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Research Report

Cocaine- and amphetamine-regulated transcript (CART) expression is differentially regulated in the hypothalamic paraventricular nucleus of lactating rats exposed to suckling or cold stimulation

Edith Sánchez[a,c], *Csaba Fekete*[b,c], *Ronald M. Lechan*[c,d], *Patricia Joseph-Bravo*[a,*]

[a]*Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, AP 510-3, Cuernavaca, Mor., C.P. 62210, México*

[b]*Department of Endocrine and Behavioral Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest H-1083, Hungary*

[c]*Tupper Research Institute and Department of Medicine, Tufts-New England Medical Center, Boston, MA 02111, USA*

[d]*Department of Neuroscience, Tufts University School of Medicine, Boston, MA 02111, USA*

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ABSTRACT

Neural stimuli, such as suckling or cold exposure, increase TRH mRNA in the paraventricular nucleus (PVN) of the rat hypothalamus, yet only suckling induces prolactin secretion. As TRH co-localizes with cocaine- and amphetamine-regulated transcript (CART) in hypophysiotropic neurons of the PVN, and CART inhibits TRH-induced prolactin release but not TRH-induced TSH release in adenohypophyseal cell cultures, we raised the possibility that differential regulation of CART gene expression in the PVN may explain the differences in prolactin secretion following each of the two stimuli. Primiparous female rats were mated and handled daily during the pre- and postpartum periods. After delivery, the litter was adjusted to 8 pups and at mid-lactation, dams were separated from their pups for 8 h and exposed to either 1 h of cold or 30 min of suckling. Long-term effects of suckling were studied by separating pups from their mothers for 24 h, followed by a 12 h period of continuous suckling. Serum TSH levels increased in response to cold exposure, while prolactin levels were increased by suckling and diminished by cold exposure. CART mRNA levels increased in rostral and mid parts of the medial parvocellular PVN following cold exposure but not after suckling stimulation. These data demonstrate a differential regulation of CART gene expression in hypophysiotropic neurons in response to stimuli that increase TRH mRNA levels, and suggest that CART activation in the PVN may contribute to the decrease in PRL release when the thyroid axis is activated by cold exposure.

* *Corresponding author.*
E-mail address: joseph@ibt.unam.mx (P. Joseph-Bravo).

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1. Introduction

Thyrotropin-releasing hormone (TRH) neurons involved in the regulation of anterior pituitary TSH and prolactin secretion (Grosvenor and Mena, 1980; Haisenleder et al., 1992) are confined to the medial and periventricular parvocellular subdivisions of the hypothalamic paraventricular nucleus (PVN), primarily organized in mid- to caudal portions of the PVN (Lechan and Toni, 1992; Fekete et al., 2000). Most of these "hypophysiotropic" TRH neurons contain a second peptide, cocaine and amphetamine-regulated transcript (CART) (Broberger, 1999; Elias et al., 2001; Fekete et al., 2000), originally identified in the striatum as a transcript upregulated by psychostimulants (Douglass et al., 1995). While CART neurons from the arcuate nucleus have been shown to exert important effects on energy homeostasis including those on the hypothalamic–pituitary–thyroid axis through their action on hypophysiotropic TRH neurons (Fekete et al., 2000; Stanley et al., 2001), the importance of the coexistence of CART with TRH in the PVN is not yet understood. Hypothyroidism regulates both TRH and CART gene expression in the PVN; reduction in circulating thyroid hormone levels leads to an increase in both TRH and CART mRNA only in the medial and periventricular parvocellular subdivisions of the PVN (Kakucska et al., 1992; Raptis et al., 2004). Nevertheless, while there is an increase in TRH in the portal blood (Rondeel et al., 1988) and TRH is a potent prolactin secretogogue (Jacobs et al., 1971; Tashjian et al., 1971; Yuan and Pan, 2002), prolactin does not rise with hypothyroidism in the rat (Jahnke et al., 1980; Tohei et al., 1997). The possibility that CART could be directly modulating pituitary secretion was corroborated in primary cultures of anterior pituitary cells from male rats; CART inhibits prolactin release after 15 min incubation (Kuriyama et al., 2004) while at longer times, prevents TRH-induced prolactin release without affecting TSH release (Raptis et al., 2004).

Included among the physiologic stimuli associated with activation of hypophysiotropic TRH neurons are suckling and cold exposure that induce TRH release (Arancibia et al., 1983; de Greef and Visser, 1981; Fink and Ben-Aroya, 1983; Hefco et al., 1975) and a rapid (30–60 min) and transient increase in proTRH mRNA levels in the PVN (Rage et al., 1994; Sanchez et al., 2001; Uribe et al., 1993; van Haasteren et al., 1996). However, the response of pituitary target cells is specific to the stimulus. In lactating dams, TSH is released after cold exposure, while it is modestly or not affected by suckling, and the opposite is observed for prolactin, released only after suckling but not cold stimulus (Adels et al., 1986; Haisenleder et al., 1992; Sanchez et al., 2001; van Haasteren et al., 1996). The main goal of this study was to determine whether activation of CART neurons in the PVN is stimulus-dependent, in concert with the selective pituitary response. We therefore evaluated the effects of cold exposure and acute or chronic suckling on CART mRNA expression in the PVN of lactating rats.

2. Results

2.1. *Effect of suckling or cold exposure on serum levels of PRL, TSH and corticosterone in lactating females*

No significant differences were observed in serum corticosterone levels between control animals that were housed at normal room temperature in an adjacent room in the vivarium (RT suckling controls), and those that were transported into a room adjacent to the cold room (RT, cold controls) (Fig. 1A). Suckling produced a small but insignificant increase in corticosterone levels after 30′ of suckling stimulation, while a 6-fold increase in corticosterone was observed after 1 h of cold exposure (Fig. 1A). PRL secretion increased 2.5-fold after suckling, but decreased after cold stimulation (Fig. 1B). TSH levels increased 4.3-fold only after cold exposure (Fig. 1C).

2.2. *Effect of suckling or cold exposure on CART mRNA levels in the PVN as determined by RT-PCR*

PCR amplification for CART mRNA generated two bands similar to that reported by Douglass et al. (1995) corresponding to different polyadenylation start sites on the CART transcript (Fig. 2). The ratio of doublet CART/Cyclophilin signal showed a significant increase in the PVN after 1 h of cold exposure (Fig. 2A). No differences were observed in response to 30′ of

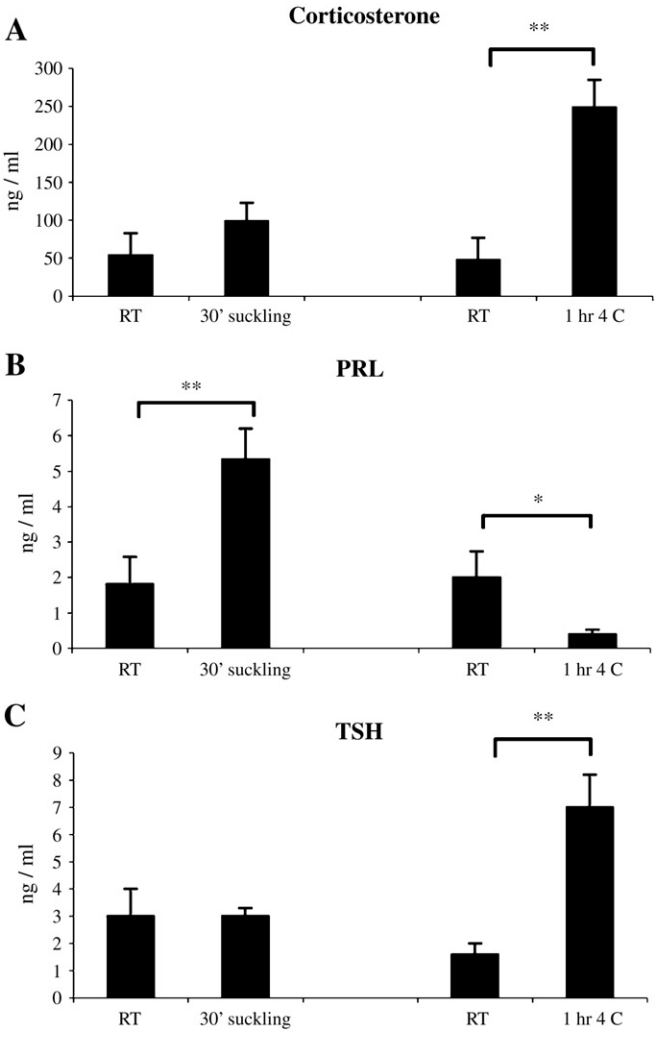

Fig. 1 – Serum hormone concentrations of control, suckling and cold stimulated lactating females quantified by radioimmunoassay (5 animals per group). Values represent mean ± SEM (*$P<0.05$ or **$P<0.001$ statistically different by Newman–Keuls *post hoc* testing).