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Full Length Research paper

L-arginine treatment alters the pattern of rat maternal aggressive response toward an intruder entering the homing cage

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Maternal aggression toward intruders is temporarily expressed during lactation in association with the rearing and protection of offspring to promote their survival and growth. This normal component of maternal behaviour that requires both the hormonal changes occur at the end of pregnancy and due to the presence of newborn for its establishment analyzed in Wistar rats. The effects of nitric oxide (NO) by L-arginine (L-arg) was analyzed when administered on days 1, 4, and 8 postpartum in order to observe the maternal aggressive response to an intruder. Data collected indicated that L-arg treated dams exhibited a significant increase of the frequency of biting on postpartum days 1 and 8. In contrast, findings also showed reductions of the frequency of sniffing on day 1. Both frontal and lateral attacks also showed significant decreases attributable to L-arg treatment. These findings suggest that the role of NO during the lactating period may be relevant for the survival and long-term behavioral development of the progeny due to the fact that it has been demonstrated that several neurotransmitters are closely regulated by NO. Therefore, this is interesting in the development of antidepressant drugs for possible human applications.

Key words: Aggressive behavior, lactating period, rats, L-arginine treatment, postpartum.

INTRODUCTION

Maternal aggressive response (MAR) toward intruders is temporarily expressed during lactation in association to the rearing and protection of offspring, to ensure their survival and growth in the nest. This normal component of maternal behaviour requires both hormonal changes occurring at the end of pregnancy and the presence of new born infants for its establishment (Kinsley and Lambert, 2006, 2008). Female mice can also show a type of territorial aggression toward other females, but this aggression is much less fierce (Parmigiani et al., 2009).

During the early postpartum of altricial species, the MAR is an adaptive and conservative behavior displayed to protect the pups from infanticide of intruders and conspecifics; MAR progressively declines to very low levels even though lactation continues (Parmigiani et al., 2009). During the first 10 days after delivery, female rats vigorously attack intruders in the nest area to protect the

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newborn pups. In order to assess the MAR in rodents, an adult intruder, male or female, was introduced into the home cage of a resident female. The frequency of sniffing, where the female approaches the intruder and sniffs its body and/or genitals; of lateral attacks, where the female moves laterally towards the intruder, usually associated with piloerection and biting the back of the intruder; of frontal attacks, where the female with the body orientated frontally lunges toward the intruder followed by biting of the intruder's head and shoulders and (the females bites any other part of the intruder's body (Giovernardi et al., 2000). Later, the decreased dependency of the pups in terms of feeding and environmental exploration may also decrease the mother's protective behaviours.

The typical MAR may be triggered by numerous endogenous stimuli, including progesterone, estradiol, and prolactin, or by cues from the pups that increase the neuronal excitability underlying the directed aggression (Gandelman and Simon, 1980; Giovernardi et al., 2000; Mayer et al., 1987; Svare and Gandelman, 1976; Svare et al., 1982; Wise, 1974). Females only express maternal aggression in association with the rearing of offspring. Females develop a specialized mechanism for the control of their adjustment.

The role of NO is definitely an interesting area of research. Nitric oxide sintetaze (NOS) is a family of tree related enzymes. All three forms of NOS occur in the brain, eNOS is found in cerebral blood vessels, iNOS is limited to glial cells. Most NO in the brain is from the presence of the enzyme NOS in brain tissue. Their formation at the synapse and is linked to the behavior of glutamate as a neurotransmitter. NO is released into the postsynaptic neuron when glutamate binds to a NMDA receptor. The binding of glutamate causes channels in the membrane to open and permit calcium ions from the fluid in the synapse to enter the postsynaptic neuron and bind calmodulin, which stimulates NOS in the neuron to convert arginine into NO and citruline (Butter and Nicholson, 2003).

Rodent aggressiveness is classified, in general, according to the emotive component and the intent of the attack, such as: offensive, territorial, irascible among males; maternal and defensive; when emotional components are minimal. Problems of aggressiveness result from the interaction between innate and acquired mechanisms acting on the basic systems to release several interrelated neurochemicals. The gas NO also has been linked to the timing of parturition in rats (Okere et al., 1996) and to the formation of olfactory memories in lactating sheep (Kendrick et al., 1997).

Herein, early MAR was used as a model to evaluate the L-arg treatment during the lactation period when maximum frequency of aggressive behavior occurs (Giovernardi et al., 2000; Numan, 1994). Moreover, this study may provide insights into the understanding of the role of NO in the regulation of human aggression associated with environmental influences, which may help diagnose some human aggressive abnormalities or degenerative diseases (Engelmann et al., 2004; Kumar and Kumar, 2010).

In this study, these investigators endeavored first to examine how the L-arg treatment affected the expression of MAR in rats when triggered toward an intruder. Next, the dynamics of NO synthesis during maternal aggression caused by behavioral MAR testing with treatment of L-arg (NO precursor) were explored. In this research early MAR was used as a model to evaluate the L-arg treatment during the lactation period that occurs when maximum frequency of aggressive behavior is noticed. (Giovernardi et al., 2000; Numan, 1994). Moreover, the study may provide insights into the understanding of the role of NO in the regulation of human aggression associated with environmental influences, which may help diagnose some human aggressive abnormalities or degenerative diseases (Engelmann et al., 2004; Kumar and Kumar, 2010). The study of NO and its role in the display of patterns of maternal aggression is an interesting topic for their potential use as an antidepressant drug.

MATHERIALS AND METHODS

Animals

All experiments were approved by Local Ethics Committees and are in accordance with the Mexican laws for animal care which comply with the guidelines for the use of animals in neuroscience and behavioral research (Gaceta Oficial del Distrito Federal, 2002; National Research Council 2003). Rats from "Harlan Sprague Dawley" (Indianapolis, USA) were maintained in a room with automatically controlled temperature at 24 ± 2°C, 50% humidity, 12 h light/dark cycle with lights on at 07:00 h, and free access to Purina chow food and water. The procedure to obtain experimental subjects was placing an adult male in an acrylic cage (60 cm × 50 cm × 20 cm) with two females (200-250 g). Later, the sperm-positive females were separated and maintained individually in acrylic maternity cages (50 cm × 40 cm × 20 cm) that were equipped with wood shavings as nesting material. After birth, pups were weighed and their sex was determined as four females and four males from each litter which were randomly distributed between dams.

L-arg administration

L-arg hydrochloride, purchased from Sigma Chemical Co., St. Louis, USA, was dissolved in a saline solution (0.9% sodium chloride) in order to obtain the following dose: 25 mg/kg. This vehicle was used as the control treatment for the control group. Injections were administrated IP at a volume of 0.001 ml/g of body weight. In the IP administration study, 25 mg/kg of L-arg was injected. Temporarily the head of the rat was inserted into an acrylic box after which a micro syringe was then inserted into the IP area. The MAR behavior was measured 15 min after the injection.

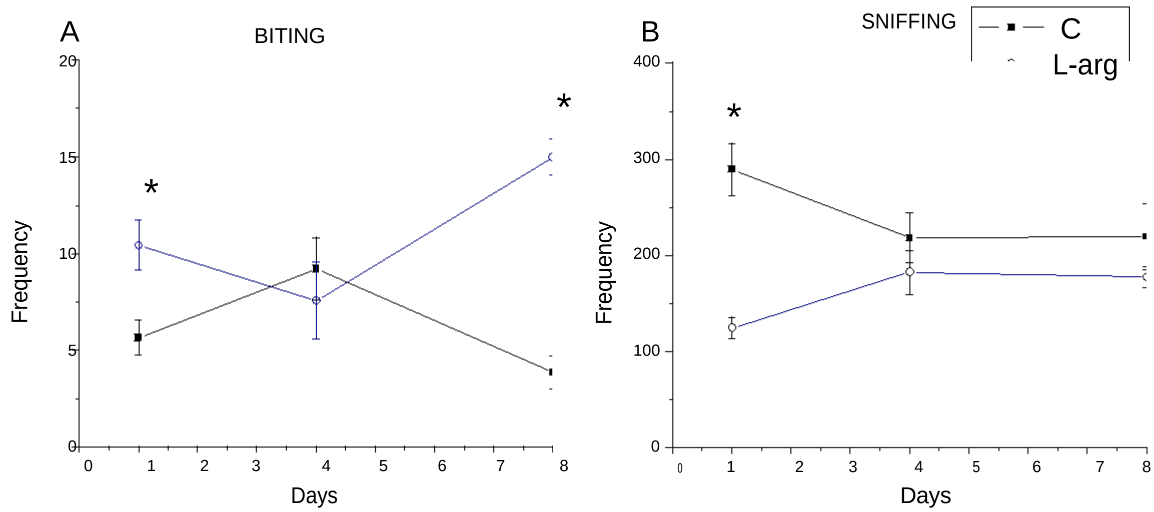

Figure.1. Means±SE frequency of biting (A) and sniffing (B) by a resident lactating female toward an intruder male. The aggression components recorded in control (C) and L-arginine treated (L-arg) lactating females. The ordinates show the frequency of MAR components recorded. * indicates significant differences between groups p<0.05.

MAR test

For MAR tests a total of 84/intruder/group males (250-300 g) were randomly assigned to one of the two groups of lactating (females) Control (C) and L-arg treated female rats. A total of 84 dam/groups, each with her own litter were used. The intruders were used once in each trial. A total of 84 trials were video recorded in C (n=14 dams/ age of testing) and L-arg treated (n=14 dams/age of testing) for independent testing of the dams at 1, 4, and 8 postpartum days. MAR was evaluated between 10:00 and 12:00 h. Before testing, the dam was habituated for 5 min to the recording environment (27ºC) by placing the mother and her litter in their own plastic living cage (50 cm × 40 cm × 20 cm) in a soundproof chamber (2.50 m × 2.50 m × 2.00 m) located next to the main laboratory area.

Each mother was observed and video recorded for a 1 min control span before MAR testing. Thereafter, an intruder was gently introduced into the home cage on the side opposite the nest location. The maternal aggressive behaviour directed at the intruder was recorded for a 10-min period. The frequency was recorded for the MAR components previously described by (Mayer and Rosenblatt, 1987; Miczek and O´Donnell, 1978; Miczek et al., 2002). These behaviors were defined as follows: 1) sniffing, the mother approaches the intruder while emit sniffing movements; 2) biting, the dam grips the body of the intruder and bites him hard with her teeth; 3) frontal and 4) lateral attacks.

Experimental data were analyzed with the Statistical Package for Social Science (SPSS), version 17.0. The scored differences of MAR components were compared with a two-way ANOVA with independent measurements, and 2(L-arg treatment) for 3 days of testing. Post hoc comparisons for particular days of the study were evaluated with the Bonferroni test. A probability of ≤0.05 was considered statistically significant.

RESULTS

The two-way ANOVA comparisons of the frequency of biting from the dams oriented toward the intruder, showed significant changes associated to the L-arg treat-ments [$F_{(1,78)}$ = 3.473, p<0.027)]. *Post hoc* comparisons showed significantly increase frequencies of biting from the L-arg treated dams on days 1 and 8 (p<0.05), (Figure 1A).

The ANOVA comparisons of C and L-arg treated females showed significant changes for sniffing frequency [$F_{(1,78)}$ = 4.990, p<0.009)]. The frequency of sniffing, decrease at day 1 (p=0.035) and non significant differences were noted on days 4 and 8 of testing (Figure 1B).

The two-way ANOVA comparisons of the frequency of frontal attacks data showed significant interaction between treatment and days of testing. [$F_{(2,78)}$ =6.551, p = 0.002] was observed (Figures 1A and 1B). *Pos hoc* comparison showed a significant decrease in the frequencies of frontal attack in the L-arg treated damson days 1 and 4 of testing (p<0.05), (Figures 2A and 2B).

The two-way ANOVA comparisons of the frequency of lateral attacks data showed significant interaction between treatment and days of testing [$F_{(2,78)}$ =84.632, p = 0.0001] was observed (Figure 2B). *Pos hoc* comparison showed significant decreases in the frequency of lateral attacks in the L-arg treated dams on day 8 of testing (p<0.05), (Figures 2A and 2B).

DISCUSSION

Findings suggest that L-arg alters the mechanism that underlies the MAR, because of the onset of a higher

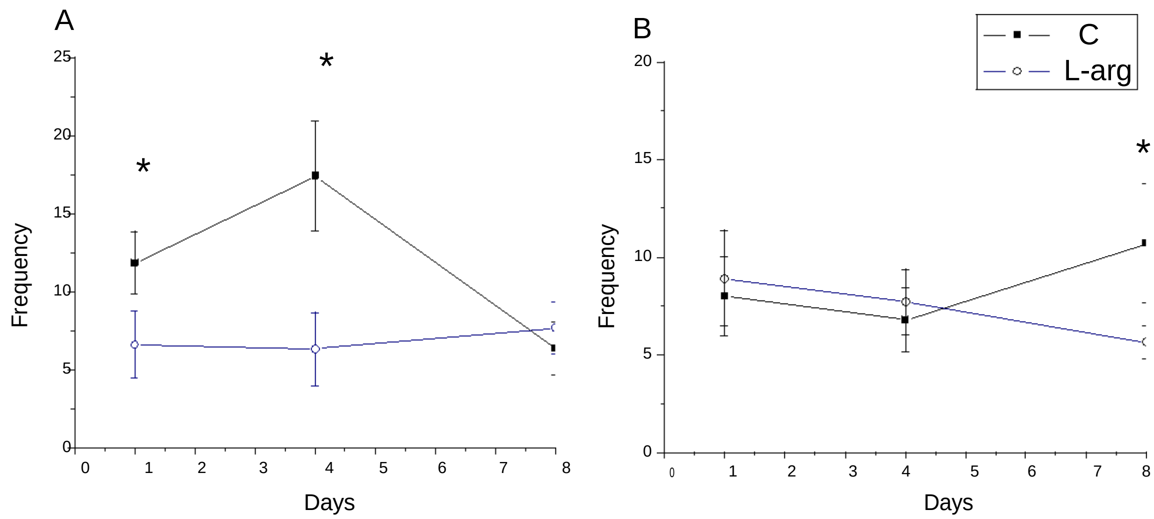

Figure 2. Means±SE frequency of frontal (A) and lateral (B) attacks by a resident lactating female toward an intruder male. The aggression parameters recorded in control (C) and L-arg treated lactating females. The ordinates show the frequency of MAR components recorded. * indicates significant differences between groups p<0.05.

frequency of biting and sniffing directed toward the intruder male due to the administration of L-arg (Figure 1). The increased aggression in L-arg treated mothers may be associated with the induced fear and anxiety brought on by the presence of the intruder (Workman et al., 2008). The L-arg treatments appears to be influential on the neural system that underlies the expression of behavioural and endocrine responses to stress (Butler and Nicholson, 2003; de Sousa-Santos et al., 2004; Douglas, 2005; Engelmann et al., 2004; Zhang et al., 2004) also observed this in undernourished rats (Perez-Torrero and Salas, 2007; Salas et al., 2003). Furthermore, data indicated that L-arg does not alter lateral attacks on days 1 and 4 of testing, possibly due to NO functional alterations in the neuronal system that modulates emotional behavior such as MAR (Miczeck and O´Donnell, 1978; Miczeck et al., 2002). Earlier studies have demonstrated that brain regions such as the hippocampus, septum, preoptic area, amygdale, and dorsal periaqueductal gray matter are involved in aggressive and defensive behaviours (de Almeida and Lucion, 1997). In addition, several studies of neuronal activity labeling (Fos-IR) suggest that alterations occur during the lactating period, influencing behavior, hormonal changes and neural activity (Davis and Marler, 2004).

Alterations observed in the pattern of MAR can be related to disorders in the amygdala level in the physiological mechanisms that regulate neuronal excitability and thereby interfere with emotional behavior (Tottenham, 2009; Walker et al., 2003). Findings suggest that the administration of L-arg and the presence of an intruder in nest area, influence brain functions at critical stages that are essential for the performance of maternal behavioural patterns which in turn may be relevant for the survival of the progeny. The L-arg treatment may alter NO synthesis or activity in neuronal structures implicated in maternal aggression (Giovernardi et al., 2000). Moreover, potentially producing increases in novelty-induced behavior due to L-arg. This suggests that this physiological stress response is mediated via activation of the nitrergic system. The role played by NO in the brain is no simple matter. Its formation occurs at the synapse and is linked to the action of glutamate as a neurotransmitter which is then released into the postsynaptic neuron (Butler and Nicholson, 2003). These findings may be related to several neurotransmitter activities including serotonin, glutamate, dopamine (DA), γ-aminobutiric acid (GABA) which are ultimately regulated by NO (Wegener and Volke, 2010). In addition, NO can inactivate the rating limiting enzymes in the synthesis of 5-HT, tryptophan hydroxylase (Kuhn and Arthur, 1996, 1997) and different types of antidepressants have been found to modulate the hippocampal NO level *in vivo*. Consequently, NO system is of great interest for use as an antidepressant and anxiolytic drug action in acute therapy, as well as in prophylaxis (Wegener and Volke, 2010).

The roughly inverse changes in patterns of behavior observed are change possible due to different circulating substances of neurodoendocrine system that adjusts the display of distinct parameters of aggressive behavior patterns. These alterations may be related to changes of circulating hormones, such as prolactin, oxytocin, and potentiating in a selective manner affecting the components of aggressive behavior. This being the early lactating period; it is also the most critical stage to induce alterations of circulating hormones as has been aforementioned. Taken as a whole, these results indicate that there are different hormone contents that regulate specific parameters of the aggressive pattern (Kennett et al., 2009; Valdez et al., 2009). Further research is necessary to understand the role of the hormonal profile in the MAR associated with the role of the nitrergic system. Due to the fact that physiological roles of NO remain relatively unclear, continuing studies into the effects of NO should be carried out. This topic continues to be an interesting matter for the future development of antidepressant drugs to be used for possible human applications.

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