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Induction of antinociceptive tolerance to the chronic intrathecal administration of apelin-13 in rat

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ABSTRACT

Pain represents a major contributing factor to the individual's quality of life. Although pain killers as opioids, endogenous or exogenous peptides can decrease pain perception, the chronic use of them leads to antinociceptive tolerance. It has been demonstrated that neuropeptide apelin has potent antinociceptive effect. However, the possibility of the induction of its antinociceptive tolerance has not yet been clarified.

The tail-flick test was used to assess the nociceptive threshold. All experiments were carried out on male Wistar rats which received intrathecal apelin for 7 days. To determine the role of apelin and opioid receptors on the development of apelin analgesic tolerance, their receptor antagonists (F-13 A and naloxone, respectively) were injected simultaneously with apelin. The lumbar spinal cord was assayed to determine apelin receptor levels by the western blotting method. Plasma corticosterone levels were assayed using ELISA.

Results showed that apelin (3 μg/rat) induced strong thermal antinociception. In addition, chronic apelin produced tolerance to its antinociceptive effect and down regulated spinal apelin receptor. F-13 A and naloxone could inhibit apelin tolerance development. The corticosterone levels did not change following drug administration.

Taken together, the data indicated that apelin like other analgesic drugs leads to the induction of side effects such as analgesic tolerance which is mediated partly via the apelin and opioid receptors activation.

1. Introduction

To date various classes of painkillers, including opioids such as morphine, heroin, codeine, methadone (Lledo-Fernandez and Banks, 2011), nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, naproxen and indomethacin (Warden, 2010) have been identified to get rid of pain. Unfortunately, continuous usage of these drugs causes analgesic tolerance, respiratory depression and stomach ulcer, respectively (Lynch and Watson, 2006). Tolerance is a process which goes on by neuroadaptations and reduction of drug effects due to receptor down regulation (Pradhan et al., 2010).

Apelin is an endogenous peptide which isolated from bovine stomach extract first in 1998. The apelin receptor (APJ), as G-protein-coupled receptors (GPCRs) with 377 amino-acid, cloned from human genomic DNA by O'Dowd and colleagues. The receptor resembles (54% in the transmembrane regions) angiotensin II receptor type 1 (AT1), but angiotensin II does not bind to it (O'Dowd et al., 1993). However, activated or non-activated APJ may affect Ang II-AT1 signaling (Sun et al., 2011). Apelin/APJ system also are distributed in central nerve system, spanal cord (Matsumoto et al., 1996; O'Carroll et al., 2000; Reaux et al., 2001) hypothalamus, cortex, cerebellum, hippocampus, midbrain, striatum, pituitary (Lee et al., 2000; Medhurst et al., 2003), amygdale, raphe nucleus (Reaux et al., 2002) and peripheral areas such as heart, kidney, skeletal muscle, liver, lung, intestine, colon and glands (Habata et al., 1999; Hekmat et al., 2011; Hosoya et al., 2000; Kawamata et al., 2001; Najafipour et al., 2012; O'Carroll et al., 2000; Wang et al., 2004). Interestingly, apelin plays an important role in the neuronal signaling pathway (O'Carroll et al., 2000).

The apelin peptide has been also shown that stimulate hypothalamic–pituitary–adrenal (HPA) axis in vivo and in vitro studies (Newson et al., 2009; Taheri et al., 2002). It has been demonstrated that central apelin-induced elevated levels of plasma corticosterone is partially blocked by corticotrophin releasing factor (CRF) antagonist (Jaszberenyi et al., 2004). On the other hand, many studies have indicated that addictive drugs (e.g. opiates, ethanol, cannabinoids, nicotine, cocaine, amphetamines) can activate HPA axis (Armario, 2010). Lyengar et al. reported that opioid-induced alterations of HPA function is involved in morphine tolerance in rats (Iyengar et al., 1987).

Recently, it has been shown that apelin has central antinociceptive effects. In addition, μ-opioid receptor potentially involved in the analgesic effect of apelin (Lv et al., 2012b; Xu et al., 2009). Although, many

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studies have been demonstrated the unwanted side effect of analgesic tolerance in human and animals (Furlan et al., 2006), it has still remained unspecified whether antinociceptive tolerance can be induced by chronic administration of apelin-13. In addition, potential affinity of APJ with μ opioid receptor (Befort et al., 2008) and HPA axis (Bülbül et al., 2016) prompts us to evaluate the possible involvement of opioid receptor in apelin analgesic tolerance.

2. Materials and methods

2.1. Animals

The study was performed in accordance with appropriate guidelines for investigation of experimental pain in animals (Zimmermann, 1983). All experimental protocol were approved by Ethical Committee of Kerman University of Medical Sciences (EC: k.91.333). All studies were carried out on male Wistar rats (weight 200–250 g), which were housed four per cage under a 12 h night/day cycle with free access to food and water at all times. Temperature (22 ± 1 °C) and humidity were carefully controlled. Animals were handled daily, between 9:00 and 10:00 A.M., for 5 days before the experiment to adapt and minimize non-specific stress responses.

2.2. Drugs

Apelin-13 (057-18) and Apelin-13, Ala13 (F13-A, 057-29) were purchased from phoenix pharmaceuticals INC, company (USA). Naloxone was prepared from Darou Pakhsh Co. (Iran). The drugs were dissolved in artificial cerebrospinal fluid (ACSF, 148 mM NaCl, 3 mM KCl, 0.8 mM $MgCl_2$, 1.4 mM $CaCl_2$, 1.5 mM Na_2HPO_4, 0.2 mM NaH_2PO_4, 0.1 mg/ml of bovine serum albumin) (Hamann et al., 2003) and given in total volume 5 μl intrathecally (i.t.) by a microinjection syringe (Microliter™ #702, Hamilton Co., USA). Control animals were received ASCF in the equal volume (5 μl).

2.3. Antinociceptive test

In order to measure acute nociceptive sensitivity, the tail flick test was used (D'Amour and Smith, 1941).

A tail-flick apparatus with a radiant heat source was used to assess the analgesic response (type 812 Hugo Sachs Electronic, Germany). Animals were loosely restrained in a Plexiglas cylinder and radiant heat was focused from a distance of 4–7 cm on the middle third of the tail. Time of latency for each rat was measured three times and the average of these trials was considered as baseline latency before drug administration. The severity of light was adjusted to produce mean control reaction time between 2 and 4 s. The cut-off time was set at 10 s to avoid any damage to the tail. After established of baseline latencies, the rats received intrathecal administration of drugs and the reaction latency was measured 15, 30, 60, 120, 180 and 240 min after injection. The tail-flick latencies were converted to the percentage of antinociception according to the following formula:

$$\%\text{Antinociception}(\%\text{MPE}) = (\text{reaction time of test} - \text{basal reaction time}) / (\text{cut off time} - \text{basal reaction time})$$

2.4. Experimental design

First, the effects of different doses of apelin-13 (1, 3, 5 μg/rat i.t.) were evaluated on nociceptive threshold before and 15, 30, 45, 60, 180 and 240 min after injection.

Second, to evaluate the possible antinociceptive tolerance, the effective dose of apelin-13 was chronically administrated and its antinociceptive effect was assessed on days 1, 3, 5, and 7 of the experiment. ACSF was given according to the same schedule as vehicle groups. Nociceptive threshold was assessed both before and 45, 60 and 120 min after apelin-13 administration on days 1, 3, 5 and 7.

Third, to determine the role of APJ or opioid receptor signaling in the induction of apelin analgesic tolerance, APJ (F13-A) or opioid (naloxone) antagonists was injected 20 min before apelin-13 but in days that nociceptive testing was measured, apelin-13 was given first and then the antinociception was measured. After that, F13-A or naloxone was injected.

2.5. Intrathecal catheter implantation for drug delivery

Animals were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg) i.p. An intrathecal catheter (PE-10) was implanted in each rat according to a previously published method (Yaksh and Rudy, 1976). Animals with neuronal defects (e.g., paralysis) were discarded from the experiment after the catheter implantation or during drug administration. In behavioral study, the position of the cannula was confirmed at the end of the examination by administering 15 μl of 2% lidocaine which temporarily paralyzed the animals' hind limbs. In molecular study, to avoid lidocaine interference in blotting data, the lumbar area was immediately cut and the correct position of the intrathecal cannula was verified macroscopically by looking approval.

2.6. Tissue extraction and preparation

Rats were anesthetized with CO_2 and decapitated. The spinal column cut through the pelvic girdle. A 16 gauge needle inserted into the sacral vertebral canal and spinal expelled with ice-cold saline by hydraulic extrusion. The dorsal half of the lumbar cord was immediately dissected from the spinal cord which was placed on ice. Tissue samples were weighed and instantly frozen in liquid nitrogen and stored at 70 °C until assay.

2.7. Western blot analysis

To evaluate modification of apelinergic system, APJ density was measured by western blot test.

The dissected spinal tissues from rats which given 7 days of chronic intrathecal injection with apelin-13, homogenized in ice-cold buffer containing 700 μl rippa buffer (sigma; R0278), 1 mM protease inhibitors (sigma; P2714-IBTL) and 1 mM sodium orthovanadate with homogenizer (Heilscher UP200-Germany). The homogenate was centrifuged at 17,000 rpm for 15 min at 4 °C. The resulting supernatant was retained as the whole cell fraction. Protein concentrations were measured using the Bradford method (Bio-Rad Laboratories, Muenchen, Germany). Equal amounts of protein (40 μg) were separated electrophoretically on a 10% SDS-PAGE gel and transferred to nitrocellulose membranes (Hybond ECL, GE Healthcare Bio-Sciences Corp., NJ, USA). Membranes were blocked overnight at 4 °C with 5% non-fat dried milk in TBST (Tris-buffered saline with Tween 20). TBST contains 150 mM NaCl, 20 mM Tris–HCl, pH 7.5 and 0.1% Tween 20. After blocking, the membranes were probed with primary antibody [APLR: sc 33823 1:1000] for 3 h at room temperature. After washing in TBS-T (three times, 5 min), the blots were incubated for 60 min at room temperature with secondary antibody [goat anti rabbit sc 2004, 1:10,000]. All antibodies were diluted in blocking buffer (5% non-fat dried milk and TBST). The antibody-antigen complexes were detected using the ECL system and exposed to Lumi-Film chemiluminescent detection film (Roch, Germany). Lab Work analyzing software (UVP, UK) was used to analyze the intensity of the expression. β-Actin immunoblotting (antibody from Cell Signaling Technology Inc., Beverly, MA, USA; 1:1000) was used to control for loading.

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