90-d-old Rats[a]

	Control	MSG
Body weight (g)	335.9 ± 4.8	233.7 ± 7.1*
Nasoanal length (cm)	22.3 ± 0.1	18.4 ± 0.3*
Lee index	310.900 ± 0.001	333.700 ± 0.003*
Periepididymal fat pad (g/100 g BW)	0.70 ± 0.01	1.80 ± 0.01*
Plasma glucose concentration (m*M*)	5.9 ± 0.2	5.6 ± 0.2
Plasma insulin concentration (n*M*)	0.880 ± 0.12	2.510 ± 0.30*

[a]Data represent the mean ± SEM of 27 control untreated and 19 MSG-treated rats. Differences were analyzed by Student's *t* test (*$p < 0.001$).

of 16 batches of four islets isolated from eight rats to each group. Isoproterenol caused a significant increase in glucose response of pancreatic islets isolated from control rats. The isoproterenol potentiation was dose-dependent from 0.01 to 1.00 μM, causing up to 125% of enhancement, as shown in Fig. 1A. On the other hand, from 10.0 to 300.0 μM, the β-adrenergic agonist inhibited glucose response dose dependently, reaching 57%. MSG-rat pancreatic islets' released insulin, stimulated by 16.7 m*M* glucose, was inhibited by all isoproterenol concentration. Figure 1A also showed that doses of 100.0 and 300.0 μM of β-adrenergic agonist provoked maximum inhibition, 80%.

Ten micromoles of yohimbine, an α_2-adrenergic antagonist, blocked the isoproterenol potentiation and inhibition effects on insulin secretion stimulated by 16.7 m*M* glucose in the incubation of pancreatic islets from control rats, as shown in the Fig. 1B. In MSG-treated rats' islets, yohimbine increased the inhibition caused by isoproterenol (0.01–10.0 μM) in up to 50%. However, at 100.0 and 300.0 μM isoproterenol, yohimbine attenuated the inhibition of glucose-induced insulin release on MSG-rat pancreatic islets.

Discussion

Results show that neonatal treatment of male rats with MSG causes obesity, as indicated by a Lee index increase in 90-d-old MSG-rats. Lee index has been used as a predictor of body fat mass to this type of rodent obesity *(15)*. Furthermore, the high fat accumulation on epididymal tissue of MSG-animals also indicated the obesity. Epididymal fat pad weight is recommended as a simple reliable estimation of body fat in normal and obese rodents *(16)*. Confirming several studies on MSG-induced obesity *(15, 17–20)*, our MSG-adult rats are also shorter than untreated ones. It has been shown that MSG causes growth impairment in rodents. While neonatal treatment with MSG destroys several hypothalamic areas, the arcuate nucleus (ARC) is more injured. The ARC contains the neurons that secrete growth hormone releasing hormone (GHRH) to the pituitary gland, which, in turn, regulates the blood growth hormone (GH) concentration *(21)*. In current research, while MSG-treated rats show fasting normoglycemia, they present hyperinsulinemia, as shown in several studies *(22,23)*. As in other rodent obesity from different origins, the hyperinsulinemia in obese MSG-rats is attributed, at least in part, to an unbalanced autonomic nervous system (ANS) *(10,24)*. Furthermore, it has been shown that MSG-obese rats are insulin resistant *(18)*. Vagotomized MSG-treated rats showed inhibition of obesity onset and did not present hyperinsulinemia *(13)*. It has been observed that hyperinsulinemia is also generated by a low sympathetic activity of MSG-obese rats *(25–27)*. Among others receptors, membrane beta-cells have muscarinic and adrenergic receptors. It is known that muscarinic receptors, especially M3R subfamily, activated by acetylcholine, potentiate glucose-induced insulin secretion *(28,29)*. On the other hand, catecholamines, adrenaline and noradrenaline may activate α and β adrenergic receptors located on beta-cell membrane, and display opposite effects on pancreatic beta-cells. Activation of β-adrenergic receptors, especially β_2, also potentiate insulin release on rat pancreatic islets stimulated by high glucose concentration *(30, 31)*. However, when the α-adrenergic receptors, especially α_2 subfamily, are activated, the insulin secretion is blocked *(32,33)*. Results indicate that when pancreatic islets from normal rat showed a dual insulin release response to a non-specific agonist β-adrenergic isoproterenol, they potentiate at 0.01–10.0 μM concentration and inhibited at 30.0–300.0 μM in the presence of 16.7 m*M*. Isoproterenol did not change the insulin release induced by low glucose concentrations, 2.8 and 5.6 m*M* (results not shown). While studies did not indicate any effect of β-adrenergic agonist on pancreatic islets, the authors used low glucose concentrations *(31,34)*. Other studies showed that isoproterenol at low concentrations (0.30–3.0 μM) potentiate insulin release stimulated by high glucose concentrations (11.1 and 16.7 m*M*) on rat pancreatic islets *(31)*. Interestingly, rat pancreas piece, incubated by 10 μM isoproterenol, slightly inhibited the insulin release stimulated by 8.3 m*M* glucose *(35)*. Furthermore, it has also been shown that high β-adrenergic agonists' concentration inhibits chromaffin cells catecholamine secretion *(36)*, cardiomyocyte contractility *(37)*, secretion of pancreatic acinar cells, and secretion of submandibullar gland *(38)*. In all these studies, it has been indicated that β-

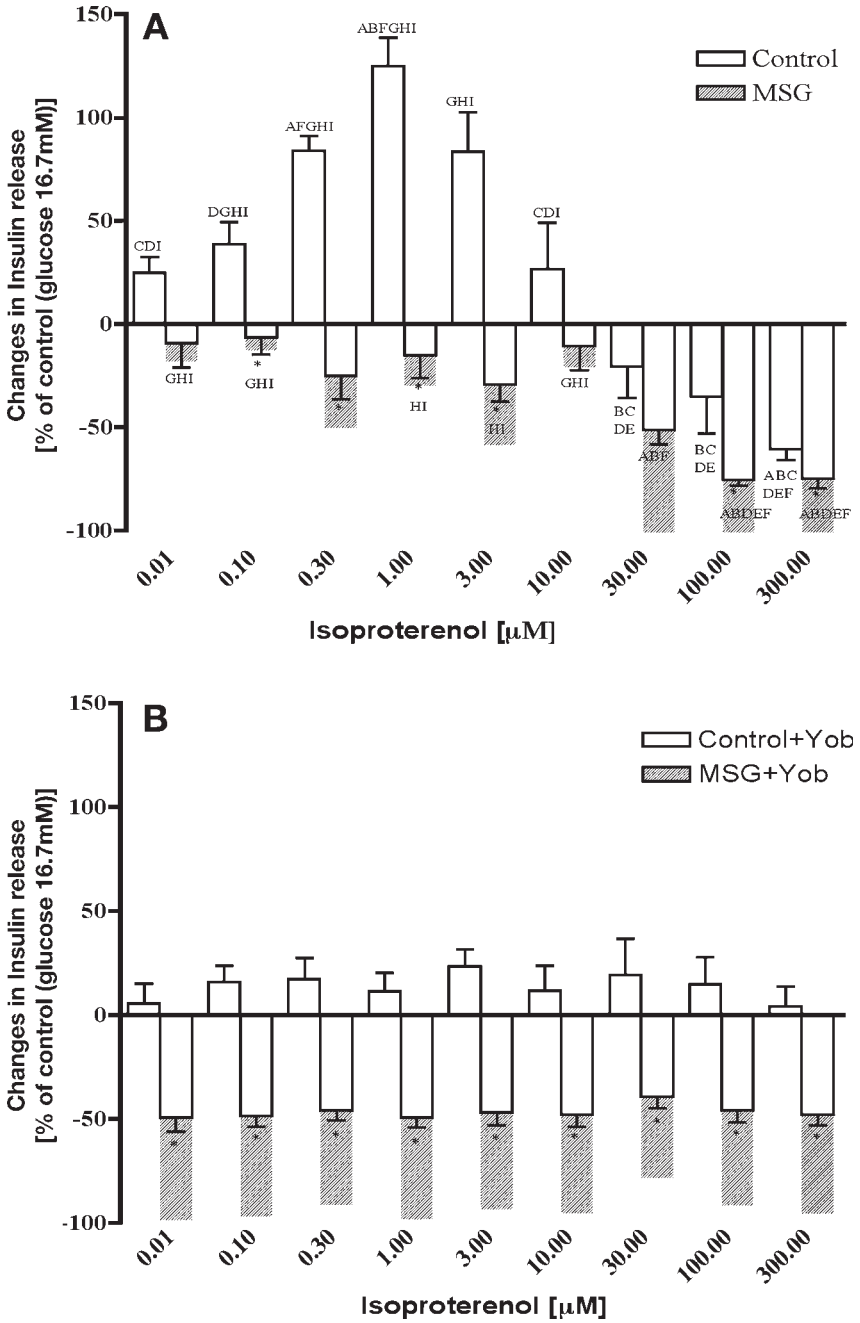

Fig. 1. Isoproterenol effects on insulin secretion stimulated by glucose in isolated pancreatic islets. (**A**) Upper panel show pancreatic islets were incubated with glucose 16.7 m*M* and at different isoproterenol concentrations. (**B**) Lower panel shows the results obtained with pancreatic islets incubated in the same conditions as the upper panel plus 10 *μM* α-adrenergic antagonist yohimbine. The islets were obtained from a pool of eight rats to groups, controls, and MSG. Bars represents the mean results from 16 batches of islets. The data are shown as percentage of increase (above zero line) or decrease (under zero line) obtained with insulin secretion stimulated by 16.7 m*M* of glucose to their respective rat groups. Lines over bar indicate the SEM. Student's *t* test was used for comparison of results between controls and MSG group at given isoproterenol concentration, in the presence or in the absence of 10 *μM* yohimbine; $*p < 0.001$. One-way ANOVA with Bonferroni post-test was used to analyze the results of each group. Letters over the bars represent statistically significant differences ($p < 0.05$) between results from intragroup, control, and MSG-rats independently, with each isoproterenol concentration, such as A = 0.01; B = 0.10; C = 0.30; D = 1.0; E = 3. 0; F = 10.0; G = 30.0; H = 100.0 and I = 300.0.

adrenergic agonist acts on coupled G protein–receptor in two types: stimulatory (G_s) and inhibitory (G_i) subfamilies. When the cells were submitted to toxin pertussis, which inhibits G_i, the G_s stimulatory effect was increased *(39)*. In addition, because rat and guinea pig pancreatic islets incubated with a 10 *μM* dose of isoproterenol were able to inhibit the glucose-induced insulin release, the authors suggest that the inhibitory effects are caused by a β-adrenoceptor stimulation promoted by high isoproterenol concentration *(40)*. Our results also show that α-adrenergic blockage with 10 *μM*

yohimbine suppressed the insulin release inhibition caused by high isoproterenol concentration (30.0–300.0 μM). It is known that beta-cells have a preponderance of β-adrenergic binding sites than β-adrenergic ones *(41)*; however, a lesser number of α-binding sites guarantees the adrenaline and noradrenaline physiological response, leading to net insulin secretion inhibition. Surprisingly, Fig. 1 shows that α_2-adrenergic antagonist yohimbine added to pancreatic islet incubations abolished the effect of isoproterenol at low concentrations. Our results suggest that isoproterenol potentiated effect is dependent on α_2-adrenergic receptor unbinding.

Current research shows that pancreatic islets from MSG-obese rats lack a potential isoproterenol effect, but they present better inhibition at high isoproterenol doses, as shown by islets from control rats. MSG-obese rats are hyperinsulinemic either during fasting or feeding conditions *(22, 23)* and their pancreatic islets are subjected to high vagus tone *(13,42)* and low sympathetic activity *(25–27)*. It has been shown that in hyperinsulinemic obese mice, ob/ob and db/db have an increased number of and a high affinity of β-adrenoceptors on the hepatocyte membrane, which is attributed to high glucocorticoids. Adrenalectomy restored the number and affinity of β-adrenoceptor in ob/ob mice, but failed to do so in obese db/db ones *(43)*. Glucocorticoid treatment also enhances the adrenoceptor number in several cellular types other than beta-cells *(44–46)*. It was found in a recent report that adrenalectomy attenuated the hyperinsulinemia and obesity onset in MSG-treated rats *(47)*. Furthermore, we may suggest that beta-cells from MSG-obese rats are equipped with a high number of β-adrenoceptor and/or high sensitivity to isoproterenol. Instead of causing glucose-induced insulin secretion, they present a net inhibition response. Also, adrenaline or noradrenaline induced a deep insulin secretion inhibition in pancreatic islets from MSG-obese rats incubated in the presence of high glucose concentration than control ones (results not shown). Mice with overexpressed β-adrenoceptor are less responsive to several β-adrenoceptor–induced functions *(48)*. The α_2-adrenoceptor blockage deeply inhibited the isoproterenol-induced insulin response in MSG-rat pancreatic islets, as reported in current work. It has been shown that β-adrenoceptors are easy desensitized by isoproterenol overstimulation in different cell types *(49,50)*. Former results has been confirmed by pretreatment with pertussis toxin, which blocked G_i protein action and increased mice's cardiac cell contraction amplitude *(37,48)*. Furthermore, pertussis toxin–treated rats presented a hyperinsulinemia during the glucose tolerance test when the glucose load was given in the presence of adrenaline or isoproterenol. It was suggested that this effect was caused by G_i protein desensitization *(39)*. Our results provide evidence that β-adrenoceptors, whose number and/or sensitivity are increased in pancreatic islets from obese animals, when stimulated, may inhibit the glucose-induced insulin release. This effect may be attributed to a rapid desensitization of G_s protein *(51,52)*. In addition, α_2-adrenoceptors binding sites, blocked by yohimbine, allow all β-adrenoceptor agonists to bind to β-adrenergic sites, which increases G_s protein desensitization and induces a high interaction with G_i protein.

In conclusion, whatever the precise mechanisms, isoproterenol causes alterations on glucose-induced insulin release identified in the current study and it suggests that β-adrenoceptors stimulation induces a net inhibition of insulin release on pancreatic islets isolated from hyperinsulinemic MSG-obese rats.

Materials and Methods

Animals and Induction of Obesity

Pregnant 70-d-old Wistar rats came from the vivarium of the State University of Maringá. After birth, the litters were equalized to six. Pups were intradermically injected with MSG (4 mg/g body weight/d) into the cervical area during the first 5 d after birth (MSG) *(25)*. Control animals were injected with equimolar saline solution. One day before weaning (21st d), males were selected for the experiments.

Rats were housed in controlled environment at 23 ± 3°C and in a 12 h light/dark cycle (07:00–19:00). Commercial chow (Nuvital, Curitiba, Brazil) and water were given to all animals *ad libitum*.

When animals were 90 d old, after anesthesia with ketamine and xylasine (55 and 8 mg/kg BW, respectively) (0.15 mL/100 g BW), the fasting rats were killed by decapitation. The Lee index — [body weight $(g)^{1/3} \div$ nasal length (cm)] × 1000 — was calculated *(15)*. The periepididymal fat pads were removed and weighed. Blood was collected to measure plasma glucose and insulin concentrations.

The Animal Ethical Committee of the University of Maringá approved all the animal experimental protocols.

Islet Preparation

Isolation of islets from rat pancreas were performed as previously described *(28)* with adaptations. Male adult Wistar rats were deeply anesthetized with ketamine and xylasine (55 and 8 mg/1 kg BW, respectively), and the abdominal wall was cut and opened. A 10 mL Hank's buffered saline solution (HBSS) containing collagenase type V (0.7 mg/mL, Sigma Chemical CO., St. Louis, MO) was injected into the common bile duct of the rat. The pancreas, swollen with the digestion solution, was quickly excised and incubated in a plastic culture bottle for 15 min at 37°C. The suspension obtained was filtered through 0.5 mm metal mesh and washed with HBSS, including 0.125% bovine serum albumin fraction V (BSA) in five continuous washings and collected.

Islet Incubation

Groups of the four Langerhans islets placed on plastic coverslips were preincubated for 60 min in 1.0 mL of Krebs–

Ringer bicarbonate-buffered solution containing 0.125% (wt/vol) bovine albumin (fraction V), and glucose 5.6 m*M*, pH 7.4. The solution was equilibrated against a mixture of CO_2 (5%) and O_2 (95%). After the adaptation (60 min) to a low glucose concentration solution, islets were submitted to incubations for further a 60 min in glucose 5.6 and 16.7 m*M* with Krebs–Ringer solutions and isoproterenol (0–300.0 μM) with or without 10 μM yohimbine. Aliquots from incubations were used to measure insulin concentration by radioimmunoassay *(4)*.

Chemicals

Human insulin marked with ^{125}I was obtained from Amershan Pharmacia (São Paulo, SP, BR). All other reagents, when not cited, were obtained from Sigma.

Statistical Analysis

Data was presented as means ± SEM. One-way ANOVA with Bonferroni post-test and Student's *t* test were performed using GraphPad Prism, version 4.00 for Windows (GraphPad Software, San Diego, CA. USA).

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