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Prior reproductive experience alters prolactin-induced macrophage responses in pregnant rats

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ABSTRACT

Reproductive experience (*i.e.*, pregnancy and lactation) induces physiological changes in mammals. A previous reproductive experience was recently shown to modulate the activity of dopaminergic hypothalamic systems while decreasing serum prolactin levels and oxidative burst activity in peritoneal macrophages. Dopamine receptor antagonists increase serum prolactin levels, and both prolactin and dopamine receptors may be involved in the modulation of macrophage activity, providing a means of communication between the nervous and immune systems. The present study evaluated the *in vitro* effects of prolactin and a dopamine D_2 receptor antagonist on the peritoneal activity of macrophages from primigravid and multigravid female rats during the third trimester of pregnancy. Oxidative bursts and phagocytosis in peritoneal macrophages were evaluated by flow cytometry. Primigravid and multigravid Wistar rats, during the third trimester of pregnancy (*i.e.*, days 17–21), were used. Peritoneal fluid samples from these rats were first incubated with prolactin (10 and 100 nM) for different periods of time. The same procedure was repeated to evaluate the effects of domperidone (10 and 100 nM) on macrophage activity. Our results showed that macrophages from multigravid rats responded more effectively to *in vitro* incubation with prolactin, especially with regard to the intensity and percentage of phagocytosis. Additionally, these effects were more pronounced after incubation periods of 30 min or 4 h. These data suggest that macrophages during a second pregnancy become more sensitive to the phagocytotic effects of prolactin.

1. Introduction

The reproductive experience is one of the most significant events in a female mammal's life. It can be defined as a rich social and hormonal experience, beginning with male interaction and mating, then pregnancy and delivery, and ultimately lactation, pup interactions, and weaning (Bridges et al., 1993; Hucke et al., 2001; Serafim and Felicio, 2002). Data indicate that primiparous or multiparous rats generally show greater resilience to stress, decreased anxiety, and improved performance in memory tasks compared with female rats that have not experienced motherhood (*i.e.*, virgin or nulliparous rats) (Kinsley et al., 1999; Macbeth and Luine, 2010; Zimberknopf et al., 2011). Moreover, neural changes remain long after the last pregnancy, persisting even into old age. Thus, the functional changes related to reproductive experience include behavioral, neural, neuroendocrine, and possibly cognitive aspects in both rodents and postpartum human mothers (Kinsley et al., 1999; Macbeth and Luine, 2010).

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For example, following a first pregnancy in women, a significant decrease in both basal and stimulated prolactin secretion is found (Brown et al., 2000; Kwa et al., 1981; Musey et al., 1987; Wang et al., 1988). In female rats, parity-related reductions of prolactin secretion have also been observed, and this phenomenon has been consistently observed in our laboratory and by others (Bridges et al., 1993; Bridges and Byrnes, 2006; Carvalho-Freitas et al., 2007; Felicio and Bridges, 1992; Mann et al., 1989; Musey et al., 1987). During pregnancy, plasma prolactin levels in multigravid rats are reduced by approximately 50% compared with primigravid rats. These effects are observed during both early pregnancy, when prolactin is secreted in large amounts, and mid-pregnancy when prolactin levels are low (Bridges and Hammer, 1992; Bridges et al., 1993; Felicio et al., 1996; Sider et al., 2003).

During lactation, multiparous females have attenuated suckling-induced prolactin secretion compared with primiparous females (Mann and Bridges, 1992). Similarly, shifts in both prolactin secretion and prolactin regulation can occur during both a second pregnancy followed by lactation and subsequent estrous cycles (Bridges and Hammer, 1992; Bridges et al., 1993; Bridges and Byrnes, 2006; Mann and Bridges, 1992). Decreased prolactin secretion in response to dopamine receptor antagonists has also been reported in ovariectomized, parous female rats (Bridges et al., 1997), and similar data were found in women (Espinosa de los Monteros et al., 1991).

The immune and neuroendocrine systems are intimately linked and involved in bidirectional communications. The relationship between prolactin and the immune system has been well established during the last two decades, opening new research avenues in the field of immunoendocrinology (Jara et al., 2011). Prolactin is secreted not only by the anterior pituitary gland, but also by many extrapituitary sites, including immune cells. Moreover, prolactin receptors can be found in many cell types, including monocytes and lymphocytes (Jara et al., 1991). Therefore, in addition to the regulation of the growth and differentiation of the mammary glands and ovaries, prolactin also plays an important role in the innate and adaptive immune response (Jara et al., 2011). Prolactin is now considered a cytokine, based on both molecular and functional evidence (Wu et al., 1996; Vera-Lastra et al., 2002).

The macrophage-mediated regulation of the immune response is manifested by various mechanisms that involve the secretion of bioactive molecules by activated macrophages, such as reactive oxygen species (ROS), tumor necrosis factor α (TNF-α), and interleukin-1β (IL-1β; Mosser and Edwards, 2008). Macrophages can be activated by numerous agents, some of which act *via* signal transduction processes that are involved in the modulation of second messengers, such as protein kinase C (PKC) and Ca^{2+}, and the activation of several transcription factors, such as STAT (signal transducer and activator of transcription) (Tripathi and Sodhi, 2007). However, little is known about the effects of prolactin on the activation of macrophages. Our group has explored this phenomenon over the past few years (Carvalho-Freitas et al., 2007, 2008, 2011; Ochoa-Amaya et al., 2010, 2011).

Hypophyseal prolactin is hypothesized to have the ability to modulate immune system function, and central dopamine systems may influence immune system competence by regulating neural prolactin receptors and possibly neural prolactin release (Torner et al., 2002).

We recently showed that hyperprolactinemia induced by the dopamine D_2 receptor antagonist domperidone led to increased oxidative bursts and phagocytosis in peritoneal macrophages from female virgin rats, and that these effects were related to dopamine and prolactin receptors on the cell surface. These events were demonstrated *in vitro*, showing enhanced macrophage activity after prolactin and domperidone incubation (Carvalho-Freitas et al., 2007, 2008). Therefore, a positive correlation between serum prolactin levels and the intensity of oxidative bursts appears likely (Carvalho-Freitas et al., 2007).

Lactation was shown to strongly affect the immune system in laboratory rats (Jaedicke et al., 2009). We recently showed that the activity of peritoneal macrophages in lactating rats may be modulated by prolactin and caused by dopamine receptor blockade (Carvalho-Freitas et al., 2011). These data suggest that reproductive experience is associated with a reduction of serum prolactin levels, and macrophages in experienced female organisms become more sensitive to the effects of prolactin. Thus, reproductive experience-induced changes in the neuroendocrine and immune systems in lactating rats appear likely (Carvalho-Freitas et al., 2011).

Normal pregnancy is associated with substantial changes in immune and endocrine signaling that are required to allow the developing fetus and placenta to survive in the environment of the maternal immune system (Murphy et al., 2006). Fetal, paternal, and placental antigens are continuously present and recognized by the maternal immune system and, rather than causing rejection of the fetoplacental graft, are tolerated and supported (Aagaard-Tillery et al., 2006). This process is coordinated through the concerted activities of maternally and placentally derived sex hormones and their associated effects on cytokine signaling and cellular immunity. Moreover, many hormones increase during pregnancy, such as corticotropin-releasing factor, adrenocorticotropic hormone, cortisol, estrogens, progesterone, prolactin, and placental lactogen (Kanik and Wilder, 2000).

Therefore, understanding the prolactin-induced modulation of the activity of immune cells during pregnancy seems important. The present study sought to study the effects of prolactin and dopamine D_2 receptor blockade on peritoneal activity in macrophages from primigravid and multigravid rats during the third trimester of pregnancy and to determine whether these responses vary as a function of the female's reproductive experience. We also measured serum prolactin levels in these animals.

2. Materials and methods

2.1. Animals

All of the *in vitro* procedures were performed with peritoneal fluid harvested from age-matched primigravid and multigravid female Wistar rats. These animals from our