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Short communication

The antinociceptive effect of intrathecal kynurenic acid and its interaction with endomorphin-1 in rats

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Abstract

Kynurenic acid as an endogenous ligand antagonizes all types of ionotropic glutamate receptors, with preferential affinity for the glycine-binding site of the *N*-methyl-D-aspartate (NMDA) receptor. The purpose of the present study was to investigate the antinociceptive potency of continuously administered kynurenic acid on carrageenan-induced thermal hyperalgesia by means of a paw withdrawal test in awake rats. The possible interaction between kynurenic acid and the endogenous μ-opioid receptor agonist peptide, endomorphin-1, was examined in the same set-up. Kynurenic acid at the higher doses (1–4 μg/min) significantly decreased the thermal hyperalgesia and increased the paw withdrawal latencies on the non-inflamed side. These doses were also associated with motor impairment on both sides. Low doses of kynurenic acid (0.01–0.1 μg/min) potentiated, but did not prolong, the antinociceptive effect of endomorphin-1 (0.1–1 μg/min) on the inflamed side. There was no sign of motor impairment during the combined treatment. These findings demonstrate that the combination of low doses of these two endogenous ligands provides effective and well-controlled antinociception without side effects. © 2002 Published by Elsevier Science B.V.

Keywords: Antinociception; Endogenous ligand; Opioid; Intrathecal infusion; NMDA receptor antagonist; Paw withdrawal test

1. Introduction

It is well known that the *N*-methyl-D-aspartate (NMDA) receptor antagonists are not particularly effective analgesics in acute nociceptive tests (Coderre and Empel, 1994; Klimscha et al., 1998), whereas a number of studies have demonstrated that these agents are effective to produce antinociception in persistent nociceptive models, such as inflammation (Yamamoto and Yaksh, 1992; Yamamoto et al., 1993).

Kynurenic acid is an endogenous tryptophan metabolite acting at the NMDA receptor complex (Ganong et al., 1983; Stone, 1993). Various data suggest a beneficial effect of kynurenic acid as an anticonvulsant (Stone, 2000), but relatively few studies have been performed on its role in pain perception (Yaksh, 1989; Hajós and Engberg, 1990; Raigorodsky and Urca, 1990; Marek et al., 1991; Yamamoto and Yaksh, 1992; Heinricher and McGaraughty, 1998; Heyliger et al., 1998; Heinricher et al., 1999). Although Ganong et al. (1983) already suggested that kynurenic acid alters pain transmission at the spinal level, to date, only three studies have been carried out to investigate the antinociceptive effect of intrathecally administered kynurenic acid in rodents, with inconsistent results (Yaksh, 1989; Raigorodsky and Urca, 1990; Yamamoto and Yaksh, 1992).

Data are available concerning the antinociceptive effect of another endogenous substance, endomorphin-1, which exhibits the highest affinity and specificity for the μ-opioid receptors. (Zadina et al., 1997). In all cases, endomorphin-1 has been revealed to exhibit an antinociceptive effect, although low efficacy, a short-lasting effect and tolerance have also been observed (Horvath, 2000). The aims of the present study were (a) to investigate how a continuous intrathecal infusion of kynurenic acid influences the thermal sensitivity of rats in an inflammatory pain model and (b) to

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analyse the possible interaction between a continuously administered mixture of kynurenic acid and endomorphin-1 at the spinal level. Some aspects of this work have been communicated in abstract form (Kekesi et al., 2001).

2. Materials and methods

The procedures involved in the animal surgery and testing were approved by the Institutional Animal Care Committee of our university. Male Wistar rats were anaesthetized with a mixture of ketamine hydrochloride (S(+)-ketamine Pfizer Med-Inform, Vienna, Austria) and xylazine (Rompun TS; Bayer, Leverkusen, Germany). An intrathecal catheter (PE-10 tubing) was inserted via the cisterna magna. We used the paw withdrawal test to measure the antinociceptive effects of the applied substances on carrageenan-induced inflammation. The baseline hindpaw withdrawal latencies (pre-carrageenan baseline values at −180 min) were measured. Unilateral inflammation was induced by

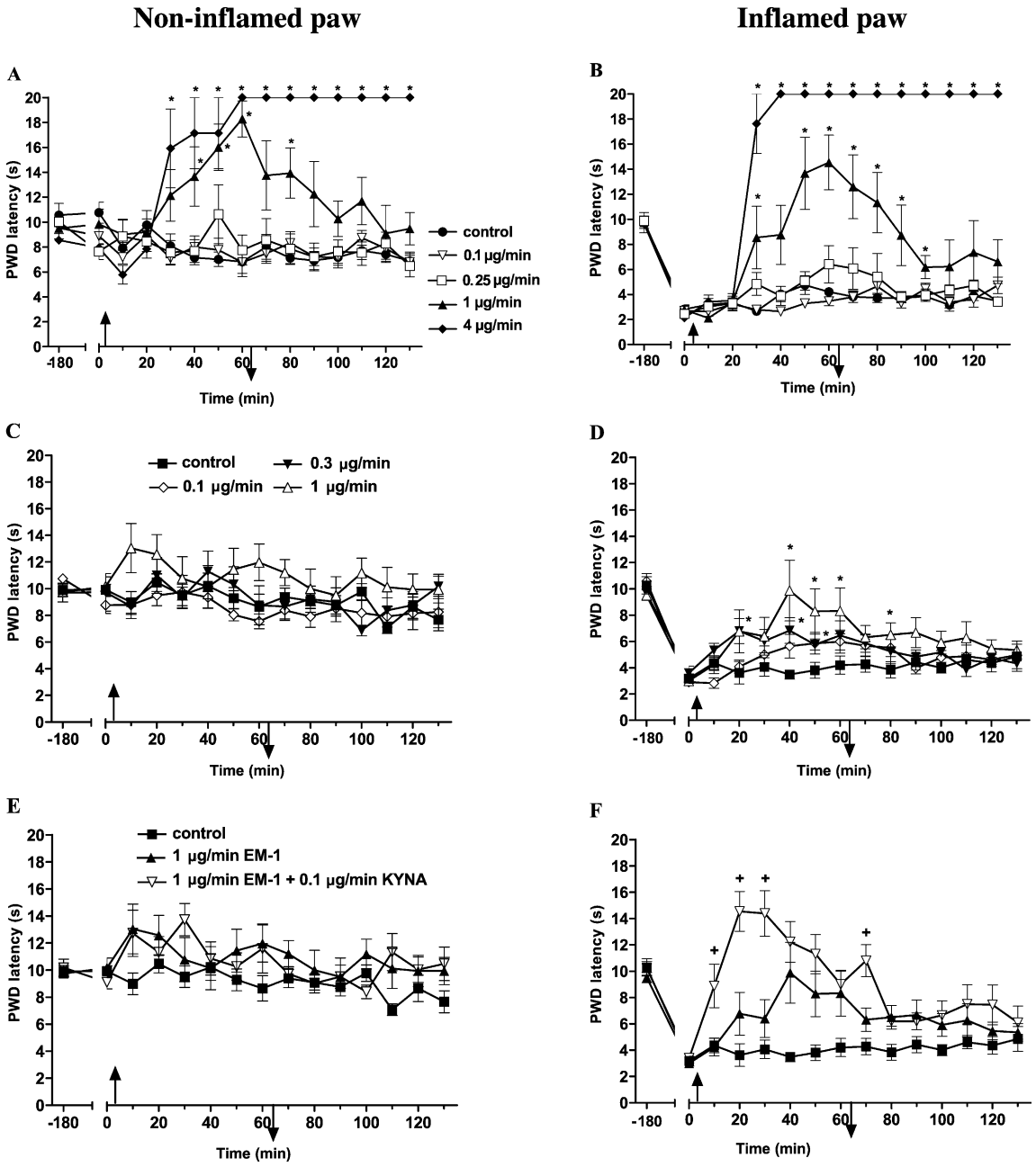

Fig. 1. Curves indicating the time–response effects of (A–B) kynurenic acid (KYNA) and (C–D) endomorphin-1 (EM-1) by themselves or (E–F) in combination. The section between the arrows represents the duration of the microinfusion. The symbol * denotes a significant difference between the results for the treated and the control group animals; the symbol + denotes a significant difference between the results for the endomorphin-1 and combination-treated groups ($P<0.05$). Values are means ± S.E.M. of the data for seven to nine animals.

intraplantar injection of 1.5 mg/0.1 ml lambda carrageenan (Sigma-Aldrich Kft., Budapest, Hungary) into one of the hindpaws. The paw withdrawal latencies were obtained again 3 h after carrageenan injection (post-carrageenan baseline values at 0 min) and at 10 min intervals subsequently for 130 min. Kynurenic acid and endomorphin-1 (Sigma-Aldrich Kft.) were administered as a continuous intrathecal infusion for 60 min in a volume of 60 μl and flushed with an additional 10 μl of 0.9% NaCl at a flow rate of 1 μl/min. Saline was used as control in all series.

One group of experiments was performed to determine the dose–response and time course of the effects of kynurenic acid (0.1–4 μg/min) and endomorphin-1 (0.1–1 μg/min) by itself. The second part of this study involved the administration of both kynurenic acid (0.01–0.1 μg/min) and endomorphin-1 (0.1–1 μg/min) in a fixed dose ratio (1:10) in order to investigate the possible interaction of the two endogenous substances on nociception.

The data are presented as means ± S.E.M. Analysis of variance (ANOVA) of data for repeated measures was used for overall effects, with the Newman–Keuls test for post hoc comparison. A probability level of 0.05 was considered significant.

3. Results

Intraplantar carrageenan injection induced inflammation of the treated paw, as evidenced by oedema, erythema and a significant decrease (from 9.9 to 2.9 s) of paw withdrawal latency within 3 h. The 1- and 4-μg/min dose of kynurenic acid in the non-inflamed paw caused a significant increase in paw withdrawal latency during the infusion and significantly decreased the hyperalgesia on the inflamed side. The dose of 4 μg/min resulted in the maximal increase (cut-off time, 20 s) in pain threshold throughout the observation period (130 min) on both sides. Dose-dependent motor impairment could also be observed. This side effect was temporary and reversible. It should be stressed that the antinociceptive effect might not have been a consequence of motor paralysis because the animals did not show any sign of pain. Since only the lowest dose did not cause motor impairment, the highest dose used for the treatment with the drug combination was 0.1 μg/min.

Endomorphin-1 also caused a dose-dependent decrease in the thermal hyperalgesia. Similarly to what was described recently (Csullog et al., 2001), intrathecally administered endomorphin-1 (0.1, 0.3 or 1 μg/min) did not alter the paw withdrawal latency significantly on the non-inflamed paw. The higher doses on the inflamed side, as compared to the control group, resulted in significant increases in paw withdrawal latency.

The combination of the two drugs did not significantly modify the nociceptive threshold at any dose for the non-inflamed paw. The highest dose combination was effective on the inflamed side because this cocktail caused an immediate, significant increase in paw withdrawal latency, and its effectiveness was significantly higher than that of 1 μg/min endomorphin-1 administered by itself (Fig. 1). The area under the curve (AUC 10–70 min) revealed a synergistic interaction between kynurenic acid and endomorphin-1 (data are not shown). Animals receiving the combinations exhibited no sign of motor dysfunction or other unusual behaviour.

4. Discussion

Our results demonstrate that when the endogenous NMDA receptor antagonist kynurenic acid is administered as a continuous intrathecal infusion by itself, it is not an appropriate antinociceptive agent because of its side effects (motor paralysis) observed at the effective antinociceptive doses. However, in low doses, kynurenic acid potentiated the antihyperalgesic effect of endomorphin-1 in this inflammatory pain model without causing side effects. Termination of the infusion was followed by a gradual decrease of the effects, suggesting that this method provides well-controlled antinociception.

The paralytic effect of kynurenic acid might be due to its action on the motoneurons similar to that of other NMDA receptor antagonists (Coderre and Empel, 1994). Although a recent review suggests a neuroprotective role of kynurenic acid, further toxicological studies need to support its safety (Stone, 2000).

An important technique employed to decrease side effects is the use of combinations of low doses of several agents that produce the same therapeutic effects as a single drug applied in a higher dose. Some studies have investigated the interactions of endomorphin-1 with different drugs to improve its efficacy (Hao et al., 1999, 2000; Wang et al., 1999; Horvath et al., 2001; Csullog et al., 2001). Our recent studies suggest that the antinociceptive effect of endomorphin-1 is potentiated by the NMDA receptor antagonist, S(+)-ketamine (Horvath et al., 2001). Since opioid and glutamate receptors are abundant in the spinal cord, the co-activation and antagonism of these receptors could have a beneficial effect on the inhibition of pain sensation at low doses which cause minimal side effects (Wiesenfeld-Hallin, 1999).

There are at least two important advantages of our results. Firstly, the antinociceptive property of the continuous infusion of this combination is well controlled. Furthermore, application of the endogenous antinociceptive ligands may have a number of advantages, e.g. fast elimination and low toxicity (Kristensen et al., 1993). Additionally, this combination might be useful to reduce the development of morphine tolerance and withdrawal symptoms (Marek et al., 1991). An important attribute of the present potentiation was the lack of side effects, suggesting that the combined drug delivery will serve in principle to enhance the therapeutic ratio of the treatment.

The data reported indicate that kynurenic acid, similarly to other excitatory amino acid receptor antagonists, has a limited therapeutic range and may, therefore, not be particularly useful alone as an analgesic agent for the treatment of clinical pain. The only, but important, exception might be the application of this drug during surgery, when the motor paralysis might be advantageous. However, the interaction of the endogenous substances kynurenic acid and endomorphin-1, which act at different receptor types, is beneficial in that it attenuates carrageenan-induced thermal hyperalgesia without causing any side effects. These results suggest an important direction for the development of pain strategies that focus on the co-administration of different endogenous ligands into the subarachnoid space.

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