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Peripherally acting NMDA receptor/glycine$_B$ site receptor antagonists inhibit morphine tolerance☆

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Abstract

The present study focused on the role of peripheral ionotropic *N*-methyl-D-aspartate (NMDA) receptors in the development of tolerance to morphine-induced antinociception. An initial experiment revealed that NMDA channel blocker memantine, and NMDA receptor/glycine$_B$ site antagonist MRZ 2/576 inhibited maximal electroshock-induced convulsions (MES) in female NMR mice with respective potency of 5.93 and 20.8 mg/kg, while other NMDA receptor/glycine$_B$ site antagonists MRZ 2/596 and MDL 105,519 were ineffective, supporting lack of CNS activity of the latter two agents. This observation was also supported by blood–brain barrier experiments in vitro. In male Swiss mice, morphine (10 mg/kg) given for 6 days twice a day (b.i.d.) produced tolerance to its antinociceptive effects in the tail-flick test. The NMDA receptor/glycine$_B$ site antagonists, MRZ 2/576 at 0.03, 0.1, 0.3 mg/kg and MRZ 2/596 at 0.1, 0.3, 3 and 10 mg/kg attenuated the development of morphine tolerance. Similarly, in male C57/Bl mice, morphine (10 mg/kg) given for 6 days b.i.d. produced tolerance to its antinociceptive effects in the tail-flick test. Like in Swiss mice, in C57/Bl mice morphine tolerance was attenuated by both MRZ 2/576 and MRZ 2/596. Another NMDA receptor/glycine$_B$ site receptor antagonist, MDL 105,519 (that very weakly penetrates to the central nervous system) also inhibited morphine tolerance at the dose of 1 but not 0.1 mg/kg. Moreover, both naloxone hydrochloride (5 and 50 mg/kg) and centrally inactive naloxone methiodide (50 mg/kg) inhibited morphine tolerance suggesting the involvement of peripheral opioid receptors in this phenomenon. The present data suggest that blockade of NMDA receptor/glycine$_B$ sites in the periphery may attenuate tolerance to the antinociceptive effects of morphine.

Keywords: Antinociception; Pain; Tolerance; Glutamate; NMDA receptor/glycine$_B$ site antagonist; Blood–brain barrier

1. Introduction

Over the last decade research has provided compelling evidence that glutamate receptors are crucially involved in phenomena related to opioid tolerance (see Mao, 1999; Price et al., 2000 for reviews). Antagonists of the ionotropic *N*-methyl-D-aspartate (NMDA) receptor complex, including memantine, the moderate affinity and highly voltage-dependent clinically used NMDA channel blocker (Parsons et al., 1999) inhibit the development of morphine tolerance (Trujillo and Akil,

☆ The referees of the present paper suggested to assess brain penetration of MRZ 2/596 using brain microdialysis. It was not possible at the time of manuscript preparation but such opportunity appeared after manuscript acceptance. The study revealed, that in rats after application of MRZ 2/596 at the dose of 30 mg/kg ip maximal concentrations in brain reach c.a. 100 nM (N=3) concentrations corrected for in vitro recovery. Thus, it is unlikely that dose used in the present study (0.05–1 mg/kg) resulted in brain levels of MRZ 2/596 that would affect NMDA receptors assuming lack of differences in blood brain barrier between rat and mice.

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1991; Marek et al., 1991; Popik et al., 2000a) (see Bespalov and Trujillo, 2002 for the recent review).

One obstacle to the introduction of NMDA receptor antagonists into clinical practice is undesired "phencyclidine side-effects" profile, that is centrally mediated (Parsons et al., 1999). In turn, NMDA receptor antagonists which weakly penetrate to the brain might have a more favorable profile. However, such compounds may not be applicable to the inhibition of tolerance to morphine-induced antinociception which is believed to be primarily of central origin (McNally and Westbrook, 1998; McNally, 1999; Ueda and Inoue, 1999). On the other hand, recent data reported by Kolesnikov and colleagues demonstrated that local (topical) application of uncompetitive NMDA receptor antagonists, (+)MK-801 or ketamine, inhibited tolerance to topically applied morphine (Kolesnikov et al., 1996; Kolesnikov and Pasternak, 1999b; Kolesnikov and Pasternak, 1999a), suggesting a peripheral component of antinociceptive morphine tolerance. Since systemic rather than local administration of compounds is more favorable from the therapeutic perspective, the aim of the present study was to investigate whether antagonism of NMDA receptors in the peripheral nervous system (PNS) would inhibit tolerance to the antinociceptive effects of systemically administered morphine. As pharmacological tools we used recently developed NMDA receptor antagonists acting at the NMDA receptor/glycine$_B$ site. These compounds reach relatively low brain levels after systemic application as compared to plasma values (MRZ 2/576: ~2% (Hesselink et al., 1999b), MDL 105,519: 0.01–0.08% (Opackajuffry et al., 1998)) or seem to be lacking CNS activity as was the case with MRZ 2/596 (see Sections 3.1.3 and 4). In contrast, the free brain levels of memantine are over 50% of plasma concentration (Hesselink et al., 1999a).

2. Methods

2.1. In vitro

2.1.1. Receptor binding

Tissue preparation was performed according to Foster and Wong (1987) with some modifications. Male Sprague-Dawley rats (200–250 g, Janvier, Le Genest-Isle, France) were decapitated and their brains were removed rapidly. Tissue was then processed as described previously (Parsons et al., 1997). The amount of protein in the final membrane preparation was determined according to Hartfree (1971) and adjusted to 250–500 μg/ml.

Membranes were suspended and incubated in 50 mM Tris–HCl, pH 8.0 for 45 min at 4 °C with a fixed [^{3}H]MDL-105,519 concentration of 2 nM. MDL-105,519 is a selective high affinity antagonist at the NMDA receptor/glycine$_B$ site and has recently been introduced as a commercially available radioligand (Amersham Biosciences, Freiburg, Germany) (Baron et al., 1996; Baron et al., 1997). Non-specific binding was defined by the addition of unlabeled glycine at 100 μM. Incubations were terminated using a Millipore filter system (Millipore, Schwalbach Germany). The samples, all in duplicate, were rinsed three times with 2.5 ml ice-cold assay buffer over glass fibre filters (Schleicher and Schuell, Dassel, Germany) under a constant vacuum. Filtration was performed as rapidly as possible (max 2 s). Following separation and rinse, the filters were placed into scintillation liquid (5 ml; Ultima Gold) and radioactivity was determined with a liquid scintillation counter (both Packard BioScience, Dreieich, Germany).

2.1.2. Patch clamp

Patch clamp recordings were made from rat hippocampal neurons, after 12–15 days in vitro, with polished glass electrodes (3–5 mΩ) in the whole cell mode at room temperature (20–22 °C) with the aid of an EPC-7 amplifier (HEKA, Lambrecht, Germany) – detailed methods described in Parsons et al. (1999).

2.1.3. BBB permeability studies

An in vitro model of the BBB has been established as a first screen for BBB permeability.

2.1.3.1. Preparation and cultivation of BBCEC. Bovine brain capillary endothelial cells (BBCEC) were isolated from brains, purified, and cultured according to Meresse et al. (1989). Briefly, after mechanical homogenization microvessels were seeded onto dishes coated with an extracellular matrix secreted by bovine corneal endothelial cells (Gospodarowicz et al., 1976). Pure colonies of endothelial cells were seeded onto gelatin-coated dishes (Corning Costar, Bodenheim, Germany) in the presence of Dulbecco's Modified Eagle's Medium (DMEM; Gibco Invitrogen GmbH, Karlsruhe, Germany) supplemented with 20% calf serum (Hyclone, Utah, USA), 2 mM glutamine, 50 μg/ml of gentamycin (Biochrom, Berlin, Germany), and bovine fibroblast growth factor (bFGF; Roche, Mannheim, Germany; 1 ng/ml added every other day). BBCEC in passage 4–6 were used for co-cultivation/transport studies.

2.1.3.2. Preparation and cultivation of rat cortical astrocytes. Astrocytes were prepared mechanically from cortices of newborn rats as described by Booher and Sensenbrenner (1972). The cell suspension was seeded onto 12-well plates (Corning, Wiesbaden, Germany) and cultivated in Dulbecco's Modified Eagle's Medium (DMEM, Gibco Invitrogen GmbH, Karlsruhe, Germany) containing 10% FCS (HyClone, Utah,