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Research report

Possible involvement of the hypothalamic pro-opiomelanocortin gene and β-endorphin expression on acute morphine withdrawal development

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ABSTRACT

We studied the effects of supraspinally administered morphine on the expression of the hypothalamic pro-opiomelanocortin (POMC) gene and β-endorphin. Mice were administered morphine intracerebroventricularly (i.c.v.) either once or 5 times for 5 days (once/day). A single morphine administration significantly increased the hypothalamic POMC gene and β-endorphin expression at 2 h after application in dose-dependent fashion; however, repeated morphine administration had no effect on the hypothalamic POMC gene and β-endorphin expression. In the immunoblot and immunohistochemical study, the increase of β-endorphin was observed in the arcuate nucleus of the hypothalamus. Moreover, the expressions of c-Fos, phosphorylated calcium/calmodulin-dependent protein kinase-IIα (pCaMK-IIα), and phosphorylated cAMP response element-binding protein (pCREB) were increased by a single i.c.v. morphine injection at various time points, but the expressions of phosphorylated extracellular signal-regulated protein kinase1/2 (pERK1/2) and phosphorylated IκB (pIκB) were not. We also found that the expressions of c-Fos, pCaMKIIα, and pCREB were co-localized with the POMC expression. Meanwhile, naloxone as well as muscimol and baclofen significantly attenuated the increases of the POMC gene expression induced by a single morphine administration. Furthermore, the pretreatment of muscimol and baclofen 10 min before morphine injection robustly attenuated the withdrawal behavior induced by a single morphine administration. These results imply that the hypothalamic POMC gene and β-endorphin expression may play an important role in the development of an acute physical dependency of morphine. In that, GABAergic neurotransmission appear to be involved in the regulation of the hypothalamic POMC gene expression induced by supraspinal morphine administration.

1. Introduction

It is generally known that the most addictive drugs cause long-lasting neurochemical changes, in which the mesolimbic and mesocortical dopaminergic pathways play a crucial role in the development of reward and positive reinforcement [30,43]. Recently, several lines of evidence pointed to an important role of endogenous opioids and their receptors in the mechanisms of drug addiction. As endogenous opioid peptides and opioid receptors have been implicated in the regulation of extracellular dopamine levels of the reward pathway, it has been widely assumed that stimulation of the activity of distinct components of the endogenous opioid system, either by opioid or other addictive drugs, may mediate their reinforcing effects [21,31,42,54].

Among the several endogenous opioid peptides, β-endorphin has long been suspected of making a major contribution to the positive reinforcement and motivational properties of several addictive substances. In early studies, it was demonstrated that endorphin, which is administered ventricularly, possess intrinsic rewarding properties [56]. Furthermore, microinjection of β-endorphin to several supraspinal regions of the mesolimbic reward system such as the NAc also produced place preferences [3]. In regard to this line, Sweep et al., also indicated that repeated administration of cocaine and heroine significantly attenuated β-endorphin expression in the various limbic areas [46,47]. Moreover, it was recently reported that acute administration of ethanol as well as psychostimulant-cocatine and amphetamine transiently increased β-endorphin neurotransmission in the rodent nucleus accumbens [38]. These previous studies supported the notion that the β-

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endorphin is involved in the development of the dependency and abuse induced by alcohol or other psychostimulants. However, it is still unclear whether β-endorphin is involved in morphine-induced dependency and withdrawal development or not. In addition, effects of a single morphine administration on the hypothalamic pro-opiomelanocortin (POMC) gene that encodes β-endorphin have not yet been characterized.

In the rodent central nervous system, POMC – one of the endogenous opioid peptide precursors that also includes adrenocorticotropin (ACTH), the β-endorphin, and melanotropin (α-, β- and γ-MSH) – is primarily located in the arcuate nucleus and the periarcuate area of the medial basal hypothalamus [4,11,19,25] and contains neurons projecting widely throughout the brain to participate in a wide range of functions such as pain control [45,52], stress responses [2] and reward responses [15,22,38]. Although POMC neurons are also located in the nucleus tractus solitarius (NTS) in the lower brain stem [4,8], the physiological role of NTS POMC on the reward circuit is not clear.

The POMC gene expression is tightly regulated by several factors such as corticotropin-releasing hormone (CRH) and glucocorticoid that are known to be among the powerful positive and negative regulators, respectively. Moreover, several signal regulatory molecules in the cytoplasm and nucleus are involved in these molecular mechanisms of the POMC gene expression. Generally, the POMC gene transcription is regulated by calcium-dependent as well as calcium-independent signaling cascades [14] including PKA, CREB, and CRH-induced c-Fos activation through the PTRE (TGACTAA) promoter region of the POMC gene in the AtT-20 cell [5,6,14]. Recently, it was demonstrated that the POMC gene could be activated by the induction of Nur77 and Nurr1, which are members of the steroid receptor superfamily expressed under stressful conditions. Nur77 is bound to the negative glucocorticoid responsive element (nGRE) existing in the POMC promoter regions by the CRH/cAMP pathway by protein kinase A (PKA) and mitogen-activated protein kinase (MAPK) pathways dependent manner [33]. We also reported that the hypothalamic POMC gene expression induced by peripheral inflammatory nociceptive stimuli is closely related with pERK as well as pCaMK-II activation [41]. Although this accumulated data has shown the underlying mechanisms of the POMC gene expression in the pituitary gland and in the hypothalamic arcuate nucleus, these studies are mainly focused on the hormonal regulation of the pituitary gland or the pain modulatory effect of the hypothalamus. In fact, the hypothalamic POMC gene and its derivate β-endorphin expression as a modulator of reward circuit activation have not been previously elucidated. In this study, therefore, we tested the hypothesis that a single morphine administration, which caused physical dependency and withdrawal symptom development, produces up-regulation of the hypothalamic POMC gene expression and increases of β-endorphin as a positive regulator on reward circuit activation. In order to initially characterize the morphine-induced POMC gene and β-endorphin response, we investigated the dose and temporal-dependent effects of an acute or repeated supraspinal administration on the POMC gene and β-endorphin expression. In addition, we characterized the possible signal molecules involved in the POMC gene expression as well as underlying mechanisms of the morphine-induced POMC gene expression.

2. Materials and methods

2.1. Animals

These experiments were approved by the Animal Care and Use Committee of Hallym University. All procedures were conducted in accordance with the 'Guide for Care and Use of Laboratory Animals' published by the National Institute of Health and also in accordance with the ethical guidelines of the International Association for the Study of Pain. We did our best to minimize the number of animals used and their suffering. Male ICR mice (MJ Ltd., Seoul, Korea) weighing 23–25 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22 ± 0.5 °C with an alternating 12 h light–dark cycle for at least 5 days before the experiments were started, and food and water were available ad libitum. The animals were allowed to adapt to the experimental conditions in the laboratory for at least 2 h before behavior testing. To reduce variation, all experiments were performed during the light phase of the cycle (10:00–17:00).

2.2. Drugs

Morphine, muscimol, naloxone, PD98059 and KN-93 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Baclofen was purchased from RBI Inc. (Natick, MA, USA). Drug doses, which were determined as critical concentrations for statistical analysis for behavioral and molecular studies were chosen based on preliminary studies. All drugs were prepared just before use.

2.3. Intracerebroventricular (i.c.v.) injection of drugs

The i.c.v. administration followed the method described by Haley and McCormick [24]. Each mouse was grasped firmly without anesthesia by the loose skin behind the head. The skin was pulled taut. A 30-G needle attached to a 25 μl syringe was inserted perpendicularly through the skull into the brain and solution was injected. The injection site was 2 mm from either side of the midline on a line drawn through the anterior base of the ears. The i.c.v. injection volumes were 5 μl, and the injection sites were verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the ventricular space. The success rate for prior injections with this technique was over 95%.

2.4. Northern blot analysis

2.4.1. Isolation of total RNA and proteins

For the analysis, the whole mouse hypothalamus and pituitary gland were dissected. Total cellular RNA was extracted from dissected tissue using a rapid guanidine thiocyanate-water saturated phenol/chloroform extraction procedure and subsequent precipitation with acidic sodium acetate [10]. Total cellular RNA in the aqueous phase was precipitated with ice-cold isopropyl alcohol. Isolated RNA samples were subjected to spectrophotometric analysis at 260 nm and 280 nm. The separated organic layer was extracted twice with an equal volume of sterilized (Millipore) water. Proteins precipitated by adding 2 volumes of absolute ethanol to the water-extracted organic phase. The protein pellets were washed twice with cold absolute ethanol and dried. The dried pellets were dissolved in a denaturing buffer (6 M guanidium chloride, 20 mM Tris–HCl [pH 8.0], and 1 mM EDTA). The protein pellets were washed twice with cold absolute ethanol and dried. The dried pellets were dissolved in a denaturing buffer (6 M guanidium chloride, 20 mM Tris–HCl [pH 8.0], and 1 mM EDTA). The protein samples were dialyzed against a renaturing buffer (20 mM Tris–HCl [pH 8.0], 100 mM NaCl, 1 mM EDTA, 5 mM dithiothreitol, 5 mM $MgCl_2$, 0.4 mM phenylmethylsulfonyl fluoride, and 20% glycerol) at 4 °C.

2.4.2. Preparation of DIG-labeled cRNA probes

The cRNA probes for POMC [11] and cyclophilin [12] were synthesized in vitro from linearized expression vectors which contained SP6 or T7 viral promoter. 1 μg of linearized plasmid was mixed with an RNA labeling mixture that contained ATP, CTP, GTP,