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Effects of corticotropin-releasing hormone receptor antagonists on the ethanol-induced increase of dynorphin A1-8 release in the rat central amygdala

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Abstract

Neurons in the central amygdala (CeA) co-express dynorphin and corticotropin-releasing hormone (CRH). Moreover, the activity of both the CRH and dynorphin systems in CeA is altered by alcohol treatments, effects suggesting interactions between the CRH and dynorphin systems. Thus, the objectives of the present study were to investigate the effects of (1) activating CRH receptors (CRHRs) by microinjection of CRH in CeA and (2) blocking CRHRs by local microinjections of CRHR antagonists in the CeA on the alcohol-induced changes in the extracellular concentrations of dynorphin A1-8 with in vivo microdialysis experiments. Microdialysis probes with a microinjection port were implanted in the CeA of alcohol-naïve Sprague–Dawley rats. Microinjections of CRH or antalarmin, a CRH receptor type 1 (CRHR1) antagonist, or anti-sauvagine-30, a CRH receptor type 2 (CRHR2) antagonist, at the level of CeA were followed by an intraperitoneal injection of either saline or 2.8 g ethanol/kg body weight. The content of dynorphin A1-8 was determined in dialyzate samples obtained prior to and following the various treatments using a specific radioimmunoassay. Activation of CRHRs in CeA induced an increase in the extracellular concentrations of dynorphin A1-8. Moreover, acute alcohol administration increased the extracellular concentrations of dynorphin A1-8 in CeA, an effect that was attenuated by blocking CRHR2 with anti-sauvagine-30 microinjection but not blocking CRHR1 with antalarmin microinjection. Therefore, the findings suggest an interaction between the CRH and dynorphin A1-8 systems at the level of CeA in response to acute alcohol exposure.

Keywords: Addiction; Ethanol; Central amygdala; Corticotropin-releasing hormone; Dynorphin; Microdialysis

Introduction

Alcohol consumption affects multiple neurobiological systems including the endogenous opioid and corticotropin-releasing hormone (CRH) systems. In addition, activation of κ-opioid and CRH receptors (CRHRs) by dynorphin and CRH peptides, respectively, has been implicated in the negative reinforcement aspects of alcohol addiction (Carlezon and Miczek, 2010; Ciccocioppo et al., 2009; Koob, 2009; Wee and Koob, 2010). Both the dynorphin and CRH systems, and their specific receptors are present in various brain regions involved in alcohol consumption including the central amygdala (CeA) (Funk et al., 2006a; Läck et al., 2006; Lam et al., 2008; Marchant et al., 2007; Roberto et al., 2010).

The dynorphin peptides, including dynorphin A1-8, bind with high affinity to κ-opioid receptors (Quirion et al., 1983). Past studies have suggested that activation of the dynorphin/κ-opioid receptor system at the level of the brain could mediate the aversive components of alcohol consumption such as intoxication (Bals-Kubik et al., 1989; Di Chiara and Imperato, 1988; Shippenberg et al., 1993; Spanagel et al., 1992). Furthermore, κ-opioid receptor agonists were shown to reduce alcohol consumption, an effect suggesting that activation of κ-opioid receptors opposes the process of alcohol reinforcement (Barson et al., 2010; Lindholm et al., 2001). Recent studies revealed that activation of the κ-opioid receptors may be a critical component of the alcohol consumption and addiction process (Carlezon and Miczek, 2010; Perreault et al., 2006, 2007; Sperling et al., 2010; Wee and Koob, 2010), and treatment of alcohol-dependent rats with a κ-opioid receptor antagonist have resulted in an attenuation of alcohol consumption (Walker and Koob, 2008; Walker et al., 2010). Interestingly, several in vivo microdialysis studies have demonstrated that acute alcohol treatment increases the

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extracellular dynorphin A1-8 concentrations in several brain regions known to influence alcohol consumption including the CeA (Jarjour et al., 2009; Lam et al., 2008; Marinelli et al., 2006). These findings provide further support for the importance of the dynorphin/κ-opioid receptor system in the process of alcohol consumption and addiction.

A number of studies have also implicated the CRH neuropeptide system in alcohol consumption. The CRH system consists of peptide ligands including the CRH peptide and the two CRHRs: CRH receptors type 1 (CRHR1) and type 2 (CRHR2) (Ciccocioppo et al., 2009; Ryabinin et al., 2002). The CRH system has been shown to have an endocrine role in the stress response as part of the hypothalamic–pituitary–adrenal axis and a role as neuromodulator in various brain regions (Charmandari et al., 2005; Ryabinin et al., 2002). Chronic alcohol studies have demonstrated that the CRH system in several brain regions including the CeA is involved in excessive alcohol consumption by alcohol-dependent rats (Funk and Koob, 2007; Funk et al., 2006a, 2007; Sabino et al., 2006). Studies have also demonstrated that foot shock, as a stressor, reinstates responding for alcohol in rats chronically trained to lever press for alcohol and is associated with increased CRH mRNA in the extended amygdala (Funk et al., 2006b). In addition, both systemic and local CeA administration of CRH antagonists block this stress-induced alcohol reinstatement (Lê et al., 2000; Liu and Weiss, 2002; Lowery et al., 2008). As well, a recent in vivo microdialysis study demonstrated that acute alcohol induced a dose- and time-dependent increase in the extracellular CRH concentrations in CeA (Lam and Gianoulakis, 2011). Blocking both CRHR1 and CRHR2 in the CeA with antagonists attenuated the alcohol-induced increase of extracellular β-endorphin concentrations, an effect suggesting an interaction of the CRH and opioid systems at the level of CeA following acute alcohol exposure (Lam and Gianoulakis, 2011). Moreover, both dynorphin and CRH are co-expressed in neurons of the CeA (Marchant et al., 2007), an effect suggesting an interaction between these neuropeptide systems in CeA.

Based on the previous studies, the goals of the current experiments were to investigate the hypotheses that (1) activation of CRHRs by CRH microinjection into CeA would enhance the extracellular concentrations of dynorphin A1-8 and (2) blocking CRHRs in CeA with antagonists would attenuate the alcohol-induced increase of the extracellular concentrations of dynorphin A1-8. To test these hypotheses, in vivo microdialysis studies were carried out. Sprague–Dawley rats were given microinjections into the CeA of either CRH or one of the CRHR antagonists: antalarmin (CRHR1 selective) or anti-sauvagine-30 (CRHR2 selective). Subsequently, rats were treated with a systemic intraperitoneal (IP) injection of either saline or 2.8 g ethanol/kg body weight. Radioimmunoassays using a specific antibody for dynorphin A1-8 were performed to determine the content of dynorphin A1-8 in the dialyzate samples collected at 30-min intervals prior to and following the various treatments.

Materials and methods

Animals

Alcohol-naïve male adult Sprague–Dawley rats were singly housed in cages in a temperature- and humidity-regulated environment on a 12-h light–dark cycle (lights on at 0800 h). Food and water were available ad libitum. Principles of laboratory animal care were followed in accordance with McGill University's Policy on the handling and treatment of laboratory animals and the guidelines of the Canadian Council of Animal Care.

Guide cannula implantation

Stereotaxic surgical guide cannula implantation was performed 1–2 weeks after the arrival of the rats to the animal facility. Stereotaxic surgery consisted of anaesthetizing the rats with isoflurane (5% induction, 2.5–3.0% maintenance) and fixing the rat's head to a stereotaxic frame (ASI instruments Inc., Warren, MI). The rat's head was shaved, sterilized with iodine, and a sagittal incision was made to expose the skull surface and bregma. The guide cannula (15 mm shaft length, Bioanalytical Systems, West Lafayette, IN) was unilaterally implanted above the CeA according to established coordinates (anterior-posterior: −2.3 mm from bregma, medial-lateral: 4.0 mm from the midline, and dorsal-ventral: 7.4 mm from the dura) and kept patent with a dummy cannula (Lam et al., 2008; Roberto et al., 2010).

Habituation

After 3–5 days' postsurgical recovery, rats were placed on three separate occasions into the same type of black, plastic containers (diameter: 31 cm and height: 30 cm) as those used for the microdialysis experiments and were habituated to experimenter handling and IP injections using saline.

In vivo microdialysis experiments

The microdialysis experimental setup has been described previously (Lam et al., 2008; Marinelli et al., 2006). On the evening prior to the microdialysis experiments, microdialysis probes with a microinjection port (1 mm polyacrylamide membrane, 320 μm outer diameter, 15 mm shaft length, Bioanalytical Systems, West Lafayette, IN) were connected to the fluorinated ethylene propylene (FEP) tubing and the syringe pump was set to pump artificial cerebral spinal fluid (aCSF) at a rate of 2.0 μL/min. Subsequently, microdialysis probes were inserted through the guide cannula and the pump rate for aCSF flow was lowered to 0.2 μL/min overnight. On the morning of the microdialysis session, the pump rate was increased to 2.0 μL/min and rats were allowed 2 h acclimatization to the new