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Prolactin-derived vasoinhibins increase anxiety- and depression-related behaviors

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Summary The hormone prolactin (PRL) regulates neuroendocrine and emotional stress responses. It is found in the hypothalamus, where the protein is partially cleaved to vasoinhibins, a family of N-terminal antiangiogenic PRL fragments ranging from 14 to 18 kDa molecular masses, with unknown effects on the stress response. Here, we show that the intracerebroventricular administration of a recombinant vasoinhibin, containing the first 123 amino acids of human PRL that correspond to a 14 kDa PRL, exerts anxiogenic and depressive-like effects detected in the elevated plus-maze, the open field, and the forced swimming tests. To investigate whether stressor exposure affects the generation of vasoinhibins in the hypothalamus, the concentrations of PRL mRNA, PRL, and vasoinhibins were evaluated in hypothalamic extracts of virgin female rats immobilized for 30 min at different time points after stress onset. The hypothalamic levels of PRL mRNA and protein were higher at 60 min but declined at 360 min to levels seen in non-stressed animals. The elevation of hypothalamic PRL did not correlate with the stress-induced increase in circulating PRL levels, nor was it modified by blocking adenohypophyseal PRL secretion with bromocriptine. A vasoinhibin having an electrophoretic migration rate corresponding to 17 kDa was detected in the hypothalamus. Despite the elevation in hypothalamic PRL, the levels of this hypothalamic vasoinhibin were similar in stressed and non-stressed rats. Stress reduced the rate

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of cleavage of PRL to this vasoinhibin as shown by the incubation of recombinant PRL with hypothalamic extracts from stressed rats. These results suggest that vasoinhibins are potent anxiogenic and depressive factors and that stress increases PRL levels in the hypothalamus partly by reducing its conversion to vasoinhibins. The reciprocal interplay between PRL and vasoinhibins may represent an effective mechanism to regulate anxiety and depression.

1. Introduction

Prolactin (PRL) regulates a wide range of biological effects in and beyond reproduction (Ben-Jonathan et al., 1996). Some of these effects occur in the brain, where PRL promotes maternal and feeding behaviors (Grattan and Kokay, 2008), suppresses fertility (Sonigo et al., 2012), stimulates neurogenesis and neuronal survival (Shingo et al., 2003), regulates neurotransmitter and neuropeptide release (Grattan and Kokay, 2008; Vega et al., 2010), and attenuates stress-induced neuroendocrine and anxiety responses (Torner et al., 2001; Torner and Neumann, 2002).

PRL is often referred to as "stress hormone" because a number of physical and emotional forms of stressors stimulate its secretion from the adenohypophysis into the circulation (Reichlin, 1988). The increase in circulating PRL is considered to be an adaptation to ensure competence of the immune system (Dorshkind and Horseman, 2000; Yu-Lee, 2002) and proper physiological and behavioral responses to stress (Torner et al., 2001; Torner and Neumann, 2002). The PRL receptor is expressed in several immune cells, in which this hormone regulates proliferation, survival, and the release of inflammatory mediators (Yu-Lee, 2002). PRL inhibits stress-induced production of immunosuppressive steroids (Cook, 1997) and the stress-induced increase of corticotropin secretion (Torner et al., 2001). Also, PRL reduces anxiety behavior, and blocking PRL receptors in the brain prevents both PRL inhibition of the hypothalamic—pituitary—adrenal (HPA) axis activation and its anxiolytic effect (Torner et al., 2001). These central actions may be mediated by circulating PRL accessing the brain after entering the cerebrospinal fluid via its receptors in the choroid plexus (Walsh et al., 1987; Mangurian et al., 1992). In addition, PRL mRNA is expressed in hypothalamic tissue (Clapp et al., 1994; Ben-Jonathan et al., 1996; Torner et al., 2004; Grattan and Kokay, 2008) and, although its neuronal localization has not been demonstrated, PRL may act as a neuropeptide to regulate stress-related responses.

Adding complexity to PRL actions is its structural polymorphism. In the hypothalamus, PRL is proteolytically cleaved to vasoinhibins (Clapp et al., 1994), a family of N-terminal PRL fragments with molecular masses ranging from 14 to 18 kDa that signal through receptors distinct from PRL receptors (Clapp and Weiner, 1992) to exert effects opposite to those of the full-length hormone on blood vessels. PRL stimulates blood vessel growth (angiogenesis), whereas vasoinhibins inhibit angiogenesis (Clapp et al., 2009). The structural diversity of vasoinhibins derives from the fact that different proteases generate the various fragments by cleaving near or within various sites of the large disulfide loop linking alpha helixes 3 and 4 of the PRL molecule (for reviews see Clapp et al., 2006, 2009). Vasoinhibins not only share blood vessel inhibitory properties but also non-vascular actions. 14 kDa Human and 16 kDa rat vasoinhibins act as proinflammatory cytokines upregulating inducible nitric oxide synthase (iNOS) in pulmonary fibroblasts (Corbacho et al., 2000), whereas PRL functions to attenuate proinflammatory cytokine-induced iNOS expression in these cells (Corbacho et al., 2003). On the other hand, both PRL and vasoinhibins stimulate the release of vasopressin by the hypothalamo-neurohypophyseal system (Mejia et al., 2003).

Since vasoinhibins are found in the hypothalamus and have actions that may or may not differ from those of PRL, here we investigated the hypothesis that vasoinhibins affect anxiety behavior and that their generation is modified under conditions of stress.

2. Methods

2.1. Reagents

A recombinant 14 kDa vasoinhibin containing the first 123 amino acids of the human PRL sequence and a tail of 7 histidines generated as described (Galfione et al., 2003) was used in behavioral tests. Recombinant rat PRL used in cleavage assays and as standard in Western blots was purchased from the National Hormone and Pituitary Program (NHPP) and Dr. A.F. Parlow (Harbor-University of California, Los Angeles Medical Center, Los Angeles, CA). PRL having a cleavage in the large disulfide loop and a 16 kDa vasoinhibin containing the first 145 amino acids of PRL were used as standards in Western blots and were generated by enzymatic proteolysis of rat PRL with a mammary gland extract enriched with cathepsin D, as reported (Clapp, 1987).

2.2. Animals

Virgin, female Wistar rats (230—250 g body weight) were maintained and treated according to local institutional guidelines and in compliance with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health. The Bioethics Committee of the Institute of Neurobiology of the National University of Mexico (UNAM) approved all animal experiments. Rats were handled daily for five to seven days before the experiment. All experiments were performed between 0900 h and 1200 h. Behavioral tests were carried out on diestrous day. In non-behavioral studies,