Neurochemistry International 108 (2017) 282–286

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Rapid communication

Upregulation of IRAS/nischarin (I_1-imidazoline receptor), a regulatory protein of μ-opioid receptor trafficking, in postmortem prefrontal cortex of long-term opiate and mixed opiate/cocaine abusers

Benjamin Keller [a, b, *], Romano La Harpe [c], Jesús A. García-Sevilla [a, b]

[a] Laboratori de Neurofarmacologia, IUNICS, Universitat de les Illes Balears (UIB), IdISBa, Palma de Mallorca, Spain
[b] Redes Temáticas de Investigación Cooperativa en Salud–Red de Trastornos Adictivos (RETICS-RTA), ISCIII, Madrid, Spain
[c] Centre Universitaire Romand de Médecine Légale, Genève, Switzerland

ARTICLE INFO

Article history:
Received 29 March 2017
Received in revised form
25 April 2017
Accepted 27 April 2017
Available online 28 April 2017

Keywords:
IRAS/nischarin
μ-Opioid receptor
Opiate dependence
Imidazoline receptors

ABSTRACT

Imidazoline receptor antisera-selected (IRAS)/nischarin, a putative I_1-imidazoline receptor, has recently been shown to regulate μ-opioid receptor (OR) trafficking and resensitisation. To study a possible involvement of this μ-OR regulator in opiate dependence, the present study assessed by Western blot analysis the contents of IRAS/nischarin and μ-OR in total homogenates and subcellular preparations of postmortem human prefrontal cortex (PFC/BA9) of long-term opiate and mixed opiate/cocaine abusers as well as of matched healthy control subjects. In the PFC/BA9 of long-term opiate/cocaine abusers (all subjects together) IRAS/nischarin content was increased (+67%, $p < 0.01$, n = 11) when compared with matched controls (n = 10). Similar increases were found for the subgroups of opiate (+72%, n = 6) and mixed opiate/cocaine (+61%, n = 5) abusers. IRAS/nischarin immunocontents were also found increased in subcellular membrane preparations (+61%, $p < 0.05$, n = 10) of PFC/BA9 from opiate addicts. In the same brain samples, the levels of μ-OR were not different to those in control subjects. Based on the increased contents in brains of opiate abusers and the reported function as μ-OR regulator, IRAS/nischarin could represent a new promising target for treatment of opiate use disorder.

1. Introduction

Chronic exposure to opiates can cause the development of drug tolerance and physical dependence as well as persistent brain neuroplasticity, but the molecular processes underlying μ-opioid receptor (μ-OR) regulation and signaling and opiate dependence are incompletely understood (Williams et al., 2013). Several studies over the last 20 years have indicated the involvement of a novel class of receptors for imidazoline compounds, the so-called imidazoline receptors (IRs; reviewed in Keller and García-Sevilla, 2015) in the modulation of opioid receptor signaling. Thus, selective ligands for the I_2-type of IRs (e.g. 2-BFI or BU224) and the putative endogenous ligand agmatine (Li et al., 1994) have been shown to potentiate the analgesic effects of morphine, and to reduce opiate tolerance and withdrawal symptoms (reviewed in Li and Zhang, 2011). The finding that I_1-IR activation by moxonidine prevented cue-induced cocaine relapse further indicated a therapeutic potential of targeting IRs in addictive disorders (Smith and Aston-Jones, 2011).

Recently, the putative human IR protein IRAS (Imidazoline Receptor Antisera-Selected) and its mouse homologue nischarin have been cloned (reviewed in Keller and García-Sevilla, 2015). IRAS knockout mice have been generated and were characterised by reduced pain threshold and response to methadone analgesia, as well as by exacerbated development of dependence to opiates (Zhang et al., 2013). Further, IRAS/nischarin has been shown to mediate the effects of agmatine on morphine dependence (Wu et al., 2005), possibly through regulation of agonist-induced μ-OR trafficking, accelerating the resensitisation of the receptor (Li et al., 2016).

Because of the role of IRs in opiate addiction, the present study quantified the content of IRAS/nischarin, a new μ-OR regulatory

* Corresponding author. Laboratory of Neuropharmacology, IUNICS, University of the Balearic Islands (UIB), Cra. Valldemossa km 7.5, E-07122 Palma de Mallorca. Spain.
E-mail address: b.keller@uib.es (B. Keller).

http://dx.doi.org/10.1016/j.neuint.2017.04.017
0197-0186/

protein (Li et al., 2016), in post-mortem human brains of well-characterised cohorts of long-term opiate and mixed opiate/cocaine abusers.

2. Materials and methods

2.1. Specimens of postmortem human brain

Brain specimens (right middle frontal gyrus, Brodmann's area 9, PFC/BA9) were obtained at autopsies (1999–2006) in the Centre Universitaire Romand de Médecine Légale-Site Genève (Switzerland), following all the legal and ethical standard procedures (García-Fuster et al., 2008). PFC/BA9 samples were stored at −80 °C and the assays done in the Laboratory of Neuropharmacology (IUNICS), University of the Balearic Islands (UIB) and after approval from the local Ethical Committee of Clinical Investigation (CEIC-CAIB). Table 1 shows the characteristics and blood and hair toxicology (Toxicology Unit, STS 390, University of Geneva) of the studied subjects. Opiate abusers died within a short period of time, as revealed by the high concentrations of 6-monoacetyl morphine (6-MAM) and free morphine or methadone in blood (Ferrer-Alcón et al., 2004a). Control subjects died in the same period of time from sudden and unexpected death (e.g. traffic and car accidents, myocardial infarction, gunshot wounds, and homicide). Toxicology in hair samples (usually scalp hair, 3–25 cm) was performed to assess opiate/cocaine exposure over the last months (see Ferrer-Alcón et al., 2004a). In opiate addicts (Table 1), hair analysis of 6-MAM (range, 0.5–90 ng/mg), morphine (range, 0.2–8.0 ng/mg) or methadone (range, 0.4–135 ng/mg) indicated a long-term abuse of opiates (6–24 months before death). In five opiate abusers, cocaine was also detected in hair (range: 0.8–8.5 ng/mg) but not in blood, indicating a continued exposure to opiates with past (last year before death) cocaine abuse (mixed opiate/cocaine abusers). Blood ethanol was detected in two opiate abusers (Table 1), but evidence of ethanol dependence was not obtained in medical records (García-Fuster et al., 2008). Other psychotropic drugs were detected in blood and/or hair samples of some subjects (residual contents of THC and MDMA, or therapeutic concentrations of some antidepressants and antipsychotics) (Table 1).

2.2. Brain preparation, immunoblot assays and quantification of target proteins

Total homogenates and subcellular fractions (F1: cytosolic, F2: membrane-associated, F3: nuclear subproteomes; ProteoExtractTM, Calbiochem/Merck, Darmstadt, FRG) of PFC/BA9 samples (100–150 mg) from control subjects (n = 10) and opiate/cocaine addicts (n = 11) were prepared as described (Ferrer-Alcón et al., 2004a; Keller and García-Sevilla, 2015). Brain proteins (total homogenates: 40 μg; F1-F3: 7.5 μg) were resolved by SDS-PAGE and standard Western blot procedures (Keller and García-Sevilla, 2015) using specific primary (epitope-affinity purified) antibodies (Ab): anti-NISCH Ab, #ab56849, lot: GR111152 (Abcam, Cambridge, UK; see details below); anti-opioid μ receptor (384–398) rabbit pAb, #PC165L, lot: D09897-1 (Calbiochem/Merck; see details below); anti-β-actin Ab, #A1978, lot: 065M4837V (Sigma-Aldrich); anti-PEA-15 Ab, #2780S, lot: 1 (Cell Signaling, Danvers, MA) (F1 marker); anti-Fas (M-20) Ab, #sc-716, lot: D2711 (Santa Cruz Biotechnology, Santa Cruz, CA) (F2 marker); anti-PARP Ab, #KP8501, lot: D00079571 (Calbiochem/Merck) (F3 marker). The origin and specificity of anti-NISCH and anti-opioid μ rceptor Abs have recently been described (Keller and García-Sevilla, 2017). The amount of immunoreactive target protein (integrated optical density, IOD) in brain samples (PFC/BA9) from opiate/cocaine abusers was compared with that of matched controls (100%) in the same gel, and normalised to the contents of β-actin. Each human brain sample was quantified in 2–4 gels and the mean value was used as a final estimate.

2.3. Data and statistical analysis

All series of data were analysed with the program GraphPad Prism, version 5.0 (GraphPad Software, Inc., San Diego, CA, USA). Results are expressed as mean ± SEM. Before statistical analysis the data were inspected for possible outliers with the Grubb's test (GraphPad Software); one outlier was detected (and discarded from further analysis) in subcellular preparations within the group of mixed cocaine/opiate abusers (deletion of the data did not significantly alter the reported results). The experimental design of this

Table 1
Demographic characteristics and toxicological data of individual long-term opiate and mixed opiate/cocaine abusers.

Paired subjects	Sex/age (years)	PMD (h)	Total blood opiates (μg/ml)	Other drugs (μg/ml) Ethanol (Eth, g/l)	Hair opiates and other drugs (ng/mg)
Opiate abusers					
1	M/36 (M/37)	3 (18)	Mor (0.7)	Clo (0.4)	Mor (0.6) + MAM (1.9) + Met (0.5)
2	M/34 (M/37)	3 (18)	Met (0.3)	None	Met (0.4) + Mor (1.8) + MAM (0.8)
3	F/33 (F/34)	16 (41)	Met (1.4)	Ven (2.5)	Met (0.4)
4	M/25 (M/20)	7 (21)	Mor (0.3)	Eth (1.77)	Mor (0.2) + MAM (1.7) + Met (135) + MDMA (6.2)
5	F/26 (F/26)	33 (10)	Met (1.4)	None	NT
6	M/28 (M/27)	42 (20)	None*	THC (0.03)	MAM (1.2) + Mor (2.8), Cod (0.2)
Mixed opiate/cocaine abusers					
7	M/34 (M/38)	86 (77)	Mor (0.9)	Eth (1.72)	Met (0.7) + MAM (0.5) + Coc (0.8)
8	M/42 (M/48)	5 (16)	Met (4.3)	None	Met (65) + Coc (0.9)
9	M/24 (M/25)	53 (52)	Mor (1.2)	THC (0.007)	Mor (0.3) + MAM (1.5) + Coc (4.5)
10	M/47 (M/51)	96 (93)	Met (3.3)	Qua (10.5)	Met (8.7) + Coc (0.9)
11	M/55 (M/56)	16 (16)	Met (0.5)	None	Met (0.5) + Mor (8) + MAM (90) + Coc (8.5)
	Addicts: 9M/2F 35 ± 3 years	33 ± 10 h	Mor (0.77 ± 0.02, n = 4) Met (1.87 ± 0.7, n = 6)		
	Controls: 8M/2F 36 ± 3 years	36 ± 8 h	None		

The characteristics of the respective matched control subject are presented in parentheses. Gender: M-male, F-female. *Death not due to opiate overdose (subject 11, homicide, gun shot). Clo, clomipramine; Coc, cocaine; Cod, codeine; Eth, ethanol; MAM, 6-monoacetylmorphine; Met, methadone (ant its metabolite 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidone); Mor, morphine; Qua, methaqualone; THC, Δ9-tetrahydrocannabinol; Ven, venlafaxine. NT: not tested.