Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 70 (2005) 1079–1087
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

IRAS, a candidate for I_1-imidazoline receptor, mediates inhibitory effect of agmatine on cellular morphine dependence

Ning Wu [a], Rui-Bin Su [a], Bo Xu [a], Xin-Qiang Lu [a], Yin Liu [a], Jian-Quan Zheng [a], John E Piletz [b], Jin Li [a,*], Bo-Yi Qin [a]

[a] *Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China*
[b] *Department of Biology, Jackson State University, Jackson, MS 39217, USA*

Received 27 May 2005; accepted 8 July 2005

Abstract

Agmatine, an endogenous ligand for the I_1-imidazoline receptor, has previously been shown to prevent morphine dependence in rats and mice. To investigate the role of imidazoline receptor antisera-selected protein (IRAS), a strong candidate for I_1R, in morphine dependence, two CHO cell lines were created, in which μ opioid receptor (MOR) was stably expressed alone (CHO-μ) or MOR and IRAS were stably co-expressed (CHO-μ/IRAS). After 48 h administration of morphine (10 μM), naloxone induced a cAMP overshoot in both cell lines, suggesting cellular morphine dependence had been produced. Agmatine (0.1–2.5 μM) concentration-dependently inhibited the naloxone-precipitated cAMP overshoot when co-pretreated with morphine in CHO-μ/IRAS, but not in CHO-μ. Agmatine at 5–100 μM also inhibited the cAMP overshoot in CHO/μ and CHO-μ/IRAS. Efaroxan, an I_1R-preferential antagonist, completely blocked the effect of agmatine on the cAMP overshoot at 0.1–2.5 μM in CHO-μ/IRAS, while partially reversing the effects of agmatine at 5–100 μM. L-type calcium channel blocker nifedipine entirely mimicked the effects of agmatine at high concentrations on forskolin-stimulated cAMP formation in CHO-μ and naloxone-precipitated cAMP overshoot in morphine-pretreated CHO-μ. Therefore, IRAS, in the co-transfected CHO-μ/IRAS cell line, appears necessary for low concentrations of agmatine to cause attenuation of cellular morphine dependence. An additional effect of agmatine at higher concentrations seems to relate to both transfected IRAS and some naive elements in CHO cells, and L-type voltage-gated calcium channels are not ruled out. This study suggests that IRAS mediates agmatine's high affinity effects on cellular morphine dependence and may play a role in opioid dependence.

Keywords: Opioid dependence; Imidazoline receptor; IRAS; Agmatine; cAMP overshoot

1. Introduction

Chronic administration of opioids such as morphine produces tolerance and dependence, which greatly limits their clinical use. The biological basis of tolerance and dependence induced by chronic exposure to opioids is considered as molecular, cellular, and neural network adaptations. The adaptation in intracellular signal transduction includes up-regulations of cAMP pathway, Ca^{2+} pathway, nitric oxide synthase (NOS) system, MAPK cascade signal pathway, and so on [1,2].

Recent studies show that agmatine, a decarboxylated product of L-arginine, enhances morphine analgesia, and attenuates chronic tolerance and dependence in vivo [3–7]. Many studies demonstrated that agmatine meets many criteria for a neurotransmitter and/or

Abbreviations: α_2-AR, α_2-adrenoceptors; Agm, agmatine; APH, aminoglycoside phosphotransferases; BU224, 2-(4,5-dihydroimidaz-2-yl)-isoquinolin; CHO, Chinese hamster ovary; CHO-μ, Chinese hamster ovary cells expressing μ opioid receptor; CHO-μ/IRAS, Chinese hamster ovary cells co-expressing μ opioid receptor and imidazoline receptor antisera-selected; CHO-μ/IRAS-Low, Chinese hamster ovary cells co-expressing μ opioid receptor and low level imidazoline receptor antisera-selected; Efa, efaroxan; GABA, γ-amino butyric acid; hIRAS, human imidazoline receptor antisera-selected protein; IBMX, 3-isobutyl-1-methylxanthine; I_1R, I_1-imidazoline receptor; I_2R; I_2-imidazoline receptor; IRAS, imidazoline receptor antisera-selected protein; Irs, imidazoline receptors; L-NAME, *N*ω-nitro-L-arginine; MK801, dizocilpine; Mor, morphine; MOR, μ opioid receptor; NE, norepinephrine; Nif, nifedipine; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; rMOR, rat μ opioid receptor; VTA, ventral tegmental area

* Corresponding author. Tel.: +86 10 66932681; fax: +86 10 68211656.
E-mail address: lijin@nic.bmi.ac.cn (J. Li).

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.07.006

neuromodulator in brain and is considered as a putative endogenous ligand for imidazoline receptors (IRs) [8]. IRs, also known as imidazoline binding sites or imidazoline recognizing sites, exist in at least two principal subtypes, I_1 and I_2 receptors [9]. Autoradiographic and immunohistochemical studies have demonstrated that I_1R is regionally distributed throughout the central nervous system [10]. Overlap in distributions of IRs and opioid receptors may be observed in some regions related to opioid antinociception and dependence, such as laminae I and II of spinal dorsal horn, nucleus of the solitary tract, midbrain periaqueductal gray, central gray, nucleus paraventricularis thalami, amygdala nucleus and striatum. It has also been reported that I_1R participates in the effects of clonidine on activation of dopaminergic neurons in VTA during morphine withdrawal [11]. Furthermore, after chronic morphine treatment of rats, the density of [^{3}H]idazoxan binding sites is decreased in forebrain and cerebellum [7]. These findings have suggested that I_1R and/or I_2R may be related to the modulation of morphine analgesia, tolerance or dependence.

Though agmatine is considered as a putative endogenous ligand for IRs, whether I_1 and/or I_2 subtypes mediate agmatine's actions on regulating morphine dependence remains unclear. In fact, it has been difficult to directly demonstrate the relationship between IRs, the especially I_1 subtype, and opioid dependence. The reason is that agents binding to I_1R also bind to α_2-adrenoceptors (α_2-ARs), and selective antagonists for I_1R are still not commercially available. Though some new ligands with high affinity and selectivity for I_1R have been synthesized recently [12–14], theses compounds are still not available commercially. Furthermore, there are no suitable cell types or animal models that express I_1R and opioid receptors in the absence of α_2-AR.

An imidazoline-binding protein, named imidazoline receptor antisera-selected protein (IRAS), was cloned from human hippocampus [15]. The amino acid sequence of human IRAS (hIRAS) is distinct from known proteins, except for a mouse homologue named Nischarin, which binds integrin α5 subunit and plays a negative role in cell migration [16]. Several lines of evidence support IRAS as a strong candidate for I_1R. First, IRAS was selected with specific anti-imidazoline receptor antiserum [15]. Second, the distribution of IRAS mRNA was positively correlated with radioligand binding density (B_{max}) for I_1R in a range of rat tissues, including brain [17]. Furthermore, transient transfection of IRAS cDNA into CHO cells resulted in the appearance of high-affinity I_1 binding sites [15].

Based on these observations, we established a stably transfected CHO cell line co-expressing MOR and IRAS. In this cell line, the role of IRAS, a strong candidate for I_1R, in cellular opioid dependence was investigated.

2. Materials and methods

2.1. Materials

The origin of hIRAS-pcDNA3.1(+) has previously been described [15]. rMOR-pcDNA3 and CHO cells were the generous gifts from Dr. L.Y. Liu-Chen (Temple University School of Medicine, Philadelphia, PA, USA). pcDNA3.1/hygro(+) was purchased from Invitrogen Corporation (Invitrogen™, Carlsbad, CA, USA). [^{3}H]diprenorphine (50 Ci/mmol), [^{3}H]clonidine (55.5 Ci/mmol), [^{3}H]yohimbine (85 Ci/mmol), and [^{3}H]L-arginine (45 Ci/mmol) were purchased from PerkinElmer Life Sciences (NEN™, Boston, MA, USA). Geneticin, lipofectamine, and RPMI 1640 medium were purchased from Invitrogen Corporation (Gibco™, Grand Island, NY, USA). Hygromycin B was purchased from Roche Diagnostics GmbH (Roche, Mannheim, Germany). Fetal bovine serum was purchased from HyClone-Pierce (HyClone®, South Logan, UT, USA). Agmatine, moxonidine, IBMX, forskolin, naloxone, clonidine, yohimbine, MK801, nifedipine and Dowex 50wx8-400 resin (H^+ form) were purchased from Sigma Chemical Company (Sigma®, St. Louis, MO, USA). Efaroxan and BU224 were purchased from Research Biochemicals International (RBI, Natick, MA, USA). Morphine was purchased from Qinghai Pharmaceutic Factory (Xining, China). The kits for cAMP assays were purchased from the National Academy of Atomic Energy of China (Beijing, China).

2.2. Generation of cell lines co-expressing MOR and IRAS and cell culture

cDNA encoding rat μ opioid receptor (rMOR) was isolated by *Hin*d III digestion from rMOR-pcDNA3 plasmid, and subcloned into the mammalian expression vector pcDNA3.1/hygro(+). Transfection of CHO cells was performed with Lipofectamine according to the manufacturer's instructions. Cells transfected with rMOR cDNA were individually isolated and cloning cultured for 4–6 weeks under the selection pressure of 200 μg/ml hygromycin B. One of the positive clones (CHO-μ) was then retransfected with the hIRAS cDNA (hIRAS-pcDNA3.1(+)) and the cells were grown for another 4–6 weeks under co-selection of 1 mg/ml geneticin and 50 μg/ml hygromycin B. After cell cloning, two positive clones (CHO-μ/IRAS and CHO-μ/IRAS-Low) were selected for study. IRAS's density of CHO-μ/IRAS-Low is one-sixth of CHO-μ/IRAS.

CHO cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 U/ml streptomycin at 37 °C with humidified atmosphere consisting of 95% air and 5% CO_2. The media for CHO-μ and CHO-μ/IRAS were the same as that for CHO cells except the former contained