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The CCK-system underpins novelty-seeking behavior in the rat: Gene expression and pharmacological analyses ☆

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Abstract

Cholecystokinin (CCK) and its receptor CCK-2R have been shown to promote emotional responsivity and behavioral sensitization to psychostimulants in the rat. An animal model has been developed based on locomotor response to a novel inescapable environment. Animals exhibiting consistent differences in locomotor response to novelty have been termed as high and low responder rats (HR and LR, respectively). This paradigm is deemed to model sensation-seeking, a personality trait closely associated with substance abuse. The present study provides genetic and pharmacological evidence that the CCK-ergic system modulates this behavior. Distinctive patterns of CCK-related gene expression in HR and LR animals occurred beyond the mesolimbic pathways. CCK gene expression was higher in hippocampus, amygdala, and prefrontal cortex, but lower in the ventral tegmental area of HR relative to LR rats. Levels of CCK-2R mRNA were more elevated in LR animals in some areas of the forebrain such as the prefrontal cortex, nucleus accumbens, and hippocampus. Additionally, CCK-2R blockade with the antagonist LY225.910 (0.5 mg/kg) removed phenotype differences in sustained exploration of novel stimuli (i.e., a novel-object) in HR and LR rats exposed to an enriched open-field test series. Finally, CCK-2R blockade also altered M_2 and 5-HT_7 receptor gene expression in the mediodorsal thalamus (a strategic structure for corticothalamic trafficking) in a phenotype-dependent manner. Taken together, the findings reported here suggest that distinct CCK-ergic function may contribute to promoting individual differences in novelty-seeking behavior.

Keywords: Cholecystokinin; CCK-2R; LY225.910; Novelty-seeking behavior; 5-HT7 receptor; M2 receptor

1. Introduction

Cholecystokinin (CCK), a gastrin-like peptide, is currently the most abundant neuropeptide found in the mammalian brain (Crawley and Corwin, 1994; Dauge and Lena, 1998). There are two CCK receptor types in the brain. The CCKA receptor (recently termed CCK-1R) is expressed at relatively low levels, while the CCKB receptor (or CCK-2R) is densely distributed in the forebrain regions. There is general agreement about the influence of CCK-2R in generating anxiety-like behaviors and emotional responsiveness (Bradwejn et al., 1991; van Megen et al., 1996; Farook et al., 2001; Chen et al., 2006). However, the anxiolytic profile of CCK-2R antagonists has been questioned (Griebel, 1999) thus raising serious concerns about the therapeutic potential of targeting the CCK-system (Abelson, 1995). Recent evidence strongly suggests that CCK-ergic neurotransmission has a role in mood regulation after repeated stressful (social defeat) experience (Becker et al., 2007).

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Dopaminergic mesocorticolimbic system is a neural substrate through which CCK may mediate all these processes. CCK coexists with dopamine in the ventral tegmental area projection onto the nucleus accumbens (Crawley, 1994). CCK regulates mesolimbic function, strongly affecting dopamine-mediated behaviors such as behavioral responsivity to psychostimulants (Higgins et al., 1994) and stress-motivated behaviors (Rotzinger et al., 2002). In addition to serving other functions, dopamine-mediated transmission in the mesoaccumbal region has been implicated in the encoding of novel events (Horvitz, 2000), adaptative processing to cope with changing environmental demands (Le Moal and Simon, 1991), and novelty-seeking behavior (Piazza et al., 1991;Piazza and Le Moal, 1998). Although the grade of novelty or familiarity with context-dependent experimental conditions influences the manner CCK mediates dopamine-mediated behaviors (Ladurelle et al., 1995; Hökfelt et al., 2002), only one study gives support to a correlation between the expression of the CCK gene in the tegmental ventral area and novelty-seeking behavior (Lucas et al., 1998).

The term "novelty-seeking" portrays a behavior in the rat characterized by the vigor of the emotional response to a novel, inescapable environment. This trait has relevance in psychiatry research as it is thought to model some aspects of sensation-seeking behavior in humans (Piazza and Le Moal, 1998; Dellu et al., 1996; Cain et al., 2005), a personality trait closely associated with drug abuse and related mental illnesses (Zuckerman and Neeb, 1979). Rats categorized into the high and low ranges of exploration when exposed to an inescapable novel environment (namely HR and LR animals, respectively) differ in the rate at which they self-administer low doses of psychotropic drugs (HR rats higher self-administration than LR rats) (Piazza et al., 1989). Most importantly, the HR and LR model is of relevance in understanding the neurobiological bases of emotional responsiveness (Kabbaj, 2004). When compared to LR rats, HR exhibit higher behavioral activation following administration of psychostimulants, self-administer drugs of abuse at higher rates, and exhibit exaggerated emotional and stress responsivity after experiencing stressful, conflicting situations (Dellu et al., 1996; Kabbaj et al., 2000; Kabbaj, 2004). Prior work from our lab suggests the mediation of CCK in the capacity of emotional self-adjustment of HR and LR rats after repetitive anxiety-like training using unconditioned (novelty-based) tests (Ballaz et al., 2007a). These considerations led us to expand our knowledge of the involvement of CCK in novelty-seeking behavior by using a gene expression and behavioral approach. Additionally, we speculated about a putative interplay between the CCK-system and receptors like 5-HT7 and M2 as they contribute to novelty-seeking behavior and behavioral flexibility, respectively (Ballaz et al., 2007b; Seeger et al., 2004).

2. Experimental procedures

2.1. Subjects

The present studies followed the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institute of Health guidelines on laboratory animal use and care (Publication No. 80-23). The University of Michigan Committee on Use and Care of Animals (UCUCA) approved the present study. Adequate measures were taken to minimize the number of animals used in the present study and their suffering. Male Sprague–Dawley rats from Charles River (Wilmington, MA), weighing 250–300 g at the beginning were used. Rats were acclimated in the housing unit for at least 10 days before any experimental procedure. They were housed two per cage on a 12 h light/dark cycle (lights on at 7 a.m.) with access to food and water *ad libitum*. Stabulation remained unchanged until rats were sacrificed. Behavioral testing was conducted during the light cycle.

2.2. Locomotor activity test

Rats were briefly handled by the experimenter for the 2 days prior to testing to ensure habituation. On the test day, rats were transferred to an adjacent, unfamiliar room where locomotor activity was recorded for 1 h. The animals were placed in Plexiglas® cages ($40 \times 20.5 \times 20.5$ cm) identical to the home cage (but with a novel grate floor) and were arranged in racks flanked by photo-beam cells to track horizontal displacement and rearing. Testing began when the rat was placed in the activity cage. All movements were processed by a central computer connected to the device using suitable software (Ratmove®, MBNI-University of Michigan, Ann Arbor). Because these measures lie upon a continuum of responses following a normal distribution, rats were assigned to the high responder (HR) group if the locomotor activity score was at least 1 SD above the mean locomotor activity score for each randomly selected subject sample or the low responder (LR) for 1 SD below the mean (Rosario and Abercrombie, 1999). As a result, the HR and LR group of rats were defined as those exhibiting locomotor scores in the upper and lower thirds of the sample, respectively.

2.3. In situ hybridization histochemistry (ISH)

To characterize phenotype differences in gene expression, brains were obtained from a cohort of rats tested only for HR–LR categorization and euthanatized 2 weeks after the locomotor activity test. In a second set of ISH experiments, brain samples were obtained from rats behaviorally tested for 1 week and sacrificed 1 day after the last test session. Brains were quickly frozen in *n*-meth-