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The mu-opioid receptor-selective peptide antagonists, antanal-1 and antanal-2, produce anticonvulsant effects in mice

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ABSTRACT

The activation of the mu-opioid receptors (MOR) in the central nervous system has a proconvulsant effect and seizures are a common side effect of high doses of short acting opioids, like morphine or fentanyl. However, the correct assessment of the role of MOR blockade in the initiation and propagation of epilepsy was hampered by the lack of potent and selective MOR antagonists. In this study we aimed at characterizing the effect of MOR blockade on the seizure threshold in mice using recently developed selective antagonists antanal-1 and antanal-2 and a classical MOR antagonist, β-funaltrexamine (β-FNA). The effect of the centrally administered MOR antagonists was characterized in the maximal electroshock seizure threshold (MEST), the 6 Hz psychomotor seizure threshold and the intravenous pentylenetetrazole (PTZ) seizure threshold test in mice. The acute effect of the studied compounds on skeletal muscular strength in mice was quantified in the grip-strength test. Antanal-1 and antanal-2 (30 and 50 nmol/mouse, i.c.v.), but not β-FNA significantly increased the seizure threshold in the MEST test in mice. In the 6-Hz test, all tested MOR antagonists significantly increased the psychomotor seizure threshold and the most potent anticonvulsant effect was observed for antanal-2 (2, 10 and 30 nmol/mouse, i.c.v.). The i.c.v. administration of β-FNA (10 and 30 nmol/mouse, i.c.v.), antanal-1 and antanal-2 (both 30, 50 and 100 nmol/mouse, i.c.v.) did not produce any significant effect on PTZ seizure threshold, the generalized clonus or the forelimbs tonus. All tested compounds did not affect muscle strength, as determined in the grip strength test. Our study demonstrated that the novel MOR-selective antagonists antanal-1 and antanal-2 displayed a potent and dose-dependent anticonvulsant action involving non-GABA-ergic, but some other pathways and mechanisms in animal models of epileptic seizures. We suggest that antanals are promising drug templates for future therapeutics, which may be used in the treatment of epilepsy in humans.

1. Introduction

Mu (MOR), delta (DOR) and kappa (KOR) opioid receptors, and their endogenous ligands constitute the endogenous opioid system, which plays a crucial role in many physiological processes. Numerous studies showed that the endogenous opioid system, MOR in particular, is an essential structural and functional component of pain signaling pathways [for review see: Janecka et al. (2004)]. Exogenously administered natural and synthetic opiates have thus been used for centuries to treat pain. It has also been demonstrated that MOR presence in the cardiovascular and respiratory systems is indispensable to maintain homeostasis. The possible anti-depressant (Fichna et al., 2007b) and immunomodulatory (McCarthy et al., 2001) role of MOR ligands have been suggested based on the in vitro and the in vivo studies.

Epilepsies are common neurological diseases, with the prevalence of 1–2% and approximately 50 million people affected worldwide (McNamara, 1999). As estimated by Loacker et al. (2007), at least one new antiepileptic drug becomes available for clinical treatment each year. However, the efficacy of antiepileptics remains questionable, as only two-thirds of patients are seizure-free under pharmacological treatment (Kwan and Brodie, 2000; Kwan and Sander, 2004), while the spectrum and intensity of side-effects remain high.

There is a growing body of evidence suggesting a possible involvement of opioid system in epileptogenesis. Myoclonus and seizures were reported as the most common and most dangerous side effects of high

Abbreviations: ANOVA, analysis of variance; BBB, blood–brain barrier; β-FNA, β-funaltrexamine; CNS, central nervous system; CS_{50}, the current strength required to produce an endpoint in 50% of the mice challenged; DMSO, dimethyl sulfoxide; DOR, delta opioid receptor; GABA, γ-aminobutyric acid; KOR, kappa opioid receptor; MEST, maximal electroshock seizure threshold; MOR, mu opioid receptor; PTZ, pentylenetetrazole; SEM, standard error of the mean.

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doses of short acting opioids, like morphine or fentanyl (Hagen and Swanson, 1997; Rao et al., 1982). Moreover, Bartenstein et al. (1994) observed changes in opioid receptor morphology and function associated with epilepsy and Saboory et al. (2007) pointed at modulatory role of opioids on neuronal excitability and neuroexcitation as possible side-effect of opioid treatment. Numerous studies showed that endogenous opioid peptides and their synthetic analogs are also involved in convulsive and non-convulsive epilepsy (Frenk, 1983; Lasoń et al., 1994; Lee et al., 1989; Ramabadran and Bansinath, 1990). It was demonstrated that seizures or the administration of epileptogenic compounds may induce changes in opioid peptide levels and mRNA expression in animal (Lasoń et al., 1983, 1994; Mazarati et al., 1999) and human (Bartenstein et al., 1994; Cheng and Xie, 1990) brain and pituitary.

It is commonly accepted that the activation of MOR, but not DOR in the central nervous system (CNS) has a net excitatory, proconvulsant effect (Jones et al., 1994; Skyers et al., 2003) often accompanied by behavioral manifestation of non-convulsive activities, such as myoclonic twitches or stereotypies (Frenk, 1983). However, the correct assessment of the role of MOR blockade in the initiation and propagation of epilepsy was hampered by the lack of potent and selective MOR antagonists. Therefore there is a significant discrepancy in the published data and their implication in epileptogenesis remains unknown.

In order to further elucidate the role of MOR blockade in epileptogenesis, we characterized the effect of two novel MOR antagonists, antanal-1 and antanal-2, on the seizure threshold and muscular strength in mice. Our recent studies demonstrated that antanal-1 and antanal-2 block MOR in the central and peripheral nervous systems with extraordinary potency and selectivity (Fichna et al., 2007a, 2010) and may be used as valuable tools to examine MOR pharmacology. Here we aimed at evaluating whether MOR blockade may be beneficial in the course of epilepsy and investigated the possible application of the MOR-selective antagonists for the future clinical treatment in humans.

2. Material and methods

2.1. Animals

Experimentally naïve male albino Swiss mice (Laboratory Animals Breeding, Słaboszów, Poland) weighing 22–30 g were used in all experiments. The animals were housed in Makrolon cages under strictly controlled laboratory conditions (ambient temperature, 22–23 °C, relative humidity, 45–55%, 12 h light/dark cycle, lights on at 6:00 a.m.). Chow pellets (Agropol S.J., Motycz, Poland) and tap water were available *ad libitum*. All experiments were performed between 8:00 and 16:00 h to minimize circadian influences, after at least 7 days of acclimatization. Each animal was used only once. The experimental protocol was approved by the Local Ethics Committee at the Medical University of Lublin. All procedures involving animals and their care were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and Polish legislation acts concerning animal experimentation.

2.2. Drug administration

Antanal-1 and antanal-2 were synthesized in the Department of Biomolecular Chemistry, Medical University of Lodz, Poland according to the methods described earlier (Fichna et al., 2007a). All other drugs and reagents, unless stated otherwise, were obtained from Sigma-Aldrich (Poznan, Poland). β-Funaltrexamine hydrochloride (β-FNA), antanal-1 and antanal-2 were dissolved in 100% dimethyl sulfoxide (DMSO, ICN Biomedicals, Inc., Aurora, OH, USA) and administered intracerebroventricularly (i.c.v.) in a volume of 5 μl/animal, using a 10-μl Hamilton syringe. The injection was performed according to a modified method described by Lipman and Spencer (1980). All compounds were injected 5 min prior to the respective test. Control animals received an i.c.v. injection of a vehicle.

The doses of β-FNA and the antanals used in this study were chosen based on preliminary experiments in order to maintain similar upper and lower limits of observed effects.

2.3. Maximal electroshock seizure threshold (MEST) test in mice

The seizures were induced by applying a sine-wave alternating current (maximal output voltage 500 V, 50 Hz for 0.2 s) via transcorneal electrodes. The stimuli were delivered by a rodent shocker (type 221; Hugo Sachs Elektronik, Freiburg, Germany). A drop of ocular anesthetic (1% solution of tetracaine hydrochloride) was applied into each eye 1 min before stimulation. Transcorneal electrodes were soaked in 0.9% saline to provide a good electrical contact. During stimulation mice were restrained manually and immediately after the stimulation they were placed in a Plexiglas arena (37 cm × 21 cm × 14 cm) for behavioral observation for the presence or absence of seizure activity. Tonic hindlimb extension was taken as an endpoint.

The thresholds for maximal electroconvulsions were assessed in accordance with an 'up-and-down' method described by Kimball et al. (1957). In this method, current intensity was lowered or raised by 0.06-log intervals depending on whether the previously stimulated animal did or did not exert seizure activity, respectively. Each mouse was stimulated only once at any given current intensity. The data obtained in groups of 19–20 animals were used to determine the threshold current causing an endpoint in 50% of mice (CS_{50} with confidence limits for 95% probability). For further methodological detail see Giardina and Gasior (2009).

2.4. The 6 Hz psychomotor seizure threshold test in mice

In order to determine the psychomotor seizure thresholds, square-wave alternating current stimuli (0.2-ms duration pulses at 6 Hz for 3 s) were applied via saline-soaked corneal electrodes using a Grass model CCU 1A constant current unit coupled to a Grass S48 stimulator (Grass Instruments Co., West Warwick, RI, USA). Ocular anesthetic (1% solution of tetracaine hydrochloride) was applied to the cornea 1 min before the stimulation. During stimulation mice were restrained manually and immediately after the stimulation they were placed in a Plexiglas arena (37 cm × 21 cm × 14 cm) for behavioral observation. The seizures induced by 6 Hz stimulation were characterized by immobility or stun posture, which was frequently followed by rearing, forelimb clonus, twitching of the vibrissae and elevated or Straub tail (Barton et al., 2001, 2003). Lack of the features listed above or the resumption of normal exploratory behavior within 10 s after stimulation were considered as the absence of seizures. The current intensity values were chosen according to an above-mentioned method (Kimball et al., 1957). The data obtained in groups of 19–20 animals were used to determine the threshold current causing 6 Hz-induced seizures in 50% of mice (CS_{50} with confidence limits for 95% probability).

2.5. The intravenous pentylenetetrazole (PTZ) seizure threshold test in mice

Five minutes following the i.c.v. administration of a respective compound or vehicle, mice were placed in a cylindrical plastic restrainer (12-cm long, 3-cm inner diameter) and a 27-gauge needle (Sterican®, B. Braun Melsungen, Melsungen, Germany) was inserted into the lateral tail vein. The needle was attached by polyethylene tubing (PE20RW, Plastics One Inc., Roanoke, VA, USA) to a plastic syringe, which was mounted in a syringe pump (model Physio 22, Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany). The correct needle placement in the tail vein was verified by the appearance of blood in the tubing. The syringe contained a 1%