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Original article

Methyl-orvinol—Dual activity opioid receptor ligand inhibits gastrointestinal transit and alleviates abdominal pain in the mouse models mimicking diarrhea-predominant irritable bowel syndrome

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ABSTRACT

Background: Diarrhea-predominant irritable bowel syndrome (IBS-D) is a functional disorder of the gastrointestinal (GI) tract. The major IBS-D symptoms include diarrhea, abdominal pain and discomfort. High density of opioid receptors (ORs) in the GI tract and their participation in the maintenance of GI homeostasis make ORs ligands an attractive option for developing new anti-IBS-D treatments.
The aim of this study was to characterize the effect of methyl-orvinol on the GI motility and secretion and in mouse models mimicking symptoms of IBS-D.
Methods: In vitro, the effects of methyl-orvinol on electrical field stimulated smooth muscle contractility and epithelial ion transport were characterized in the mouse colon. *In vivo*, the following tests were used to determine methyl-orvinol effect on mouse GI motility: colonic bead expulsion, whole GI transit and fecal pellet output. An antinociceptive action of methyl-orvinol was assessed in the mouse model of visceral pain induced by mustard oil.
Results: Methyl-orvinol (10^{-10} to 10^{-6} M) inhibited colonic smooth muscle contractions in a concentration-dependent manner. This effect was reversed by naloxone (non-selective opioid antagonist) and β-funaltrexamine (selective MOP antagonist). Experiments with a selective KOP receptor agonist, U50488 revealed that methyl-orvinol is a KOP receptor antagonist in the GI tract. Methyl-orvinol enhanced epithelial ion transport. *In vivo*, methyl-orvinol inhibited colonic bead expulsion and prolonged GI transit. Methyl-orvinol improved hypermotility and reduced abdominal pain in the mouse models mimicking IBS-D symptoms.
Conclusion: Methyl-orvinol could become a promising drug candidate in chronic therapy of functional GI diseases such as IBS-D.

Introduction

Endogenous opioid system is composed of opioid peptides and cell surface receptors. Opioid receptors (ORs) are divided into three subtypes μ (mu, MOR), δ (delta, DOR) and κ (kappa, KOR). In the human body ORs are widely distributed in the central and peripheral nervous system and non-neuronal tissues (mainly in the gastrointestinal (GI) tract). In the GI tract ORs are present on smooth muscle cells, at the terminals of sympathetic and sensory peripheral neurons and immune cells, where they are involved in the maintenance of homeostasis through modulation of the intestinal motility, but also fluid and electrolyte secretion/absorption and immune response [1,2]. The role of ORs agonists in pain modulation is well known and determined in numerous animal models of pain. This antinociceptive effect is mediated through either central or peripheral ORs. There are several differences in the molecular mechanism of action between types of ORs. It was reported, that activation of MOR and DOR results in inhibition of adenylate cyclase, decrease of cAMP and reduced protein kinase A activation (what leads to reduction of neuronal excitability). Moreover, activation of MOR and DOR results in inhibition of Ca^{2+} channels and activation of K^+ channels. While the

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action mediated by KOR is limited to reduction of neurotransmitters release, a result of inhibition of Ca^{2+} channels located on the nerve terminals [2].

In recent years, researchers have investigated the role of the endogenous opioid system in the pathophysiology of GI diseases. For instance, it was evidenced that the relative mRNA expression of MOR and KOR is decreased in patients with IBS-D [3]. IBS-D is a chronic relapsing functional GI disorder, which affects up to 4% of population. IBS-D patients suffer from diarrhea, abdominal pain, cramping, and bloating (for review see: [4]). Their quality of life is poor during symptoms exacerbation. Moreover, patients with IBS-D have psychosocial disturbances and more frequently suffer from depression or affective disorders. Currently available anti-IBS-D therapeutics only alleviate symptoms of the disease. Therefore, ORs appear to be promising pharmacological targets in IBS-D therapy.

Orvinols (6,14-ethenotetrahydrooripavine derivatives), developed by Kenneth Bentley and his group in 1960s, belong to a large group of thebaine derivatives [5]. Orvinols are strong antinociceptive agents (comparable or even more effective than morphine). They were initially designed in the hope of reducing adverse effects common for opioids (i.e. morphine), such as: tolerance and addiction development, respiratory depression or constipation. The primary effects of orvinols are mediated through one or both, MOR and KOR [6].

The structure of orvinols and their interesting pharmacological profile have gained the attention of numerous researchers. It was observed that already minor modifications in certain parts in the structure result in significant changes in their action. The pharmacological profile of orvinols has been characterized in various assays including early work in isolated tissues such as vas deferens isolated from rats and rabbits and more recently in [^{35}S] GTPγS assays [6]. While binding affinity remains high for each ORs, efficacy at MOR and KOR, is strongly influenced by the N-substituent (typically methyl or cyclopropylmethyl groups) and the groups attached to C_{20} [7]. Similarly, Bentley et al. [5] reported that in the thevinols, 3-*O*-methyl ether analogues of the orvinols, modification of the C_{20} group significantly altered antinociceptive potential in the tail pressure test in rats.

Methyl-orvinol, the simplest tertiary alcohol member of the orvinol family, having two methyl groups attached to C_{20}, was characterized in vasa deferentia as a potent MOR and KOR ligand [6], Fig. 1. In our study we determined the effect of methyl-orvinol on GI motility, secretion and abdominal pain in mouse models mimicking IBS-D symptoms.

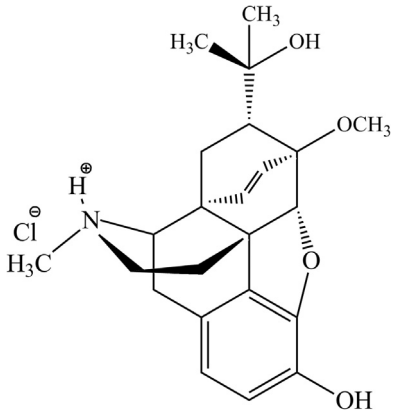

Fig. 1. The structure of methyl-orvinol.

Materials and methods

Animals

In this study, male Balb/C mice (Institute of Occupational Medicine, Łódź, Poland), weighing from 22 to 26 g were used. Mice were maintained under a 12-h light/dark cycle and at a constant temperature (22–23 °C). Mice were housed in sawdust-lined plastic transparent cages with a free access to laboratory chow and tap water. All of the experiments in this study were performed in accordance with respective national guidelines and animal use was approved by the Local Ethical Committee (48/ŁB700/DLZ/2015).

In vitro *experiments*

Isolated smooth muscle strips

Organ bath studies were performed as described previously [8]. Mice were sacrificed by cervical dislocation. Subsequently, the colon was rapidly removed and full-thickness fragments (0.5 cm) of the distal colon were kept in Krebs solution (NaCl 115.0 mM, KCl 8.0 mM, KH_2PO_4 2.0 mM, $NaHCO_3$ 25.0 mM, $MgCl_2$ 2.4 mM, $CaCl_2$ 1.3 mM, and glucose 10.0 mM). One end of each colonic fragment was attached to the bottom of the individual organ bath, another end to a FT03 force displacement transducer (Grass Technologies, West Warwick, RI, USA) using a silk thread. Each segment of the colon was placed between two electrodes in organ bath containing Krebs solution (25 ml) oxygenated with 95% O_2 and 5% CO_2 at constant temperature (37 °C). The changes in tension were amplified by a P11T amplifier (Grass Technologies, West Warwick, RI, USA) and recorded using the POLYVIEW software (Polybytes Inc., Cedar Rapids, IA, USA). Electrical field stimulation was applied by a S88X stimulator (Grass Technologies, EFS, 8 Hz, 60 V, pulse duration 0.5 ms, train duration 10 s), and delivered through electrodes placed around the tissue.

The tissue preparations were exposed to methyl-orvinol in increasing concentrations (10^{-10} to 10^{-6} M). Methyl-orvinol was added cumulatively into the organ bath for 8 min for each concentration. The effect of tested compound was recorded on a personal computer using the PolyView software (Polybytes Inc., 108 Cedar Rapids, IA, USA). At first, the mean amplitude of four twitch contractions was measured and treated as an internal control (in control experiments, the effect of the vehicle (DMSO) was assessed). The changes in smooth muscle contractions were reported as the percentage of the internal control. These assays were performed as paired with respective controls, using four organ baths in parallel.

To assess the involvement of ORs, the following OR antagonists were added 10 min prior to methyl-orvinol: naloxone (10^{-6} M, a non-selective OR antagonist) and β-funaltrexamine (β-FNA, 10^{-6} M, to block MOR).

In further *in vitro* experiments we wanted to determine if methyl-orvinol is KOR antagonist. Thus, we assessed the effect of U50488, selective KOR agonist (concentrations ranging from 10^{-10} to 10^{-6} M, added cumulatively), on smooth muscles contractility of the mouse colon, in the presence of β-FNA (10^{-6} M) (added at the beginning of the experiment), and methyl-orvinol (10^{-6} M) (added 10 min after MOR antagonist). The results were compared to contractility of tissue strips exposed to U50488 alone (10^{-10} to 10^{-6} M).

Epithelial ion transport

Epithelial ion transport was assessed according to techniques described earlier [9]. The distal colon was removed and kept at 37 °C Krebs solution (NaCl 115.0 mM, KCl 8.0 mM, KH_2PO_4 2.0 mM, $NaHCO_3$ 25.0 mM, $MgCl_2$ 2.4 mM, $CaCl_2$ 1.3 mM). Then preparations