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Kynurenic acid blocks chemogenic nociception

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Abstract—Previous studies have suggested a role of excitatory amino acids (EAA) in nociception. In the present study the effects of two antagonists of EAA-receptors on capsaicin-induced eye wipings were investigated. Intracisternally administered kynurenic acid, in contrast to intravenously administered MK-801, was found to effectively block the induced eye wipings. It is proposed that EAA, by activating non-NMDA receptors, are involved in the transmission of chemogenic nociception.

Excitatory amino acids (EAA) have been suggested to be involved in nociception. B-Type sensory neurons of the dorsal root ganglia display high glutaminase immunoreactivity, indicating a neurotransmitter function of glutamate in C-afferents (Cangro et al 1985). Moreover, neurons located in the superficial layers of the dorsal horn, a target area of the B-type sensory neurons, were found to be highly sensitive to glutamate application as well as to electrical stimulation of C-afferent fibres (Schneider & Perl 1988). Furthermore, nociceptive stimulation has been shown to elicit glutamate and aspartate release in the dorsal spinal cord (Skilling et al 1988). Studies utilizing antagonists of EAA also support a significant role of EAA in nociception. Thus, EAA antagonists have been shown to block the electrophysiological responses of spinal nociceptive neurons evoked by C-afferent stimulation (Schouenborg & Sjölund 1986) as well as to depress aversive responses to heat and mechanical pressure (Cahusac et al 1984; Aanonsen & Wilcox 1986).

During recent decades capsaicin has been used to studying various sensory mechanisms (see Nagy 1982), since it selectively activates the polymodal nociceptors at C-fibre afferents (Kenins 1982). In the present study, this property of capsaicin was utilized to study the involvement of EAA in chemogenic pain nociception.

Materials and methods

Male Sprague-Dawley rats were used. Capsaicin (Reanal, Budapest) was dissolved at a concentration of 1% (32·74 mmol L^{-1}) in saline by means of ethanol (10%) and Tween 80 (10%) and final dilutions were made up with saline. Kynurenic acid and MK-801 (Sigma) were dissolved in 0·9% NaCl (saline); the pH of the kynurenic acid solution was adjusted to 8·0. Intracisternal (i.c.) injections of kynurenic acid and capsaicin were performed under light ether anaesthesia from which rats recovered within 5 min. Control animals received saline; the injected volume was always 100 μL.

The sensitivity to chemogenic stimulus was tested by utilizing the eye wiping test as described by Jancsó (1968). Thus, one drop of 0·01% capsaicin solution was applied on the corneal surface and the number of eye wiping movements by the forepaws during 1 min was assessed.

Intracisternal injection of capsaicin (50 μg) induces insensitivity of the facial areas to nociception due to the neurotoxic effect of the drug on primary sensory C-afferents (Jancsó & Király 1981). In order to test the ability of kynurenic acid to prevent capsaicin-induced degeneration, control and kynurenic acid pretreated animals (1 μmol, 20 min) were injected with 50 μg (i.c.) of capsaicin and the chemical sensitivity was measured as an indicator for the development of degeneration.

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Results

The mean number of eye wipings in response to one drop (about 0·03 mL) of 0·01% capsaicin solution was 11·37 ± 0·96. Doses lower than 0·3 μmol of kynurenic acid did not significantly prevent capsaicin-induced eye wiping behaviour. However, this reaction was significantly reduced ($P < 0·001$) 20 min after i.c. kynurenic acid (0·3 μmol) injection. The mean numbers of wiping following i.c. injection (100 μL) of saline or kynurenic acid (0·1, 0·2 or 0·3 μmol) were 11·8 ± 1·5, 11·7 ± 2, 11·9 ± 1 and 2·8 ± 1, respectively (n = 8, 5, 5, 9). The sensitivity to capsaicin returned to the control level within 2 h after the kynurenic application. Higher doses of kynurenic acid (0·5–1 μmol) totally blocked the eye wiping responses, although a blepharospasm could be observed (n = 6). Doses of kynurenic acid higher than 0·5 μmol, also abolished the corneal reflex and frequently caused a slightly incoordinated movement (but not akinesia). In contrast to kynurenic acid, MK-801 (3 mg kg^{-1}, intravenously), a specific and non-competitive antagonist of EAA acting on *N*-methyl-D-aspartate (NMDA) receptors, failed to affect capsaicin-induced eye wipings significantly (n = 4; data not shown).

In a pilot study the influence of kynurenic acid on the toxicity of capsaicin was investigated. Kynurenic acid (1 μmol i.c.) 20 min before injection of capsaicin (50 μg i.c.) did not attenuate the acute toxic effects (e.g. apnoeic reaction) of capsaicin. The same dose of kynurenic acid also did not interfere with the development of to co??al capsaicin insensitivity induced by intracisternal capsaicin (data not shown; n = 8).

Discussion

The results of the present study, that kynurenic acid, which appears to be a pure antagonist for several of EAA receptors without affecting other types of receptors (Perkins & Stone 1982; Stone & Connick 1985), significantly reduced the eye wipings capsaicin-induced pain, are in line with previous observations demonstrating a functional role of EAA in nociception. The site and mode of action of kynurenic acid on the chemogenic response is not clear. Capsaicin has been demonstrated to stimulate primary unmyelinated C-fibre afferents and to induce release of tachykinins from these neurons (see Nagy 1982 for review). In addition, capsaicin displays a selective neurotoxicity towards unmyelinated C-fibre afferents (Jancsó & Király 1981) and causes long-lasting depletion of various neuropeptides in the spinal cord and in the trigeminal caudal nucleus (Cuello et al 1981; Jancsó et al 1981). C-Fibres innervating the cornea specifically and directly project to the trigeminal caudal nucleus. Considering that a relatively large portion of C-afferents display glutaminase immunoreactivity (Cangro et al 1985), and that glutamate is released during chemical nociceptive stimulation of C-fibre afferents projecting to the spinal cord (Skilling et al 1988), it may be speculated that glutamate is the transmitter for the mediation of the chemogenic effect to the trigeminal caudal nucleus.

At variance with kynurenic acid, the non-competitive and specific NMDA-receptor antagonist MK-801 failed to significantly affect capsaicin-induced eye wiping. This clearly shows that NMDA-receptors are not primarily involved in the analgesic effect of kynurenic acid.

Since EAA antagonists are known to prevent neurotoxic effects of EAA (Foster et al 1984; Rothman & Olney 1987), the ability of kynurenic acid to prevent capsaicin-induced neurotoxicity was investigated. In our functional tests, kynurenic acid failed to influence capsaicin-induced toxicity, i.e. intracisternal application of capsaicin induced chemical nociceptive insensitivity similar to those found in control animals, indicating that the mode of action by which capsaicin causes neurodegeneration is basically different from that of EAA.

In conclusion, the results of the present study strongly support the view that EAA are involved in nociceptive sensory mechanisms. With respect to the trigeminal sensory system it appears that non-NMDA receptors are essential for the C-fibre mediated chemogenic transmission.

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