Original Paper

NeuroImmunoModulation

Neuroimmunomodulation 2008;15:131–139
DOI: 10.1159/000148196

Received: January 16, 2008
Accepted after revision: May 14, 2008
Published online: August 5, 2008

# In vitro Macrophage Activity: Biphasic Effect of Prolactin and Indirect Evidence of Dopaminergic Modulation

Maria I.R. Carvalho-Freitas Elaine C. Rodrigues-Costa Antonia G. Nasello
João Palermo-Neto Luciano F. Felicio

Department of Pathology, School of Veterinary Medicine, University of São Paulo, São Paulo, Brazil

**Key Words**
In vitro · Flow cytometry · Domperidone

**Abstract**
***Objective:*** Prolactin (PRL), a peptide hormone produced by the pituitary gland, is involved in the interaction between the neuroendocrine and immune system. Since dopamine receptor antagonists increase serum levels of PRL, both PRL and dopamine receptors might be involved in the modulation of macrophage activity, providing means of communication between the nervous and immune systems. This study evaluated the effects of PRL and the dopamine antagonist domperidone (DOMP) on macrophage activity of female rats. ***Methods:*** Oxidative burst and phagocytosis of peritoneal macrophages were evaluated by flow cytometry. Samples of peritoneal liquid from female rats were first incubated with PRL (10 and 100 nM) for different periods. The same procedure was repeated to evaluate the effects of DOMP (10 and 100 nM). ***Results:*** In vitro incubation of macrophages with 10 nM DOMP decreased oxidative burst, after 30 min, whereas the PMA-induced burst was decreased by DOMP 10 nM after 2 and 4 h. Treatment with PRL (10 and 100 nM) for 30 min decreased oxidative burst and rate of phagocytosis (10 nM). After 2 h of incubation, 10 nM PRL decreased oxidative burst and phagocytosis intensity, but increased the rate of phagocytosis. On the other hand, after 4 h, PRL 10 and 100 nM increased oxidative burst and the rate of phagocytosis, but decreased intensity of phagocytosis. ***Conclusions:*** These observations suggest that macrophage functions are regulated by an endogenous dopaminergic tone. Our data also suggest that both PRL and dopamine exert their action by acting directly on the peritoneal macrophage.



## Introduction

Prolactin (PRL), a polypeptide hormone secreted by the acidophilic cells of the anterior pituitary gland, produces pronounced physiological effects on growth, reproduction and osmoregulation. The physiological control of PRL secretion is mainly inhibitory, and the activation of pituitary dopamine D2 receptors seems to be the most important inhibitory mechanism controlling the synthesis and release of PRL. Consistently, blockade of dopamine D2 receptors increases serum PRL levels [1–8].

Evidence indicates the participation of PRL in the regulation of humoral and cell-mediated immune responses [9–12]. The field of neuroimmunomodulation was advanced by the discovery that certain pituitary hormones might also be regarded as cytokines, since they can also

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com


1021–7401/08/0152–0131$24.50/0

Accessible online at:
www.karger.com/nim

Dr. Maria Isabel Roth de Carvalho-Freitas, PhD
Av. Prof. Dr. Orlando Marques de Paiva, 87
Cidade Universitária 05508-900, São Paulo (Brazil)
Tel. +55 11 3091 7934, Fax +55 11 3091 7829, E-Mail rothvet@yahoo.com.br

be released within the immune system, thus regulating its function. Indeed, data taken from human immune system studies have convincingly demonstrated that PRL, in addition to exerting its endocrine control over the immune system, also acts as a cytokine. As a cytokine, PRL is released within the immune system and regulates the lymphocyte responses by paracrine and autocrine mechanisms [13].

In this context, studies suggest an immunoregulatory role for PRL in rodents. In animals, hypophysectomy results in decreased antibody titers against sheep red blood cells as well as depressed delayed hypersensitivity reaction to chlorodinitrobenzene and the development of adjuvant arthritis after treatment with Freund's complete adjuvant [14]. Bromocryptine-induced hypoprolactinemia in mice injected with *Listeria monocytogenes* is associated with impaired lymphocyte proliferation and decreased production of macrophage-activating factors by T lymphocytes resulting in increased mortality [15]. Furthermore, a PRL-like molecule is secreted following Con A stimulation of murine lymphocytes [16]. Both a PRL-like mRNA and its product have been detected in human B lymphoblastoid cell lines [17, 18]. Moreover, it has been demonstrated that a PRL-like protein was secreted by peripheral blood mononuclear cells, suggesting that it binds to PRL receptors and migrates to the nucleus acting as a comitogen and autocrine regulator of cell growth [19]. These findings support the concept that locally generated neuropeptides can modulate immune function.

It is known that the immune system is regulated, to a great extent, by the central as well as the peripheral sympathetic nervous system [20]. This is primarily achieved by several neurotransmitters, neuropeptides, hormones and cytokines which interact with different immune effector cells and thereby ultimately regulate the homeostatic response of an individual to disease and other environmental stressors [21]. Recently, it has been reported that among these neural mediators, catecholamines, particularly central and peripheral dopamine, play a significant role in immune regulation [22]. The functional influence of dopamine on the immune system is further strengthened by the presence of (1) its receptors in immune cells [23, 24], (2) a specific endogenous dopamine transport system in leukocytes [23, 25] and (3) endogenous synthesis of this monoamine in leukocytes [26, 27]. The latter feature justifies designing experiments using in vitro blockade of dopamine receptors of immune cells.

It is also known that dopaminergic agents have immunoregulatory actions. For instance, the dopamine antagonist chlorpromazine inhibits delayed-type hypersensitivity responses [28] and prevents lethal endotoxinemia in mice [29]. It was observed that veralipride, a dopamine antagonist, inhibits macrophage Fcγ receptor expression [30].

Administration of the dopamine antagonist metoclopramide after trauma-hemorrhage restored the depressed cell-mediated immune functions [31]. However, these results suggest that the beneficial immunomodulatory effects of metoclopramide treatment after trauma-hemorrhage are mediated via upregulation of PRL. Since metoclopramide increases plasma levels of the PRL, the authors suggest that this drug should be considered a safe and useful adjunct for treating immunodepression in trauma victims [31]. Moreover, a positive correlation was recently observed between serum levels of PRL and the intensity of oxidative burst by peritoneal macrophage of female rats treated with domperidone (DOMP), an antagonist of dopamine D2 receptors. DOMP-treated animals showed increased serum levels of PRL and simultaneous increase in peritoneal macrophage oxidative burst, suggesting an indirect participation of hyperprolactinemia, induced by this treatment in peritoneal macrophage activity of female rats [8].

Hence, the in vivo effects of a dopamine receptor antagonist on macrophage activity might be due to the blockade of dopamine receptors on the macrophage surface, to the effects of the drug on the synthesis and release of PRL or to the combination of both effects. The present study was undertaken to investigate separately the in vitro effects of PRL and the dopamine receptor antagonism on the oxidative burst and phagocytosis by peritoneal macrophages of rats.

## Materials and Methods

*Animals*

All in vitro procedures were performed with the peritoneal liquid harvested from virgin Wistar female rats, 90–120 days old. These animals from our own colony were randomly housed in polypropylene cages in groups of 5 animals per cage under a 12/12 h light-dark cycle (lights on at 6.00 h) and in temperature-controlled (23–25°C) rooms. Food and water were available ad libitum throughout the experiment. The animals were housed and used in accordance with the guidelines of the Committee on Care and Use of Animal Resources of the School of Veterinary Medicine, University of São Paulo; these guidelines are similar to those of the United States National Research Council. We attempted to minimize the number of rats used, and every effort was made to ensure that no rat suffered unnecessarily.

*Flow Cytometry*

Macrophages were obtained by peritoneal lavage using 10.0 ml of phosphate-buffered saline (PBS, pH 7.2–7.4). The peritoneal fluid was collected into plastic tubes, and kept in an ice bath. Macrophages were subsequently counted using a Neubauer chamber and Trypan blue dye. The number of cells was adjusted to $2.0 \times 10^6$ cells/ml; only cell suspensions with 90% or more viability were used.

A flow cytometer (FACSCalibur; Becton Dickinson Immunocytometry Systems, San Jose, Calif., USA) interfaced with a Macintosh G4 computer was used [32]. Data from 10,000 events were collected in list mode and analyzed in Cell Quest Pro software (Becton Dickinson Immunocytometry Systems). Cell populations were identified based on their properties on forward scatter versus side scatter plots, mechanically sorted and evaluated through light microscopy after staining with Giemsa dye. Data from peritoneal macrophage were collected applying gates that sorted out lymphocyte and monocyte clusters. Fluorescence data were collected on log scale. Green fluorescence from 2′,7′-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Eugene, Oreg., USA) and DCFH + phorbol myristate acetate (PMA; Sigma, St. Louis, Mo., USA) were measured at 530 ± 30 nm (FL1 detector); red fluorescence from propidium iodide-labeled *Staphylococcus aureus* (PI-*S. aureus;* Sigma) was measured at 585 ± 42 nm (FL2 detector). PI-*S. aureus* and DCFH-DA fluorescence were analyzed after compensation to correct for any signal crossover.

Oxidative Burst and Phagocytosis

The substances used for triggering the oxidative burst were PMA (100 ng) and *S. aureus* ($2.4 \times 10^9$ bacteria/ml). Quantification of oxidative burst and phagocytosis was estimated as suggested elsewhere [33] by mean DCFH-DA and PI-*S. aureus* fluorescence cells, respectively. Briefly, 100 μl of peritoneal macrophage ($2.0 \times 10^5$ cells/100 μl) was mixed with 200 μl of DCFH-DA (0.3 mM), 100 μl of PI-*S. aureus* or PMA in different polypropylene tubes, according to table 1. Samples were incubated with different reagents under agitation at 37°C for 30 min, 2 or 4 h (table 1). Reactions were stopped by adding 2.0 ml of cold EDTA solution (3 mM) in order to terminate phagocytosis. Samples were then centrifuged (250 *g* for 10 min) and the cell pellets resuspended in 0.5 ml of cold PBS for flow cytometry. Direct measurements of mean fluorescence in green and red channels were recorded as oxidative burst and phagocytosis, respectively, as proposed elsewhere [33]. Quantification of phagocytosis and oxidative burst were estimated by mean PI-*S. aureus* and DCFH-DA fluorescence/cell, respectively. Data on intensity of fluorescence for both macrophage oxidative burst and phagocytosis were expressed as means of their respective controls.

*In vitro Effects of PRL or DOMP*

A total of 6 experiments were made to study the in vitro effects of PRL and DOMP for different periods of incubation on macrophage activity, and in each experiment 7 animals were used. In order to investigate the in vitro effects of PRL 10 and 100 nM after 30 min, the animals were euthanized and resident peritoneal macrophages were harvested by a lavage with PBS solution (pH 7.2–7.4). A pool of cells was made and then adjusted to $2.0 \times 10^6$ cells/ml. These cells were distributed into 3 groups, control, PRL 10 nM and PRL 100 nM, and then incubated with the reagents, according to table 1. The same procedure was repeated to test in vitro effects of PRL 10 and 100 nM after 2 and 4 h of incubation on macrophage activity, and to test in vitro effects of DOMP 10 and 100 nM after 30 min, 2 and 4 h. According to table 1, total volume in each tube was always adjusted to 1,000 μl by adding 100 μl of PBS (control groups) or PRL or DOMP in order to obtain their final experimental concentrations. Oxidative burst and phagocytosis were evaluated by flow cytometry, as described above.

**Table 1.** Reagents used in flow cytometry technique

| Reagents/tubes | A | B | C | D | E |
|---|---|---|---|---|---|
| PBS, μl | 900 | 700 | 600 | 600 | 800 |
| DCFH-DA, μl | – | 200 | 200 | 200 | – |
| PMA, μl | – | – | 100 | – | – |
| PI-*S. aureus*, μl | – | – | – | 100 | 100 |

$2.0 \times 10^5$ cells/100 μl were incubated, during 30 min, 2 or 4 h, at 37°C. Tubes of control groups: A = basal cell fluorescence; B = spontaneous oxidative burst; C = PMA-induced oxidative burst; D = phagocytosis-induced oxidative burst; E = percent and intensity of phagocytosis.

*Statistical Analysis*

Bartlett's test was previously performed to evaluate whether data should be analyzed by parametric or nonparametric tests. All data were parametric, thus they were analyzed by ANOVA followed by the Tukey test. $p < 0.05$ was considered to show significant differences for all comparisons made. Results are presented as means + SD and are expressed as a percentage of control.

## Results

*In vitro Effects of PRL (10 and 100 nM) on Macrophage Activity*

After 30 min, in vitro incubation of macrophage with PRL in both tested concentrations decreased spontaneous oxidative burst (fig. 1). Concerning phagocytosis of labeled *S. aureus*, when macrophages were incubated with PRL 10 nM, the rate of phagocytosis decreased compared with the control group. After 2 h of in vitro incubation with PRL, there were no modifications regarding spontaneous oxidative burst. Nevertheless, PMA-induced oxidative burst was reduced after macrophage incubation with PRL 10 nM, compared with control and PRL 100 nM groups. Macrophages incubated with PRL 10 nM for 2 h showed increased percent of phagocytosis of labeled *S. aureus*, but simultaneously decreased intensity of phagocytosis.

After 4 h of in vitro incubation with PRL, there were no modifications regarding spontaneous oxidative burst.

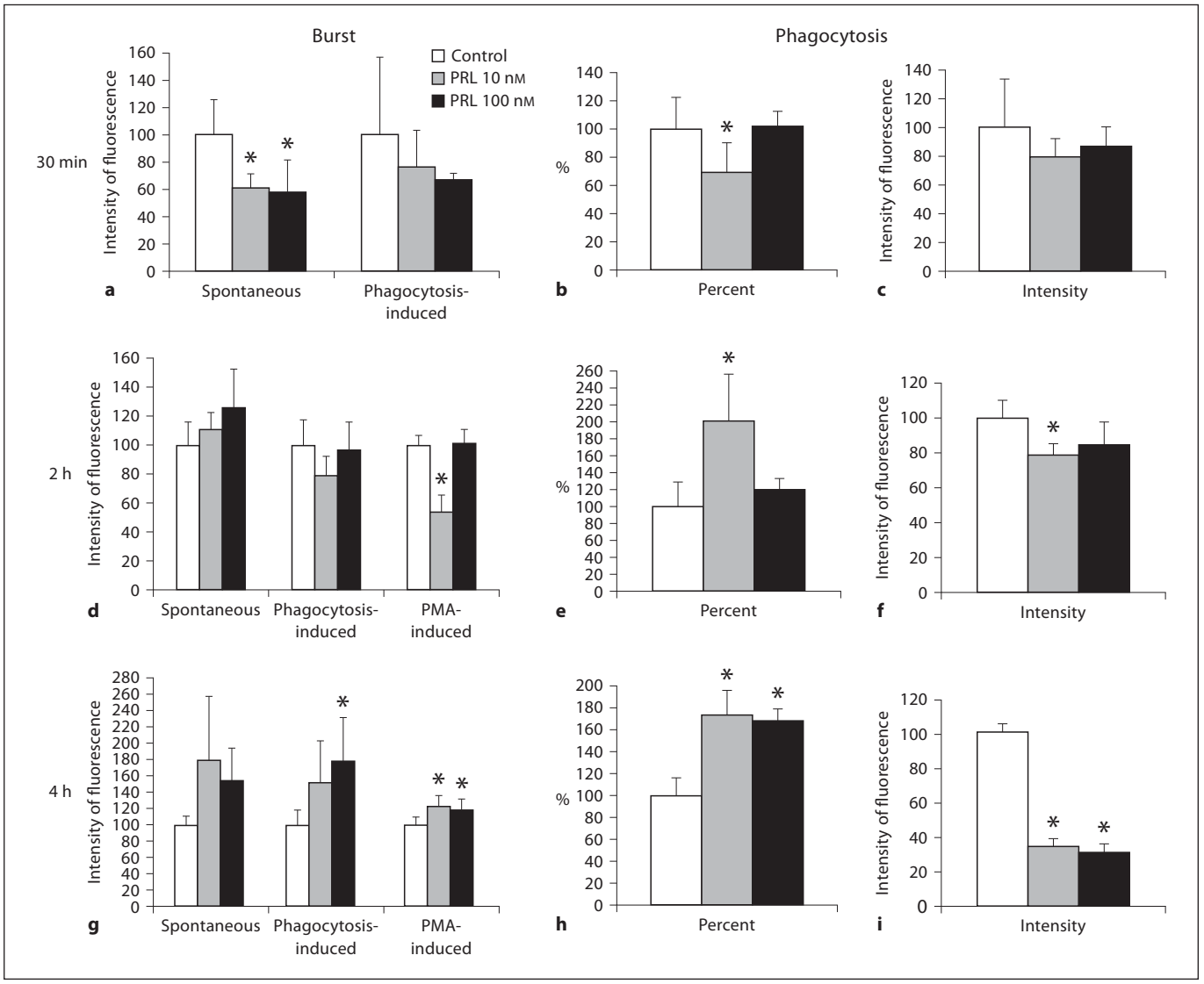


**Fig. 1.** Effects of PRL in vitro (10 or 100 nM) upon macrophage activity: oxidative burst (**a**), percent of phagocytosis (**b**) and intensity of phagocytosis (**c**) after 30 min of incubation; oxidative burst (**d**), percent of phagocytosis (**e**) and intensity of phagocytosis (**f**) after 2 h of incubation; oxidative burst (**g**), percent of phagocytosis (**h**) and intensity of phagocytosis (**i**) after 4 h. Values are means + SD and are expressed as a percentage of the PBS control. * $p < 0.05$, ANOVA, Tukey test.

Nevertheless, PMA-induced oxidative burst was increased after macrophage incubation with PRL 10 and 100 nM, compared with the control group. Phagocytosis-induced oxidative burst was increased in macrophages of the PRL 100 nM group, compared with the control group. Macrophages incubated with both concentrations of PRL for 4 h showed increased percent of phagocytosis of labeled *S. aureus,* but simultaneously decreased intensity of phagocytosis.

*In vitro Effects of DOMP (10 and 100 nM) on Macrophage Activity*

After 30 min, macrophages incubated with DOMP 10 nM showed reduction in spontaneous oxidative burst (fig. 2). After 2 and 4 h, macrophages incubated with DOMP 10 nM showed reduction in PMA-induced oxidative burst. On the other hand, parameters related with phagocytosis of labeled *S. aureus* were not altered after 30 min, 2 or 4 h of in vitro incubation with DOMP.

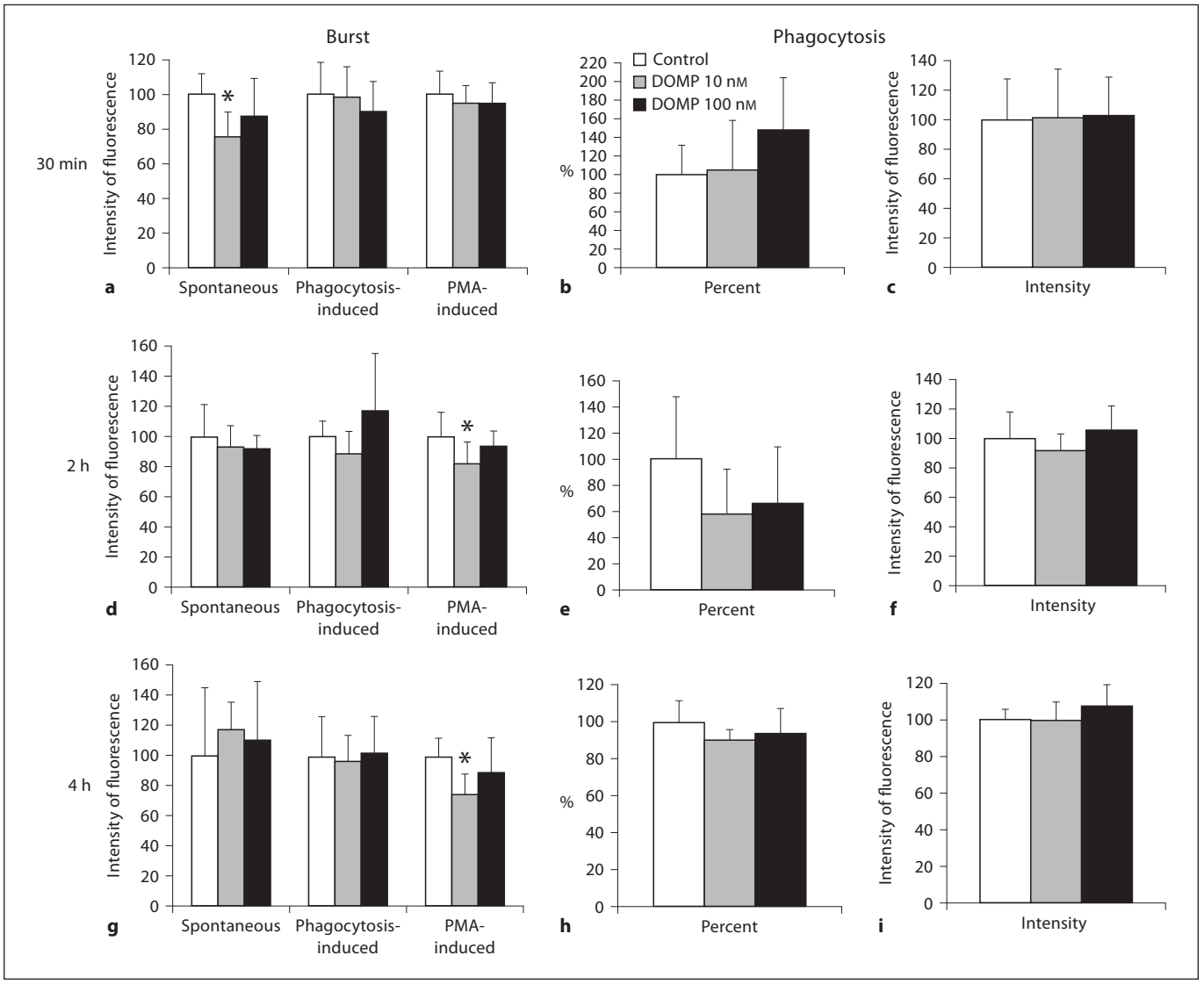


**Fig. 2.** Effects of DOMP in vitro (10 or 100 nM) upon macrophage activity: oxidative burst (**a**), percent of phagocytosis (**b**) and intensity of phagocytosis (**c**) after 30 min of incubation; oxidative burst (**d**), percent of phagocytosis (**e**) and intensity of phagocytosis (**f**) after 2 h of incubation; oxidative burst (**g**), percent of phagocytosis (**h**) and intensity of phagocytosis (**i**) after 4 h. Values are means + SD and are expressed as a percentage of the PBS control. * $p < 0.05$, ANOVA, Tukey test.

## Discussion

The immune system is regulated, to a great extent, by the central nervous system as well as by the peripheral sympathetic nervous system [20]. This is primarily achieved by several hormones, cytokines and neurotransmitters which interact with different immune effector cells, ultimately regulating the response of an individual to disease and other environmental stressors [21]. PRL has multiple physiological functions, playing a role in lactation, reproduction, growth and development, water and electrolyte balance, as well as immunoregulation [34–37]. This peptide hormone has been shown to stimulate T and B cells, natural killer cells, macrophages, neutrophils, CD34+ hematopoietic cells and antigen-presenting dendritic cells [38–41].

Among the neural mediators, controlling synthesis and release of PRL, dopamine plays a significant role.

Thus, dopamine receptor antagonists induce increases in PRL serum levels as a consequence of this mechanism of action. It has been consistently shown that dopamine antagonists modulate immune responses [8, 31, 42–44] and the catecholaminergic tone modulates macrophage activity in vitro [45, 46]. Importantly, the culture of resident peritoneal cells includes lymphocytes, which can release both PRL [16] and dopamine [26, 27]. The latter feature justifies designing experiments using in vitro blockade of DA receptors of immune cells.

The aim of this work was to directly and separately evaluate the effects of PRL and dopamine antagonism on macrophage activity. Thus, in vitro incubation of both PRL and the dopamine antagonist DOMP were tested in different concentrations and after different times of incubation.

Concerning the 30-min in vitro incubation period with PRL 10 and 100 nM, macrophage activity generally tended to decrease. Peritoneal macrophage incubated with 10 nM PRL for 2 h showed decreased PMA-induced oxidative bursts, increased percent of phagocytosis and decreased intensity of phagocytosis. When the period of incubation with PRL in both tested concentrations was 4 h, there were increased PMA-induced oxidative bursts, phagocytosis-induced oxidative bursts (PRL 100 nM), as well as increased percent of phagocytosis and decreased intensity of phagocytosis. Therefore, in vitro effects of PRL depend on the concentration and the period of incubation in macrophage activity. These PRL effects are consistent with its in vivo actions [36].

PRL acts through its receptors (PRL-R), which have been shown to be present in immunocompetent cells in the mouse [47–49]. Other reports using ligand-binding methods have indicated that PRL-R is present in peripheral blood lymphocytes, spleen and thymus cells [49, 50] as well as in large granular lymphocytes [51]. Isolated peritoneal macrophages constitutively express high-intensity PRL-R, suggesting that cells from the immune system may respond to either pituitary PRL or PRL-like molecules [48].

In this context, the role of PRL as a mediator of physical activity-induced stimulation of macrophage phagocytosis was investigated. Thus, peritoneal macrophages from BALB/c mice were incubated for 30 min with 1.1 ng/ml (basal concentration in these animals), 2.2 ng/ml (the concentration observed in plasma after swimming until exhaustion), 8, 16 and 22,000 ng/ml of PRL. Incubation of peritoneal macrophages with a concentration of PRL similar to that observed in plasma immediately after physical activity stress stimulates phagocytic capacity. This stimulation was also observed after incubation of macrophages with the higher concentrations of PRL. Therefore, these findings indicate that PRL acts as a mediator of macrophage phagocytosis after 30 min of incubation [52], as it was found in the present work.

The activation of rainbow trout *(Oncorhynchus mykiss)* phagocytic cells by PRL was investigated in vitro elsewhere. Those macrophages, incubated with 10–100 ng PRL/ml, showed significantly enhanced production of superoxide anion compared with control macrophages (without hormone). In addition, cells treated with PRL also showed increased phagocytic activity and phagocytic index. These results indicate that PRL stimulates in vitro in the macrophages of this fish, similar to what we found in rat macrophage [53]. Hence, the role of PRL on macrophage activity is important from an evolutionary view and seems to be conserved in fishes and mammals.

It is well known that cells of the monocyte/macrophage lineage play an important role in the host's defense against various microbial infections and tumors [54, 55]. Macrophage-mediated microbe death is manifested by a variety of mechanisms, involving secretion of bioactive molecules, such as nitric oxide (NO), and generation of reactive oxygen intermediates, such as $H_2O_2$ [56, 57]. Macrophages can be activated by a number of agents, some of which act via signal transduction processes involving the modulation of various second messengers like protein kinase C (PKC) and $Ca^{2+}$ [58, 59].

In this context, the effects of in vitro treatment of murine macrophages with PRL have been reported on the production of regulatory molecules such as nitric oxide (NO) and the modulation of PKC activity in these cells. The production of NO by macrophages was enhanced after simultaneous treatment with PRL and LPS. However, NO production decreased when macrophages were treated with PRL and the $Ca^{2+}$ blocker, nifedipine, suggesting a role of $Ca^{2+}$ in the activation of macrophages with PRL [60].

Therefore, one can suggest that the in vitro effects of PRL described in the present study are due to the activation of PRL-R on the peritoneal macrophage surface; PRL-induced activation of PRL-R ultimately leads to macrophage activity in a time-dependent fashion, by gene transcription activation or through second messenger involvement, such as PKC or $Ca^{2+}$. The biphasic effects of PRL have been described previously in vivo. Short- and long-term treatments with this hormone usually generate opposite behavioral and neurochemical effects [36].

In vitro effects on macrophage activity of different concentrations of DOMP, in different periods of incubation, were also tested. Spontaneous oxidative burst was decreased by DOMP 10 nM after 30 min of incubation. After 2 or 4 h, DOMP 10 nM in vitro was able to reduce PMA-induced oxidative burst.

Indeed, there is evidence of specific dopamine receptors on macrophage surface [23, 26], as well as in other immune cells [22, 61–63]. Norepinephrine and dopamine are neurotransmitters and stress hormones known to affect macrophage functions [64–67]. Importantly, since macrophages accumulate catecholamines into functional pools and lymphocytes themselves produce catecholamines [68, 69], an autocrine or paracrine regulatory loop may exist. This loop would attenuate the functions of macrophages, such as hydrogen peroxide production, according to the conditions and requirements of the inflammatory site [46].

The biphasic effects of dopamine have already been reported. In wall lizards, the 3 catecholamines dopamine, norepinephrine and adrenaline stimulated macrophage phagocytosis at lower concentrations ($10^{-11}$ to $10^{-15}$ M), whereas they suppressed phagocytic activity at higher concentrations ($10^{-7}$ to $10^{-5}$ M), suggesting the existence of a concentration-related differential effect of catecholamines on macrophage function [70]. The stimulatory effect of catecholamines on phagocytic activity of peritoneal macrophages has been demonstrated in chickens in which β-adrenergic antagonists and dopamine receptor antagonists blocked the norepinephrine and dopamine action, respectively [71].

Similarly, intracellular cAMP level by phosphodiesterase activity stimulators, imidazole and phenylimidothiazole, or an inhibitor agent, theophylline, enhanced or reduced the phagocytosis by murine peritoneal macrophages, respectively [72].

Hence, as reported in the present study, both PRL and the blockade of dopamine receptor using DOMP modulate macrophage activity: phagocytosis and oxidative burst. Also, the results showed that shorter and longer periods of incubation, especially with PRL, differently alter macrophage activity. Therefore, it is possible to suggest that both PRL and dopamine can modulate macrophage activity. Consequently, in vivo effects of DOMP may be due to both its direct effects on macrophage, shutting down the dopaminergic tone of the cell culture and its regulatory effects on synthesis and release of pituitary PRL, which can also modulate macrophagic activity.

## Acknowledgments

This research was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grants awarded to Luciano F. Felicio (No. 05/56833-4) and João Palermo-Neto (No. 04/14128-0). Maria I.R. Carvalho-Freitas was supported by a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

## References

1 Ben-Jonathan N, Arbogast LA, Hyde JF: Neuroendocrine regulation of prolactin release. Prog Neurobiol 1989;33:399–447.
2 Laduron PM, Leysen JE: Domperidone, a specific in vitro dopamine antagonist, devoid of in vivo central dopaminergic activity. Biochem Pharmacol 1979;28:2161–2165.
3 Vanzeler ML, Felicio LF, Nasello AG: Effects of chronic domperidone treatment on rat conditioned avoidance behavior. Braz J Med Biol Res 1990;23:865–868.
4 Nasello AG, Vanzeler ML, Felicio LF: A comparison of bromopride and domperidone effects on rat conditioned avoidance and motor activity. Pharmacol Toxicol 1991;68:46–50.
5 Felicio LF, Bridges RS: Domperidone induces a probenecid-sensitive rise in immunoreactive prolactin in cerebroventricular perfusates in female rats. Brain Res 1992;573:133–138.
6 Nasello AG, Tieppo CA, Felicio LF: Apomorphine-induced yawning in the rat: influence of fasting and time of day. Physiol Behav 1995;57:967–971.
7 Nasello AG, Vanzeler ML, Madureira EH, Felicio LF: Effects of acute and long-term domperidone treatment on prolactin and gonadal hormone levels and sexual behavior of male and female rats. Pharmacol Biochem Behav 1997;58:1089–1094.
8 Carvalho-Freitas MIR, Anselmo-Franci JA, Teodorov E, Nasello AG, Palermo-Neto J, Felicio LF: Reproductive experience modifies dopaminergic function, serum levels of prolactin, and macrophage activity in female rats. Life Sci 2007;81:128–136.
9 Russell DH, Kibler R, Matrisian L, Larson DF, Poulos B, Magun BE: Prolactin receptors on human T and B lymphocytes: antagonism of prolactin binding by cyclosporine. J Immunol 1985;134:3027–3031.
10 Spangelo BL, Hall NR, Ross PC, Goldstein AL: Stimulation of in vivo antibody production and concanavalin-A-induced mouse spleen cell mitogenesis by prolactin. Immunopharmacology 1987;14:11–20.
11 Leite-de-Moraes MC, Touraine P, Kelly PA, Kuttenn F, Dardenne M: Prolactin receptor expression in lymphocytes from patients with hyperprolactinemia or acromegaly. J Endocrinol 1995;147:353–359.
12 Ben-Jonathan N, Mershon JL, Allen DL, Steinmetz RW: Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. Endocr Rev 1996;17:639–669.
13 Matera L: Endocrine, paracrine and autocrine actions of prolactin on immune cells. Life Sci 1996;59:599–614.
14 Nagy E, Berczi I: Immunodeficiency in hypophysectomized rats. Acta Endocrinol 1978;89:530–537.

15 Bernton EW, Meltzer MS, Holaday JW: Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. Science 1988;22:401–404.
16 Montgomery DW, Zukoski CF, Shah GN, Buckley AR, Pacholczyk T, Russell DH: Concanavalin A-stimulated murine splenocytes produce a factor with prolactin-like bioactivity and immunoreactivity. Biochem Biophys Res Commun 1987;145:692–698.
17 DiMattia GE, Gellersen B, Bohnet HG, Friesen HG: A human B-lymphoblastoid cell line produces prolactin. Endocrinology 1988; 122:2508–2517.
18 Baglia LA, Cruz D, Shaw JE: An Epstein-Barr virus-negative Burkitt lymphoma cell line (sfRamos) secretes a prolactin-like protein during continuous growth in serum-free medium. Endocrinology 1991;128:2266–2272.
19 Sabharwal P, Glaser R, Lafuse W, Varma S, Liu Q, Arkins S, Kooijman R, Kutz L, Kelley KW, Malarkey WB: Prolactin synthesized and secreted by human peripheral blood mononuclear cells: an autocrine growth factor for lymphoproliferation. Proc Natl Acad Sci USA 1992;89:7713–7716.
20 Blalock JE: A molecular basis for bidirectional communication between the immune and neuroendocrine systems. Physiol Rev 1989;69:1–32.
21 Weigent DA, Blalock JE: Interactions between the neuroendocrine and immune systems: common hormones and receptors. Immunol Rev 1987;100:79–108.
22 Basu S, Dasgupta PS: Dopamine, a neurotransmitter, influences the immune system. J Neuroimmunol 2000;102:113–124.
23 Basu S, Dasgupta PS, Lahiri T, Roychowdhury J: Uptake and biodistribution of dopamine in bone marrow, spleen and lymph nodes of normal and tumor bearing mice. Life Sci 1993;53:415–424.
24 Ricci A, Amenta F: Dopamine D5 receptors in human peripheral blood lymphocytes: a radio-ligand binding study. J Neuroimmunol 1994;53:1–7.
25 Bondy B, Ackenheil M, Ruppert T: Spiperone binding in lymphocytes: part of a dopamine uptake system? Ann NY Acad Sci 1992;650: 221–225.
26 Bergquist J, Tarkowski A, Ekman R, Ewing A: Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. Proc Natl Acad Sci USA 1994;91:12912–12916.
27 Cosentino M, Marino F, Bombelli R, Ferrari M, Lecchini S, Frigo G: Endogenous catecholamine synthesis, metabolism, storage and uptake in human neutrophils. Life Sci 1999;64:975–981.
28 Roudebush RE, Berry PL, Layman NK, Butler LD, Bryant HU: Dissociation of immunosuppression by chlorpromazine and trifluoperazine from pharmacologic activities as dopamine antagonists. Int J Immunopharmacol 1991;13:961–968.
29 Gadina M, Bertini R, Mengozzi M, Zandalasini M, Mantovani A, Ghezzi P: Protective effect of chlorpromazine on endotoxin toxicity and TNF production in glucocorticoid-sensitive and glucocorticoid-resistant models of endotoxic shock. J Exp Med 1991;173: 1305–1310.
30 Gomez F, Ruiz P, Briceno F, Rivera C, Lopez R: Macrophage Fcγ receptors expression is altered by treatment with dopaminergic drugs. Clin Immunol 1999;90:375–387.
31 Knöferl MW, Angele MK, Ayala A, Cioffi WG, Bland KI, Chaudry IH: Insight into the mechanism by which metoclopramide improves immune functions after trauma-hemorrhage. Am J Physiol Cell Physiol 2000; 279:C72–C80.
32 Fonseca ES, Sakai M, Carvalho-Freitas MI, Palermo-Neto J: Naloxone treatment prevents prenatal stress effects on peritoneal macrophage activity in mice offspring. Neuroendocrinology 2005;81:322–328.
33 Hasui M, Hirabayashi Y, Kobayashi Y: Simultaneous measurement by flow cytometry of phagocytosis and hydrogen peroxide production of neutrophils in whole blood. J Immunol Methods 1989;117:53–58.
34 Bridges RS, Felicio LF, Pellerin LJ, Stuer AM, Mann PE: Prior parity reduces post-coital diurnal and nocturnal prolactin surges in rats. Life Sci 1993;53:439–445.
35 Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA: Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev 1998;19:225–268.
36 Cruz-Casallas PE, Nasello AG, Hucke EE, Felicio LF: Dual modulation of male sexual behavior in rats by central prolactin: relationship with in vivo striatal dopaminergic activity. Psychoneuroendocrinology 1999; 24:681–693.
37 Yokoyama Y, Kitchens WC, Toth B, Schwacha MG, Bland KI, Chaudry IH: Upregulation of hepatic prolactin receptor gene expression by 17β-estradiol following trauma-hemorrhage. J App Physiol 2003;95: 2530–2536.
38 Kooijman R, Hooghe-Peters EL, Hooghe R: Prolactin, growth hormone, and insulin-like growth factor-I in the immune system. Adv Immunol 1996;63:377–454.
39 Dogusan Z, Hooghe R, Verdood P, Hooghe-Peters EL: Cytokine-like effects of prolactin in human mononuclear and polymorphonuclear leukocytes. J Neuroimmunol 2001;120: 58–66.
40 Matera L, Mori M, Galetto A: Effect of prolactin on the antigen presenting function of monocyte-derived dendritic cells. Lupus 2001;10:728–734.
41 Yu-Lee LY: Prolactin modulation of immune and inflammatory responses. Recent Prog Horm Res 2002;57:435–455.
42 Kleeb SR, Xavier JG, Frussa-Filho R, Dagli MLZ: Effect of haloperidol on the development of the solid Ehrlich tumor in mice. Life Sci 1997;60:PL69–PL74.
43 Kleeb SR, dos S Rizzo M, Dagli MLZ, Frussa-Filho R: Haloperidol increases spreading and nitric oxide production in macrophages from tumor-bearing mice: a possible mechanism for its antitumoral effect. Int J Immunopharmacol 1999;21:575–580.
44 Lourenço GA, Dorce VA, Palermo-Neto J: Haloperidol treatments increased macrophage activity in male and female rats: influence of corticosterone and prolactin serum levels. Eur Neuropsychopharmacol 2005;15: 271–277.
45 Chi DS, Qui M, Krishnaswamy G, Li C, Stone W: Regulation of nitric oxide production from macrophages by lipopolysaccharide and catecholamines. Nitric Oxide 2003; 8:127–132.
46 Kondomerkos DJ, Kalamidas SA, Kotoulas OB: In vitro effects of hormones and autacoids on the hydrogen peroxide production and the morphology of endotoxin-activated rat peritoneal macrophages. Histol Histopathol 2003;18:55–65.
47 Gagnerault MC, Touraine P, Savino W, Kelly PA, Dardenne M: Expression of prolactin receptors in murine lymphoid cells in normal and autoimmune situations. J Immunol 1993;150:5673–5681.
48 Gala RR, Shevach EM: Influence of prolactin and growth hormone on the activation of dwarf mouse lymphocytes in vivo. Proc Soc Exp Biol Med 1993;204:224–230.
49 Hawkins TA, Gala RR, Dunbar JC: The lymphocyte and macrophage profile in the pancreas and spleen of NOD mice: percentage of interleukin-2 and prolactin receptors on immunocompetent cell subsets. J Reprod Immunol 1996;32:55–71.
50 Bellussi G, Muccioli G, Ghe C, Di Carlo R: Prolactin binding sites in human erythrocytes and lymphocytes. Life Sci 1987;41:951–959.
51 Matera L, Muccioli G, Cesano A, Bellussi G, Genazzani E: Prolactin receptors on large granular lymphocytes: dual regulation by cyclosporin A. Brain Behav Immun 1988;2: 1–10.
52 Ortega E, Forner MA, Barriga C: Effect of prolactin on the in vitro phagocytic capacity of macrophages. Comp Immunol Microbiol Infect Dis 1996;19:139–146.
53 Sakai M, Kobayashi M, Kawauchi H: In vitro activation of fish phagocytic cells by GH, prolactin and somatolactin. J Endocrinol 1996;151:113–118.
54 Adams DO, Hamilton TA: The cell biology of macrophage activation. Annu Rev Immunol 1984;2:283–318.

55 Germain RN, Margulies DH: The biochemistry and cell biology of antigen processing and presentation. Annu Rev Immunol 1993; 11:403–450.
56 Unanue ER, Allen PM: The basis for the immunoregulatory role of macrophages and other accessory cells. Science 1987;236:551–557.
57 Warwick-Davies J, Lowrie DB, Cole PJ: Growth hormone is a human macrophage activating factor: priming of human monocytes for enhanced release of $H_2O_2$. J Immunol 1995;154:1909–1918.
58 Adams DO, Hamilton TA: Molecular transductional mechanisms by which IFN γ and other signals regulate macrophage development. Immunol Rev 1987;97:5–27.
59 Keller R: The macrophage response to infectious agents: mechanisms of macrophage activation and tumor cell killing. Res Immunol 1993;144:271–273; discussion 294–298.
60 Kumar A, Singh SM, Sodhi A: Effect of prolactin on nitric oxide and interleukin-1 production of murine peritoneal macrophages: role of $Ca^{2+}$ and protein kinase C. Int J Immunopharmacol 1997;19:129–133.
61 Ricci A, Veglio F, Amenta F: Radioligand binding characterization of putative dopamine D3 receptor in human peripheral blood lymphocytes with [3H] 7-OH-DPAT. J Neuroimmunol 1995;58:139–144.
62 Kwak YT, Koo MS, Choi CH, Sunwoo I: Change of dopamine receptor mRNA expression in lymphocyte of schizophrenic patients. BMC Med Genet 2001;2:3.
63 McKenna F, McLaughlin PJ, Lewis BJ, Sibbring GC, Cummerson JA, Bowen-Jones D, Moots RJ: Dopamine receptor expression on human T- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. J Neuroimmunol 2002; 132:34–40.
64 Miles BA, Lafuse WP, Zwilling BS: Binding of α-adrenergic receptors stimulates the anti-mycobacterial activity of murine peritoneal macrophages. J Neuroimmunol 1996; 71:19–24.
65 Hasko G, Shanley TP, Egnaczyk G, Nemeth ZH, Salzman AL, Vizi ES, Szabo C: Exogenous and endogenous catecholamines inhibit the production of macrophage inflammatory protein (MIP) 1α via a β adrenoreceptor mediated mechanism. Br J Pharmacol 1998; 125:1297–1303.
66 Kohut ML, Davis JM, Jackson DA, Colbert LH, Strasner A, Essig DA, Pan RR, Ghaffar A, Mayer EP: The role of stress hormones in exercise-induced suppression of alveolar macrophage antiviral function. J Neuroimmunol 1998;81:193–200.
67 Ortega E, Garcia JJ, De La Fuente M: Modulation of adherence and chemotaxis of macrophages by norepinephrine: influence of ageing. Mol Cell Biochem 2000;203:113–117.
68 Spengler RN, Chensue SW, Giacherio DA, Blenk N, Kunkel SL: Endogenous norepinephrine regulates tumor necrosis factor-α production from macrophages in vitro. J Immunol 1994;15:3024–3031.
69 Musso NR, Brenci S, Setti M, Indiveri F, Lotti G: Catecholamine content and in vitro catecholamine synthesis in peripheral human lymphocytes. J Clin Endocrinol Metab 1996; 81:3553–3557.
70 Roy B, Rai U: Dual mode of catecholamine action on splenic macrophage phagocytosis in wall lizard, *Hemidactylus flaviviridis*. Gen Comp Endocrinol 2004;136:180–191.
71 Ali RA, Qureshi MA, McCorkle FM: Profile of chicken macrophage functions after exposure to catecholamines in vitro. Immunopharmacol Immunotoxicol 1994;16:611–625.
72 Lima AO, Javierre MQ, Da Silva W, Camara DS: Immunological phagocytosis: effects of drugs on phosphodiesterase activity. Experientia 1974;30:945–946.