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Excitatory amino acid receptor involvement in peripheral nociceptive transmission in rats

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Abstract

The involvement of excitatory amino acid receptors in peripheral nociceptive processing was assessed in two separate experiments. In the first, one knee joint cavity of rats was injected with 0.1 ml of L-glutamate (0.001 mM; 0.1 mM; 1.0 mM), L-aspartate (0.001 mM; 0.1 mM; 1.0 mM), L-arginine (0.1 mM) or different combinations of these amino acids. The animals tested for paw withdrawal latency to radiant heat and withdrawal threshold to von Frey filaments at different time points. Combinations of glutamate/aspartate, aspartate/arginine or glutamate/aspartate/arginine when injected into the joint, in the absence of any other treatment, reduced the paw withdrawal latency and withdrawal threshold immediately after the injection and persisting up to 5 h indicating the development of hyperalgesia and allodynia. Subsequent intra-articular injection of either an NMDA or a non-NMDA glutamate receptor antagonist ((±)-2-amino-7-phosphonoheptanoic acid (AP7), 0.2 mM) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 0.1 mM) attenuated the thermal hyperalgesia and the mechanical allodynia produced by glutamate/aspartate/arginine. On the other hand, in a second experiment intra-articular injection of AP7, ketamine or CNQX reversed the hyperalgesia and allodynia produced by injection of a mixture of kaolin and carrageenan into the joint. These receptor antagonists, however, did not have an effect on the joint edema. These findings provide evidence for a potential role of peripheral NMDA and non-NMDA receptors in nociceptive transmission.

Keywords: NMDA receptor; Non-NMDA glutamate receptor; Nociception; Hyperalgesia; Allodynia; Inflammation; Pain

1. Introduction

The excitatory amino acids, glutamate and aspartate, are ubiquitously distributed in mammalian tissues. It has been well documented that excitatory amino acids mediate most of the excitatory synaptic transmission in the spinal cord (Gerber et al., 1991; Jeftinija, 1989). Accumulating evidence indicates that excitatory amino acids and their receptors play a significant role in central nociceptive transmission, modulation and the sensitization that underlies allodynia and hyperalgesia (Coderre and Melzack, 1991; Dougherty and Willis, 1991; Haley et al., 1990; Seltzer et al., 1991). Support for this argument comes from studies in rats and mice in which selective excitatory amino acid receptor antagonists have been shown to be antinociceptive (Ault and Hildebrand, 1993b; Cahusac et al., 1984; Coderre and Van Empel, 1994). The involvement of NMDA and non-NMDA receptors in the spinal processing of afferent activity after kaolin and carrageenan-induced inflammation of the knee joint has been studied in rats and cats (Schaible et al., 1991; Sluka and Westlund, 1993b). The concentration of glutamate and aspartate in the spinal cord dorsal horn has been shown to increase after induction of acute arthritis in rats and monkeys (Sluka and Westlund, 1992; Sorkin et al., 1992), whereas administration of NMDA as well as non-NMDA receptor antagonists in the dorsal horn through a microdialysis fiber blocks the thermal hyperalgesia associated with the joint inflammation (Sluka and Westlund, 1993b).

While these results show that hyperalgesia is dependent on activation of central glutamate and aspartate receptors, the peripheral content of glutamate in fine joint afferents in the medial articular nerve is also increased during the development of acute arthritis in monkeys (Westlund et al., 1992). Little is known, however, about the involvement of

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excitatory amino acids in peripheral nociceptive transduction. It has been shown that primary afferent fibers in the dorsal root are depolarized by excitatory amino acids acting at kainate receptors and that the action of kainate is limited to the C-afferent fibers (Agrawal and Evans, 1986). Ault and Hildebrand (1993a,c) investigated the effect of kainate and related excitatory amino acids on ventral root reflexes, using the rat isolated spinal cord-tail preparation, and they have confirmed the existence of functional kainate receptors on nociceptive afferents. More recently, it has been demonstrated that activation of the NMDA and non-NMDA glutamate receptors in glabrous skin of the rat hindpaw by injecting different glutamate agonists resulted in the development of mechanical allodynia and hyperalgesia (Zhou et al., 1996).

It is well known that activation of excitatory amino acid receptors stimulates various intracellular second messenger systems. Recent evidence implicates a contribution of nitric oxide (NO) to hyperalgesia and nociceptive processing in the spinal cord (Meller and Gebhart, 1993). In the periphery, administration of nitric oxide synthase receptor antagonists blocks the edema associated with intraplantar injection of bradykinin or substance P and activation of the intracellular cascade events producing NO results in antinociception (Duarte et al., 1990; Meller et al., 1990).

The aim of the present study was to investigate the role of knee joint excitatory amino acid receptors in the peripheral modulation of nociceptive transmission. Thus, we tested the paw withdrawal latency and withdrawal threshold to radiant heat and mechanical stimuli respectively before and after intra-articular injection of glutamate and aspartate as well as arginine (a substrate for NO synthesis) in an attempt to provide evidence for the involvement of excitatory amino acid receptors and their agonists in the development of hyperalgesia and allodynia. Subsequently, the ability of intra-articular injection of glutamate receptor antagonists to reduce the effect of injected amino acids or kaolin and carrageenan was also tested. The data support a role of peripheral excitatory amino acid receptors in mediating nociception associated with acute arthritis in rats. Preliminary results have been presented in abstract form (Westlund et al., 1995).

2. Materials and methods

2.1. Experimental animals

2.1.1. Intra-articular amino acids

Male Sprague-Dawley rats (250–350 g) were used in this study. The animals were housed in a room with a constant ambient temperature of 22°C and a 12-h light/dark cycle. They had free access to food and water. Initially, all rats were anesthetized with a short-acting barbiturate (Brevital, 60 mg/kg). Animals were divided into 13 experimental groups. Each group was injected with 0.1 ml of one or more amino acids (dissolved in phosphate buffer and adjusted to a final pH of 7.4) or vehicle into the left knee joint cavity. Seven groups of rats received a single injection of amino acids in the absence of any other agent. These included either L-glutamate (Glu) (0.001 mM, $n = 4$; 0.1 mM, $n = 6$; 1 mM, $n = 6$), L-aspartate (Asp) (0.001 mM, $n = 4$; 0.1 mM, $n = 6$; 1 mM, $n = 6$) or arginine (Arg) (0.1 mM, $n = 6$). Four other groups ($n = 6$/group) received a combination of either Glu/Asp, Asp/Arg, Glu/Arg or Asp/Glu/Arg at 0.1 mM each. All amino acid combinations were contained in a total volume of 0.1 ml. Two additional groups of rats were injected with Asp/Glu/Arg and treated at the same time with the NMDA or non-NMDA receptor antagonists, (±)-2-amino-7-phosphonoheptanoic acid (AP7) or CNQX (0.2 mM and 0.1 mM, respectively). The vehicle control group received an injection of phosphate buffer (PB) (0.1 M; pH: 7.4).

2.1.2. Intra-articular kaolin and carrageenan

Knee joint inflammation was induced in three additional groups of animals anesthetized with a short-acting barbiturate by injecting 0.1 ml of a mixture of 3% kaolin and carrageenan into the knee joint cavity. To test for mechanical allodynia in arthritic animals, von Frey filaments were applied on the area of skin around the knee joint. To investigate further the involvement of peripheral glutamate NMDA and non-NMDA receptors in joint inflammation, two groups of animals ($n = 6$/group) were treated 3 h after induction of arthritis with an injection of AP7, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) or ketamine (0.2 mM, 0.1 mM and 0.1 mM, respectively) into the knee joint. To control for the effect of the drug injection, a different group of arthritic animals was injected intra-articularly with PB ($n = 6$) at the same time point.

2.2. Paw withdrawal latency to radiant heat

All rats were tested for thermal hyperalgesia by measuring the paw withdrawal latency to radiant heat, as described by Hargreaves et al. (1988). Animals were placed in small plastic cages elevated on a glass plate. After an adaptation period of 15–20 min, radiant heat was applied to the plantar surface of each hindlimb by focusing a high intensity light through an aperture (1.0×0.8 cm) onto the lower surface of the glass plate. Radiant heat was applied until the animal lifted its paw from the glass. The time from onset of radiant heat application to withdrawal of the rat's hindpaw was defined as the paw withdrawal latency. Both hindpaws were tested independently for three trials with a 5 min interval between each trial. The average paw withdrawal latency in the three trials of the injected limb was compared to that of the untreated limb of the same groups and to the treated limb of the vehicle group. Paw withdrawal latency was always tested prior to any injection, immediately after and for up to 8 h after injection of the amino acids. For arthritic rats, paw withdrawal latency to radiant heat was measured just before and at 4, 5, 6, 7

and 8 h after induction of arthritis. Presumably, this demonstrates secondary hyperalgesia because the testing was done on the hindpaw remote from the joint inflammation site (Hardy et al., 1967).

2.3. Paw withdrawal response to repeated mechanical stimuli

Animals were tested for allodynia to innocuous mechanical stimuli using von Frey filaments with bending forces ranging from 30 mN to more than 100 mN. Mechanical stimulation applied with the range of filaments tested produces a sense of touch or slight pressure when applied to human skin. Before testing, the rats were placed in small clear cages elevated on a mesh grid. For groups of animals injected intra-articularly with the amino acids, von Frey filaments were applied through the mesh grid to the plantar surface of the foot until the filaments bent slightly. A single trial of stimuli consisted of 3 applications per second. Three trials separated by 5 min intervals were performed on each hindpaw. This procedure was repeated using each of the von Frey filaments in ascending order. To quantify the hindpaw sensitivity to innocuous stimuli, the withdrawal threshold was determined to be the bending force of the lowest strength von Frey filament from which the animal withdrew its limb in 50% or more of the trials.

2.4. Assessment of inflammation

The role of peripherally injected amino acids in producing joint inflammation was also investigated. The degree of inflammation was assessed by the extent of joint swelling and the increase in joint temperature prior to and at 8 h after injection of the substances tested. To determine the extent of swelling, knee joint circumference was measured with a flexible tape measure around the center of the joint while the limb was held in extension. Thermographic readings were obtained using a liquid crystal tablet placed on the medial surface of the joint with the rat lying in a supine position.

2.5. Statistics

Data are expressed as the mean ± S.E.M. In normally distributed populations, significant changes in paw withdrawal latency and withdrawal threshold within groups over time were determined with one-way repeated measures analysis of variance (ANOVA) of the raw data followed by a Scheffé test for post-hoc analysis. In non-normally distributed populations, significant changes were determined using Friedman's analysis of variance on ranks. Comparisons of paw withdrawal latency and withdrawal threshold to radiant heat before and after the injections at all testing times were done using paired *t*-tests. Statistical significance between groups was assessed using a rank sum test. $P < 0.05$ was considered significant.

3. Results

Behavioral tests showed that injection of PB into the knee joint cavity produced no change from baseline or from untreated side values in the paw withdrawal latency to radiant heat or withdrawal threshold to innocuous mechanical stimuli. The responses after intra-articular injection of Glu or Asp at each dose given were comparable to those of the control PB group and to those of the contralateral (untreated) side or to baseline values obtained before the injection. Similarly, no changes in paw withdrawal latency or withdrawal threshold were noted following the intra-articular injection of either Arg or a combination of Glu/Arg when compared to baseline values or to the values of the control PB group at each time point. Interestingly, 2 rats out of 4 injected with the lowest concentration of either Glu or Asp (0.001 mM) exhibited some behavioral changes such as curled toes or decreased weight bearing on the injected side. These changes were not seen with higher concentrations, and they represent mild pain-related behaviors (Attal et al., 1990).

3.1. Effect of intra-articular injection of different combinations of amino acids on paw withdrawal latency to radiant heat and withdrawal threshold to repeated mechanical stimuli

Fig. 1A illustrates the paw withdrawal latency to radiant heat on the treated side in all groups at 10 min and 30 min, and at 1, 2, 4, 5, 6 and 8 h after the injection. Mean latencies for the injected paws were significantly less than baseline and PB group values. By 6 h, withdrawal times returned to baseline values. The combinations of Asp/Glu, Asp/Arg or Asp/Glu/Arg injected into the knee joints of normal rats resulted in a decrease in paw withdrawal latency indicative of secondary hyperalgesia beginning 10 min after the injection. This decrease was maintained for up to 5 h, at which time the paw withdrawal latency returned to baseline levels. Rats showed significant differences in paw withdrawal latency to radiant heat at 10 min after injection of the amino acids into the knee joint as compared to baseline values ($P < 0.05$). Over 5 h, withdrawal latencies of the paw from thermal stimuli were also significantly decreased by the intra-articular injection of the different amino acid combinations: Asp/Glu, $P < 0.001$; Asp/Arg, $P < 0.001$; Asp/Glu/Arg, $P < 0.0001$.

There was an overall statistically significant difference between the paw withdrawal latency values obtained for the paws injected with Asp/Glu, Asp/Arg or Asp/Glu/Arg versus the PB group ($P < 0.001$).

Prior to intra-articular injection of the amino acids, there was no significant difference in the withdrawal thresholds to mechanical stimulation between the treated and untreated hindpaws. A 50% withdrawal rate occurred when a 90 mN von Frey filament was used. In all of the trials, the withdrawal reaction consisted of a quick with-

drawal, eventually followed by a brief burst of scratching or grooming behavior. After the injection, a side-to-side difference was noted at different testing time intervals. Fig. 1B shows that 10 min after injection of either Asp/Glu, Asp/Arg or Asp/Glu/Arg, the threshold on the experimental side was significantly lower than the pre-treatment values ($P < 0.001$). At this time, the mean withdrawal threshold on the treated side had decreased significantly (Asp/Glu, $P < 0.0001$; Asp/Arg, $P < 0.0002$; Asp/Glu/Arg, $P < 0.0001$) and remained so for 4 h. By 5 h, no significant difference was noted when compared to the baseline or to the vehicle control group values except for the Asp/Glu/Arg group.

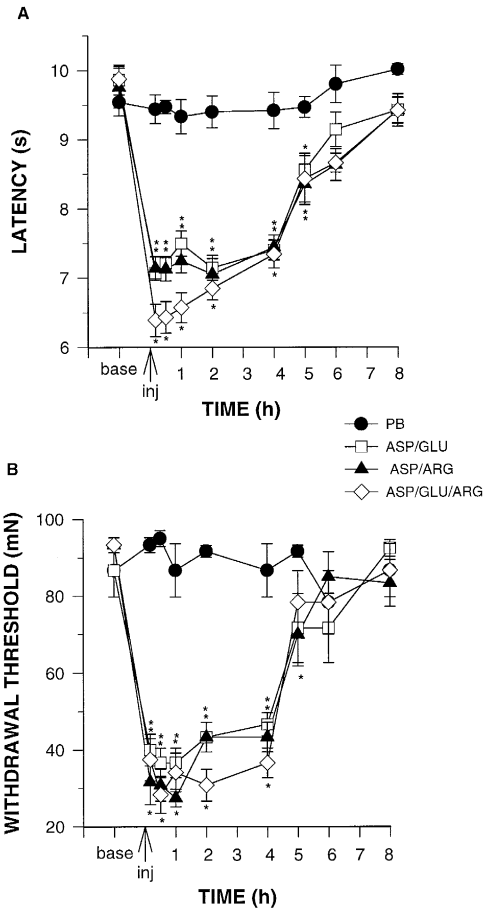

Fig. 1. Paw withdrawal latency (PWL) to radiant heat (A) and withdrawal threshold (WT) to innocuous mechanical stimuli (B) (mean ± S.E.M.) in rats treated intra-articularly with Asp/Glu ($n = 6$), Asp/Arg ($n = 6$), Asp/Glu/Arg ($n = 6$) or with phosphate buffer solution ($n = 6$). The mean withdrawal threshold and paw withdrawal latency of the injected paws are plotted against the time-course (h) of the experiments. Baseline paw withdrawal latency and withdrawal threshold from noxious and innocuous stimuli were measured immediately prior to any injection. Animals were tested at 10 and 30 min and 1, 2, 4, 5, 6 and 8 h after injection. Thermal hyperalgesia and mechanical allodynia peaked 10 min after the injection and lasted up to 5 h. * Significantly different from baseline values, $P < 0.05$.

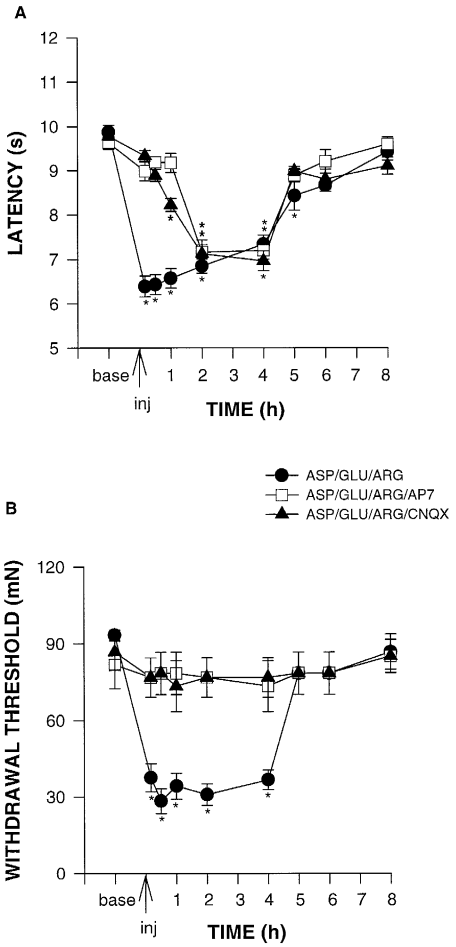

Fig. 2. Effects of intra-articular injection of AP7 and CNQX on paw withdrawal latency to radiant heat stimulation (A) and withdrawal threshold to innocuous mechanical stimulation (B) in rats treated intra-articularly with a mixture of Asp/Glu/Arg ($n = 6$/group). When AP7 and CNQX were injected with the single bolus of Asp/Glu/Arg, AP7 attenuated the development of hyperalgesia for 1 h after injection and CNQX for 30 min. The threshold for all groups was decreased at 120 min and returned to baseline value at 5 h in animals injected with AP7 and CNQX. The paw withdrawal latency returned to baseline level by 6 h after the injection of Asp/Glu/Arg. AP7 and CNQX were completely effective in preventing the mechanical allodynia observed with intra-articular injection of Asp/Glu/Arg shown in (B). * Significantly different from baseline values, $P < 0.05$.

3.2. Effects of AP7 and CNQX on the development of heat hyperalgesia and mechanical allodynia produced by injection of Asp / Glu / Arg

Fig. 2 illustrates the paw withdrawal latency to radiant heat (A) and the withdrawal threshold to innocuous mechanical stimuli (B) for rats treated with Asp/Glu/Arg, Asp/Glu/Arg/AP7 or Asp/Glu/Arg/CNQX. As illustrated in Fig. 2A, intra-articular injection of Asp/Glu/Arg reduced the paw withdrawal latency. However, the addition of AP7 or CNQX initially prevented the reduction in

latency of paw withdrawal to thermal stimuli and thus produced a significant antihyperalgesic effect. When measured 10 and 30 min after injection, no statistically significant difference in the paw withdrawal latency on the experimental side was observed for the animals injected with AP7 as compared to the vehicle control and baseline values. CNQX also produced a significant antihyperalgesic effect when administered with the three amino acids.

The attenuation of paw withdrawal latency by the single injection of CNQX was maintained for up to 2 h in contrast to decreases in paw withdrawal values obtained from animals injected with Asp/Glu/Arg alone. Two hours after drug treatment, all animals became hyperalgesic. The paw withdrawal latency returned to baseline levels 6 h after injection in all groups.

Fig. 2B illustrates the effects of either AP7 or CNQX on the withdrawal threshold to innocuous stimuli using von Frey filaments after injecting the knee joint with Asp/Glu/Arg. AP7 and CNQX prevented the reduction in the withdrawal threshold when compared to values obtained from Glu/Asp/Arg-treated rats at all time points.

3.3. Effect of peripheral administration of amino acids on the development of inflammation

No marked swelling of the knee joints was observed following intra-articular injection of any combination of the amino acids as assessed by knee joint circumference measurements. Similarly, no change in the joint temperature was observed after the amino acid injections as compared to baseline and untreated side values.

3.4. Effect of intra-articular injection of kaolin and carrageenan on heat hyperalgesia and mechanical allodynia

Rats injected with a mixture of kaolin and carrageenan exhibit limping, guarding of the limb and decreased weight bearing on the inflamed side starting 3–4 h after induction of arthritis (Sluka and Westlund, 1993a). Prior to the kaolin and carrageenan injection, the mean paw withdrawal latencies ($\pm$S.E.M.) to radiant heat and the mean withdrawal thresholds to repetitive mechanical stimulation of the treated paws were 9.66 ± 0.19 s and 88.33 ± 7.23 mN respectively. The time-courses of changes in the paw withdrawal latency to thermal stimuli and of changes in withdrawal thresholds to mechanical stimuli after induction of arthritis are illustrated in Fig. 3A and B, respectively. A decrease in paw withdrawal latency of the inflamed paw was noted in the arthritic control group upon the first test, at 4 h after induction of arthritis, and was maintained throughout the testing period, lasting 8 h. In arthritic rats, the marked side-to-side differences in their paw withdrawal latencies to radiant heat and thresholds to mechanical stimuli at 4 h lasted for the entire testing period. The mean paw withdrawal latencies ($\pm$S.E.M.) for the untreated and the inflamed paws were significantly

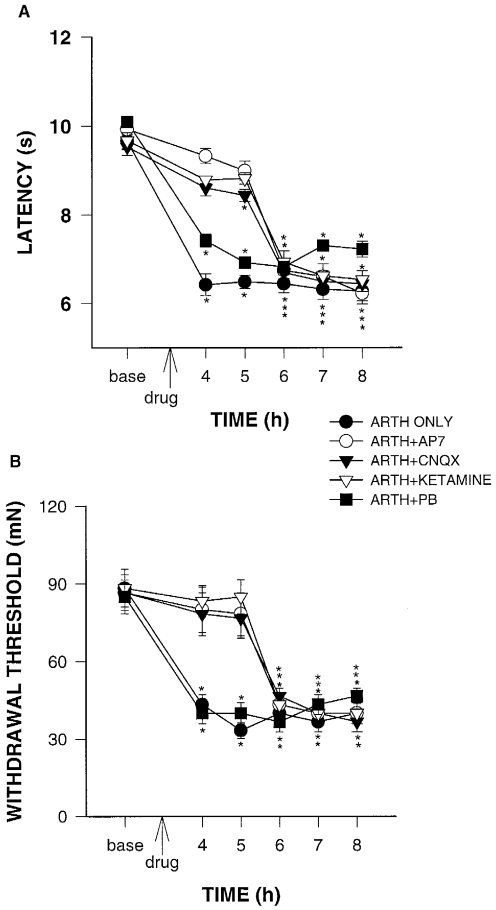

Fig. 3. Time-course of the mean withdrawal latencies to radiant heat (A) and to innocuous mechanical stimuli (B) after intra-articular injection of PB, AP7, CNQX and ketamine in rats injected with kaolin and carrageenan (immediately after baseline testing). Intra-articular injection of PB had no effect on the paw withdrawal latency as well as withdrawal threshold of arthritic animals. AP7 and ketamine attenuated the development of both heat hyperalgesia and mechanical allodynia associated with joint inflammation up to 2 h. The CNQX-treated group showed no significant difference in paw withdrawal latency from baseline values at the 1 h time point and mechanical allodynia was attenuated for 2 h. * Significantly different from baseline values, $P < 0.05$.

different ($P < 0.002$). Similarly, the mean withdrawal threshold to innocuous mechanical stimulation on the experimental side was significantly lower as compared to the baseline and the untreated limb values ($P < 0.001$).

3.5. Effects of peripherally administered PB, AP7, CNQX and ketamine on thermal hyperalgesia and mechanical allodynia induced by acute arthritis

Intra-articular injection of PB 3 h post-induction of arthritis did not change the paw latency to either the radiant heat or to mechanical stimuli when compared to baseline values. However, an injection of AP7, CNQX or ketamine into the synovial cavity of the rat knee at the same time point attenuated both heat hyperalgesia and mechanical allodynia. Fig. 3 illustrates the effects of these

glutamate receptor antagonists on the paw withdrawal latency to radiant heat (A) and the withdrawal threshold to mechanical stimuli (B). One hour after a single administration of AP7, CNQX or ketamine, the paw withdrawal latency to radiant heat was not significantly different from baseline levels but animals became hyperalgesic after 2 h, indicating an escape from the protective effects of the receptor antagonists. Similarly, the withdrawal threshold to repetitive innocuous mechanical stimuli remained at baseline after administration of the receptor antagonists. The protective effect lasted for only 2 h. The AP7 and CNQX had no effect on the edema produced by injection of the mixture of kaolin and carrageenan into the knee joint.

4. Discussion

The present study examined the effect of peripheral administration of several amino acids (Asp, Glu and Arg) that have been associated with central nociceptive transmission. The effects of intra-articular administration of these amino acids on the paw withdrawal latency to radiant heat and the threshold for withdrawal from repetitive mechanical stimuli were investigated in awake behaving rats. Amino acids were injected either separately or in different combinations into the knee joint cavity during a brief period of anesthesia. Excitatory amino acids when administered individually at any concentration in the range of 0.001 to 1 mM failed to produce heat hyperalgesia or mechanical allodynia at any time after the injection. Similarly, the injection of arginine alone at 0.1 mM did not produce any nociceptive behavior. However, a major finding of these experiments is that when certain combinations of amino acids (Asp/Glu, Asp/Arg or Asp/Glu/Arg) were given, a significant decrease was observed in paw withdrawal latency to radiant heat and in withdrawal threshold to repetitive innocuous mechanical stimulation on the experimental side. Conversely, the combination of Glu/Arg had no effect on paw withdrawal latency or withdrawal threshold when injected into the knee joint. These results suggest that in the periphery Asp, Glu and Arg, in certain combinations, act as chemical mediators of nociception, producing an excitatory action lasting up to 5 h. Moreover, since peripheral administration of NMDA and non-NMDA receptor antagonists directly into the joint were shown in these experiments to have an antihyperalgesic effect, the data suggest that both NMDA and non-NMDA receptors are activated in the joint. These results provide the first indication that excitatory amino acid receptors may exist either on the peripheral terminals of the primary afferent fibers in the knee joint or on other cellular elements in the joint, such as synoviocytes and immune cells. Activation of these receptors may directly or indirectly induce behavioral hyperalgesia and allodynia in rats, presumably by sensitizing nociceptors and subsequently inducing changes in the dorsal horn. Control measurements on the contralateral limb suggested that the amino acids did not escape the knee joint in sufficient amounts to have systemic effects.

The role of excitatory amino acid receptors in the peripheral nervous system has not been extensively studied. Indeed it has been assumed that glutamate receptors are absent from peripheral nerves of vertebrates (Wolf and Keilhoff, 1983) until recently. However, new evidence for expression of excitatory amino acid receptors by peripheral neurons has been provided. It has been demonstrated that kainic acid and several other excitatory amino acids depolarize sensory nerve fibers in the dorsal roots of newborn rats and that this effect is unique to primary afferent C-fibers (Agrawal and Evans, 1986). It is speculated that this is due to the presence of specific glutamate receptors, since the effect can be blocked by glutamate receptor antagonists. Moreover, Ault and Hildebrand (1993a) have demonstrated the existence of functional kainate receptors on nociceptive afferents since kainate was found to act at peripheral kainate receptors to stimulate nociceptive-like responses.

In accordance with our study, Hargreaves and his colleagues have demonstrated that intraplantar administration of L-glutamate reduced the paw withdrawal latency to radiant heat 15 min after injection while intraplantar injection of either NMDA or non-NMDA receptor antagonists, MK-801 or CNQX respectively, reduced the thermal hyperalgesic response induced by carrageenan injection into the paw (Jackson et al., 1995). Similarly, activation of NMDA, AMPA and kainate glutamate receptors by specific agonists in glabrous skin of rat hindpaw resulted in primary mechanical hyperalgesia and allodynia (Zhou et al., 1996). These observations indicate that excitatory amino acid receptors, whether in deep or cutaneous tissues, may play a role in the peripheral control of inflammation. However, in our study a single intra-articular injection of L-glutamate did not produce secondary behavioral hyperalgesia or allodynia. Possible explanations suggested for the difference in results between the two studies are methodological and injection site differences or receptor saturation. It is worthy to note that the concentration of each amino acid used in our study was not optimal to produce a response independently of the other. The possible existence of different receptor transduction mechanisms peripherally in the joint and the skin should also be considered (Yu et al., 1993).

More studies to support the existence of excitatory amino acid receptors peripherally comes from Lovinger and Weight (1988) who found that a substantial proportion of dorsal root ganglia neurons from adult rats express conventional NMDA receptors, which show voltage-dependent block by Mg^{2+} and potentiation by glycine. Huettner (1990) has also shown that rapid application of glutamate, quisqualate and AMPA evokes a transient current in a proportion of dorsal root ganglia neurons and desensitizes cells to subsequent application of kainate and do-

moate. While glutamate receptors exist on central terminals and cell bodies of primary afferent fibers, our findings may provide further evidence for the existence of such receptors on their peripheral terminals.

The role of L-arginine, a NO precursor, in peripheral nociception, is not well established. In the spinal cord, it is proposed that NO plays a significant role in nociceptive processing (Meller and Gebhart, 1993). The results of the present study have shown that L-arginine, when combined with Asp or Asp/Glu, participates in producing a decrease in paw withdrawal latency to radiant heat, as well as in the withdrawal threshold to innocuous mechanical stimuli. It has been shown that NMDA receptor activation centrally increases intracellular calcium, which in turn activates NO synthase to produce NO from free arginine (Meller et al., 1992). This finding is in agreement with the present study in which interaction of several amino acids is required to enhance nociceptive reflexes. More evidence to support our findings comes from studies by Holthusen and Arndt (1994) who observed that NO acts algesically in humans when applied intracutaneously, presumably by exciting cutaneous nociceptors. In contrast to the present study, Duarte et al. (1990) have provided evidence for the involvement of NO in peripheral analgesia by demonstrating that nitroprusside, a non-enzymatic generator of NO, possesses antinociceptive activity in a pain model produced by intraplantar injection of prostaglandins or carrageenan. Moreover, they have shown that L-arginine causes analgesia when applied in rats with carrageenan-induced hyperalgesia of the hindpaw. However, since the analgesia induced by sodium nitroprusside was not blocked by L-NMMA, an inhibitor of the formation of NO from L-arginine, this raises the possibility of a non-specific effect of nitroprusside since no direct evidence for NO involvement was provided. L-Arginine had an analgesic effect on the hyperalgesia produced by carrageenan but not by prostaglandins, suggesting that NO produces differential effects depending on the type of receptors involved.

Another piece of evidence supporting the role of NO in mediating nociceptive transmission and peripheral inflammation in arthritic animals comes from our studies where it has been demonstrated that intra-articular injection of L-NAME, 4.5 h after induction of inflammation by injection of kaolin and carrageenan, blocked the heat hyperalgesia for the whole testing period (4 h). L-NAME has also been shown to prevent a further increase in swelling, assessed by measuring the joint circumference, as well as joint temperature (Lawand et al., 1997).

Based on these findings, we propose the following to explain the results obtained in the present study: application of either Glu or Asp to the knee joint may cause a brief depolarization of the primary afferent units that inactivates within a few seconds due to rapid desensitization of the glutamate receptors. This explanation can be supported by recent electrophysiological studies in rats where intra-arterial injection of either L-glutamate (10^{-6} up to 10^{-2} mM) or L-aspartate (10^{-3} up to 1 mM) have been shown to produce a brief excitation of the primary articular afferents limited to the duration of injection (Lawand et al., 1996). When Asp/Glu, Asp/Arg or Asp/Glu/Arg are given, the rats developed a maintained hyperalgesia and allodynia which is measurable at a remote site on the plantar surface (secondary hyperalgesia). Interestingly, the administration of Glu/Arg into the knee joints of rats was ineffective in producing more than minimal nociceptive behavior. In these experiments, the arginine had an additive effect to that of Asp and the Asp/Glu mixture but not to that of Glu. Arginine is known to depolarize peripheral nerve fibers directly or indirectly by being taken up by the nerve endings, and it is presumably converted into NO since NO synthase is shown to be localized in the dorsal root ganglia (Aoki et al., 1993). Studies on an invertebrate nervous system show that L-arginine induces neuronal depolarization, and simultaneous application of several amino acids has an additive effect (Ichinose and McAdoo, 1985). In this study, when Glu was combined with arginine, non-NMDA receptors may become activated but then desensitized rapidly. However, when Arg is combined with Asp or Asp/Glu, NMDA receptors are also activated, producing a sensitization of the nociceptors that may involve second messengers such as NO to modulate neuronal signaling. Therefore, activation of NMDA receptors by aspartate seems to be required for the development of hyperalgesia or allodynia. However, it seems likely that an interaction between different glutamate receptor subtypes, NMDA, non-NMDA and metabotropic, is necessary to activate nociceptors and to maintain a steady state of hyperalgesia. On the other hand, it should be pointed out that these results cannot be accounted for by a non-specific or central effect of these amino acids since paw withdrawal latency and withdrawal thresholds of the untreated hindpaw were unaffected. The ability of AP7, ketamine or CNQX injected into the knee joint to block the hyperalgesia and allodynia strongly implicates both excitatory amino acid receptors in the knee joint in mediating these responses. It is also interesting to note that peripheral administration of different combinations of amino acids did not produce any increase of the knee joint circumference or joint temperature.

In the present study, the antinociceptive effects of peripherally administered NMDA and non-NMDA receptor antagonists on heat hyperalgesia and mechanical allodynia that developed in an experimental model of acute arthritis were also studied. In this model, a decrease in paw withdrawal latency to radiant heat and in the withdrawal threshold to repetitive mechanical stimuli was observed throughout the 4–8-h time period after induction of arthritis by intra-articular injection of kaolin and carrageenan, in agreement with previous studies (Sluka and Westlund, 1993a). Another major finding of this study is that the intra-articular injection of the different NMDA receptor antagonists (AP7, ketamine), as well as a non-NMDA

receptor antagonist (CNQX), after the development of kaolin and carrageenan-induced inflammation (3 h) resulted in a return of the mean withdrawal thresholds to noxious and innocuous stimuli back to baseline levels. These results cannot be accounted for by a central effect of the receptor antagonists, specifically ketamine which is a lipophilic systemically active anesthetic agent, since withdrawal latencies of the untreated hindpaw were not altered from baseline by this treatment. In the present study, the NMDA and non-NMDA receptor antagonists were given locally into the knee joint cavity, and their effect on the treated paw was immediate and short lasting. Although we cannot rule out the possibility of a central effect due to systemic leakage of the drugs, any small leakage would presumably not be enough to produce a significant effect, since the volume of the injection was small and confined to a closed cavity. Evidence to support this argument comes from Jackson et al. (1995) in studies in which they have demonstrated that the same dose and volume of the NMDA and non-NMDA receptor antagonists, which produced an antinociceptive effect when applied to the formalin inflamed paw, had no significant effect on PWL to radiant heat when injected systemically. In our study, the effects of these receptor antagonists were presumably mediated by blocking excitatory amino acid receptors present within the joint capsule. It is possible that movement of the inflamed joint releases intracellular glutamate from immune cells in the knee capsule, and/or from primary afferent fibers, promoting C-fiber activation. A documented physiological process that could be involved is stimulation of C-fibers by glutamate released from inflammatory cells, such as mast cells and macrophages (Piani et al., 1991). Alternatively, the excitatory amino acids could cause release of cytokines, histamine or 5-HT and these could excite the nociceptors. Additionally, because C-fibers release glutamate and neuropeptides, such as substance P, at central terminals (Salt and Hill, 1983), it seems likely that glutamate is also released from nerve terminals in the periphery, leading to modulation of transmitter release from other afferent fiber endings (Jackson et al., 1995; Jeftinija et al., 1991). It is well known that following peripheral inflammation, neuropeptides are released by the peripheral terminals of the primary afferent fibers (Lam and Ferrell, 1989; Yaksh, 1988). Westlund et al. (1992) observed an increase in glutamate content in the medial articular nerve during the development of acute inflammation, and so more glutamate may be available for release in arthritis than in the normal state. Our recent studies have also shown that in an arthritis model, action potentials (dorsal root reflexes) travel down the primary afferents toward the periphery in all fiber types, including C fibers, and would provide a mechanism for glutamate release into the joint (Rees et al., 1994; Sluka et al., 1995). In conclusion, these findings provide support for the existence of functional NMDA as well as non-NMDA excitatory amino acid receptors on peripheral terminals of articular primary afferent fibers that could potentially play a role in nociception and neurogenic inflammation. Attenuation of pain-related behaviors by intra-articular application of NMDA and non-NMDA excitatory amino acid antagonists after full development of the knee joint inflammation suggests a novel and viable alternative for pharmacological reduction of joint pain associated with inflammation.

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