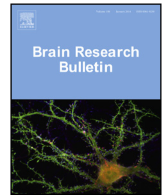
Brain Research Bulletin 117 (2015) 32–38

Contents lists available at ScienceDirect

Brain Research Bulletin

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



Research report

A new potent analgesic agent with reduced liability to produce morphine tolerance

Kornel Kiraly[a,1], Francesca Felicia Caputi[b,1], Adrienn Hanuska[a], Erzsébet Kató[a], Mihaly Balogh[a], László Köles[a], Martina Palmisano[b], Pal Riba[a], Sándor Hosztafi[c], Patrizia Romualdi[b], Sanzio Candeletti[b], Péter Ferdinandy[a], Susanna Fürst[a], Mahmoud Al-Khrasani[a,*]

[a] Department of Pharmacology and Pharmacotherapy, Semmelweis University, Nagyvárad tér 4, H-1089 Budapest, Hungary
[b] Department of Pharmacy and Biotechnology (FaBiT), Alma Mater Studiorum, University of Bologna, Via Irnerio 48, Bologna 40126, Italy
[c] Department of Pharmaceutical Chemistry, Semmelweis University, Hőgyes Endre u., 9. H-1092 Budapest, Hungary

ARTICLE INFO

Article history:
Received 4 June 2015
Received in revised form 22 July 2015
Accepted 24 July 2015
Available online 30 July 2015

Keywords:
14-*O*-Methylmorphine-6-sulfate
Analgesia
Tolerance
Glutamatergic transmission
MOP receptor down-regulation

ABSTRACT

The therapeutic use of opioids is limited by the development of tolerance to the analgesic effect and the cellular and molecular mechanisms underlying this phenomenon are still not completely understood. For this reason the search for new analgesic derivatives, endowed with lower tolerance, is always an active field. The newly synthesized 14-*O*-Methylmorphine-6-sulfate (14-*O*-MeM6SU) shows high efficacy in *in vitro* assays and a strong analgesic action in the rat tail flick test. The aim of present work was to investigate: the analgesic effect of 14-*O*-MeM6SU in mouse tail-flick test; the tolerance to analgesic effect of 14-*O*-MeM6SU compared to morphine in mice, the effects of test compounds on glutamatergic neurotransmission by measuring spontaneous excitatory postsynaptic currents (sEPSCs) of layer V pyramidal cells from rat prefrontal cortices; and the effect of acute and chronic 14-*O*-MeM6SU treatments on opioid receptor gene expression in SH-SY5Y neuroblastoma cells expressing μ-opioid (MOP) and nociceptin/opioid receptor-like 1 (NOP) receptors.

14-*O*-MeM6SU was 17 times more potent than morphine in analgesia and had long duration of action in analgesic dose equipotent to morphine. Mice were treated subcutaneously (s.c.) either with 200 μmol/kg morphine or with 14-*O*-MeM6SU (12 μmol/kg) twice daily for three days. The magnitude of tolerance or cross-tolerance indicated by the shift in antinociceptive ED_{50} measured was greater for morphine compared to 14-*O*-MeM6SU. Subsequent to behavioral testing, patch-clamp experiments in layer V pyramidal neurons of rat prefrontal cortical slices in the presence of bicuculline were performed. Both 14-*O*-MeM6SU (0.1 μM) and morphine (1 μM) decreased the frequency of sEPSCs, indicating reduction of glutamate release. The effect of the novel compound was reversed by the opioid receptor antagonist naloxone, indicating an opioid mediated action. In contrast, the amplitude was not affected. Finally, gene expression data showed a dose dependent down-regulation of MOP receptor after 24 h and 48 h exposure to 14-*O*-MeM6SU. Interestingly, no changes were detected for NOP receptor gene expression. The specific lack of this effect could be related to the lower tolerance development to analgesic effect of 14-*O*-MeM6SU. Furthermore, 14-*O*-MeM6SU displayed high intrinsic efficacy possibly an important factor in the observed effects. Further, the observed inhibition of glutamatergic signaling might be attributed also to the reduction of opioid tolerance. Based on our results the development of a new clinically important, safe analgesic agent might be possible.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Nociceptive modulation involves several neurotransmitter systems such as opioidergic, cannabinoidergic, adrenergic, gabaergic, serotonergic, cholinergic, dopaminergic and glutamatergic ones. (Cobacho et al., 2014; Ferreira and Menescal-de-Oliveira, 2014; Pan et al., 2008; Raposo et al., 2015; Thomson et al., 2006; Tuboly et al., 2015). Drugs affecting these systems may modulate the pain

* Corresponding author at: Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Nagyvárad tér 4, H-1089 Budapest, Hungary. Fax: +36 1 2104412.
E-mail address: al-khrasani.mahmoud@med.semmelweis-univ.hu (M. Al-Khrasani).

[1] These authors equally contributed to the work.

http://dx.doi.org/10.1016/j.brainresbull.2015.07.005
0361-9230/© 2015 Elsevier Inc. All rights reserved.

transmission at periphery, spinal and brain level. Among these agents opioid receptor agonists such as morphine are still the gold standards for the treatment of both acute and chronic severe pain. Although the opioid medicines are the strongest analgesics their prolonged and repetitive treatment may result in a significant reduction in the analgesic effect (i.e., tolerance) of used agents. Thus, opioid tolerance has become a major clinical problem and generated a continuous effort to find major analgesics with less tolerance developing potency and these investigations have been going on for many years. Opioid receptors belong to the superfamily of the G-protein coupled receptors. There are several mechanisms responsible for the development of opioid tolerance. These mechanisms might involve receptor desensitization, internalization, second messenger switch, receptor dimerization, changes in the endogenous opioid system and neuronal network functions, etc (Allouche et al., 2014; Crain and Shen, 1996; George et al., 2000; He and Lee, 1997; Jordan and Devi, 1999; Kiraly et al., 2006; Lee et al., 1980; Riba et al., 2002; Szentirmay et al., 2013; Tulunay et al., 1981).

Opioid drug research has been going on to develop potent and type-selective opioid agents having reduced liability to produce analgesic tolerance though we still have a poor understanding of the mechanisms responsible for opioid tolerance development. Recently, we have synthesized and developed 14-*O*-Methylmorphine-6-sulfate (14-*O*-MeM6SU) having high efficacy in *in vitro* assays and strong analgesic action on rat tail flick test compared to morphine (Lacko et al., 2012).

Whether long term systemic administrations of 14-*O*-MeM6SU influence its analgesic action has not been investigated. Also, the cross tolerance with morphine was not studied. Therefore one of the objectives of the present work was to determine the capability of 14-*O*-MeM6SU to produce analgesic tolerance in mice chronically treated with morphine or 14-*O*-MeM6SU. Prefrontal cortical neurons beside their role in the emotional components of pain are also critically involved in withdrawal, reward and tolerance development as indicated by in *in vivo* and *in vitro* studies (Cao et al., 2014; Wu et al., 2013). Thus we have also measured the effect of 14-*O*-MeM6SU on spontaneous excitatory postsynaptic currents (sEPSCs) in layer V pyramidal cells of rat prefrontal cortical slices, compared to morphine to clarify how the new compound may affect glutamatergic transmission.

Furthermore, nociceptin/opioid receptor-like 1 (NOP) receptor is the receptor for the endogenous peptide nociceptin that has been suggested to exert a functional antagonism toward the classical opioid system, at least at suspraspinal level (Mogil and Pasternak, 2001; Zaratin et al., 2004). At this regard, an involvement of the nociceptin/NOP receptor system in the mechanisms underlying the development of tolerance to the analgesic effect of morphine has been reported (Chung et al., 2006; Ueda et al., 1997; Zaratin et al., 2004. Therefore, we also explored the possibility that 14-*O*-MeM6SU might affect MOP and NOP receptors gene expression, in comparison with morphine, in an *in vitro* model represented by the neuroblastoma cell line SH-SY5Y that constitutively expresses both receptors (Caputi et al., 2013, 2014).

2. Materials and methods

2.1. Animals

Male NMRI mice (18–28 g; Toxicoop, Hungary) and Wistar/Wistar rats (Animal House of Semmelweis University, 9–12 days old) were used in this study. All animals were kept in group of 8 animals/cage in animal room of 12/12 h light-dark cycle and 20 ± 2 °C temperature. Standard rodent food and water were available ad libitum. Experiments were performed in accordance to the local animal care committee, the Ethical Board of Semmelweis University, Budapest, Hungary, based on the Declaration of the European Communities Council Directives (86/609/ECC). Permissions No. 22.1/605/001/2010 and PEI/001/276-4/2013.

2.2. Cell culture

Human SH-SY5Y neuroblastoma cells, purchased from ICLC-IST (Genoa, Italy), were used, cultured in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% (v/v) fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. In all experiments, cells were allowed to reach 80% of confluence before starting treatments. Before cell exposure, growth medium was changed and replaced with a fresh medium. Cells were exposed to increasing concentrations of 14-*O*-MeM6SU.

2.3. Drugs

14-*O*-Methylmorphine-6-sulfate (14-*O*-MeM6SU) was synthesized by S. Hosztafi as previously reported (Lacko et al., 2012). Morphine hydrochloride (ICN, Tiszavasvári, Hungary) and all other chemicals were of analytical grade and purchased from standard commercial sources. Drugs or saline delivered subcutaneously (s.c.) in a volume of 10 ml/ kg body weight. The saline (s.c.)-injected mice acted as the control group. Drugs were dissolved in 0.9% solution of NaCl. Experiments were performed in a blinded way to the drugs and doses applied. In whole cell voltage clamp experiments stock solutions (10 or 100 mM) were prepared with distilled water and were dissolved further in artificial cerebrospinal fluid (ACSF).

2.4. Analgesic test (mouse tail-flick)

Radiant heat tail flick test was used to assess antinociceptive effect of test compounds. The assay was carried out as described by Tulunay and Takemori (Tulunay and Takemori, 1974) using ITC Life Sciences equipment. Briefly, the light intensitiy was adjusted to set the control tail-flick latency between 1.3 and 2.8 s. Mice failed to respond within this range were excluded from the experiments. Cut-off time was set to 6 s to avoid tissue damage. A baseline latency was measured before and 30, 60, 120, 180 min after s.c. drug or saline administration. A saline treated group was used for each experiment as a negative control.

Maximal possible effect (MPE)% was calculated for each mouse as follows: [Latency after treatment–Basal Latency]/[Cut off–Basal Latency] × 100%. For each dose a separate group of animals (n = 5–12) was used.

2.5. Induction of tolerance

Mice were rendered tolerant to morphine hydrochloride (200 μmol/kg) or 14-*O*-MeM6SU (12 μmol/kg) by twice daily (7 AM and 7 PM) s.c. injections for 3 days. Saline injections (10 ml/kg twice daily) were used in the control animals. The degree of tolerance was determined as the ratio of the ED_{50} value of the agonist in morphine or 14-*O*-MeM6SU injected mice to that in saline injected mice. The experiment was done on the fourth day as described previously (Szentirmay et al., 2013). Treatment dose of 14-*O*-MeM6SU was calculated by using the following equation: [morphine dose] × [14-*O*-MeM6SU naive ED_{50}]/[morphine naive ED_{50}].

2.6. Dose-response relationships

Dose response curves for each drug were determined on the fourth day, following three days of chronic s.c. saline or drug treatments. Dose response curves for morphine were constructed

Download English Version:

https://daneshyari.com/en/article/4318681

Download Persian Version:

https://daneshyari.com/article/4318681

Daneshyari.com