Endocrine, *vol. 30, no. 2, 191–196, October 2006* 0969–711X/06/30:191–196/$30.00
ENDO (Online) ISSN 1559-0100

Pancreatic Islets from Hypothalamic Obese Rats Maintain K^+_{ATP} Channel-Dependent but Not -Independent Pathways on Glucose-Induced Insulin Release Process

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One of the main features of obesity is hyperinsulinemia, which is related to insulin oversecretion. Glucose is by far the major physiological stimulator of insulin secretion. Glucose promotes an increase in the ATP/ADP ratio, which inactivates ATP-sensitive K^+ channels (K^+_{ATP}) and induces beta cell depolarization with consequent calcium influx. Increased intracellular calcium concentration triggers insulin exocytosis. K^+_{ATP} channel function is important for K^+_{ATP} channel-dependent pathways involved in glucose-stimulated insulin secretion (GSIS). However, K^+_{ATP} channel-independent pathway has been identified and it has been found that this pathway sustains GSIS. Both pathways are critical to better GSIS control. GSIS was studied in pancreatic islets from hyperinsulinemic adult obese rats obtained by monosodium L-glutamate (MSG) neonatal treatment. Islets from MSG-obese rats were more glucose responsive than control ones. Diazoxide, a drug which maintains the K^+_{ATP} channels open without interfering with cell metabolism, blocked GSIS in islets from both groups. High extracellular potassium concentration plus diazoxide was used to study an alternative to the K^+_{ATP} channel pathway; in these conditions islets from MSG-obese rats did not respond, while islets from control animals showed enhanced GSIS. Results indicate that MSG-obese rats oversecreted insulin, even though the K^+_{ATP} channel-independent pathway is impaired in their beta cells.

Key Words: Pancreatic islets; MSG obese rats; K^+_{ATP} channels; insulin release; diazoxide.

Introduction

Glucose is by far the most important physiological regulator of pancreatic beta cells' functions. The ATP/ADP ratio is understood to couple glucose metabolism with the closing of ATP-sensitive K^+ (K_{ATP}) channels and the ionic events leading to the exocytosis of insulin *(1)*. The mechanisms by which glucose controls pancreatic beta cells' function are complex *(2,3)*. A number of studies have established that a metabolic control of ionic events in beta cells is a critical step in secretion–stimulus coupling *(4)*, and assigned the K^+_{ATP} channel in the plasma membrane a pivotal role in this control *(5)*. The essential role of K^+_{ATP} channels is strikingly illustrated by the inhibition of insulin release brought about by diazoxide. This drug selectively opens K^+_{ATP} channels and does not cause depolarization of the plasma membrane or inhibition of Ca^{2+} influxes; the metabolism of glucose is unchanged *(6)*. There is evidence that glucose can control insulin release independently from its action on K^+_{ATP} channels *(7)*. A second pathway of regulation by glucose is tested by treating beta cells with diazoxide to hold K^+_{ATP} channels open along with a high extracellular K^+ concentration that depolarizes the membrane and restores Ca^{+2} influx. This condition does not allow glucose to induce increased intracellular calcium ($[Ca^{+2}]_i$), but amplifies the action of the ion on the insulin-releasing process *(8)*.

Obesity is associated with abnormal control of insulin secretion in animals and humans. Hyperinsulinemia and peripheral insulin resistance are characteristic of human and animal obesity. Although the precise mechanisms that cause obesity are not yet understood, beta cells' functions are impaired and glucose-induced insulin release is enhanced in animal experimental obesity models *(9,10)*. Studies in obese hyperinsulinemic rodents, ob/ob mice, and Zucker rats, or in GK rats, who present non-insulin-dependent diabetes mellitus (NIDDM) showed that their beta cells do not display any alteration in K^+_{ATP} channel function *(11,12)*. These studies suggest that insulin oversecretion cannot be attributed to K^+_{ATP} channel dysfunction of beta cells from obese animals. In the presence of diazoxide and a depolarizing extracellular concentration of potassium, glucose was still

Received July 17, 2006; Revised September 2, 2006; Accepted October 2, 2006.

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Table 1
Effects of Neonatal Treatment with MSG

	Control	MSG
Basal insulin (ng/mL)	0.25 ± 0.02	1.29 ± 0.09*
Basal glycemia (m*M*)	5.07 ± 0.13	5.21 ± 0.07
Periepididymal fat (g/100g bw)	0.77 ± 0.03	1.94 ± 0.09*
Retroperitoneal fat (g/100g bw)	0.76 ± 0.06	1.96 ± 0.08*

Data present media ± SEM. Eight to 10 animals from both groups were used to obtain these results. The glucose and insulin blood levels were evaluated after fasting overnight. Data from fat pads were expressed in relation to body weight. Differences were analyzed by Student's *t* test (*$p < 0.001$).

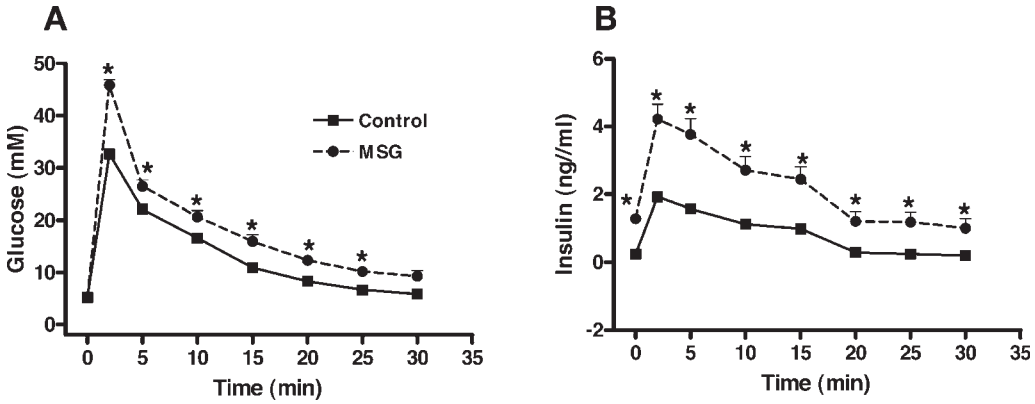

Fig. 1. Glucose tolerance test (ivGTT). Each symbol represents the mean from 8 to 10 animals from both groups. Lines over the symbol represent the SEM. The increment of glycemia is shown in panel **A** and insulinemia in panel **B**; both data were obtained after glucose load (1 g/kg). Differences were analyzed by Student's *t* test (*$p < 0.05$).

able to induce insulin secretion in pancreatic islets from lean rats and humans *(13,14)*. Even in islets from obese ob/ob mice and Zucker rats, the alternative pathway remained intact *(15,16)*. However, in islets from diabetic GK rats, reduced activity of this K^+_{ATP} channel-independent pathway was observed *(17,18)*.

Monosodium L-glutamate (MSG) administered during the neonatal period induces obesity with marked hyperinsulinemia and insulin resistance in adult animals *(19)*. These major abnormalities have been attributed to a disruption in the insulin-secretion control in pancreatic islets. Islets isolated from MSG rats presented higher insulin release when incubated in the presence of elevated glucose concentration. In spite of presenting higher fasting levels of insulin in plasma, MSG rats are normoglycemic *(20)*. Furthermore, these obese adult animals are not considered diabetic. The purpose of the present work is to evaluate the K^+_{ATP} channel-dependent and -independent pathways on glucose-induced insulin secretion in pancreatic islets isolated from MSG-obese rats.

Results

Effects of Neonatal Treatment with MSG in Adult Rats

Table 1 shows that MSG rats presented a 152% increase in the periepididymal and retroperitoneal fat pad when compared to untreated rats ($p < 0.001$). Fasting blood levels of insulin were 400% highest in MSG rats than in control animals ($p < 0.001$). In spite of higher levels of insulin, MSG rats presented blood glucose levels similar to control animals.

Glucose Tolerance Test

The increment of glycemia is presented in Fig. 1A, while the insulin increment is shown in Fig. 1B. The ivGTT is a technique that simulates the fed state and reveals that MSG rats are glucose intolerant. Plasma glucose increments were 154% higher in MSG rats when compared to control animals ($p < 0.001$), throughout the ivGTT. Blood insulin levels were 70% higher at 2, 5, 10, and 15 min, compared to the same times in control animals ($p < 0.001$).

Insulin Secretion in Isolated Pancreatic Islets

Glucose Response

Glucose exerted more evident insulinotropic effect in islets from MSG rats. The release of insulin was 40% greater, with 8.0–16.7 m*M* of glucose in islets from MSG rats, than in the control ones ($p < 0.001$), as shown in Fig. 2.

Diazoxide Response

Diazoxide 250 μM, a K^+_{ATP} channel opener, which avoided depolarization of beta cells, inhibited by 85% the glucose

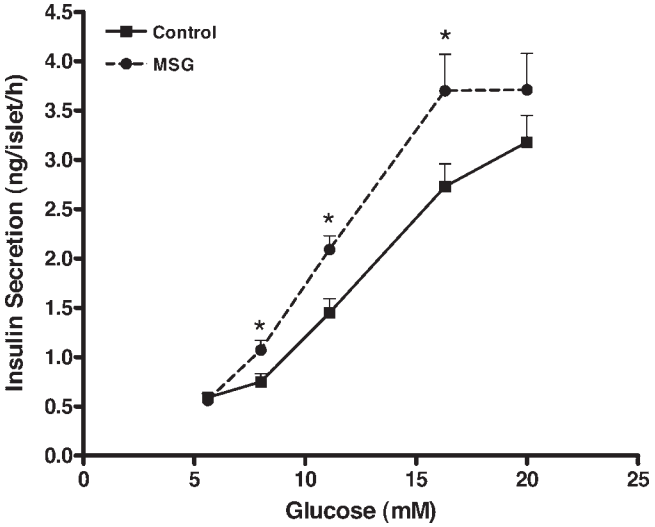

Fig. 2. Insulin secretion by islets in different glucose concentrations. Each symbol presents the mean from 20 batches of four islets incubated for 60 min in 1 mL of medium containing different glucose concentrations. Lines over the symbol represent the SEM. Differences were analyzed by Student's *t* test (*$p < 0.05$).

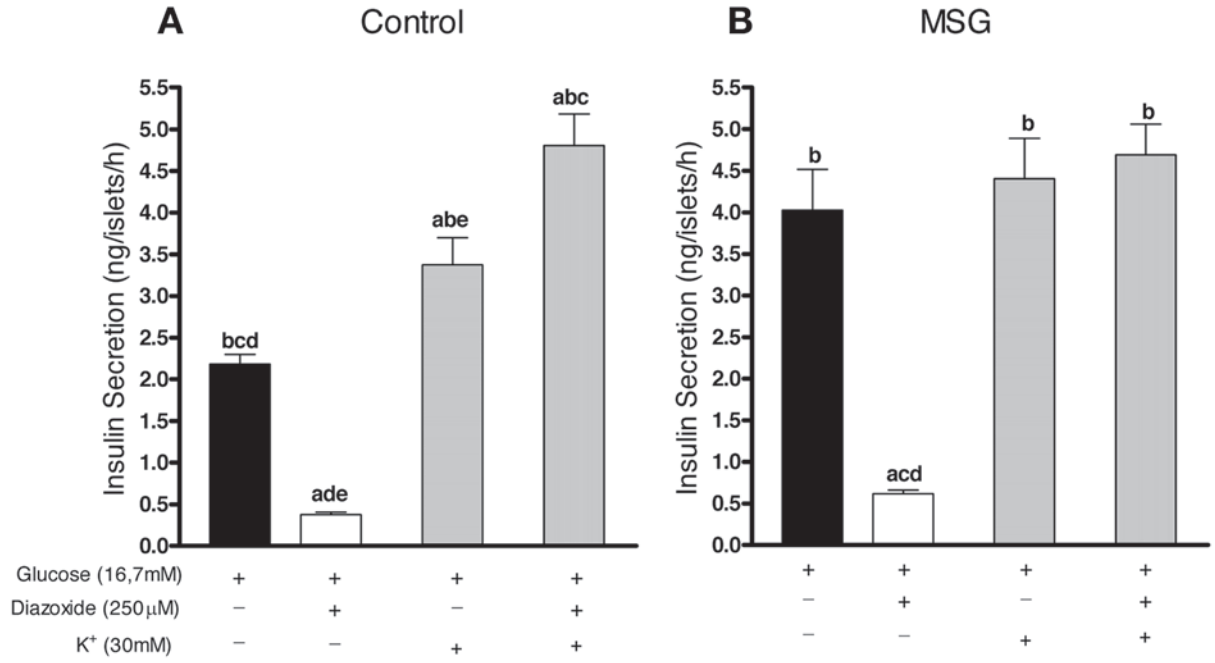

Fig. 3. Effects of diazoxide and high K^+ on induced insulin secretion in isolated pancreatic islets. Batches of four islets were incubated for 60 min in 1 mL of medium containing glucose, K^+, and diazoxide, explained in Methods. Values are mean ± SEM for 20 batches of islets. Panel **A** presents the effects in islets from lean rats and panel **B** shows the effects in islets from MSG-obese rats. The letters over the bars represent significant (ANOVA) differences with $p < 0.05$ between: *a*, glucose; *b*, glucose + diazoxide; *c*, glucose + K^+, and *d*, glucose + K^+ + diazoxide.

16.7 m*M* response in pancreatic islets from both animal groups (Figs. 3A,B). The effect of diazoxide completely eliminated glucose response when compared to the secretion induced by glucose 5.6 m*M*, as shown in Fig. 2.

High Extracellular Potassium Response

High extracellular potassium concentration (30 m*M*) impaired K^+ out fluxes and induced depolarization in beta cells. Under these conditions (Fig. 3A), insulin secretion was increased by 55% when compared to the effect of glucose 16.7 m*M* alone in islets from control rats ($p < 0.001$). The stimulatory effect of high potassium upon insulin secretion was not observed in islets from MSG rats, as shown in Fig. 3B.

Glucose Response in the Presence of Diazoxide and High Extracellular Potassium

With the K^+_{ATP} channel opened by diazoxide and inactivation of other K^+ channels by 30 m*M* of KCl, glucose 16.7 m*M* was able to increase insulin secretion by 120% and 43%

when compared to glucose alone and glucose in the presence of high potassium, respectively ($p < 0.01$), in islets from control rats. The same experimental conditions did not affect the insulin secretion stimulated by glucose or glucose plus high potassium in islets from MSG-rats, as shown in Fig. 3B.

Discussion

Neonatal treatment with MSG caused obesity in adult rats with 90 d of age, as estimated by fat accumulation in epididymal and retroperitoneal pads. Hyperinsulinemia was also observed in MSG-obese rats, without high blood glucose concentration. MSG treatment lesioned a major part of neurons localized in the hypothalamic arcuate nucleus (ARC), disarranging an important area involved in body weight control *(21)*. Obese hyperinsulinemic rodents having a genetic defect, such as Zucker rats and ob/ob mice, present with hyperphagia *(22)*, while MSG-obese rats show normo- or hypophagia *(19,23)*. However, MSG-rats accumulate increased fat in tissues *(24,25)*. Up to 210 d of age, MSG-obese animals do not develop hyperglycemia, while presenting insulin resistance *(26,27)*. MSG-obese rats present with fasting and fed hyperinsulinemia, as evoked during the ivGTT test. The same results were obtained by other authors *(28,29)*.

Hyperinsulinemia in MSG-obese rats has been attributed to insulin oversecretion *(20,30)*. As we showed, pancreatic islets from MSG-obese rats are more responsive to high glucose concentrations than islets from lean rats. However, at glucose 5.6 m*M*, which has been considered the glucose concentration of fasting, the stimulated insulin release was similar in both islet groups. Other factors, such as high parasympathetic activity, have been suggested to be involved in the cause of hyperinsulinemia in fasting, observed in MSG-obese rats *(20)*. Islets isolated from Zucker rats and ob/ob mice showed increased insulin release when stimulated by low and high glucose concentrations *(31,32)*. A higher response to glucose has also been observed in islets isolated from hypothalamic obese rats, such as those lesioned in the ventromedial area (VMH) *(33)*. Much experimental evidence supports the proposal that glucose stimulates the beta cell metabolism to trigger mechanisms that lead to the exocytosis of insulin granules *(34–37)*. It can be suggested that, independently of their origin, experimental obese rodents present alterations in the regulation of glucose-induced insulin secretion, at least partly as a result of changes in early stages of the glycolytic pathway. ATP levels have been known to couple glucose metabolism with the closing of K^+_{ATP} channel. The resulting decrease in potassium conductance leads to depolarization of the membrane with subsequent opening of voltage-dependent calcium channels. An increase in intracellular Ca^{2+} starts the insulin exocytosis *(38)*. The islets from obese Zucker rats and ob/ob mice respond to diazoxide and blockade of K^+_{ATP} channels in a manner similar to that shown by islets from lean normal rodents *(16,39)*. Glucose-induced insulin secretion was inhibited by diazoxide 250 μM in islets of MSG-obese rats with the same magnitude as in the islets of lean rats *(16)*. It has been established that 250 μM of diazoxide concentration is enough to ensure that all K^+_{ATP} channel are opened, preventing the insulin exocytosis stimulated by glucose. Our results indicate that the insulin secretion is also greatly dependent on K^+_{ATP} channel function in islets of MSG-obese rats. As in other obese rodents, the insulin oversecretion could be related to abnormalities in glycolytic pathways more than in K^+_{ATP} channel dysfunctions.

High K^+ membrane permeability is very important to determine the resting potential of beta cells. Although this permeability decreases when glucose concentration is raised, the membrane potential of beta cells remains very sensitive to changes in the equilibrium potential of K^+. Increasing extracellular potassium moves the equilibrium potential of K^+ to less-negative values, which leads a sustained depolarization. Under these conditions, $[Ca^{+2}]_i$ and insulin release are increased, as observed in the blockage of K^+_{ATP} channel *(40)*. As expected, high extracellular potassium enhanced the glucose-induced insulin release in islets from lean rats; however, the effect of depolarization with high potassium was not recorded in islets from MSG-obese rats. Our results suggest that beta cells from MSG-obese rats have abnormalities in the oscillations of membrane potential, when this islets are stimulated by glucose, as showed also in ob/ob mice *(41)*.

It has been demonstrated that insulin release stimulated by glucose can bypass the K^+_{ATP} channel pathway. Islets from normal mice, rats, and humans treated with diazoxide in the presence of depolarizing extracellular potassium concentrations still secrete insulin when stimulated by glucose. In these conditions, the magnitude of the secretion is high in comparison with glucose insulinotropic effect by itself or glucose combined with high extracellular potassium concentration *(13,14,42)*. These results support the suggestion that glucose-induced insulin secretion is a resultant from pathway-dependent and -independent K^+_{ATP} channel functions. However, the mechanisms by which glucose can control insulin release independently from changes in K^+_{ATP} channel activity and changes in beta cells' membrane potential requires stimulation of beta cells' metabolism, but does not involve increases in intracellular calcium, cAMP levels, inositol phosphate levels, or PKC activity *(7)*. Alternatively, ATP/ADP ratio controls insulin release by regulating both the activity of K^+_{ATP} channel and a much later step of stimulus-secretion coupling. The latter pathway seems to be related to the effect of glucose on the amplification of the effectiveness in intracellular calcium rise, which was provoked by the K^+_{ATP} channel pathway. The possibility that insulin oversecretion, observed in obesity, could be related to K^+_{ATP} channel alternative pathway in glucose-induced insulin secretion was tested in islets of MSG-obese rats.

Our results confirmed that islets isolated from lean rats used this pathway, while islets of MSG-obese did not. Islets from obese ob/ob mice and Zucker rats have no changes in this mechanism *(15,16)*. However, islets from diabetic GK rats, which present a reduction in glucose-induced insulin release, showed a decrease in K^+_{ATP} channel alternative pathway function *(11)*. Even though MSG-obese rats present hyperinsulinemia and normoglycemia, their pancreatic islets showed normal functioning of the K^+_{ATP} channel pathway, but absence of function in K^+_{ATP} channel-independent pathway. Alternatively, to solve this paradox, it is possible to speculate that beta cells from MSG-obese rats have enhanced glycolytic metabolism, allowing a decrease of the threshold for glucose-stimulated insulin release, through the K^+_{ATP} channel pathway function. Patients with hyperinsulinemia caused by mutations in glycolytic enzymes respond well to diazoxide *(43,44)*.

Regardless of the evidence that for precise control of insulin blood levels, beta cell must be set to trigger and amplify pathways (dependent K^+_{ATP} channel) and amplifying pathways (independent K^+_{ATP} channel) in glucose-stimulated insulin release process, our findings indicate that pancreatic islets isolated from MSG-obese rats are able to sustain insulin oversecretion, even without using K^+_{ATP} channel-independent pathway.

Materials and Methods

Animals and Obesity

Neonate Wistar rats received monosodium-L-glutamate (MSG) injections intradermally during the 5 first days of life, with a dose of 4 g/kg of body weight (BW). Control animals received equimolar saline solution. Both groups of animals were weaned at 21 d of age. All animals were housed under control conditions of the luminosity cycle of 12 h light–dark (07:00 to 19:00 h) and temperature of 21 ± 2°C. Water and standard rodent chow (Nuvital-Curitiba-Brazil) were supplied *ad libitum*. Only males were used in the experimental protocols. At 90 d, rats from both groups were anesthetized with ketamine and xylasine (55 and 8 mg/kg BW, respectively) and sacrificed by cervical dislocation. Periepididymal and retroperitoneal fat pad were removed, washed, and weighed to estimate the obesity induced by MSG treatment.

Pancreatic Islets Isolation

Isolation of islets from the rats' pancreas was performed as previously described *(45)* with adaptations. Rats from another sample were anesthetized had their abdominal wall cut and open. 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 rats. The pancreas, swollen with the collagenase solution, was quickly excised and incubated in a plastic culture bottle for 15 min at 37°C. The suspension obtained was filtered with a 0.5 mm metal mesh and washed with HBSS, including 0.12% bovine serum albumin fraction V (BSA) in five continuous washings. The islets were collected with the aid of a microscope. At least three rats were used to obtain a pancreatic rat pool for each animal group.

Islets Incubation

Batches of four islets were preincubated in the presence of 1 mL of normal Krebs solution containing 120 m*M* NaCl; 4.8 m*M* KCl; 2.5 m*M* $CaCl_2$; 1.2 m*M* $MgCl_2$, and 24 m*M* $NaHCO_3$. This solution was gassed with O_2/CO_2 (95/5%) to maintain pH 7.4 and supplemented with bovine albumin fraction V (0.12%) for 60 min in the presence of glucose 5.6 m*M*. After this period, the islets were submitted for another 60 min of incubation in glucose stimulatory condition (16.7 m*M*) with normal or high K^+ (30 m*M*) Krebs solution. Both experimental conditions were also evaluated in the presence or absence of diazoxide (250 μM). When concentration of KCl was increased to 30 m*M*, NaCl concentration was decreased to 94.8 m*M* to maintain isoosmolarity. In another protocol, the islets were submitted to 60 min of incubation in the presence of normal Krebs with different glucose concentrations (5.6, 8.0, 11.1, 16.7, and 20.0 m*M*). Samples of incubation media were taken and stored frozen until assay to measure the secreted insulin by radioimmunoassay (RIA) *(46)*.

Glucose Tolerance Test

Under anesthesia, a silicone cannula was implanted into the remaining rats' left jugular and attached to the animals' back. To avoid blood clotting, heparinized saline (50 IU heparin in saline 0.9%/mL) was previously injected into the cannula. Intravenous glucose tolerance tests (ivGTT) were performed at 8:00 after a 12 h fast (19:00–07:00) and without any anesthesia rats were injected with a glucose load (1 g glucose/kg BW) through the cannula. Blood samples (300 μL) were collected from the same cannula sequentially before glucose load (*t*0) and 5 (*t*5), 10 (*t*10), 15 (*t*15), 20 (*t*20), 25 (*t*25), and 30 (*t*30) min after the injection of glucose *(47)*. They were then centrifuged, and the plasma was separated and stored at 20°C for posterior dosage of glucose concentration by the glucose oxidase method (Kit-Bio Diagnostic Chemistry Industry®) and insulin by RIA. The State University of Maringá Ethical Committee for Animal Experiments approved the described protocols.

Chemicals

[^{125}I]Insulin human recombinant was acquired from Pharmacia (São Paulo, BR). Routine reagents were purchased from Sigma unless otherwise specified.

Statistical Analysis

Results were given as mean ± SEM. Data were submitted to Student's *t* test or variance analysis (ANOVA). In the case of analyses with a significant *F*, the differences

between means were evaluated by Bonferroni *t* test. *p* values less than 0.05 were considered statistically significant. The tests were performed using GraphPad Prism version 3.02 for Windows (GraphPad Software®).

Acknowledgment

We gratefully acknowledge financial support CNPq (Brazilian Council for Scientific and Technological Development).

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