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Research report

Effects of cholecystokinin-8 on morphine-induced spatial reference memory impairment in mice

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HIGHLIGHTS

- Spatial reference memory is impaired in morphine dependency mice.
- CCK-8 improved morphine-induced spatial memory impairment.
- CCK-8 enhanced learning ability and spatial reference memory.
- CCK-8 reversed spine density decrease in CA1 of morphine dependency mice.

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ABSTRACT

Acute and chronic exposure to opiate drugs impaired various types of memory processes. To date, there is no preventive treatment for opiate-induced memory impairment and the related mechanism is still unclear. CCK-8 is the most potent endogenous anti-opioid peptide and has been shown to exert memory-enhancing effect, but the effect of CCK-8 on morphine-induced memory impairment has not been reported. By using Morris water maze, we found that escape latency to the hidden platform in navigation test was not influenced, but performance in the probe test was seriously poor in morphine dependency mice. Amnesia induced by chronic morphine treatment was significantly alleviated by pre-treatment with CCK-8 (0.01, 0.1 and 1 μg, i.c.v.), and CCK-8 (0.1 and 1 μg, i.c.v.) treatment alone could improve performance in either navigation or probe test. Furthermore, Golgi-Cox staining analysis revealed that pre-treatment with CCK-8 (1 μg, i.c.v.) reversed spine density decreased in CA1 region of hippocampus in morphine dependency mice, and CCK-8 (1 μg, i.c.v.) alone obviously increased spine density in CA1. Our findings conclude spine density change in CA1 region of hippocampus may be the structural plasticity mechanism which is responsible for enhancing effect of CCK-8 on spatial reference memory. Therefore, CCK-8 could effectively improve memory impairment in morphine dependency mice.

1. Introduction

Morphine has been widely used in the clinical management of pain in the past decades, but the strong dependent potential greatly limited its clinical practice. Morphine-induced associative memory is viewed as a pathological process of learning, involving cell-signaling and synaptic mechanisms similar to those implicated in neural models of learning and memory [1,2]. Accumulating evidence also has demonstrated that acute and chronic administration of opioids has produced impaired effects on physiological memory processes. Various studies reported that pre-training administration of morphine impaired acquisition or retention of memory in passive-avoidance [3], Y-maze [4,5], or water maze paradigms [6,7]. However, the effects of chronic exposure to morphine on spatial memory are still controversial. It has been demonstrated that chronic morphine treatment produced residual working memory impairment [8], and could influence spatial learning processes and impair the formation and retrieval of spatial memory [5,9]. Other studies have reported that morphine dependency did not impair learning ability, but partially impaired retention of spatial reference memory in rats [10].

Cholecystokinin (CCK), a neuropeptide, exists in several forms of different sizes, including 4, 8, 33, 39, and 58 amino acids. CCK-8 is the most potent endogenous anti-opioid peptide, and has been shown to exert a wide range of biological activities on several systems, including the central and peripheral nervous system. It has been shown to modulate the release of neurotransmitters, such as dopamine (DA) and gamma-aminobutyric acid (GABA), and possibly acts as a neurotransmitter/modulator [11–13]. In some

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disease models, CCK-8 attenuated thermal, tactile allodynia and has an anti-inflammatory effect [14,15]. Endogenous CCK attenuated morphine induced antinociception [16,17], but has a distinct analgesic effect in tail flick test when given exogenously. And, a study indicated that endogenous CCK plays an important role in morphine-induced hyperalgesia and antinociceptive tolerance [18,19].

Furthermore, CCK-8 is hypothesized to effectively regulate various types of memory [20,21]. CCK-8 has been shown an improve effect on spatial learning and memory [22,23], and facilitates changes in glutamatergic synaptic transmission of the hippocampus and augmenting long-term potentiation in hippocampal slices [24,25]. In our previous studies, we found that pre-treatment with CCK-8 significantly inhibited the acquisition and reinstatement of morphine induced conditioned place preference (CPP) [26] and attenuated behavioral signs of morphine abstinence syndrome in rats [27]. The results revealed that CCK-8 play a vital modulatory role in morphine reward memory. However, effect of CCK-8 on morphine induced spatial memory impairment and the related synaptic plasticity mechanism is not clear.

The present study was undertaken in order to investigate the effect of CCK-8 on spatial reference memory and memory impairment induced by chronic morphine treatment. Spine density was observed to explore structural plasticity mechanism for enhancing effect of CCK-8 on memory processes.

2. Materials and methods

2.1. Animals

Two-hundred-and-forty-nine Kunming strain male mice were obtained from the Centre of Laboratory Animal Science at the Hebei Medical University. Animal care and the experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The mice weighed 20–25 g upon arrival in the laboratory and were habituated for 3 days prior to the experiments. Furthermore, male mice were used in the present study to avoid the effect of estrogen on morphine dependence. Constant temperature (21 ± 2 °C), humidity (about 60%) and a 12 h light/dark cycle (lights on at 7:00 am) were maintained throughout the experiments. Food and water were provided ad libitum except for the periods of behavioral testing in MWM. All protocols in this study were approved by the Local Committee of Animal Use and Protection of the Hebei Medical University.

2.2. Drugs

Morphine hydrochloride was obtained from Shenyang First Pharmaceutical Factory (Liaoning, China). CCK-8 was purchased from Sigma, Ltd. (MA, USA). CCK-8 was resuspended to a concentration of 1 mg/ml in 1% ammonia saline solution. The working solution of CCK-8 was diluted in saline to the concentrations of 0.5, 0.05, 0.005 and 0.0005 mg/ml immediately before use.

2.2.1. Morphine treatment

Thirty mice were randomly assigned to two groups, and subcutaneously injected with saline or morphine twice per day at an interval of 12 h for 6 consecutive days. The morphine dose was progressively increased as follows: day 1, 20 mg/kg; day 2, 40 mg/kg; day 3, 60 mg/kg; day 4, 80 mg/kg; days 5 and 6, 100 mg/kg; as described by previous study [28]. Then, the animals were adapted, trained, and tested in the Morris water maze for the next 7 days, during which 100 mg/kg morphine was injected to maintain chronic drug treatment. In order to avoid the effect of acute morphine treatment on locomotion of mice, all behavioral trainings and tests were performed 2 h after the morning injection each day (Fig. 1B).

2.2.2. CCK-8 treatment

Ninety mice were randomly assigned to six groups as normal control group, sham-operated group and four different doses of CCK-8 group. Mice in sham-operated or different doses of CCK-8 group were intracerebroventricularly injected with saline (2 μl) or CCK-8 (0.001, 0.01, 0.1 and 1 μg) twice per day at an interval of 12 h for 13 consecutive days. Animals were adapted, trained, and tested from day 7 to day 13. All behavioral trainings and tests were performed 2 h after the morning injection of CCK-8 or saline each day. The doses of CCK-8 were determined based on a previous study and our preliminary results and the vehicle for CCK-8 was saline.

2.2.3. CCK-8 + morphine treatment

In this part, seventy-five mice in five groups were involved. To examine the effect of CCK-8 on morphine dependency-induced spatial reference memory impairment, mice were microinjected with CCK-8 (0.001, 0.01, 0.1, and 1 μg, i.c.v.) or saline 15 min before morphine treatment.

2.3. Morris water maze test

The water maze was a black circular pool with a diameter of 120 cm and a height of 50 cm, filled with 21 ± 1 °C water to a depth of 20 cm. A hidden circular platform (6 cm in diameter) was located in the center of the southwest quadrant (the target quadrant), submerged 1.0 cm beneath the surface of the water. Four equal quadrants and release points were designed as shown in Fig. 1A. Fixed maze visual cues were hung above the four quadrants around the maze. The animals' motions were recorded by a camera above the center of the maze, animal escape latency, traveled distance and swimming speed were measured automatically using the Animal Video Analysis System (JLBeh Soft-tech Co. Ltd., Shanghai, China).

The behavioral procedure was performed as previously described to test spatial reference memory [28]. On day 1, mice performed a 60 s free swimming trial for adaptation at the release point in the pool without the platform. On days 2–6, the platform was placed 1 cm under the water surface. The training procedure included four trials per day with four different starting positions (sequence of starting position in days 2–6: Day 2: N, E, SE, NW; Day 3: SE, N, NW, E; Day 4: NW, SE, E, N; Day 5: E, NW, N, SE; Day 6: N, SE, E, NW). In each trial, animals were given a maximum of 60 s to find the platform. After mounting the platform, the mice were allowed to remain there for 30 s. If the mouse failed to find the platform in 60 s, it was placed on the platform and allowed to rest for 30 s. Then, the mice were placed in a holding cage for 5–10 min until the start of the next trial. The escape latencies and swimming speeds of each group were recorded.

On day 7, a probe trial consisting of a 60 s free swim period without the platform was performed to test spatial memory, and mice were placed into the water at the release point. The swimming time spent and distance traveled in each quadrant, crossing latency and swimming speed were recorded.

In order to assess the possibility of drug interference with animal sensory and motor coordination or the animal motivation, the capability of animal to escape to a visible platform was tested after probe test. In this session, platform was elevated above the water surface with a flag and placed in the different positions in four quadrants. Mice were given four trials for visuo-motor coordination on the visible platform and escape latency were recorded and measured by the Animal Video Analysis System.

2.4. Golgi-Cox staining

Fifty-four mice were used for this part of study. Animals were randomly assigned to four groups as follows: saline group, morphine group, CCK-8 group and CCK-8 + morphine group. Mice were treated with morphine twice per day at an interval of 12 h for 5 consecutive days (day 1, 20 mg/kg; day 2, 40 mg/kg; day 3, 60 mg/kg; day 4, 80 mg/kg; day 5, 100 mg/kg, s.c.), on day 6, mice was treated with 100 mg/kg morphine only once. Saline (2 μl) or CCK-8 (1 μg, i.c.v.) was given before each saline or morphine injection.

Mice were aneasthetized with 20% urethane and perfused with saline 2 h after the last drug treatment. Then whole brains were dissected and processed using a modified version of the single-section Golgi-impregnation method. Firstly, brains were dropped into Golgi-Cox solution for 14 days in dark (refresh solution every 2 days), and then transferred to 30% sucrose for 3 days. Coronal sections 200 μm thick were cut by use of a Vibratome in a bath of 6% sucrose and stored in this solution. For Golgi-Cox staining, the sections were rinsed in distilled water, dehydrated, cleared, and then mounted onto gelatinized slides and cover slipped for observation.

In order to be selected for analysis, Golgi impregnated hippocampal cells had to possess the following characteristics: (1) location within the dorsal portion of the CA1 hippocampal fields for pyramidal cells, (2) relative isolation from neighboring impregnated cells in order to allow identification of dendrites which emanated from specific cells, and (3) dark and consistent impregnation throughout the extent of the neuron. These selection criteria were employed in an effort to analyze neurons from the same populations within and between groups.

2.5. Surgery and microinjections

Surgical implantation of cannulae was used for the intracerebroventricular injection of CCK-8, and conducted in an aseptic environment. Mice were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and placed in the stereotaxic apparatus (Benchmark™ Stereotaxic Instruments, USA). We drilled a hole on the skull surface corresponding to the lateral ventricle, targeted above the left or right lateral ventricle (ML, ±0.94 mm; AP, −0.22 mm). A stainless steel guide cannula was implanted 2.2 mm ventrally beneath 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. Animals were treated with penicillin (1000 u/day i.m.) for 3 days and allowed to recover for at least 7 days. Each microinjection was made with a 10 μl syringe (Hamilton, USA) attached to PE tubing connected to the injection cannula and was given at a rate of 0.5 μl/min in a volume of 2 μl using a syringe pump (KD Scientific, USA). The injection cannula extended 0.2 mm beyond the guide cannula and was left in place for 5 min following microinjections to minimize backflow of the drug.