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Research Report

Transient up-regulation of cocaine- and amphetamine-regulated transcript peptide (CART) immunoreactivity following ethanol withdrawal in rat hypothalamus

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ABSTRACT

We investigated the profile of CART immunoreactivity in some discrete hypothalamic nuclei following chronic ethanol treatment and withdrawal conditions. Adult, male, Sprague–Dawley rats were fed with liquid diet (pair-fed) or liquid diet containing ethanol (ethanol-fed) for 15 days. Thereafter, all the animals were given access to ethanol free nutritionally balanced liquid diet and killed at 0, 24, 48 and 72 h post-withdrawal, and their brains processed for immunocytochemistry using monoclonal antibodies against CART. CART-immunoreactive fibers, but not the cells, were significantly increased in the paraventricular nucleus (PVN). However, the profile of CART-immunoreactive cells and/or fibers in the periventricular area (PeA), arcuate nucleus (ARC), perifornical area inclusive of lateral hypothalamus (LH) and tuber cinereum (TC), dorsomedial (DMH), and ventromedial (VMH) hypothalamus at the 0 h ethanol withdrawal time point was quite similar to that in the pair-fed control rats. Twenty-four hours following ethanol withdrawal, the immunoreactivity in all these areas was dramatically increased. While significant reduction in CART immunoreactivity was noticed in the PVN, PeA, ARC and VMH at 48 h, immunoreactive profile was restored to normal by 72 h post-ethanol withdrawal. The immunoreactive profile in the LH, TC and DMH resembled that of the pair-fed groups at 48 and 72 h post-withdrawal intervals. However, CART-immunoreactive profile in the supraoptic nucleus did not respond to the chronic ethanol treatment and/or withdrawal. We suggest that transient up-regulation of CART in some discrete hypothalamic nuclei following ethanol withdrawal, at least in part, may contribute to the pathogenesis of ethanol withdrawal-induced symptoms like anxiety and anorexia.

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1. Introduction

Spiess et al. (1981) isolated and partially sequenced the cocaine- and amphetamine-regulated transcript peptide (CART) from ovine hypothalamus, which was then named a somatostatin-like peptide. Fourteen years later, using polymerase chain reaction, CART cDNA was isolated from rat brain as a transcript whose expression was found to be regulated by injection of the psychostimulant cocaine or amphetamine (Douglass et al., 1995). The CART gene is composed of 3 exons and 2 introns, with rat and mouse having alternatively spliced variants resulting in the production of two proteins namely long and short forms containing 129 and 116 amino acids respectively (Douglass et al., 1995; Adams et al., 1999). The C-terminal end of CART, consisting of 48 amino acid residues and three disulfide bonds, is thought to constitute the biologically active part of the molecule, and to date, several CART peptide fragments have been identified (Kuhar and Yoho, 1999; Thim et al., 1999). Among them, two fragments CART 55–102 and 62–102 were isolated and sequenced from the hypothalamus and pituitary of rat (Gautvik et al., 1996; Kristensen et al., 1998; Thim et al., 1998, 1999; Bannon et al., 2001). While the peptide was found to be one of the most abundant region specific mRNA in the hypothalamus (Gautvik et al., 1996), in situ hybridization studies showed its localization in several discrete nuclei in the brain (Douglass et al., 1995; Couceyro et al., 1997). It translates into the peptides with neurotransmitter functions in the brain of rat (Kuhar and Dall Vechia, 1999, Vicentic et al., 2006).

In terms of function, the role of CART as an anorectic peptide is well established (Kristensen et al., 1998). Thereafter, its role in the regulation of pain, arousal, startle response, regulation of calcium channels and neuroendocrine hormone secretion has been demonstrated (Bannon et al., 2001; Yermolaieva et al., 2001; Smith et al., 2004; Damaj et al., 2004, 2006). CART has also been reported to play a crucial role in anxiogenic-like activity (Kask et al., 2000; Asakawa et al., 2001) and stress-related responses (Chaki et al., 2003; Koylu et al., 2006; Stanek, 2006). The role of CART in the nucleus accumbens (Acb) in drug reward and reinforcement has already been suggested (Koylu et al., 1998; Smith et al., 1999; Jaworski and Jones, 2006). CART is believed to play a role in drug abuse, addiction and recognized as a potential therapeutic target for drug development (Hunter and Kuhar, 2003; Kuhar et al., 2005; Vicentic and Jones, 2007).

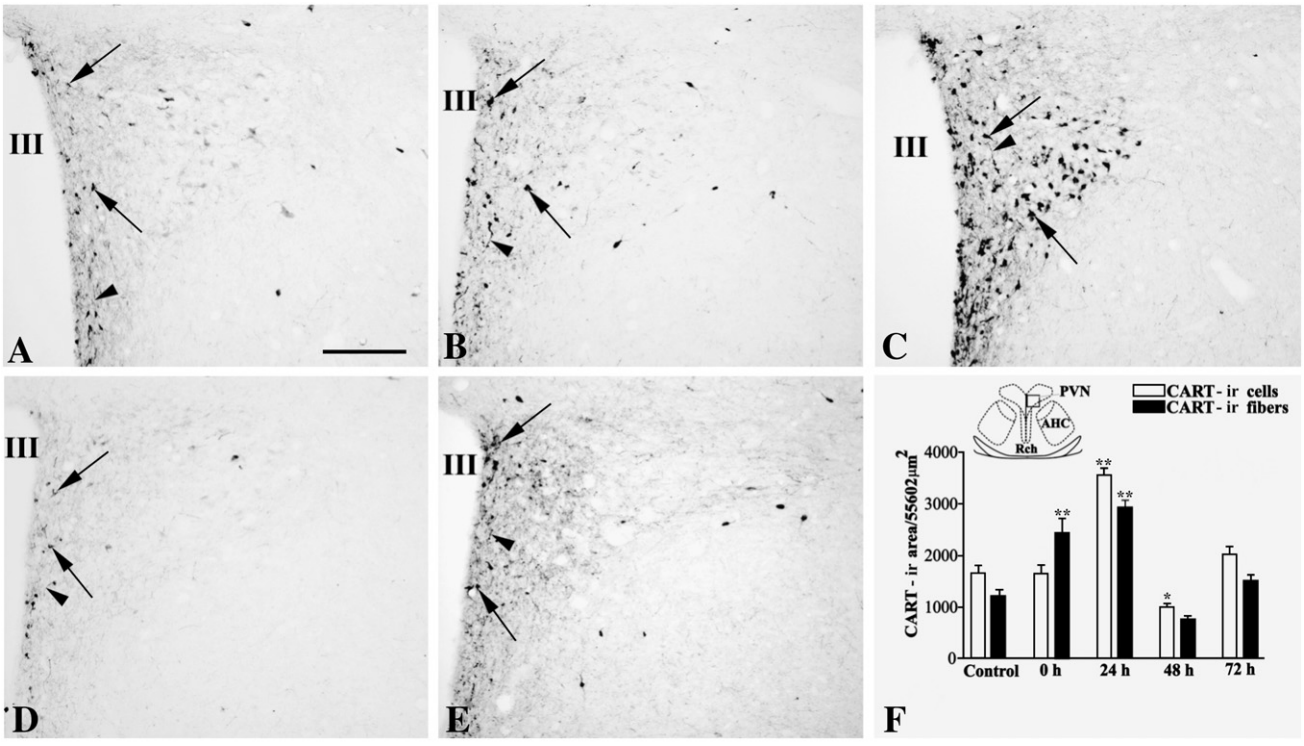

Fig. 1 – Photomicrographs showing CART-immunoreactive (CART-ir) cells (arrows) and fibers (arrow-heads) in the hypothalamic paraventricular nucleus (PVN) of the pair-fed control (A) and ethanol-withdrawn rats at the 0 (B), 24 (C), 48 (D) and 72 h (E) time points. Note a dramatic increase in CART immunoreactivity in the PVN 24 h post-withdrawal compared to the normal and 0 h withdrawal time point rats. CART immunoreactivity showed a rapid decline in the 48 h ethanol-withdrawn animals and attained normal base line levels by 72 h. Diagram F, represents the semiquantitative morphometric analysis of CART immunoreactivity in the PVN of normal and ethanol-withdrawn animals at different time points. The outline of the transverse section through the brain (co-ordinates: bregma −1.80 mm, Paxinos and Watson, 1998) indicates the region of the PVN (square, not to scale) from which the measurements were collated. AHC, central part of anterior hypothalamic area; Rch, retrochiasmatic nucleus. The bar values are shown as the mean ± S.E.M. for 6–8 rats. *P < 0.01 and **P < 0.001 vs respective control group. Scale bar = 200 μm.