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The effect of pituitary adenylate cyclase-activating polypeptide on elevated plus maze behavior and hypothermia induced by morphine withdrawal

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ABSTRACT

The aim of the present investigation was to study the effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on morphine withdrawal-induced behavioral changes and hypothermia in male CFLP mice. Elevated plus maze (EPM) and jump tests were used to assess naloxone-precipitated morphine withdrawal-induced behavior responses. Different doses of subcutaneous (s.c.) naloxone, (0.1 and 0.2 mg/kg, respectively) were used to precipitate the emotional and psychical aspects of withdrawal on EPM and 1 mg/kg (s.c.) was used to induce the somatic withdrawal signs such as jumping, and the changes in body temperature. In our EPM studies, naloxone proved to be anxiolytic in mice treated with morphine. Chronic intracerebroventricular (i.c.v.) administration of PACAP alone had no significant effect on withdrawal-induced anxiolysis and total activity at doses of 500 ng and 1 μg. At dose of 500 ng, however, PACAP significantly counteracted the reduced motor activity in the EPM test in mice treated with morphine and diminished the hypothermia and shortened jump latency induced by naloxone in mice treated with morphine.

These findings indicate that anxiolytic-like behavior may be mediated via a PACAP-involved pathway and PACAP may play an important role in chronic morphine withdrawal-induced hypothermia as well.

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) was originally isolated from ovine hypothalamus by its potent activity in stimulating cAMP production in rat anterior pituitary cells (Arimura, 1992). PACAP is a neuropeptide and member of a vasoactive intestinal polypeptide (VIP) superfamily that includes several peptides (VIP, secretin, helodermin, glucagon, peptide histidin isoleucine, galanin, etc.) (Christophe, 1993; Gourlet et al., 1998). The peptide has two amidated forms: PACAP-38 and PACAP-27 (Miyata et al., 1990). The presence of PACAP has been detected in hypothalamus, medial and ventral areas of the diencephalon, central thalamic nuclei, amygdala, bed nucleus of stria terminalis, septum, hippocampus, cingulate and entorhinal cortex, substantia nigra, nucleus accumbens, globus pallidus and sacral spinal cord (Dietl et al., 1990; Joo et al., 2004). Two receptor classes have been described for PACAP in mammalian tissues: type I and type II. Type II was further divided into three subclasses: PAC1, VPAC1 and VPAC2. PAC1 receptors are more selective for PACAP than VIP, but VPAC1 and VPAC2 receptors show similar affinity for PACAP-27, PACAP-38 and VIP (Cauvin et al., 1990; Gourlet et al., 1996). Type I receptors stimulate both adenylate cyclase and phospholipase C, thus being coupled to dual transduction pathways, involving interactions with G proteins of both Gs- and Gq-type. PACAP-38 and PACAP-27 were also effective in increasing cAMP release, cellular cAMP content, and total cAMP production in a dose-dependent manner in common carp pituitary cells (Xiao et al., 2002) and in rat neuroepithelial cells (Zhou et al., 2001).

Other behavioral studies examined the PACAP effect on motor stimulation and conditioned place preference (CPP) induced by morphine (Marquez et al., 2009); analgesic tolerance to morphine (Mácsai et al., 2002); mechanical hyperalgesia and thermal allodynia (Sándor et al., 2010). Our earlier experiments demonstrated that PACAP diminished the antinociceptive effect of acute morphine and enhanced the analgesic tolerance to morphine (Mácsai et al., 2002). In a recent report, acute PACAP administration increased the amount of time that animals spent in the open arm/total time rate in morphine treated mice compared to morphine treated mice in the absence of PACAP in the EPM (Szakács

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et al., 2010). Low doses of PACAP (0.03 and 0.3 μg), which had no effect on basal motor activity, enhanced morphine-induced (5 mg/kg, s.c.) motor stimulation and PACAP-deficient mice exhibited reduced morphine-induced motor stimulation (Marquez et al., 2009). Morphine-induced CPP following a single alternate-day saline/morphine (10 mg/kg, s.c.) conditioning was blunted in PACAP-deficient mice compared to their wild type littermates (Marquez et al., 2009). In spite of intense research, the mechanisms of PACAP action on morphine withdrawal-induced behavior responses and motor activity changes have not been clarified yet. The goal of the present study was to examine the effects of PACAP on naloxone precipitated morphine withdrawal in two experimental paradigms.

2. Materials and methods

2.1. Animals

Male CFLP white mice (30 ± 5 g of weight) of an outbred strain (Domaszék, Hungary) were used. They were kept under a standard light–dark cycle (lights on between 07.00 and 19.00 h) with food and water available *ad libitum*. The animals were kept and treated according to the rules of the Ethical Committee for the Protection of Animals in Research (Faculty of Medicine, University of Szeged, Hungary).

2.2. Surgery

For intracerebroventricular (i.c.v.) cannulation, the mice were anesthetized with intraperitoneal (i.p.) injection of sodium pentobarbital (Nembutal®, Phylaxia-Sanofi, Budapest, Hungary; 50 mg/kg), and a polyethylene cannula was inserted into the right lateral cerebral ventricle and cemented to the skull with cyanoacrylate-containing instant glue. The experiments were started 4 days after i.c.v. cannulation. Upon conclusion of the experiments, 10 μl of methylene blue were injected into the cerebral ventricle of the decapitated animals and the position of the cannula was inspected visually. After injection methylene blue spread throughout the ventricular space was used to verify that the whole amount of PACAP got into the ventricle. Mice with improper cannula placement were excluded from the statistical analysis.

2.3. Drugs

For i.c.v. treatments PACAP-38 (synthesized by Gábor Tóth using solid-phase peptide synthesis) were dissolved in artificial cerebrospinal fluid (aCSF) and injected in a volume of 2 μl. For testing the morphine effects and the somatic signs of withdrawal, morphine–HCl (Sigma–Aldrich) and naloxone–HCl (Sigma–Aldrich) were used. Control mice received saline s.c. and aCSF i.c.v.

2.4. Assessment of naloxone-precipitated withdrawal jumping in mice treated with graded doses of morphine

Precipitated withdrawal jump latency was measured in mice treated with morphine in the presence and absence of PACAP after naloxone (1 mg/kg, s.c.) administration. Immediately after the naloxone or saline injection, mice were placed on a circular platform. The precipitated abstinence syndrome was measured by scoring the latency to the appearance of stereotyped jumping from a circular platform 35 cm in diameter and 70 cm high (Azarov et al., 1992). A cutoff time of 15 min was used. The rectal body temperatures and body weights of all animals were also measured 15, 30, 60 min after naloxone injection, and changes in both parameters were calculated.

2.4.1. Influence of PACAP on naloxone-precipitated morphine withdrawal symptoms

We used twice daily injections of graded doses of morphine (09.00 and 19.00 h.) as follows: day 1: 20 mg/kg, day 2: 40 mg/kg, day 3: 60 mg/kg, day 4: 80 mg/kg or saline (Contet et al., 2008). Mice were also treated once a day (09.30 h) with either PACAP (500 ng/2 μl) or aCSF i.c.v. On the test day (day 5) animals received in the morning a single morphine injection (100 mg/kg s.c., 9.00 h) or saline (s.c.). Ninety minutes later aCSF or PACAP was given (10.30 h, i.c.v.). Withdrawal signs were evoked by naloxone administration (1 mg/kg, s.c.) 2 h after the final morphine treatment.

Precipitated morphine withdrawal syndrome was induced by 1 mg/kg naloxone-HCl as described by Azarov et al. (1992)). For treatment of specific groups please see legends to figures (Figs. 1 and 2) and Table 1.

2.5. Elevated plus maze (EPM)

The elevated plus maze (EPM) is an accepted model for examining anxiety-like behavior in mice (Lister, 1987). Conditions that decrease time spent in the open arms are associated with anxiety-like behavior, whereas increased time spent in the open arms is associated with an anxiolytic effect. The EPM apparatus consists of four arms (87-mm wide, 155-mm long) elevated 63.8 cm above the ground, with two arms enclosed by 16.3-cm-high opaque walls and illuminated with 60 W light situated 1 m above the maze. The combination of height, luminosity and open space is assumed to induce anxiety-like behavior in the animal. Behavioral testing was conducted between 11.00 and 13.00 h. Mice were carried to the experimental room in their home cages and habituated to the laboratory for at least 30 min before testing. Only one EPM apparatus per testing room was present. The apparatus was thoroughly cleaned between mice. Mice were placed in the center of the maze facing toward an enclosed arm and there behavioral activity were recorded for 10 min (Schulteis et al., 1998). These behavioral parameters were monitored: duration of time spent in the open arms which was defined as all four legs having crossed the entrance line to one of the open arms and total activity which was defined as the total number of crosses between any two arms.

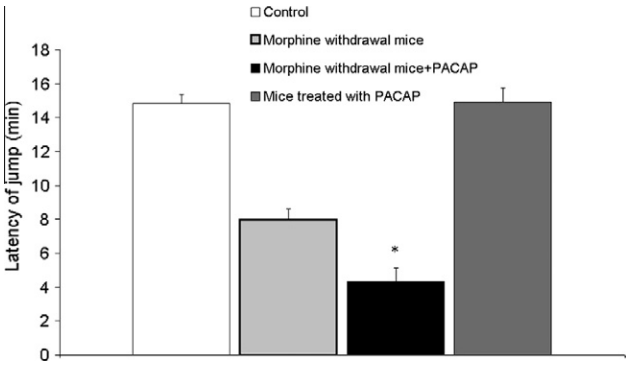

Fig. 1. Assessment of naloxone-precipitated withdrawal jumping in mice treated with graded doses of morphine. Graded doses of morphine (mg/kg, s.c. per injection) or saline were given twice daily for 5 days (day 1, 20; day 2, 40; day 3, 60; day 4, 80). Mice were also treated once a day with either PACAP (500 ng/2 μl) or aCSF i.c.v. 30 min after morphine injection. On day 5, 30 min prior to test either PACAP or aCSF was injected. Naloxone (1 mg/kg, s.c.) or saline was administered 2 h after the final injection of morphine at a dose of 100 mg/kg, and the jump latency was immediately measured. Therefore, we injected morphine or saline 2 h; PACAP or aCSF 30 min; naloxone or saline 0 min prior to jumping behavior. Number of mice: control: 9, morphine withdrawal mice: 10, morphine withdrawal mice + PACAP: 9, mice treated with PACAP: 9. Bars represent the latency of jump, vertical lines on the top of the bars denote S.E.M., $^{*}p < 0.05$ vs. mice given morphine and naloxone.