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Short communication

Pharmacological characterization of AMPA-induced biting behaviour in mice

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Abstract

The spinal cord dorsal horn contains neural mechanisms which can greatly facilitate pain. It is well established that excitatory amino acids, aspartate and glutamate, are involved in the spinal transmission of nociceptive information and in the development of hyperalgesia. In the present study, intrathecal (i.t.) administration of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), a structural analog of L-glutamate, produced a dose-dependent behavioural syndrome characterized by caudally directed biting in mice. We demonstrated that peripheral pre-administration of the AMPA receptor antagonists 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*F*)quinoxaline (NBQX, 10–100 mg/kg s.c.) and 1-(4-aminophenyl)-3-methylcarbamoyl-4-methyl-3,4-dihydro-7,8-methylene-dioxy-5*H*-2,3-benzodiazepine-HCl (GYKI 53655, 3–10 mg/kg s.c.), and also of the NMDA receptor antagonist 5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK 801, 0.3–1 mg/kg s.c.) reversed this effect. These findings suggest that the hyperalgesia induced by the i.t. injection of AMPA in mice involves the activation of both NMDA and non-NMDA excitatory amino acid receptor sites.

Keywords: NMDA receptor; AMPA receptor; Spinal cord; Hyperalgesia; (Mouse)

1. Introduction

The transmission of nociceptive signals from peripheral nociceptors to secondary neurons in the dorsal horn of the spinal cord involves several transmitter systems. Among them, the excitatory amino acids, L-glutamate and L-aspartate, are generally regarded as the neurotransmitters released by the primary afferent fibres (Graham et al., 1967).

The intrathecal (i.t.) injection of excitatory amino acid receptor agonists, acting at both NMDA and non-NMDA receptors, gives rise to a behavioural response of pain, consisting of caudally directed biting and scratching, which is due to stimulation of excitatory amino acid receptor complexes in the dorsal spinal horns (Mjellem et al., 1993; Aanonsen and Wilcox, 1987).

However, the hypersensitivity of second-order spinal neurons may play an important role in inflammatory pain. Even though the precise mechanism of spinal hypersensitivity is not clear, there is an increased amount of evidence that neuromodulation of second-order spinal neurons by glutamate is essential for the induction of spinal hypersensitivity associated with inflammatory hyperalgesia (Aanonsen et al., 1990). In addition, it has been reported that ionotropic receptors rather than a glutamate metabotropic receptor seem to be involved in spinal hyperalgesia (Fagg and Massieu, 1991).

Evidence has accumulated that suggests the existence of at least three ionotropic excitatory amino acid receptor subtypes (Cunningham et al., 1994). They are classified as NMDA, kainate and AMPA, according to their sensitivities to these agonists. The algesic action of i.t. administration of NMDA and kainate has been extensively investigated (Aanonsen and Wilcox, 1987). Since the generalized behavioural response to excitatory amino acids can be differentiated pharmacologically, we investigated the excitatory amino acid receptor subtype(s) involved in α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)-induced biting behaviour in mice, by evaluating the ability of different excitatory amino acid receptor antagonists to counteract the behavioural effects induced by the i.t. administration of AMPA. Drugs which antagonize the described hyperalgesic effect of spinally released

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glutamate or block its release from primary sensory neurons may constitute a new class of analgesic.

2. Materials and methods

2.1. Animals

Male CD1 mice (Charles River, Italy) weighing 20–22 g were used. Animals were provided with standard laboratory pellets (Mucedola, Settimo Milanese, Italy) and with tap water ad libitum. They were housed 20 to a cage (55 × 37.5 × 19.5 cm) in a regulated environment (21 ± 1°C, 50–55% relative humidity, 12-h light-dark cycle, light on at 07:00 h) for a period of at least 5 days before the beginning of the experiments.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines, in compliance with national and international laws and policies (EEC Council Directive 86/609 = J L 358, 1, Dec. 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

2.2. Methods

The i.t. technique was adapted from Hylden and Wilcox (1980), with minor modifications. A lumbar puncture was performed, using a 27-gauge needle inserted between L5 and L6. A cutaneous incision (1 cm) was made, at least 1 h before the injection, under frozen CO_2 anaesthesia. Puncture of the dura was reliably indicated by a flick of the tail. Preliminary experiments were done with injection of Evans blue dye to confirm that the distribution of the dye was consistently intradural.

For AMPA administration the animal was held firmly by the pelvic girdle and i.t. injected, and was then placed in a Perspex cage (27 × 21 × 14 cm). The amount of time the animal spent biting during the first minute was recorded by an observer unaware of the given treatment.

For antagonism experiments, doses of test compounds were administered s.c. 15 min before AMPA (30 ng/10 μl) injection and were as follows: NBQX (10, 30 and 100 mg/kg), MK 801 (0.1, 0.3 and 1 mg/kg) and GYKI 53655 (1, 3 and 10 mg/kg). 10–14 animals each dose were used.

2.3. Drugs

The following drugs were used: α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid $2H_2O$ (AMPA, Tocris Neuramin), 5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK 801, RBI), 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*F*)quinoxaline (NBQX) and 1-(4-aminophenyl)-3-methylcarbamoyl-4-methyl-3,4-dihydro-7,8-methylene-dioxy-5*H*-2,3-benzodiazepine-HCl (GYKI 53655), both were synthesized in the Department of Chemistry at Boehringer Ingelheim. AMPA and GYKI 53655 were dissolved in a few drops of 1 N HCl and diluted with 50 nM Tris, MK 801 was dissolved in saline and NBQX in a few drops of 1 N NaOH and diluted with 50 nM Tris.

Table 1
Dose-response curve for AMPA-induced biting in mice

Dose (ng/10 μl i.t.)	Number of animals	Time spent in biting (s) (mean ± S.E.M.)
7.5	10	6.3 ± 3.9
15	18	20.0 ± 4.7
30	20	34.2 ± 3.9
60	20	38.8 ± 4.2

2.4. Statistical analysis

Results are expressed in seconds as mean ± S.E. of time spent biting. Data were analysed by one-way analysis of variance followed by Dunnett's *t*-test. The statistical eval-

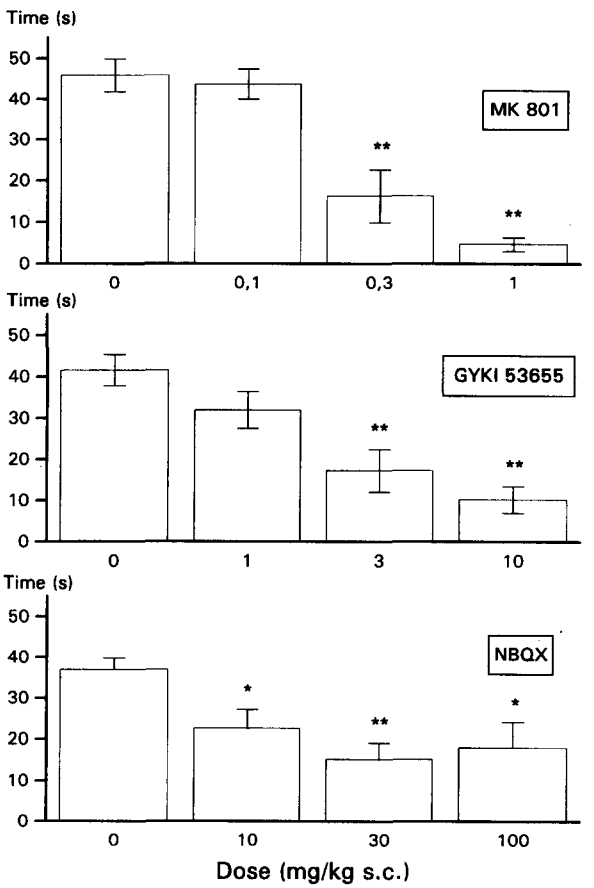

Fig. 1. The effect of the excitatory amino acid receptor antagonists on the biting behaviour induced by i.t. injection of AMPA in mice. Data are means ± S.E. from 10–14 animals in each dose group and represent the time (s) spent biting after AMPA administration. Compounds were administered s.c. 15 min before AMPA. One-way analysis of variance followed by Dunnett's *t*-test. * $P < 0.05$ and ** $P < 0.01$ compared to vehicle-pretreated group.

uation was carried out with the program system SAS (SAS Institute, Cary, NC), version 6.07 on a DEC computer, o.s. VMS.

3. Results

The i.t. injection of AMPA (7.5–60 ng/10 l) dose dependently produced a behaviour of caudally directed biting which started a few seconds after administration and lasted for a maximum of 1 min after AMPA injection (Table 1). Vehicle injections were without effects. The dose of 30 ng of AMPA, i.e. a submaximal dose, was selected according to the dose-response curve and was used for testing the effects of antagonists.

When the AMPA competitive receptor antagonist NBQX was administered at 10, 30 and 100 mg/kg s.c. 15 min before 30 ng i.t. of AMPA, a non-dose-dependent, but significant reduction of AMPA-induced biting behaviour was found. The administration of the non-competitive AMPA receptor antagonist GYKI 53655 (1, 3 and 10 mg/kg s.c.) and the non-competitive NMDA receptor antagonist MK 801 (0.1, 0.3 and 1 mg/kg s.c.) induced a dose-dependent reduction of biting evoked by AMPA (Fig. 1).

4. Discussion

Mammalian motor neurons possess glutamate receptors on their cell surface. Glutamatergic neurotransmission is believed to be important in several pathways in the human motor system including the corticospinal tracts and excitatory interneuronal pathways in the spinal cord (Shaw et al., 1994).

The present series of experiments suggest that the hyperalgesia produced by AMPA, measured as biting behaviour after i.t. injection in mice, may be mediated at the level of the spinal cord by excitatory amino acid receptors belonging to both NMDA and non-NMDA subtypes (Watkins et al., 1994). In particular, our study has demonstrated that NMDA receptors are involved in AMPA-induced hyperalgesia in mice, since MK 801 (an NMDA channel blocker; Shaw et al., 1994) abolished this hyperalgesic state. A possible AMPA-induced release of glutamate, which subsequently activates NMDA receptor in the spinal cord, might explain the antagonism observed with MK 801. In addition, the present experiments suggest that non-NMDA excitatory amino acid receptors are also critical, since NBQX, an AMPA competitive receptor antagonist, and GYKI 53655, a non-competitive AMPA receptor antagonist (Cumberbatch et al., 1994; Chizh et al., 1994) also prevented AMPA-induced hyperalgesia (Cumberbatch et al., 1994; Chizh et al., 1994).

In conclusion, the findings reported here suggest that facilitation of spinal cord dorsal horn excitability and resultant hyperalgesia in mice appear to be mediated by excitatory amino acids acting at both NMDA and non-NMDA excitatory amino acid receptors on NMDA receptor-containing neurons. Thus, drugs acting as antagonists at excitatory amino acid receptors may constitute a new pharmacological treatment for hypersensitivity associated with inflammatory hyperalgesia.

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