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Neuroendocrine, Metabolic, and Immune Functions during the Acute Phase Response of Inflammatory Stress in Monosodium L-Glutamate-Damaged, Hyperadipose Male Rat

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Key Words
ACTH · Glucocorticoid · Cytokines · Leptin · Insulin

Abstract
In rats, neonatal treatment with monosodium L-glutamate (MSG) induces several metabolic and neuroendocrine abnormalities, which result in hyperadiposity. No data exist, however, regarding neuroendocrine, immune and metabolic responses to acute endotoxemia in the MSG-damaged rat. We studied the consequences of MSG treatment during the acute phase response of inflammatory stress. Neonatal male rats were treated with MSG or vehicle (controls, CTR) and studied at age 90 days. Pituitary, adrenal, adipo-insular axis, immune, metabolic and gonadal functions were explored before and up to 5 h after single sub-lethal i.p. injection of bacterial lipopolysaccharide (LPS; 150 μg/kg). Our results showed that, during the acute phase response of inflammatory stress in MSG rats: (1) the corticotrope-adrenal, leptin, insulin and triglyceride responses were higher than in CTR rats, (2) pro-inflammatory (TNFα) cytokine response was impaired and anti-inflammatory (IL-10) cytokine response was normal, and (3) changes in peripheral estradiol and testosterone levels after LPS varied as in CTR rats. These data indicate that metabolic and neroendocrine-immune functions are altered in MSG-damaged rats. Our study also suggests that the enhanced corticotrope-corticoadrenal activity in MSG animals could be responsible, at least in part, for the immune and metabolic derangements characterizing hypothalamic obesity.

Introduction

The effects of monosodium L-glutamate (MSG) administration to neonatal animals have been described [1, 2]. This treatment induces morphological, behavioral, and endocrine abnormalities, such as growth disturbances, self-mutilation, pseudo-obesity and hypogonadism [2–4]. Several studies have documented a severe loss of catecholaminergic and peptidergic neurons in retina and hypothalamic arcuate nucleus (ARC) [4–6]. The neuronal loss impacts on several functions, including energy balance [7–9], pituitary [10–12] and adrenal [13] activity. A conspicuous effect of MSG-induced hypothalamic damage is an enhanced response of the median eminence neuron terminals to specific and non-specific stimuli [10–12]. The extensive brain damage has been used to explain the alteration of several neuroendocrine functions in this model [14–16].

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Acute inflammatory stress affects anterior pituitary hormone secretion indirectly by affecting the relevant hypothalamic centres [17–21], which are deranged in the MSG animal model [4–6]. The ARC is a pivotal structure involved in the regulation of food intake and energy expenditure [22] and cells of this region are sensitive to leptin [22]. Amongst other effects, neonatal MSG treatment of rats affects adipocyte function, such that the animals are hyperleptinemic [23] due to both hyperadiposity and enlarged adipocyte size [24]. Moreover, MSG rats are partly refractory to leptin inhibition of food intake and body weight gain [25], and adrenocortical leptin resistance seems to be directly related to enhanced glucocorticoid production [13, 26].

The aim of the present study was to investigate whether MSG-induced hypothalamic damage has any impact on neuroendocrine, immune and metabolic functions during the acute phase of response to inflammatory stress.

Material and Methods

Animals and Treatment

Adult male and female Sprague-Dawley rats were allowed to mate in colony cages in a room with light (lights on from 7:00 to 19:00 h) and temperature (22°C) control. Rat chow and water were available ad libitum. Pregnant rats were transferred to individual cages. Beginning on day 2 after parturition, newborn pups were injected i.p. with either 4 mg/g BW MSG (Sigma Chemical CO., St. Louis, Mo.) dissolved in sterile 0.9% NaCl or 10% NaCl (litter-mate controls; CTR) once every 2 days until 10 days of age [12]. Rats were weaned and sexed at 21 days of age. Body weight and food intake of male rats was recorded daily. CTR and MSG rats were used for experimentation on day 90 of life with mean (± SEM) body weights of 473.03 ± 10.01 and 363.64 ± 8.55 g for CTR and MSG groups, respectively ($p < 0.05$; n = 30 per group). MSG-injected animals were screened for effectiveness of treatment by macroscopic observation of degeneration of the optic nerves at the time of euthanasia. Animals were euthanized according to protocols for animal use, in agreement with NIH Guidelines for care and use of experimental animals. All experimentation received approval from our Institutional Animal Care Committees.

Experimental Design

All rats were implanted with i.v. catheters, under light ketamine anesthesia, and were then left undisturbed in individual cages, with food and water available ad libitum for 48 h prior to experimentation. On the morning (8:00 h) of the experimental day, the rats were bled before treatment (2 and 5 min pre-treatment; sample time 0 h) and at 1, 2, 3, 4 and 5 h after i.p. injection of bacterial lipopolysaccharide (LPS; 150 μg/kg) [27]. This protocol is similar to the one previously validated by our group, although with minor modifications [28]. Blood samples taken were replaced by a similar volume of red blood cells resuspended in artificial plasma. Plasma samples were split in several aliquots and kept frozen (–80°C) until assayed. Plasma concentrations of glucose [27], triglycerides [27], ACTH [29], corticosterone (B) [30], leptin [27], insulin [31], TNFα [28], IL-10 [28], estradiol [32] and testosterone [32] were determined by specific, previously validated assays.

Statistics

Data are expressed as means (± SEM) and were analyzed by two-way ANOVA with repeated measures, followed by Student-Newman-Keul's test for comparison of means. Results of the area under the curve (AUC) of metabolite levels were analyzed by two-way ANOVA [33].

Results

Carbohydrate and Lipid Responses to Endotoxin in Normal and Hyperadipose Rats

Table 1 shows the results of circulating glucose and triglyceride concentrations before and several times after LPS injection. As shown, circulating glucose levels were similar across groups, and control and treated animals displayed no significant time-dependent changes in plasma glucose concentrations (AUC 0.71 ± 0.06 vs. 0.79 ± 0.08 g/l·5 h, for CTR and MSG rats, respectively). As shown in table 1, basal triglyceride levels were also similar in both groups of animals. In CTR rats, lipid levels were significantly ($p < 0.05$ vs. respective basal levels) reduced at 1 and 2 h after LPS, and returned to pre-treatment values at 3 h after treatment. Conversely, in MSG rats, lipid levels showed a significant ($p < 0.05$) increase in a time-related fashion between 1 and 5 h post-LPS. The AUC of triglyceride values was significantly ($p < 0.01$) higher in MSG (7.17 ± 0.55 g/l·5 h) than in CTR (0.01 ± 0.005 g/l·5 h) rats.

Corticotrope-Adrenal Axis Response to LPS in CTR and MSG Rats

Basal and the early peak (1 and 2 h post-LPS) plasma ACTH levels (fig. 1a), were similar in both groups. Values 3 and 4 h post-LPS were also similar in CTR and MSG, but at 5 h plasma ACTH concentrations were higher ($p < 0.05$) in MSG treated animals than in CTR animals. The AUC of circulating ACTH concentrations was higher ($p < 0.05$) in MSG treated rats (819.27 ± 48.15 pg/ml·5 h) than in CTR (493.31 ± 22.77 pg/ml·5 h) rats.

Figure 1b shows that both groups developed a significant time-dependent adrenal response to endotoxin treatment. As depicted, corticosterone levels were greater ($p < 0.05$) in MSG-treated than in CTR animals 0, 1, 2, 3 and 5 h relative to treatment. As with ACTH, the AUC of

Table 1. Circulating glucose and triglyceride levels

	Glucose, g/l		Triglyceride, g/l	
	CTR	MSG	CTR	MSG
0 h	1.36 ± 0.13	1.24 ± 0.06	1.76 ± 0.05	1.43 ± 0.29
1 h	1.52 ± 0.11	1.26 ± 0.08	1.31 ± 0.17^{+}	1.92 ± 0.39^{+}
2 h	1.23 ± 0.07	1.01 ± 0.11	1.34 ± 0.16^{+}	2.05 ± 0.29$^{+,*}$
3 h	1.11 ± 0.11	1.04 ± 0.05	1.62 ± 0.25	2.62 ± 0.40$^{+,*}$
4 h	1.16 ± 0.08	1.15 ± 0.06	1.44 ± 0.17	3.22 ± 0.29$^{+,*}$
5 h	1.21 ± 0.05	1.22 ± 0.07	1.34 ± 0.26	3.15 ± 0.59$^{+,*}$

Levels shown are before (time 0 h) and several hours after LPS i.p. administration in control (CTR) and hypothalamo-damaged (MSG) male rats. Values are the mean ± SEM (n = 7 rats per group).

$^{+}$ $p < 0.05$ vs. time 0 h values in the same group; * $p < 0.05$ vs. CTR values on similar time.

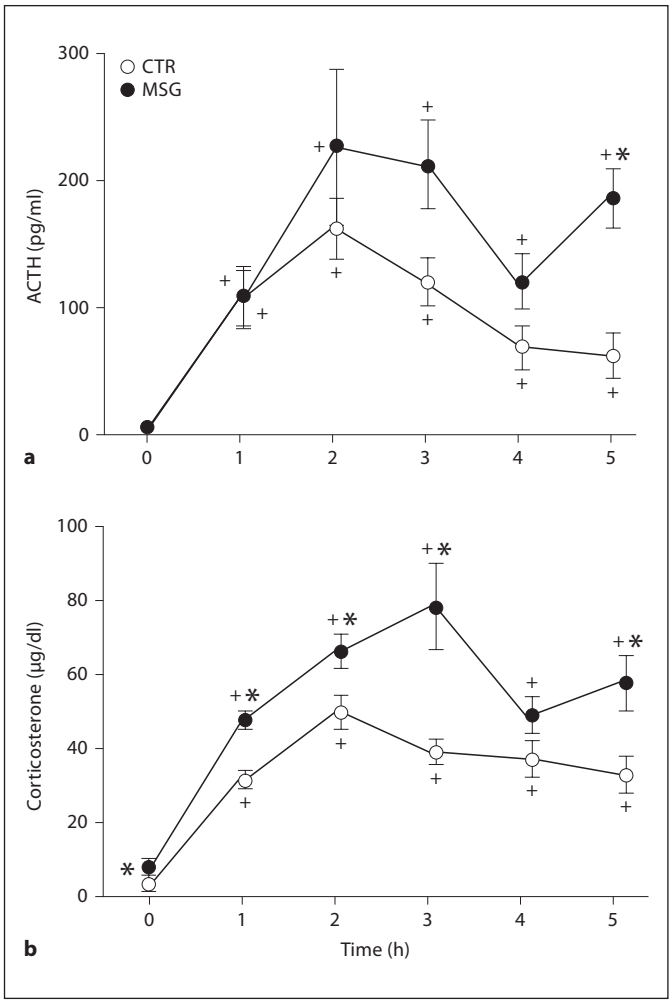

Fig. 1. Plasma circulating levels of ACTH (**a**) and corticosterone (**b**) before (time 0 h) and several hours after i.p. bacterial lipopolysaccharide administration (LPS) in normal (CTR) and MSG-damaged male rats. Values are the mean ± SEM (n = 7 rats per group). $^{+}$ $p < 0.05$ vs. time 0 h values in the same group; * $p < 0.05$ vs. CTR values on similar time.

plasma corticosterone levels were significantly ($p < 0.05$) higher in MSG vs. CTR rats (266.51 ± 16.12 vs. 177.34 ± 8.83 μg/dl·5h, respectively).

Pro- and Anti-Inflammatory Cytokine Responses to Endotoxemia

Figure 2a shows circulating TNFα levels before and after LPS administration in both groups. Although no difference among groups was found in basal values, circulating TNFα levels were significantly ($p < 0.05$) lower in MSG than in CTR rats 1 h post-LPS. After 2 h, circulating levels of TNFα were similar in both groups. Also the AUC of circulating pro-inflammatory cytokine levels was significantly ($p < 0.05$) lower in MSG-treated (9,027.14 ± 376.11 pg/ml·5 h) than in CTR (14,437.73 ± 645.57 pg/ml·5 h) rats.

Figure 2b shows the pattern of IL-10 levels before and post-LPS treatment. LPS increased levels in both groups, but the response was longer in the CTR animals ($p < 0.05$). AUCs of IL-10 plasma concentrations were similar in both groups (457.78 ± 44.71 and 440.27 ± 40.13 pg/ml·5 h, in CTR and MSG rats, respectively).

Adipo-Insular Axis Response to LPS in Normal and Hyperadipose Rats

Figure 3a shows plasma leptin concentrations before and after LPS or vehicle treatment. MSG-treated rats were hyperleptinemic in the basal condition, and 1, 2, 3 and 5 h post-LPS ($p < 0.05$ vs. respective CTR values). Plasma leptin levels were elevated in both groups following LPS treatment, being significantly ($p < 0.05$) higher than the respective basal values 3 h after treatment (both groups) and 5 h after treatment (MSG rats only). AUC of plasma leptin levels were higher ($p < 0.03$) in MSG treated rats (69.66 ± 5.65 ng/ml·5 h) than in CTR rats (38.74 ± 2.81 ng/ml·5 h).

Figure 3b shows plasma insulin concentrations, which were significantly ($p < 0.05$) higher than baseline in both groups 3, 4 and 5 h post-LPS. As with plasma leptin values, basal insulin levels and levels at 2, 3 and 4 h after LPS treatment were significantly ($p < 0.05$) higher in MSG-

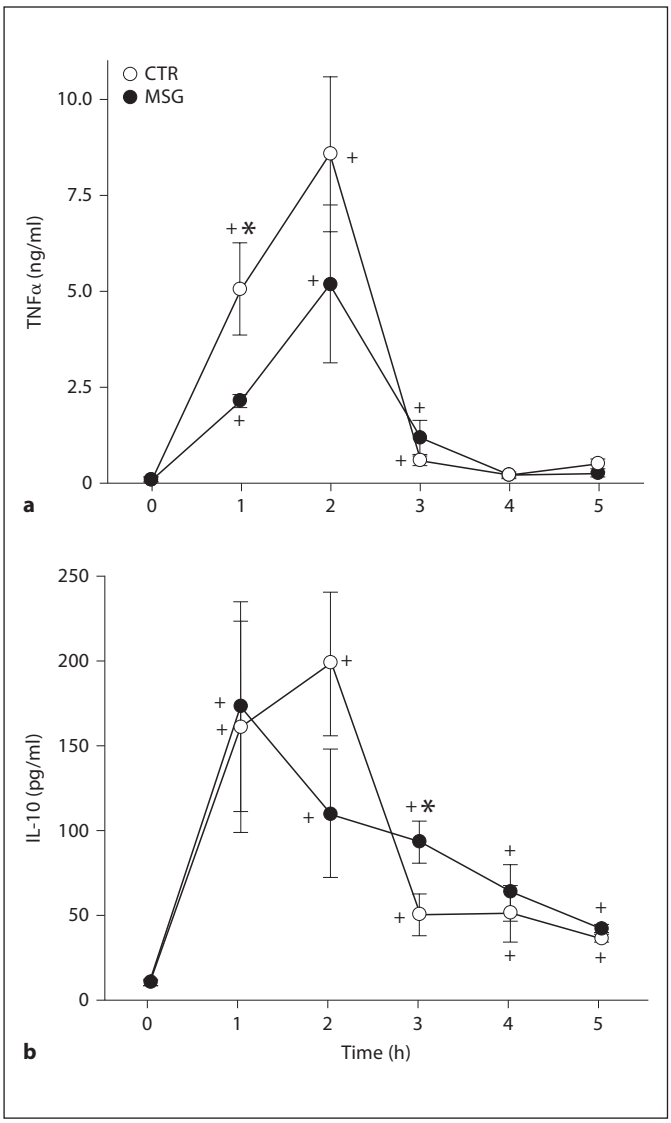

Fig. 2. Peripheral levels of TNFα (**a**) and interleukin-10 (**b**) before (time 0 h) and several hours after i.p. LPS treatment in CTR and MSG-damaged male rats. Values are the mean ± SEM (n = 7 rats per group). $^{+}$ $p < 0.05$ vs. time 0 h values in the same group; * $p < 0.05$ vs. CTR values on similar time.

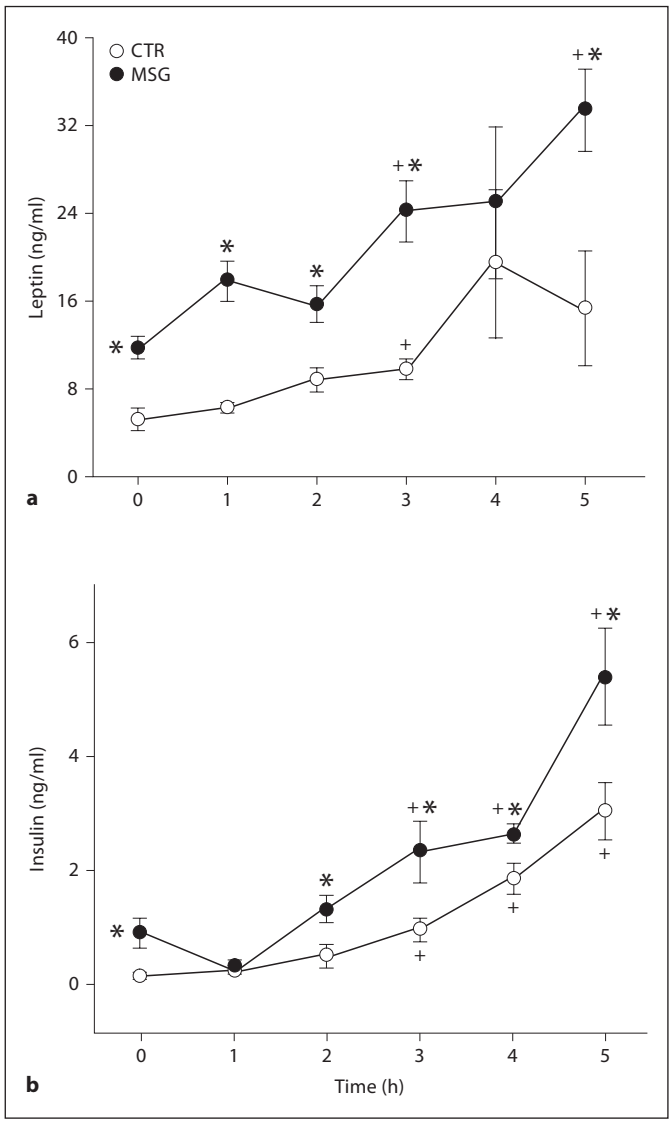

Fig. 3. Circulating concentrations of insulin (**a**) and leptin (**b**) before (time 0 h) and after (1–5 h) i.p. bacterial endotoxin injection in CTR and MSG-damaged male rats. Values are the mean ± SEM (n = 7 rats per group). $^{+}$ $p < 0.05$ vs. time 0 h values in the same group; * $p < 0.05$ vs. CTR values on similar time.

treated rats than in CTR rats. AUC for insulin concentrations were higher ($p < 0.05$) in MSG-treated rats (8.04 ± 0.74 ng/ml·5 h) than in CTR (5.79 ± 0.65 ng/ml·5 h) animals.

Changes in Sex-Steroid Circulating Levels during Endotoxic Shock

Plasma concentrations of E2 were significantly ($p < 0.05$) lower in MSG-treated rats than in CTR rats at only 1 experimental timepoint, being 2 h post-LPS (fig. 4a). In MSG-treated rats, plasma E2 levels were significantly ($p < 0.05$) higher than basal values 4 and 5 h post-LPS treatment. In CTR rats, plasma E2 levels were higher ($p < 0.05$) than basal at the 5 h timepoint. There were no differences between groups in AUC for plasma E2 levels (67.81 ± 4.59 vs. 67.23 ± 6.88 pg/ml·5 h, in CTR and MSG-treated rats, respectively).

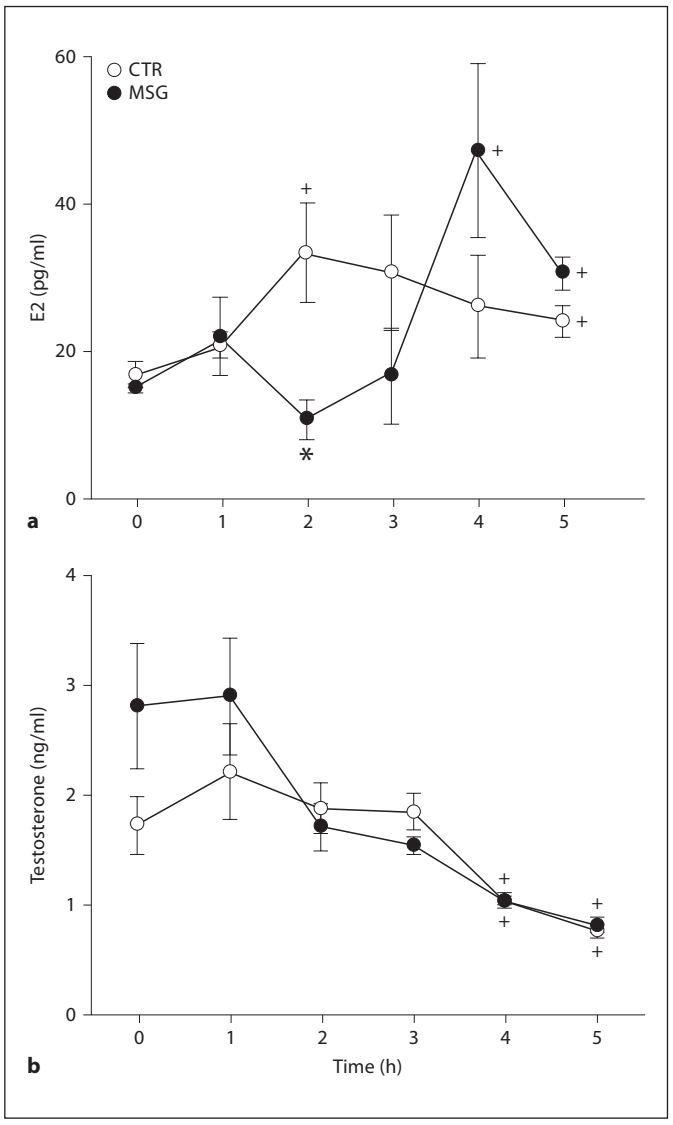

Fig. 4. Plasma levels of estradiol (**a**) and testosterone (**b**) before (time 0 h) and several hours after i.p. LPS administration in CTR and MSG-damaged male rats. Values are the mean ± SEM (n = 7 rats per group). $^{+}$ $p < 0.05$ vs. time 0 h values in the same group; * $p < 0.05$ vs. CTR values on similar time.

Plasma testosterone levels were similar in both groups throughout the experiment (fig. 4b). In both groups, samples taken on times 4 and 5 h showed values that were significantly ($p < 0.05$) lower than the respective basal values. AUC for plasma androgen concentrations were lower ($p < 0.01$) in MSG treated rats (–3.31 ± 0.19 ng/ml·5 h) than in CTR (0.81 ± 0.04 ng/ml·5 h) rats.

Discussion

Whereas the MSG rat model has been extensively studied, the responses to inflammatory stress have not been examined previously. The present study indicates that MSG-induced neonatal hypothalamic damage results in an overall alteration of neuroendocrine, immune and metabolic functions in adulthood.

We have reported previously that HPA axis hyperactivity in MSG rats is due, at least in part, to leptin resistance of the hypothalamo-pituitary-adrenal (HPA) axis. In fact, as a consequence of prolonged hyperleptinemia [13] a down-regulation of adrenal Ob-Rb expression [26] takes place. This abnormality contributes with the characteristic corticoadrenal hyperactivity [34] and reduced clearance rate of glucocorticoid in MSG animals [35]. The adult phenotype in animals treated neonatally with MSG seems to be directly dependent on elevated corticosteroid levels. Transient correction of corticoadrenal hyperfunction restores the leptin inhibitory effect on ACTH-stimulated corticosterone secretion by isolated adrenal cells in MSG treated animals [13]. In addition, we have found that bilateral adrenalectomy normalizes adipose mass and function [30]. In the present study, we have shown that HPA axis hyperactivity in MSG rats is also evident during the acute-phase response to inflammatory stress. LPS-induced glucocorticoid hypersecretion could also be responsible for the impaired TNFα response to endotoxin stimulus observed in MSG animals, since glucocorticoids are inhibitors of cytokine-secreting immune cells [36]. Although the adipocyte is a source of TNFα [37], the relatively obese MSG-treated rats had normal basal circulating levels of this cytokine, which is similar to results from mice treated neonatally with MSG [38]. Conversely, MSG-treated rats displayed normal IL-10 responses to LPS, thus indicating that their anti-inflammatory response remained despite treatment.

Regarding the adipo-insular axis response to LPS, it is well known that this stimulus enhances the secretion of leptin [28] and insulin [39] in normal rats. We found that the response was exacerbated in MSG rats. Moreover, hyperleptinemic (MSG) rats also displayed hyperinsulinemia in basal condition. These results concur with those from previous in vivo studies from our laboratory [30], and with in vitro studies suggesting an enhanced parasympathetic regulation of pancreatic activity [40]. Moreover, hyperinsulinemia and glucose intolerance in MSG rats can be overridden after normalization of circulating glucocorticoid levels by either adrenalectomy,

combined with corticosterone replacement therapy, or bilateral adrenal enucleation [13, 24, 30].

We have previously observed [13] that hyperleptinemia and enhanced glucocorticoid secretion precede (age 30 days) the establishment of a significant hyperinsulinemia (age 90 days) [41 and present data]. Thus the increased adipogenic signal (glucocorticoid) [42] seems to play a key role in the pathophysiological events characterizing the phenotype of MSG rats. Long-term exposure to high circulating leptin levels not only induces leptin resistance (e.g. at the adrenal level [30]) but could also alter insulin signaling [43], leading to insulin resistance in MSG rats [24]. In relation to the intriguing lack of peripheral glucose response to increased insulin levels post-LPS treatment, it should be noted that LPS treatment enhances the secretion of IL-1 and TNFα and increases pancreatic insulin secretion. In addition, glucagon is released as a counter-regulatory signal and the ratio of peripheral insulin:glucagon concentrations remains similar to that displayed by saline-treated rats. IL-1 is considered a key element in the induction of fever and hyperinsulinemia in LPS-treated rats, with unchanged glycemic status [44]. Moreover, in endotoxemic animals there is an increase in glucocorticoid levels that precedes the change in insulin levels, so the former could be a causative factor that operates to maintain glucose homeostasis [45]. This may be a phenomenon that is vital to the survival of an individual during shock. Additionally, it has been showed that MSG rats develop reduced lipolytic and enhanced lipogenic activities across time [46]. These observations could explain the increase in triglycerides levels found shortly after LPS [47–49] treatment in MSG rats, being a protective mechanism against infection [50].

Regarding the variations in circulating sex-steroid levels post-LPS, we observed that both groups showed similar patterns of change. These were characterized by a time-dependent increase in estradiol, and concomitant reduction in testosterone peripheral levels. These results agree with previous data [51], and provide an evidence of increased testicular aromatization of the androgen to estrogen during endotoxemia [52, 53]. Thus, our study provides evidence that testicular aromatase activity remains intact in MSG rats. This creates benefits for the body's overall defense mechanism by allowing an enhanced, estradiol-dependent, immune response to infection [54].

In conclusion, our data demonstrate that many of the defense mechanisms of the host that are crucial for the survival of an organism during endotoxemia, are disturbed in this model of neonatal MSG treatment which leads to obesity in rats. Because the main site of MSG action is the ARC, cells that produce NPY [26], POMC [55] and GHRH [56] may be impaired. The animal model is characterized, amongst other things, by hypophagia, hyperadiposity, reduced body weight, stunted growth and several behavioral abnormalities [13, 23, 26, 57]. Some of the disrupted mechanisms reported in the present study could be due to perturbation of NPY cell function [58–60].

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