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14-*O*-Methylmorphine: A Novel Selective Mu-Opioid Receptor Agonist with High Efficacy and Affinity

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ABSTRACT

14-*O*-methyl (14-*O*-Me) group in morphine-6-*O*-sulfate (M6SU) or oxymorphone has been reported to be essential for enhanced affinity, potency and antinociceptive effect of these opioids. Herein we report on the pharmacological properties (potency, affinity and efficacy) of the new compound, 14-*O*-methylmorphine (14-*O*-MeM) in *in vitro*. Additionally, we also investigated the antinociceptive effect of the novel compound, as well as its inhibitory action on gastrointestinal transit in *in vivo*. The potency and efficacy of test compound were measured by [^{35}S]GTPγS binding, isolated mouse vas deferens (MVD) and rat vas deferens (RVD) assays. The affinity of 14-*O*-MeM for opioid receptors was assessed by radioligand binding and MVD assays. The antinociceptive and gastrointestinal effects of the novel compound were evaluated in the rat tail-flick test and charcoal meal test, respectively. Morphine, DAMGO, Ile^{5,6} deltorphin II, deltorphin II and U-69593 were used as reference compounds.

14-*O*-MeM showed higher efficacy (E_{max}) and potency (EC_{50}) than morphine in MVD, RVD or [^{35}S]GTPγS binding. In addition, 14-*O*-MeM compared to morphine showed higher affinity for μ-opioid receptor (MOR). *In vivo*, in rat tail-flick test 14-*O*-MeM proved to be stronger antinociceptive agent than morphine after peripheral or central administration. Additionally, both compounds inhibited the gastrointestinal peristalsis. However, when the antinociceptive and antitransit doses for each test compound are compared, 14-*O*-MeM proved to have slightly more favorable pharmacological profile.

Our results affirm that 14-*O*-MeM, an opioid of high efficacy and affinity for MOR can be considered as a novel analgesic agent of potential clinical value.

1. Introduction

The opium-derived analgesic morphine is widely used in clinic to manage moderate to severe pain and considered to be the prototypical non-peptide opioid agonist, with a high selectivity for the μ-opioid receptor (MOR) subtype. Besides μ-opioid receptors, mammalians are also hosting κ-opioid receptors (KOR) and δ-opioid receptors (DOR). These receptors are G-protein-coupled receptors (GPCR) and they are expressed at central and peripheral relay points of nociceptive transmission (Fürst, 1999) and activated by endogenous or exogenous opioids. Upon their activation besides antinociceptive effect other measurable unwanted actions like respiratory depression, sedation or constipation are evoked (Debono et al., 2013; Koob et al., 1998).

One of the main goals of opioid researchers is to find opioid ligands of better pharmacological profiles than that of the currently available. 14-methoxy analogues of oxymorphone (14-*O*-methyloxymorphone) or morphine-6-*O*-sulfate have been reported to have higher affinity for opioid receptors and enhanced antinociceptive action compared to the parent compounds (Fürst et al., 2005; Khalefa et al., 2013; Lacko et al., 2012; Spetea et al., 2004). To the best of our knowledge, the *in vitro* and *in vivo* pharmacological profile of 14-methoxy analogues of morphine, particularly, 14-*O*-methylmorphine (Fig. 1) has not been reported yet.

Therefore, the aim of the present work was to synthesize 14-*O*-methylmorphine, assess its receptor preference (selectivity and affinity) for opioid receptors in biological (MVD, mouse *vas deferens*) and biochemical (equilibrium competition binding) assays. Further aim was to

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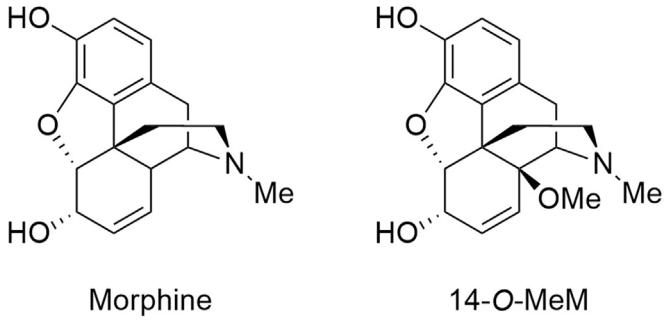

Fig. 1. The structure of morphine and 14-*O*-methylmorphine (14-*O*-MeM).

determine the potency and efficacy of 14-*O*-methylmorphine and compare them to the parent compound, morphine and to other, selective μ-, δ- and κ-opioid receptor agonists, such as [D-Ala^2,N-Me-Phe^4,Gly-ol^5]enkephalin (DAMGO), deltorphin II and U-69593, respectively, in mouse and rat *vas deferens* and functional [^{35}S]GTPγS binding. Further objective was to determine the antinociceptive effect of 14-*O*-methylmorphine, as well as its inhibitory effect on gastrointestinal transit applying rat tail-flick and charcoal meal assays, respectively and compare them to the effects of morphine.

2. Materials and methods

2.1. Animals

Male NMRI mice (35–45 g) for experiments designed for MVD and male Wistar rats for rat tail-flick test (140–240 g) and for RVD and gastrointestinal charcoal meal test (160–260 g) were used. Mice and rats were obtained from Toxi-Coop Zrt. (Budapest, Hungary) and the Animal House of Semmelweis University (Budapest, Hungary), respectively. Animals were housed in the local animal house of the Department of Pharmacology and Pharmacotherapy, Semmelweis University (Budapest, Hungary).

For *in vitro* receptor binding assays male and female Wistar rats (250–300 g body weight) and male guinea pigs (~400–700 g body weight, LAL/HA/BR strain) were used. Rats were purchased from and housed in the local animal house of the Biological Research Centre of the Hungarian Academy of Sciences (Szeged, Hungary), while guinea pigs were purchased from and housed in LAB-ÁLL Bt. (Budapest, Hungary).

The animals were kept in a temperature controlled room (21–24 °C) under a 12:12 light and dark cycle and were provided with water and food *ad libitum*. All housing and experiments were handled in accordance with the European Communities Council Directives (2010/63/EU), the Hungarian Act for the Protection of Animals in Research (XXVIII.tv. 32.§) and local animal care committee (PEI/001/276-4/2013). All the researchers did the best effort to minimize the number of animals and their suffering.

2.2. Chemicals

14-*O*-methylmorphine (Fig. 1) was synthesized as described under Section 2.3. Tris-HCl, EGTA, NaCl, $MgCl_2 \times 6H_2O$, GDP, the GTP analogue GTPγS, the DOR and KOR antagonist naltrindole and norbinaltorphimine, respectively and the KOR agonist U-69593 were purchased from Sigma-Aldrich (Budapest, Hungary). The MOR selective antagonist cyprodime was provided by Dr. Helmut Schmidhammer (Department of Pharmacy, University of Innsbruck, Austria) and the MOR agonist enkephalin analogue Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol (DAMGO) and the DOR selective agonist deltorphin II (Delt II) were obtained from Bachem Holding AG (Bubendorf, Switzerland) and Tocris Bioscience (through Biomedica Hungária Kft., Budapest, Hungary). The selective DOR agonist Ile^{5,6}-deltorphin II (IleDelt II) was synthesized in the Laboratory of Chemical Biology group of the Biological Research Centre of the Hungarian Academy of Sciences (Szeged, Hungary). The non-selective opioid receptor antagonist naloxone was kindly provided by the company Endo Laboratories DuPont de Nemours (Wilmington, DE, USA). Morphine hydrochloride was obtained from (Alkaloida-ICN, Tiszavasvári, Hungary). Ligands were dissolved in water and were stored in 1 mM stock solution at 20 °C for *in vitro* tests. Ligands used for *in vivo* assays were dissolved in saline prior to the experiments.

The radiolabeled GTP analogue, [^{35}S]GTPγS (specific activity: 1000 Ci/mmol) was purchased from Hartmann Analytic (through Izotóp Intézet Kft., Budapest, Hungary). [^{3}H]DAMGO (specific activity: 38.8 Ci/mmol), [^{3}H]IleDelt II (specific activity: 19,6 Ci/mmol) were radiolabeled by the Laboratory of Chemical Biology group in BRC (Szeged, Hungary). [^{3}H]U-69593 (specific activity: 43,6 Ci/mmol) were purchased from PerkinElmer (through Per-Form Hungária Kft., Budapest, Hungary). The UltimaGold™ MV aqueous scintillation cocktail was purchased from PerkinElmer (through Per-Form Hungária Kft., Budapest, Hungary).

2.3. Chemistry

14-*O*-methylmorphine was synthesized as described previously (Lacko et al., 2012). Briefly, 14-OH-codeinone was used as the starting material (Fig. 2). O-methylation was carried out by dimethyl sulfate in the presence of sodium hydride in *N,N*-dimethylformamide (Kobylecki et al., 1982; Razdan and Ghosh, 1980). 14-*O*-methylcodeinone was selectively demethylated in the 3-*O* position by refluxing in aqueous hydrogen bromide (Schmidhammer et al., 1990). The resulting 14-*O*-methylmorphinone was reduced by sodium borohydride in methanol to give 14-*O*-methylmorphine.

NMR data: Mp.: 221–223 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.62 (d, *J* = 8.1 Hz, H-2, 1H), 6.48 (d, *J* = 8.1 Hz, H-1, 1H), 5.88 (d, *J* = 9.9 Hz, H-7, 1H), 5.47 (dd, *J* = 9.9, 3.2 Hz, H-8, 1H), 4.87 (d, *J* = 6.4 Hz, H-5, 1H), 4.60 (m, H-6, 1H), 3.20 (s, 14-OMe, 3H), 2.44 (s, NMe, 3H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 144.6, 138.6, 137.8, 132.8, 129.0, 126.2, 119.5, 117.1, 90.2, 74.9, 66.0, 57.4, 50.6, 47.6, 46.0, 43.3, 30.6, 29.9, 22.6 ppm.

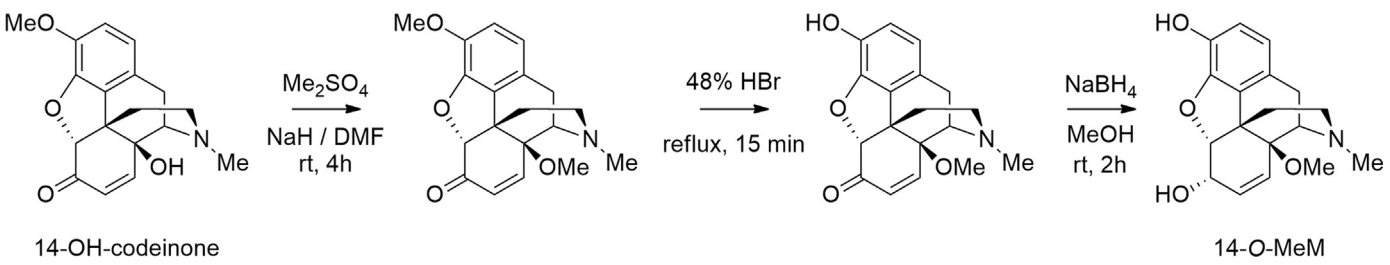

Fig. 2. The synthesis of 14-*O*-methylmorphine (14-*O*-MeM). For further information see Section 2.3.

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