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PROLACTIN FRACTIONS FROM LACTATING RATS ELICIT EFFECTS UPON SENSORY SPINAL CORD CELLS OF MALE RATS

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Abstract—Recently it has been suggested that the neurohormone prolactin (PRL) could act on the afferent nociceptive neurons. Indeed, PRL sensitizes transient receptor potential vanilloid 1 (TRPV1) channels present in nociceptive C-fibers and consequently reduces the pain threshold in a model of inflammatory pain. Accordingly, high plasma PRL levels in non-lactating females have been associated with several painful conditions (e.g. migraine). Paradoxically, an increase of PRL secretion during lactation induced a reduction in pain sensitivity. This difference could be attributed to the fact that PRL secreted from the adenopituitary (AP) is transformed into several molecular variants by the suckling stimulation. In order to test this hypothesis, the present study set out to investigate whether PRL from AP of suckled (S) or non-suckled (NS) lactating rats affects the activity of the male Wistar wide dynamic range (WDR) neurons. The WDR neurons are located in the dorsal horn of the spinal cord and receive input from the first-order neurons (Ab-, Ad- and C-fibers). Spinal administration of prolactin variant from NS rats (NS-PRL) or prolactin variant from S rats (S-PRL) had no effect on the neuronal activity of non-nociceptive Ab-fibers. However, the activities of nociceptive Ad-fibers and C-fibers were: (i) increased by NS-PRL and (ii) diminished by S-PRL. Either NS-PRL or S-PRL enhanced the post-discharge activity. Taken together, these results suggest that PRL from S or NS lactating rats could either facilitate or depress the nociceptive responses of spinal dorsal horn cells, depending on the physiological state of the rats.

Key words: lactation, prolactin, nociception, WDR neurons, rats.

INTRODUCTION

Since its discovery, considerable literature has built up describing more than 300 biological actions of prolactin (PRL) Ben-Jonathan et al., 1996; Bole-Feysot et al., 1998; Grattan and Kokay, 2008). Recent behavioral studies have shown that PRL generated during peripheral inflammation could act as a key mediator to reduce the pain threshold (Scotland et al., 2011). Furthermore, in cultured trigeminal nociceptive neurons, exogenous PRL is able to sensitize transient receptor potential vanilloid 1 (TRPV1) channels and consequently a potential pro-nociceptive effect was suggested (Diogenes et al., 2006).

Indeed, several authors propose that an increase of PRL secretion could contribute to certain pain disorders, such as migraines (Silberstein and Merriam, 1993), rheumatoid arthritis (Chikanza et al., 1993), and mastodynia (Theunissen et al., 2005). Rushen et al. (1993) suggested that an increase of prolactin secretion by suckling paradoxically reduces pain sensitivity. We hypothesize that under non-lactating conditions prolactin might enhance sensory perception, while this might not happen during lactation, due to suckling-induced transformation of the prolactin being secreted from the anterior pituitary. Indeed PRL secreted from the adenopituitary (AP) is transformed into several molecular variants by suckling stimulation (Grosvenor and Mena, 1992). Furthermore PRL has as a number of molecular isoforms produced by posttranslational modifications (Sinha, 1992), and we could propose that this molecular heterogeneity is one of the mechanisms involved in the pleiotropic activity of these peptides in addition to that suggested by Grattan and Kokay (2008) who in part attributed this pleiotropic activity to the regulation of expression of PRL receptors.

Prolactin variants are secreted under different physiological conditions (Mena et al., 2010), and it is known that functional interactions and cytological differences exist among pituitary lactotrophs within the anterior pituitary gland (Dymshitz and Ben-Jonathan, 1991). For instance, molecular isoforms present in the AP of the lactating rats are transformed by suckling stimulation (Mena et al., 1992). This transformation of PRL involves changes in the solubility of the protein that

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Abbreviations: AP, adenopituitary; ELISA, enzyme-linked immunosorbant assay; NS, non-suckled; NS-PRL, prolactin variant from non-sucked rats; NR, non-denaturing; PRL, prolactin; RF, receptive field; S, suckled; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; S-PRL, prolactin variant from suckled rats; TBS, Tris-buffered saline; TRPV1, transient receptor potential vanilloid 1; TTBS, TBS with 0.5% Tween 20; WDR, wide dynamic range neurons.

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confers a longer half-life in the circulation and potentially different biological actions (Mena et al., 1992).

In order to test the potential effect of PRL from the AP of suckled (S) or non-suckled (NS) rats on the neuronal activity of nociceptive fibers, we recorded in anaesthetized male rats the nociceptive responses evoked on the wide dynamic range (WDR) neurons. The WDR neurons are large, second-order neurons that are widely distributed in the dorsal horn spinal cord and receive input from the first-order non-nociceptive (Ab-fibers) and nociceptive (Ad- and C-fibers) neurons (Mendell, 1966; Willis, 1987, 1988). These cells represent an important connector between primary afferent nociceptive fibers and higher nociceptive centers. Our results show that spinal administration of NS-PRL or S-PRL had no effect on the neuronal activity corresponding to the activation of non-nociceptive Ab-fibers (latency between 0 and 20 ms). However, the firing of Ad-fibers (latency between 20 and 90 ms) and C-fibers (90–350 ms) (both nocicceptive) were: (i) increased by NS-PRL and (ii) diminished by S-PRL. Finally, either NS-PRL or S-PRL enhanced the post-discharge activity (350–800 ms). These findings provide a basis for addressing the physiological relevance of the prolactin variants induced by suckling and might help to explain the differences observed in several experiments related to nociception.

EXPERIMENTAL PROCEDURES

Animals

Experiments were carried out on Wistar rats from the Neurobiology Institute Animal House. The animals were housed individually in plastic cages in a special, temperature-controlled room (22 ± 2 °C) and given *ad libitum* access to food (Purina Chow, Ralston Purina Co., Chicago, IL, USA) and water. In the case of primiparous lactating rats, the animals were in a room with a reversed light–dark cycle (lights on at 6:00 h; 14 h light, 10 h darkness). The primiparous rats weighed 250 g at mating, they gained weight during pregnancy and lactation, and they weighed an average of 300 g during experimentation, as did the male rats used in the electrophysiological experiments.

All experimental procedures were approved by our Institutional Ethics Committee, and they were in accordance with the IASP ethical guidelines (Zimmermann, 1983) and the guidelines contained in the NIH guide for the Care and Use of Laboratory animals (80–23, revised in 1996). All efforts were made to limit distress and to use only the number of animals necessary to produce reliable scientific data.

General methods for prolactin extraction

Surgical procedures and preparation of concentrated conditioned media. On postpartum days (10–12), the pups (8–10 pups per litter) from primiparous lactating rats were removed from groups of mothers at 7:00 h, and 6 h later their pups were or were not returned for suckling for 15 min. At the end of the non-suckling (NS) or suckling (S) periods, the mothers were killed by decapitation after light ether anesthesia. The AP was collected using a fine forceps as originally described by Boockfor and Frawley (1987), i.e. the central region around the neurointermediate lobe was dissected and incubated. The AP fragments corresponding to the central pituitary region from S and NS lactating rats were used to prepare the conditioned media. These fragments were incubated in individual flasks containing 300 µl of Earle's medium. Flasks containing the pituitary fragments were gassed with 95% O_2, 5% CO_2, sealed with rubber stoppers, and incubated at 37 °C for 1 h in a water bath shaker (American Optical, Buffalo, NY, USA). After incubation, conditioned media were concentrated, desalted in a Centricon micro-concentrator (Centripep, Millipore, Bredford, MA, USA), and stored frozen until assayed. The PRL concentration in the conditioned media was determined by the enzyme-linked immunoabsorbant assay (ELISA) method (Section 'ELISA').

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The samples of conditioned media from N and NS rats were analyzed by SDS–PAGE in 1.0-mm thick, 6-cm long, 12.5% gels using the buffer system of Laemmli (1970) and Bradford (1976) in a mini Protean III cell (Bio-Rad, California, USA). Samples were electrophoresed under non-denaturing (NR) conditions. The gels were then cut and divided into six fractions (Fig. 1); the proteins in each fraction were electrophoretically eluted, dialyzed, lyophilized, reconstituted, and washed (ProteoSpin, Detergent Clean Up Micro Kit, Norgen, ON, Canada), and assayed by ELISA for PRL content (Fig. 1).

Each fraction of NS-PRL and S-PRL was adjusted to have 20 µg of protein per 100 µl of deionized water, and 20 µl of each solution was administrated at the same spinal cord level (L4–L5) as the electrophysiological recording (see Section 'Western blotting').

ELISA. The PRL concentrations in conditioned media and fractions were determined by the ELISA method as modified by Signorella and Hymer (1984). Briefly, 96-well microtiter plates (Immulon 2HB, Chantilly, VA, USA) were coated overnight at 4 °C with 10 ng of rat PRL in 100 µl of 1 M carbonate buffer, pH 10.3. The plates were washed with TPBS (0.01 M sodium phosphate, 0.15 mM NaCl, 0.05% v/v Tween-20, pH 7). This washing procedure was performed after each incubation step. For the standard curve, serial dilutions of rat PRL (NHPP-NIH) (0.06–64 ng/ml) in TPBS were incubated for 16 h with 100 µl primary anti-rPRL polyclonal antiserum (1:40,000; NHPP-NIH) in TPBS containing 1% (w/v) non-fat dry milk (Bio-Rad). Samples and standards (100 µl) were then added to the coated wells and incubated for 2 h at room temperature. Secondary goat anti-rabbit IgG peroxidase conjugate (Bio-Rad) was then added (1:3000 in TPBS with 1% non-fat dry milk) and incubated for 2 h at room temperature. Bound secondary antibodies were detected by reaction with