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Dual Effects of Hyperprolactinemia on Carrageenan-Induced Inflammatory Paw Edema in Rats

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Key Words
Prolactin · Domperidone · Dopamine · Inflammation

Abstract
Objectives: The effects of short-term 5-day and long-term 30-day hyperprolactinemia induced by domperidone (1.7 mg/kg/day, s.c.) or ectopic pituitary graft on the acute inflammatory response induced by carrageenan were evaluated in male rats. Both models of hyperprolactinemia effectively increased serum prolactin (PRL) levels. ***Methods:*** The volume in milliliters of inflammatory edema was measured by plethysmography 1, 2, 3, 4, 6, 8 and 24 h after carrageenan injection. The areas under the inflammatory time-response curves were compared. Additionally, the effects of hyperprolactinemia on body weight and serum corticosterone levels were evaluated. ***Results:*** In both domperidone-treated and pituitary graft-implanted animals, short-term 5-day hyperprolactinemia increased the inflammatory response, while long-term 30-day hyperprolactinemia had anti-inflammatory effects. Body weight was not affected by either short- or long-term hyperprolactinemia. ***Conclusion:*** These results show that PRL has biphasic effects on the carrageenan-induced inflammatory response.

Introduction

Prolactin (PRL) is functionally involved in osmoregulation, growth and development, reproduction and metabolism of carbohydrates and lipids. The list of effects attributed to this hormone includes actions on the reproductive system [1–3], modulation of salt and water transport [4], induction of maternal behavior [5, 6] and regulation of sexual behavior [2, 7, 8]. This hormone also has reported effects on the immune system in mammals [9–11]. PRL has the ability to promote cell growth and differentiation in several tissues and has been shown to restore immune competence in hypophysectomized mammals [12–14]. Conversely, inhibition of endogenous PRL secretion with bromocriptine results in immunosuppression [14, 15].

PRL is unique among the adenohypophyseal hormones, and its effects depend on dose, time of administration and the physiological status of the animal [16]. PRL exerts various effects on seemingly unrelated target organs and can have opposite effects on the same tissue. For instance, in vitro macrophage activity varies according to time of incubation and concentration of PRL [11]. Moreover, current evidence indicates that the neuroendocrine system has significant regulatory effects on im-

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mune-inflammatory reactions; for example, PRL stimulates the production of leukocytes, including lymphocytes, to sustain immunocompetence. Thus, the neuroendocrine regulation of the inflammatory response is important for immune homeostasis [17].

PRL is also able to influence the development of inflammatory processes, evaluated in the rat carrageenan-induced foot edema model [18]. Briefly, the acute inflammatory reaction is characterized by exudation of fluid and plasma proteins, leading to formation of local edema consisting of leukocyte-dependent and leukocyte-independent components [19]. These vascular changes are produced by different mediators that act mainly by increasing the microvascular permeability to macromolecules in the postcapillary venules, thus enhancing plasma protein efflux [20, 21]. Carrageenan causes a reproducible inflammatory reaction and remains the standard irritant for examining acute inflammation and anti-inflammatory drugs [22].

Recently, we found that 5 days of domperidone-induced hyperprolactinemia increased the volume of inflammatory edema, suggesting that short-term hyperprolactinemia has proinflammatory effects. Because such an effect was not observed in long-term hyperprolactinemic animals, PRL-induced tolerance seems likely. Those data suggested that short-term hyperprolactinemia may act as a protective factor in rats subjected to acute stress, and that hyperprolactinemia and stress interact differentially according to the time elapsed [23].

The present study investigated the short- and long-term effects of hyperprolactinemia on a carrageenan-induced inflammatory response.

Materials and Methods

Animals

Male Wistar rats aged 80–110 days and weighing 240–285 g were used. The animals were housed at constant temperature (23 ± 2°C) and humidity (70%) under a fixed 12-hour light/dark cycle (lights on at 06.00 h) with free access to food and water. All procedures were performed in strict accordance with the guidelines of the Animal Committee of the Colegio Brasileiro de Experimentação Animal and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. We attempted to minimize the number of rats used, and every effort was made to ensure that no rat suffered unnecessarily.

Experimental Hyperprolactinemia

Hyperprolactinemia was induced by domperidone administration or by pituitary graft.

Domperidone Administration

Hyperprolactinemia was induced by domperidone administration. Although domperidone does not cross the blood-brain barrier, it acts on the hypophysis, increasing PRL secretion [24, 25]. Domperidone was administered at a dose of 1.7 mg/kg (s.c.) 3 times per day (07.30, 15.00 and 22.00 h). The duration of treatment was 8 consecutive days or 33 consecutive days [23].

Induction of Hyperprolactinemia by Pituitary Graft

Three pituitary glands obtained from adult female rats were implanted under the kidney capsule. The capsule was nicked, and through this small hole the 3 pituitaries from the female rat donors were pushed medially to the inferior pole using a blunt probe. Pituitaries implanted in such a fashion soon exhibit excellent vascularity. Control rats of the same age were sham operated by opening the kidney pocket. The completeness of graft acceptance was determined for each animal by necropsy at the end of the experiment.

Carrageenan-Induced Paw Edema

Paw edema was induced by a subcutaneous injection of 0.1 ml of 1% carrageenan per rat into the left hind paw. The volume of the induced edema was measured with a plethysmometer (model 7150, Ugo Basile). Measurements were made immediately before and 1, 2, 3, 4, 6, 8 and 24 h after carrageenan injection to determine differences in paw volume up to the tibiotarsal joint [23, 26–28].

Drugs

Association anti-infection polyvalent (Pentabiótico, Wyeth) was composed of 600,000 UI of penicillin G-benzathine, 300,000 UI of crystalline penicillin G-potassium and 250 mg of streptomycin base-sulfate and was dissolved in distilled water to a volume of 5 ml. The solution was administered at a dose of 0.003 ml (i.m.) per animal, once after ectopic pituitary implant surgery.

Carrageenan (κ-carrageenan, Sigma), an inducer of inflammatory responses, was suspended in 0.5% Ringer's solution and subcutaneously injected (subplantar injection) into the cell tissue of the left paw. Sodium chloride was used as the physiologic saline solution. The dopamine D_2 receptor-specific antagonist domperidone [5-chloro-1-(1-[3-(2,3-dihydro-2-oxo-1H-benzimida-zolyl) propyl]-4-piperidinyl)-1,3-dihidro-2H-benzimidazol-2-one; Cilag, Janssen, Brazil] was suspended in saline solution (0.9% Tween 80; approximately 1:0.002, v:v) and administered subcutaneously at a dose of 1.7 mg/kg 3 times per day at 07.30, 15.00 and 22.00 h. Domperidone was dissolved in volumes that permitted injections of 1.0 ml/kg body weight. The control group of rats received volumes of vehicle equal to those of the experimental groups (1.0 ml/kg).

Serum PRL and Corticosterone Level Quantification (Radioimmunoassay)

Trunk blood samples were collected in tubes and centrifuged (250 *g* for 20 min). Sera were frozen at –20°C until being assayed for PRL and corticosterone content by means of radioimmunoassay. Serum PRL concentrations were determined by double-antibody radioimmunoassay using specific kits provided by the National Institute of Diabetes and Digestive and Kidney Diseases (Baltimore, Md., USA). The antiserum for PRL was anti-rat PRL-S9, and the reference preparations were PRL-RP3. Assay sensitiv-

ity and the intra-assay coefficient of variation for PRL were 1.5 ng and 5.5%, respectively. PRL levels were reported as ng/ml [10]. Serum corticosterone levels were measured in duplicate using a Universal Coat-a-Count kit (DPC, Los Angeles, Calif., USA). Assay sensitivity was 1.77 ng/ml, and the intra-assay coefficient of variation was 3.98%.

Experimental Design

Experiment 1

Experiment 1 examined the effects of 5-day domperidone-induced hyperprolactinemia on carrageenan-induced inflammatory paw edema in rats.

Twenty-four male Wistar rats were divided into a control group (n = 12) and an experimental group (n = 12). The experimental group was treated with domperidone (1.7 mg/kg, s.c.) 3 times per day (07.30, 15.30 and 22.00 h) for 8 consecutive days. Animals in the control group received injections of vehicle following the same schedule. At 08.00 h on day 6 of treatment, all animals were transferred to an experimentation room that was maintained at a stable temperature (25°C). The left tibiotarsal articulation was then marked with an indelible marker, and the volume of the corresponding limb was measured using plethysmography. At 10.00 h on the same day (2 h and 30 min after the first domperidone injection), both groups received carrageenan (subcutaneously) in the left plantar pad. The volume of the same limb was measured again 1, 2, 3, 4, 6, 8 and 24 h after the injection of the irritant. The volume of inflammatory edema was calculated by comparing the volume at each studied time point with the volume of the paw before carrageenan injection.

The domperidone administration protocol was followed without interruptions until 07.30 h on day 8. On the 8th day, 1.5 h after the last injection of domperidone or vehicle, all animals were weighed and decapitated, and their blood was collected for evaluation of serum corticosterone and PRL levels.

Experiment 2

Experiment 2 investigated the effects of 5-day ectopic pituitary graft-induced hyperprolactinemia on carrageenan-induced inflammatory paw edema.

Twenty-seven male rats were randomly divided into a control group (n = 12) and an experimental group (n = 15). The animals belonging to the experimental group received 2 pituitary glands under the left renal capsule. The control group was subjected to the same surgical procedure, but they received striated muscle rather than pituitary glands (sham surgery). At 08.00 h on day 6 after the surgery, the animals were transferred to the experimental room. The left tibiotarsal articulation was then marked with indelible marker, and the volume of the corresponding limb was measured using plethysmography. At 10.00 h, both groups received carrageenan in the subcutaneous tissue of the left plantar pad, and the volume of the same limb was measured again 1, 2, 3, 4, 6, 8 and 24 h after the injection of the irritant. The volume of inflammatory edema was calculated by comparing the volume at each studied time point with the volume of the paw before carrageenan injection.

On the 8th day, all animals were decapitated, and their blood was collected to determine serum PRL and corticosterone levels. Necropsy was then performed to verify the presence of the ectopic pituitary glands, and the left adrenal gland was dissected, removed and weighed. Data from animals in which the gross presence of the implanted glands was not observed were excluded from the analysis. Kidneys with confirmed presence of the pituitary glands were fixed with Bouin's solution, and histological slices were made to evaluate the viability of the implant.

Experiment 3

Experiment 3 examined the effects of 30-day domperidone-induced hyperprolactinemia on carrageenan-induced inflammatory paw edema in rats.

Twenty-four male rats were randomly divided into a control group (n = 12) and an experimental group (n = 12). The experimental group was treated with domperidone for 33 days as described above. Animals in the control group received injections of vehicle. At 08.00 h on the 31st day of treatment, all animals were transferred to the experimental room, which was maintained at a stable temperature of 25°C. The left tibiotarsal articulation was then marked with indelible marker, and the volume of the corresponding limb was measured using plethysmography. At 10.00 h on the same day (2 h and 30 min after domperidone injection), both groups received carrageenan in the subcutaneous cellular tissue in the left plantar pad. The volume of the same limb was measured again 1, 2, 3, 4, 6, 8 and 24 h after the injection of the irritant. The volume of inflammatory edema was calculated by comparing the volume at each studied time point with the volume of the paw before carrageenan injection.

The domperidone administration protocol was continued without interruption until 07.30 h on the 33rd day. One hour and 30 min after the last injection, all of the animals were decapitated, and their blood was collected to determine serum hormone levels. Additionally, body weights were measured throughout the 30 days of treatment.

Experiment 4

Experiment 4 examined the effects of 30-day ectopic pituitary graft-induced hyperprolactinemia on carrageenan-induced inflammatory paw edema in rats.

Nineteen male Wistar rats were divided into a control group (n = 10) and an experimental group (n = 9). The experimental group received an implant of 2 pituitary glands under the renal capsule. The control group was subjected to the same surgical procedure, but they received striated muscle rather than pituitary glands (sham surgery). On the 31st day, the animals received the same manipulation and procedures as the animals from experiment 2. On the 33rd day, all animals were decapitated, and their blood was collected to determine serum corticosterone and PRL levels. Necropsy was then performed to verify the presence of the ectopic pituitary glands. Data from animals in which the gross presence of the implanted glands was not observed were excluded from the analysis. Variations in body weight during the 30 days of treatment were also analyzed.

Statistical Analysis

Because the inflammatory edema data did not show a normal distribution at every time point, the area under the curve of the inflammatory volume was calculated individually and compared between groups using an unpaired Student's t test. Serum hormone levels were analyzed using the Mann-Whitney U test. Values of $p < 0.05$ were considered statistically significant. Overall variations in body weight during the experimental period were analyzed using Student's t test.

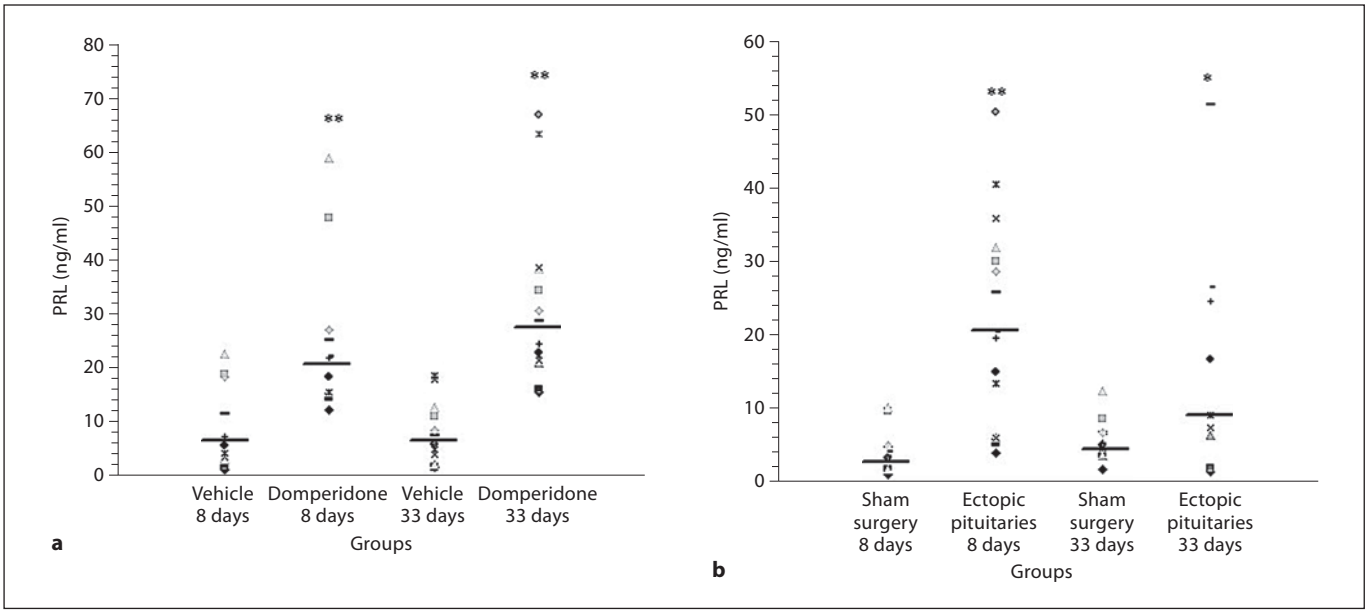

Fig. 1. Serum PRL levels in rats treated with domperidone (**a**) or implanted with ectopic pituitaries (**b**). The dispersion graphs represent the variation from the median (horizontal line) for each group. * $p < 0.05$, ** $p < 0.01$ compared to the control groups.

Table 1. Body weight of rats treated for 33 days with domperidone or vehicle or implanted with pituitary glands under the renal capsule

	Domperidone	Vehicle	Ectopic pituitary gland implant	Sham surgery
Rats, n	12	12	9	10
Initial weight, g	255.4 ± 6.9	260.3 ± 7.0	243.0 ± 10.2	240.0 ± 8.6
Weight on day 8, g	259.5 ± 7.8	265.7 ± 6.9	251.0 ± 9.1	260.5 ± 7.0
Weight on day 33, g	278.2 ± 7.4	291.9 ± 9.0	328.3 ± 8.2	341.0 ± 7.1

Values are shown as means ± standard errors. No significant differences were observed between experimental groups and their corresponding controls ($p > 0.05$, unpaired t test).

Table 2. Serum corticosterone levels in rats 8 or 33 days after the start of treatment with domperidone or pituitary graft implant under the renal capsule

Duration of treatment	Domperidone	Vehicle	Ectopic pituitary gland implant	Sham surgery
8 days	299.6 ± 43.1 (n = 12)	289.8 ± 47.7 (n = 12)	259.5 ± 41.6 (n = 12)	199.8 ± 27.3 (n = 12)
33 days	237.4 ± 20.8 (n = 12)	242.4 ± 28.6 (n = 12)	255.9 ± 50.3 (n = 9)	258.9 ± 45.1 (n = 12)

Values are shown as means ± standard errors (in ng/ml).

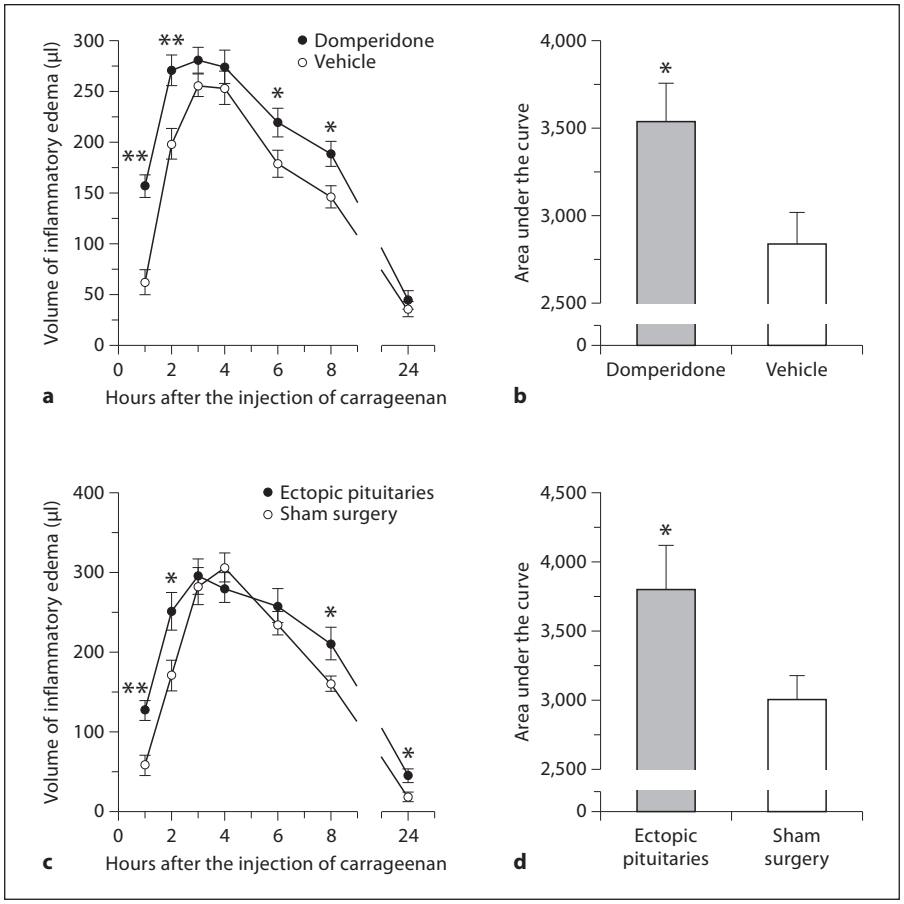

Fig. 2. Effects of 5-day domperidone-induced hyperprolactinemia (**a**, **b**) and 5-day ectopic pituitary graft-induced hyperprolactinemia (**c**, **d**) on carrageenan-induced inflammatory paw edema. **a**, **c** Kinetics. **b**, **d** Area under the curve. Data are expressed as means ± standard errors. * $p < 0.05$, ** $p < 0.01$.

Results

Subcutaneous administration of domperidone (1.7 mg/kg, s.c., 2 times per day) was shown to effectively increase circulating PRL levels (fig. 1). The variations in body weight during the 30 days of treatment are shown in table 1. Body weight was not affected by domperidone treatment or pituitary graft at any of the evaluated time points ($p > 0.05$). Serum corticosterone levels were not significantly different between groups ($p > 0.05$; table 2).

Experiment 1

The volume of inflammatory edema was greatest in rats subjected to domperidone treatment for 5 days (1.7 mg/kg, s.c., 3 times per day). The area under the curve of the volume of inflammatory edema was larger in hyperprolactinemic animals than in controls ($p < 0.05$; fig. 2a, b). Therefore, animals treated with domperidone exhibited a greater edematogenic response than the control group.

Experiment 2

Figure 2c, d shows the inflammatory volumes induced by carrageenan in rats with 2 ectopic pituitary glands and in the sham-operated group. The areas under the curve were different between the groups ($p < 0.05$). Inflammatory paw edema was significantly greater on day 5 in hyperprolactinemic animals.

Experiment 3

Animals treated for 30 days with domperidone consistently showed significantly lower volumes of inflammatory edema than those that received vehicle, as demonstrated by the area-under-the-curve analysis ($p < 0.01$; fig. 3a, b).

Experiment 4

The area under the curve for animals with 30-day graft-induced hyperprolactinemia was significantly smaller than the area observed for control sham-operated animals ($p < 0.05$; fig. 3c, d).

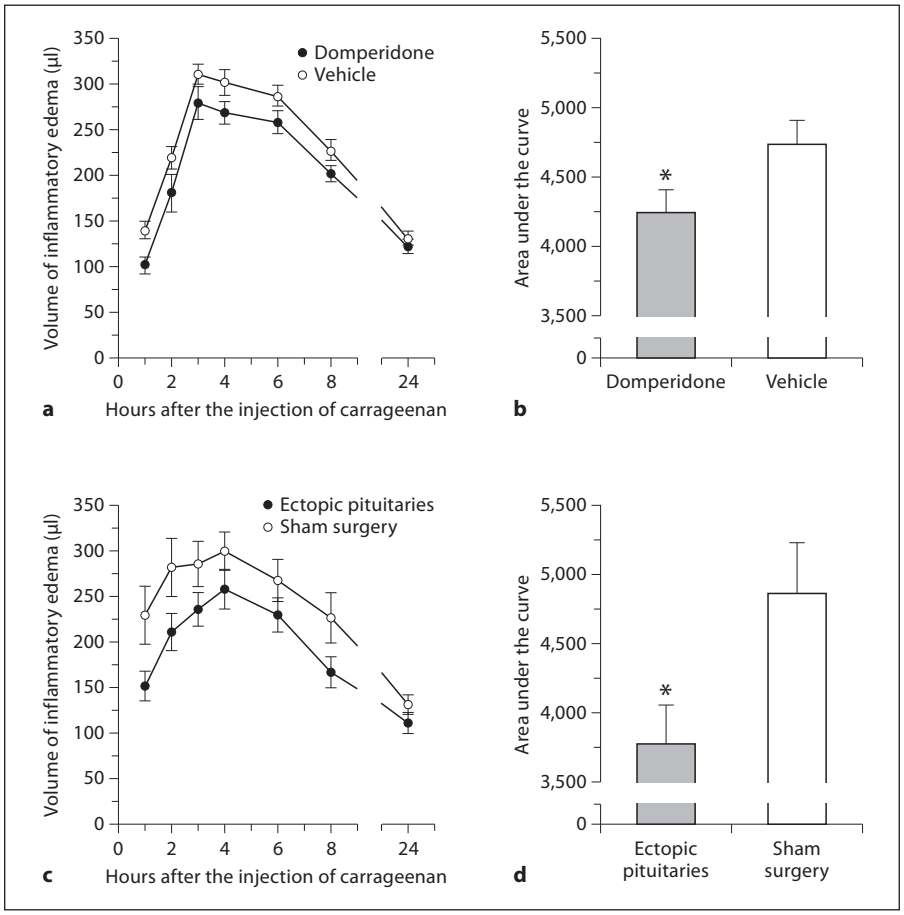

Fig. 3. Effects of 30-day domperidone-induced hyperprolactinemia (**a**, **b**) and 30-day ectopic pituitary graft-induced hyperprolactinemia (**c**, **d**) on carrageenan-induced inflammatory paw edema. **a**, **c** Kinetics. **b**, **d** Area under the curve. Data are expressed as means ± standard errors. * $p < 0.05$.

Discussion

The present study suggests that short-term hyperprolactinemia has proinflammatory effects, while long-term hyperprolactinemia has opposite effects on the edematogenic activity of carrageenan. Domperidone administration is well known to induce hyperprolactinemia. This effect is attributable to domperidone blockade of pituitary dopamine D_2 receptors [24, 25, 29–33].

The implantation of pituitary glands under the renal capsule is also a widely used model for studying the effects of PRL [8, 34, 35]. These ectopic glands, once free from hypothalamic inhibition, secrete high amounts of PRL and minimal or undetectable amounts of other hormones, such as adrenocorticotropic hormone, thyroid-stimulating hormone, growth hormone, follicle-stimulating hormone and luteinizing hormone [34]. This increased ectopic PRL release lasts from a few days [7] to several months after surgery [1]. PRL is secreted by the ectopic pituitary glands and has a molecular weight similar to the circulating isoform secreted by the eutopic pituitary [36]. Ectopic-originating PRL also exerts negative feedback on the secretion of PRL by the eutopic gland [36–38], confirming its chemical, biological and physiological similarity to eutopic PRL [39].

In the present study, both the administration of domperidone and the implantation of pituitary glands under the renal capsule increased serum PRL levels. Animals were considered to be hyperprolactinemic when serum PRL levels were significantly higher than those in controls. The PRL levels observed in these models were 2–5 times higher than in controls. Hyperprolactinemia is considered to be pathological when PRL levels are 50–100 times higher than the basal levels of normal individuals, and this applies to both humans and other animal models, such as inoculation of tumor cells that secrete PRL [40–43]. Therefore, both domperidone- and graft-induced hyperprolactinemia were considered moderate and within a physiological range of variability.

The results of the present study can be interpreted as corresponding to the effects of short and long periods of moderate hyperprolactinemia. Previous work has shown that domperidone-induced hyperprolactinemia varies according to the duration of treatment. Animals treated for 5 days have been reported to show higher serum PRL levels than those treated for 30 or 60 days [44]. In the present study, PRL levels of animals treated for 30 days showed no significant differences from animals treated for 5 days. The possibility that tolerance of dopamine D_2 receptors occurred because of prolonged blockade by domperidone was not evident in the present results. The present protocol of domperidone administration may have avoided the development of tolerance to the drug.

Regarding body weight evaluation, several reports suggest that conditions of hyperprolactinemia increase the body weight of females, but not males [45–47]. However, PRL is known to directly stimulate food consumption in both male and female animals [48–52]. Animals with an ectopic pituitary graft and the sham-operated animals gained more weight than those treated only with domperidone or saline for 30 days. These differences may be attributable to the stress caused by the daily injections.

Thus, the present results correspond to the effects of short and long periods of moderate hyperprolactinemia induced by the administration of domperidone or by pituitary graft; moreover, the results suggest that short- and long-term hyperprolactinemia have proinflammatory and anti-inflammatory effects, respectively.

A short 5-day period of hyperprolactinemia, induced by domperidone administration or by pituitary graft implantation under the renal capsule, had proinflammatory effects. In both models of hyperprolactinemia, the significant differences appeared at the beginning and end of the inflammatory process. The proinflammatory effect of hyperprolactinemia induced by the administration of ovine PRL was observed previously [53]. However, the data in that study revealed significant differences only in the final phase of the edematogenic response. Therefore, the stimulation of prostaglandin release, proposed by these authors as the possible mechanism of action for PRL, is not supported by the present results. The stimulatory effect of acute hyperprolactinemia on the edematogenic response may be attributable to the effects of PRL on the release and synthesis of histamine, serotonin and bradykinin [18, 53].

Moreover, it was demonstrated that PRL within physiological concentrations (100 ng/ml) may selectively increase TNF-α and IL-12 release upon lipopolysaccharide stimulation. On the other hand, IL-10 synthesis, which inhibits proinflammatory reactions by downregulating the production of IL-12 and TNF-α, was virtually unaffected within elevated levels of PRL up to 200 ng/ml; cytokines such as TNF-α and IL-12 can be considered essential mediators of inflammation. However, high PRL concentrations (300 ng/ml) may activate a negative feedback system through IL-10, thus limiting the proinflammatory reactivity [54]. Therefore, it is reasonable to suggest that proinflammatory responses may be attributable to the effects of acute hyperprolactinemia induced by either domperidone or pituitary grafts.

A long 30-day period of hyperprolactinemia had opposite effects to those observed in animals submitted to short-term 5-day hyperprolactinemia. Similar biphasic effects have been reported for the behavioral and neurochemical responses to PRL. Short and long periods of hyperprolactinemia produce facilitation and inhibition of sexual behavior, respectively [3, 8]. Central administration of ovine PRL can have opposite effects on striatal dopaminergic activity. Thus, a single injection of PRL increased striatal extracellular concentrations of the dopamine metabolites homovanillic acid and dihydroxyphenylacetic acid in vivo, whereas relatively long-term 5-day treatment decreased in vivo striatal concentrations of these metabolites [3].

Furthermore, it was recently shown that PRL can modulate macrophage activity, i.e. phagocytosis and oxidative burst. Those results showed that shorter (30 min) and longer (4 h) periods of in vitro incubation with PRL differentially alter macrophage activity. After 30 min of in vitro incubation, macrophage activity generally tended to decrease. However, when the period of incubation with PRL was 4 h, there were increased oxidative bursts, as well as an increased percentage of phagocytosis and decreased intensity of phagocytosis. Therefore, the in vitro effects of PRL on macrophage activity depend on the period of incubation [11].

Finally, corticosterone serum concentrations were not significantly different between the groups in the present study. These results differ from those reported in another study [55], which observed increased plasma corticosterone levels in animals with hyperprolactinemia. Since in this study, corticosterone levels were measured just once, possible changes that would occur throughout the inflammatory period may have been missed. On the other hand, it was demonstrated that PRL protects against trauma-hemorrhage by reducing plasma levels of corticosterone and IL-6, enhancing splenocyte proliferation and function and increasing survival of animals with septic shock [56, 57], thus indicating that PRL protects

against inflammation and improves dysfunctional immune responses under conditions of severe stress. A reciprocal relationship is also found between high serum corticosterone and low PRL levels after a burn injury [58].

Some autoimmune diseases involving disturbances in the balance of proinflammatory and anti-inflammatory processes have been described, and increased circulating PRL levels are suspected of being involved in their pathogenesis [59]. The role of PRL in the development of autoimmune diseases, such as systemic lupus erythematosus or rheumatoid arthritis, is currently a matter of great discussion [59–61].

In conclusion, although the mechanisms involved are not yet known, PRL can exert both anti-inflammatory and proinflammatory effects, depending on the cell type, the tissue, the physiological state of the organ [62] and, as confirmed by the present data, the duration of hyperprolactinemia. Otherwise, it was demonstrated elsewhere that low levels of circulating PRL are necessary for maintaining normal immunocompetence [63, 64]. However, our results revealed that acute hyperprolactinemia may increase proinflammatory immune responses, which might suggest immune dysfunctions. Further research is necessary to better explain the different physiological hyperprolactinemic states and their association with pathophysiological, proinflammatory immune responses.

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