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Research report

The inimitable kynurenic acid: The roles of different ionotropic receptors in the action of kynurenic acid at a spinal level

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ABSTRACT

Kynurenic acid (KYNA) is a neuroactive metabolite that interacts with NMDA, AMPA/kainate and alpha 7 nicotinic receptors. The goal of this study was to clarify the roles of these receptors in the action of KYNA at a spinal level by using highly specific receptor antagonists alone or in triple combinations.

Chronic osteoarthritis-like joint pain was induced with monosodium-iodoacetate in male Wistar rats. Mechanical allodynia and motor function were quantified. In the first series we determined the dose–response and time course effects of intrathecally administered KYNA (10–100 μg), D-(−)-2-amino-5-phosphonopentanoic acid (AP5; an NMDA receptor antagonist; 10–200 μg), methyllycaconitine (MLA; an alpha 7 nicotinic receptor antagonist; 100–200 μg) and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzoquinoxaline-7-sulfonamide (NBQX; an AMPA/kainate receptor antagonist; 1–20 μg). In the second series, four different triple combinations of MLA, AP5 and NBQX were investigated.

Intrathecal administration of KYNA caused a dose-dependent motor impairment and antinociception. The highly specific NMDA receptor antagonist AP5 caused a motor impairment and antinociception with lower potency. High doses of NBQX resulted in significant antinociception with a slight motor impairment, while only the highest dose of MLA gave rise to significant antinociception with a slight motor impairment. After the coadministration of these ligands as combinations, no potentiation was observed. It may be supposed that the effects of KYNA are primarily due to the inhibition of NMDA receptors at both glycine and phencyclidine (PCP) binding sites, and not to the interactions at the different ionotropic receptors, but the mechanisms behind its high bio-efficiency are still unknown.

1. Introduction

Degradation of the essential amino acid, tryptophan, along the kynurenine pathway yields several neuroactive intermediates, among them kynurenic acid (4-oxo-1H-quinoline-2-carboxylic acid; KYNA) (Moroni et al., 1988; Schwarcz and Pellicciari, 2002; Vecsei and Beal, 1991). This is found in low concentrations (10–150 nM) both centrally and peripherally and is synthesized in the central nervous system (CNS), including the spinal cord, predominantly by glial cells (Moroni et al., 1988; Nemeth et al., 2005; Pawlak et al., 2000; Schwarcz and Pellicciari, 2002; Turski and Schwarcz, 1988; Urbanska et al., 2000). The most intensely labeled neurons observed in the spinal cord were the large motoneurons of the ventral horn, while the preganglionic thoracic spinal neurons displayed moderate to intensive staining, and lightly labeled neurons were observed to be sparsely distributed in layers 1–5 of the dorsal horn of the spinal cord (Kapoor et al., 1997).

KYNA is a neuroactive metabolite that interacts with a multitude of molecular targets in the CNS. It is an excitatory amino acid (EAA) receptor antagonist with preferential activity at the N-methyl-D-aspartate (NMDA) receptors, acting at the glycine (half-maximal inhibitory concentration: IC_{50} ~20 μM) and the NMDA recognition sites (IC_{50} ~200 μM) of the NMDA receptor complex (Carpenedo

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et al., 2001; Ganong et al., 1983; Hilmas et al., 2001; Rozsa et al., 2008; Stone, 1993; Vecsei et al., 2013). In higher concentrations (0.1–1 mM) it also antagonizes the AMPA and kainate receptors, and KYNA is a potent noncompetitive antagonist at alpha 7 nicotinic acetylcholine (ACh) receptors (nAChRs) (IC_{50} ~7 μM) too (Hilmas et al., 2001; Prescott et al., 2006; Stone, 1993, 2000). Finally, with EC_{50} in the micromolar concentration range, KYNA also activates the orphan G-protein receptor 35 (GPR35) (Cosi et al., 2011; Moroni et al., 2012; Szalardy et al., 2012; Wang et al., 2006).

One of the key transmitters of central sensitization in arthritic pain is glutamate that activates NMDA and non-NMDA receptors on spinal cord neurons (Schaible et al., 2002). Some earlier data suggest that the intrathecal (IT) administration of KYNA led to antinociception and motor impairments (Horvath and Kekesi, 2006; Kekesi et al., 2002; Raigorodsky and Urca, 1990; Yaksh, 1989; Yamamoto and Yaksh, 1992; Zhang et al., 2003). Since the different ionotropic glutamate and ACh receptors play roles in these effects (Khan et al., 1994; Monaghan and Cotman, 1985; Raigorodsky and Urca, 1990; Ren et al., 1992; Young et al., 2008), the goal of the present study was to clarify the significance of these receptors in the effects of KYNA at a spinal level. Our concept was to use highly specific NMDA, AMPA/kainate and alpha 7 nicotinic receptor antagonists alone or in triple combinations in order to imitate KYNA effects. There are several synthetic ligands which have higher affinities and potencies at these receptors than those of KYNA, *e.g.* methyllycaconitine citrate (MLA), an alpha 7 nicotinic receptor antagonist; D-(−)-2-amino-5-phosphonopentanoic acid (AP5), an NMDA receptor antagonist; and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzoquinoxaline-7-sulfonamide disodium salt (NBQX), an AMPA/kainate receptor antagonist (Davies et al., 1999; Davies and Watkins, 1982; Jin, 1997; Vazquez et al., 1992). We hypothesized that if these ligands alone or in combinations can simulate the antinociceptive and paralytic effects of KYNA, we can propose the roles of the different receptors in its action at a spinal level. Since the utter paralysis of the highest dose of KYNA may simulate the effects of local anesthetic drugs, we additionally wished to rule out any effects on voltage-gated sodium channels (VGSCs). The effects on motor neurons after the perineural administration of KYNA were therefore also investigated.

2. Materials and methods

The procedures involved in the animal surgery and testing were approved by the Hungarian Ethical Committee for Animal Research (registration numbers: XIV./02080/000/2009. and XIV./3754/2012). Animal suffering and the number of animals per group were kept at a minimum attention to the guidelines of pain research in animals. Animals were maintained on a 12 h light/dark cycle under conditions of controlled temperature (22 ± 1 °C) and humidity (55 ± 10%) with *ad libitum* food and water access. At the time of testing animals weighed 315 ± 4.0 g.

2.1. Drugs

The following drugs were used: ketamine hydrochloride (Calypsol, Richter Gedeon RT, Budapest, Hungary), xylazine hydrochloride (CP-Xylazin 2%, CP-Pharma, Burgdorf, Germany), gentamycin (Sanofi-Aventis, Budapest, Hungary), monosodium iodoacetate (MIA) (Sigma–Aldrich; Budapest, Hungary), KYNA, MLA, AP5 (all purchased from TOCRIS, Budapest, Hungary) and morphine hydrochloride (Teva Zrt, Debrecen, Hungary). KYNA was dissolved in 0.1 M NaOH. The excess NAOH was back-titrated with 0.1 M HCl to neutral pH and the volume was adjusted with physiological saline. All the other drugs were dissolved in saline. The applied doses were based on previous studies and the maximal doses were limited by the solubility. Since there was no difference between the effects of the vehicle of KYNA and physiological saline, the latter was used as control. IT administered drugs were injected over 120 s in a volume of 10 μl, followed by a 10 μl flush of physiological saline.

2.2. IT catheterization

Male Wistar rats (315 ± 4.0 g; $n = 134$) were anesthetized with a mixture of ketamine hydrochloride and xylazine (72 and 8 mg/kg intraperitoneally, respectively). An IT catheter (PE-10 tubing; Intramedic, Clay Adams; Becton Dickinson; Parsippany, NJ; I.D. 0.28 mm; O.D. 0.61 mm) was inserted *via* the cisterna magna and passed 8.5 cm caudally into the subarachnoid space (Yaksh and Rudy, 1976), which served to place the catheter tip between vertebrae Th12 and L2, corresponding to the spinal cord segments (L3–L6) that innervate the hindpaws (Dobos et al., 2003). After the surgery, the animals were injected with gentamycin (10 mg/kg, subcutaneously) to prevent infection, and were housed individually. Animals with postoperative neurologic deficits or those that did not exhibit paralysis of one of the hindpaws after the administration of 100 μg lidocaine were excluded (about 10%) (Dobos et al., 2003).

2.3. Monosodium iodoacetate-induced osteoarthritis

One day after IT catheterization, osteoarthritis was induced by injecting MIA (1–1 mg/30 μl in the morning and again in the afternoon on the same day) into the tibiotarsal joint of one of the hind legs as described earlier (Peter-Szabo et al., 2007). MIA treatment was given to gently restrained conscious animals, using a 27-gauge needle, without anesthesia so as to exclude any drug interaction. These injections did not elicit signs of major distress. A period of 7 days was allowed for osteoarthritis to develop, which has consistently been shown to cause severe end-stage cartilage destruction, resulting in osteoarthritis-like joint pain (Bove et al., 2003; Kalbhen, 1987).

2.4. Behavioral nociceptive testings

Mechanical allodynia was determined by using a dynamic plantar aesthesiometer (Ugo Basile, Comerio, Italy). Prior to baseline testing, each rat was habituated to a testing box with a wire-mesh grid floor for at least 20 min. Measurements were made with a straight metal filament that exerts an increasing upward force at a constant rate (6.25 g/s) with a maximum cut-off force of 50 g. The filament was placed under the plantar surface of the hindpaw. Measurement was stopped when the paw was withdrawn (PWD), and results were expressed as paw withdrawal thresholds in grams; the IT drug effect was determined both on the MIA-injected ipsilateral and contralateral hindpaws.

2.5. Testing of motor function

To quantify the changes in motor function, the placing-stepping reflex was evoked by drawing back the dorsal surface of both hindpaws with a brush. Normal animals responded to these stimuli with active placing (no paresis). The reflex was scored as follows: 0, normal; 1, a slight deficit; 2, a moderate deficit; 3, no response. The motor function was determined both on the ipsilateral and contralateral hindpaws.

2.6. Experimental paradigm for IT experiments

All animals were assessed for their baseline sensitivity to mechanical stimuli and for their tibiotarsal joint diameter 1 day