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CHOLECYSTOKININ OCTAPEPTIDE INDUCES ENDOGENOUS OPIOID-DEPENDENT ANXIOLYTIC EFFECTS IN MORPHINE-WITHDRAWAL RATS

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Abstract—Cholecystokinin octapeptide (CCK-8), a brain–gut peptide, plays an important role in several opioid addictive behaviors. We previously reported that CCK-8 attenuated the expression and reinstatement of morphine-induced conditioned place preference. The possible effects of CCK-8 on the negative affective components of drug abstinence are not clear. There are no studies evaluating the effect of CCK-8 on emotional symptoms, such as anxiety, in morphine-withdrawal animals. We investigated the effects of CCK-8 on the anxiety-like behavior in morphine-withdrawal rats using an elevated plus-maze. Morphine withdrawal elicited time-dependent anxiety-like behaviors with peak effects on day 10 (5 days after induction of morphine dependence). Treatment with CCK-8 (0.1 and 1 μg, i.c.v.) blocked this anxiety in a dose-dependent fashion. A CCK1 receptor antagonist (L-364,718, 10 μg, i.c.v.) blocked the effect of CCK-8. Mu-opioid receptor antagonism with CTAP (10 μg, i.c.v.) decreased the 'anxiolytic' effect. CCK-8 inhibited anxiety-like behaviors in morphine-withdrawal rats by up-regulating endogenous opioids *via* the CCK1 receptor in rats. This study clearly identifies a distinct function of CCK-8 and a potential medication target of central CCK1 receptors for drugs aimed at ameliorating drug addiction.

Key words: cholecystokinin octapeptide, CCK receptor, morphine withdrawal, anxiety, elevated plus-maze.

INTRODUCTION

Therapeutic use of morphine predates recorded history. However, its clinical application is limited by the potential for drug dependence. A number of emotional symptoms, such as bipolar disorder, hyperirritability, major depression and anxiety, emerge after drug abstinence (Majewska, 1996; Goeldner et al., 2011; Radke and Gewirtz, 2012). These negative emotional states are most commonly reported after compulsive and continued use and relapse followed by drug abstinence (Spanagel and Weiss, 1999). Thus, alleviating or preventing these negative emotional states might be useful in the treatment of relapse.

Accumulating data suggest that non-opioid neuronal transmitters may be important targets for morphine withdrawal and relapse therapy (Lu et al., 2000; Van Bockstaele and Valentino, 2013). Cholecystokinin (CCK), a neuropeptide, is composed of varying numbers of amino acids, depending on the post-translational modification of the CCK gene product. CCK has been demonstrated to exert a wide range of biological activities by acting on CCK receptors distributed widely throughout the CNS (Crawley and Corwin, 1994; Moran and Schwartz, 1994). In humans, it has been suggested that CCK administration causes nausea and anxiety (Adams et al., 1995). CCK tetrapeptide (CCK-4) is routinely used to induce anxiety and is commonly used in research to induce panic attacks for the purpose of testing new anxiolytic drugs (Bourin, 1998; Toru et al., 2013). Different forms of CCKs have been demonstrated to have highly variable effects. CCKs and opioids play antagonistic roles in analgesia, and CCK octapeptide (CCK-8) is the most potent endogenous anti-opioid peptide (Baber et al., 1989; Cesselin, 1995). In our previous studies, we identified a significant inhibitory effect of CCK-8 on naloxone-precipitated withdrawal-induced conditioned place aversion (CPA) (Yu et al., 2012). It has been difficult to determine whether CCK-8 attenuates the negative affective component of drug withdrawal. There are no studies that examine the effect of CCK-8 on emotional symptoms, such as anxiety, in morphine-withdrawal animals.

Two CCK receptors, CCK1 and CCK2, were identified based on the pharmacological properties and specific CCK binding (Noble et al., 1999). Previous studies have reported that blockade of the CCK2 receptor inhibits development of opioid tolerance and dependence (Dourish et al., 1990; Lu et al., 2000). However, our results suggest that CCK receptor activation exerts an inhibitory effect on morphine-induced conditioned place preference (CPP) and cellular morphine dependence (Wen et al., 2012a,b, 2013). CCK-8 prevents morphine dependence at high but not low concentrations. CCK1, but not CCK2, receptor stimulation induces antinociceptive effects and prevents the development of morphine

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Abbreviations: ANOVA, analysis of variance; CCK-8, cholecystokinin octapeptide; DMSO, dimethylsulfoxide; EPM, elevated plus-maze.

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tolerance (Derrien et al., 1993; Rezayat et al., 1994, 1997). The two CCK receptors have opposing effects on different neuronal activities (Moran et al., 1986; Dauge et al., 1992). CCK-4 has been demonstrated to induce anxiety-like effects *via* the CCK2 receptor. The action of CCK receptors that respond to CCK-8 in morphine-withdrawal animals has not been determined.

The goal of this study was to assess the effects of CCK-8 on anxiety-like behavior in morphine-withdrawal rats using the elevated plus-maze (EPM) test. The subtypes of CCK receptors mediating the regulative effect of CCK-8 and the functional interaction between CCK-8 and the endogenous opioid system on morphine-withdrawal related anxiety were also investigated.

EXPERIMENTAL PROCEDURES

Animals

Four hundred and eight Wistar male rats were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). Animal care and experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The rats weighed 180–200 g upon arrival in the laboratory and were housed for 7 days prior to the experiments. Constant temperature (21 ± 2 °C), humidity (approximately 60%) and a 12-h light/dark cycle (lights on at 7:00 and off at 19:00) were maintained. Food and water were provided *ad libitum*. All protocols in this study were approved by the Local Committee of Animal Use and Protection of Hebei Medical University.

Drugs

Morphine hydrochloride was obtained from Shenyang First Pharmaceutical Factory (Liaoning, China). CCK-8 (H-Asp-Tyr-(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2), a non-selective opioid receptor antagonist (naloxone) and a selective μ-opioid receptor antagonist (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2, CTAP) were purchased from Sigma, Ltd. (MA, USA). The CCK1 receptor antagonist, L-364,718, and CCK2 receptor antagonist, L-365,260, were purchased from Tocris Bioscience (Tocris Cookson, Northpoint, UK). CCK-8 was resuspended in a vehicle consisting of 1% ammonia saline to a concentration of 1 mg/ml. CCK receptor antagonists were suspended in dimethylsulfoxide (DMSO) to a concentration of 10 mg/ml. The working solutions were made fresh before use.

Surgery and microinjections

Cannulae (RWD Life Science Co., Shenzhen, China) were surgically implanted for the intracerebroventricular injection of drugs. The rats were placed in a stereotaxic apparatus (Benchmark™ Stereotaxic Instruments, St. Louis, MO, USA) after anesthesia (intraperitoneal pentobarbital sodium, 40 mg/kg). Using the atlas by Paxinos and Watson (1998) as a guide, a single hole was drilled through the skull above the left or right lateral ventricle (AP, −0.92; ML, ±1.67) Fig. 1. A stainless steel guide cannula (innerdiameter: 0.34, outer diameter: 0.48 mm) was implanted 3.0 mm ventral from the surface of the skull. To prevent occlusion, a dummy cannula was inserted into the guide cannula. Dental cement was used to fix the guide cannula to the skull. After surgery, the rats were treated with penicillin (1000 U/day, i.m.) for 3 days and allowed to recover for at least 7 days.

Rats were gently handled for 3 days prior to the experiments to minimize stress associated with manipulation. Each microinjection was made by using a 10-μl syringe (Hamilton, USA) attached to polyethylene (PE) tubing connected to the injection cannula (innerdiameter: 0.14, outer diameter: 0.30 mm). Microinjections were administered at a rate of 0.5 μl/min and a volume of 2 μl using a syringe pump (KD Scientific, Holliston, MA, USA).

EPM testing for anxiety measurement

The maze consisted of two open arms (50 × 15 cm) and two closed arms (50 × 15 × 40 cm). The apparatus was placed at a height of 50 cm from the floor. All sides and floor surfaces of the open and closed arms were constructed from black Plexiglas. To start the test, rats were individually placed at the center of the maze facing

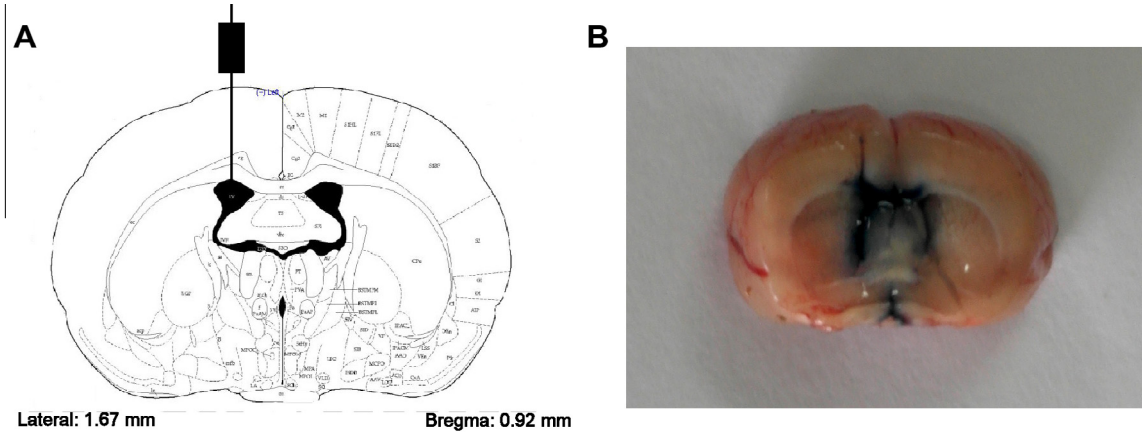

Fig. 1. Photograph indicating the placement of the cannula. (A) Stainless steel guide cannulae were implanted 3.0 mm ventral from the surface of the skull and through the skull above the left or right lateral ventricle (AP, −0.92; ML, ±1.67). (B) Cannula location for animal with correct placement is indicated by dispersion of methylene blue dye throughout the ventricles.