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Neurochemical changes after morphine, dizocilpine or riluzole in the ventral posterolateral thalamic nuclei of rats with hyperalgesia

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Abstract

A number of studies suggest the involvement of glutamate in central hyperalgesia through NMDA receptors in animal models of inflammation. Most studies analyze glutamate effects at the spinal cord level. In this work, the effects of morphine, dizocilpine and riluzole on the hyperalgesia induced by carrageenan administration in the rat paw model were investigated. The effects of morphine and riluzole on the release of glutamate and aspartate and on the concentrations of citrulline and arginine in dialysates of the ventral posterolateral nucleus of the thalamus were also examined. All three drugs decreased hyperalgesia when administered prior to carrageenan injection. Morphine decreased the glutamate concentration in dialysates of the ventral posterolateral nucleus but did not affect the concentrations of the other amino acids. The effect of morphine was observed in the absence of painful stimulation and when pressure applied to the rat paw induced a nociceptive reaction. Riluzole decreased the concentrations of glutamate and aspartate and those of citrulline and arginine in the presence or absence of painful stimulation. These experiments suggest that morphine and riluzole attenuate the hyperalgesia induced by injection of carrageenan in the rat hind paw, at least partly, by decreasing glutamate release in the ventral posterolateral thalamic nucleus.

Keywords: Hyperalgesia; Ventral posterolateral nucleus; Excitatory amino acid; Morphine; Riluzole; Dizocilpine

1. Introduction

In recent years, considerable interest has been focused on the pathophysiological changes in the peripheral and central nervous systems in response to injury causing increased pain sensitivity or hyperalgesia. Thus, it is now accepted that long-lasting nociceptive inputs evoke sustained changes in the physiological properties of dorsal horn neurons of the spinal cord (Dubner and Ruda, 1992; Meller and Gebhart, 1994). These changes can be induced by the peripheral administration of irritative substances, such as carrageenan or formalin (Woolf et al., 1994; Coderre and Melsack, 1992). It has been suggested that the NMDA receptor is implicated in the mediation of persistent pain and hyperalgesia (Woolf, 1992; Ren et al., 1992).

Studies of the mechanisms responsible for the enhanced response have emphasized the involvement of dorsal horn neurons in central sensitization, while the role of other centers has been investigated in less detail. Kayser and Guilbaud (1984) observed that peripheral noxious stimuli evoked the excitation of a proportion of the ventrobasal thalamic neurons of the rat. These investigators drew attention to the fact that non-noxious stimulation of the inflamed joint of rats enhanced the firing of these cells; both noxious and non-noxious responses were reduced by systemic morphine administration. More recently, Sherman et al. (1997) reported that intrathecal strychnine evokes thalamic responses similar to those elicited by noxious stimuli. The actions of strychnine on the spinal processing of sensory information were reflected by changes in the receptive field and in the response properties of nociceptive ventral posterolateral thalamic neurons. These experiments suggest that sensory processing in the thalamic nuclei may be affected by mechanisms similar to those

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described in the spinal cord. The involvement of glutamate receptors in sensory processing in ventral posterolateral nuclei has been proposed by several investigators (Petralia and Wenthold, 1992; Salt, 1986; Salt and Eaton, 1989).

The aims of the present investigation were to analyze the effects of morphine, dizocilpine and riluzole on the nociceptive response induced by mechanical stimulation of the inflamed paw of the rat and to examine the changes in the concentrations of aspartate, glutamate, arginine and citrulline in dialysates of the ventral posterolateral thalamic nucleus, associated with inflammatory pain induced by carrageenan in the rat paw. Citrulline and arginine were investigated as possible indicators of metabolic changes associated with glutamate release. Morphine (Sepúlveda et al., 1998) and riluzole (Chéramy et al., 1992) are known to reduce neurotransmitter release in several nervous system structures. Therefore, the effects of systemic administration of these drugs on the concentrations of the abovementioned amino acids in dialysates of the ventral posterolateral nucleus were also studied. In the current work, microdialysis in freely moving rats and capillary zone electrophoresis with laser-induced fluorescence detection were combined to measure the changes in the ventral posterolateral nucleus.

2. Materials and methods

Male albino rats of the Wistar strain (weighing 250–300 g) from the vivarium of the Faculty of Biological Sciences of Concepción University were used in all experiments. For algesiometric experiments, rats were housed in groups of six; rats used in neurochemical experiments were individually housed. All groups received food and water ad libitum; they were maintained on a 12/12-h dark–light cycle at a temperature of 20 ± 2°C. Determinations of nociceptive and neurochemical responses were carried out in the period between 9:30 and 12:00 h. Animals were used for only one experimental condition; after the experiments, the animals were killed with an overdose of ethylether. All procedures were carried out in accordance with the institutional guidelines and with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.1. Algesiometric experiments

Mechanical nociceptive thresholds were measured by the Randall–Sellito paw pressure test (Randall and Sellito, 1957). The response to a noxious mechanical stimulus was measured with an Ugo Basili algesiometer. The hind paw of the animal was positioned over the surface of the instrument, and pressure was applied at a linearly increasing rate of 16 g/s to the dorsal surface until the rat reacted by withdrawing the limb. The pressure (g) required to elicit the response was defined as the nociceptive threshold. This threshold was determined for both paws as a baseline for subsequent comparison before the injection of one paw with carrageenan. All animals received carrageenan (30 mg/kg, in a volume of 0.2 ml) injected into the plantar surface of the right paw. Vehicle or test drugs were given s.c. 30 min before the administration of carrageenan.

The effects of drugs on pain perception are expressed as the pressures applied to induce a response 3.5 or 48 h after administration of either saline (vehicle) or the drug under study.

Control rats received saline or vehicle (10% Tween 80) in a volume similar to that used in pharmacological treatments.

2.2. Microdialysis studies

Under ketamine (2 mg/kg) plus pentobarbital (60 mg/kg) intraperitoneal anesthesia, a guide cannula consisting of 10-mm long pieces of 21-gauge stainless steel tubing was stereotaxically implanted at the following coordinates: *A*: 3.14 mm rostral to the bregma, *L*: 3.3 mm lateral to the midsagital suture, and *V*: 2.6 mm ventral to the surface of the leveled skull (Paxinos and Watson, 1986). After surgery, 200,000 units of penicillin were administered intramuscularly and at least seven postsurgical days were allowed before experiments were started.

2.3. Microdialysis probes

The probes and their recovery characteristics are described elsewhere (Hernández et al., 1986). They were made of cellulose tubing (MW 12,000 cutoff) of 200 μm outside diameter and 10 μm wall thickness. A piece of this tube was sealed at one end with an epoxy plug. The open end was attached to the tip of a piece of 26-gauge stainless steel tube. A length of fused silica capillary tubing (150 μm outside diameter and 75 μm inside diameter) with a polyimide cover was inserted into the stainless steel and cellulose tube. These probes protruded 5 mm out of the guide cannula and the efficient dialysis length (length of the dialysis section of the probe) was 4.0 mm.

2.4. Drugs and reagents

Carrageenan, acetone, naloxone, fluorescein isothiocyanate isomer I, sodium chloride, potassium chloride, magnesium chloride, sodium bicarbonate, sodium carbonate and naloxone (Sigma, Saint Louis, MO, USA); morphine hydrochloride (E. Merck, Darmstadt, Germany); riluzole and dizocilpine (Research Biochemicals International, Natick, MO, USA) were used. Drugs were dis-

solved in saline (except riluzole, which was dissolved in 10% Tween 80) and administered s.c. in a volume of 1 ml/kg.

2.5. Microdialysis sessions

For studies on microdialysis, the experiments were performed with conscious unrestrained rats. The experimental groups consisted of four rats for each drug, morphine or riluzole. Control groups received saline or 10% Tween 80. Carrageenan was administered as indicated in algesiometric experiments.

Ten dialysate samples were obtained during a 5-min period before treatments were initiated. The effects induced by carrageenan injection on the amino acid concentrations were analyzed in 10 samples collected every 30 s, starting 170 min after drug or vehicle administration. The effects of a noxious mechanical stimulus applied to the inflamed paw, with an Ugo Basili algesiometer, on amino acid concentrations in dialysates were also investigated. This stimulus was applied 3.5 h after drug, saline or vehicle administration.

The probes were connected to a syringe pump and perfused at a flow rate of 1 μl/min with Ringer's solution of the following composition (mM) NaCl 135.0, KCl 3.7, $MgCl_2$ 1.0, $CaCl_2$ 1.2 and $NaHCO_3$ 10.0, and adjusted to pH 7.4 with 0.1 N HCl. Microdialysis samples (0.5 μl total sample volume) were collected every 30 s.

2.6. Neuronal origin of amino acids

Twelve hours before the experiment, microdialysis probes were inserted in the ventral posterolateral thalamic nucleus of three rats. After collection of two baseline samples, the perfusion Ringer's solution was disconnected from the microdialysis probe and the perfusion system was thoroughly washed with calcium-free Ringer's solution. The microdialysis probe was connected to a syringe pump loaded with calcium-free Ringer, and seven more samples were collected.

2.7. Derivatization procedure

The samples were reacted with 0.2 μl of a solution composed of equal volumes of 20 mM carbonate buffer at pH 9.6 and 4×10^{-4} M fluorescein isothiocyanate in acetone. After a 16-h incubation in the dark, the samples were diluted with 9 μl of carbonate buffer and injected into a homemade capillary electrophoresis-laser induced fluorescence detection instrument. Glutamate and aspartate solutions (1 mM) were prepared as standards and 1 ml of these solutions was reacted with 20 μl of 4×10^{-4} M fluorescein isothiocyanate in acetone solution. After a 16-h incubation in the dark, this mixture was used for spiking the samples. For this purpose, 9 μl of the amino acid solution was mixed with the sample and analyzed with the capillary electrophoresis-laser induced fluorescence detection instrument.

2.8. Capillary electrophoresis

The instrument consisted of a fused silica capillary (150 μm outside diameter and 25 μm inside diameter) filled with carbonate buffer. The ends of the capillary were immersed in buffer reservoirs. Each reservoir contained a Pt–Ir wire electrode connected to a high-voltage power supply. A 5-mm window was opened in the capillary by burning the polyimide cover. The collinear detector used has been described elsewhere (Hernández et al., 1991). Briefly, the 488 nm line of a tunable Argon ion laser was reflected by a dichroic mirror and focused on the window of the capillary through a 0.85 NA microscope objective. The same objective collected the fluorescence. After appropriate filtering and with the aid of an eyepiece, the fluorescence was focused on the light-sensitive window of a model 928, Multialkali, Hamamatsu photomultiplier tube (PMT) operated at 700 V. The signal generated by the PMT was sent to a Pentium II MMX/233 MHz for data acquisition with ONICE software. The sample was hydrodynamically injected into the anodic end of the capillary by applying a −19 psi pulse of 0.2 s duration at the cathodic end. Electrophoresis was carried out at 20 kV. The peaks were identified by migration time and by spiking of the sample with standard solutions (for details, see Sepúlveda et al., 1998).

2.9. Histological studies

After the experiments, the animals were killed with an overdose of ethylether. Their brains were perfused through the heart, then dissected out, fixed in formalin for 5 days, subsequently frozen and the probe tracts were localized on the wet unstained sections by the birefringence method.

2.10. Statistical analysis

Responses to mechanical stimuli were analyzed as differences from the baseline scores prior to carrageenan injections. Data are presented as means ± S.E.M. Statistical analysis of nociceptive responses was done with the one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls multiple comparison tests. Differences with P values < 0.05 or less were considered statistically significant.

The data of neurochemical experiments were analyzed by two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor. Differences with P values < 0.05 or less were considered statistically significant. All neurochemical data are shown as means ± S.E.M. of amino acid concentrations (μM).

3. Results

3.1. Effects of morphine, dizocilpine and riluzole on the hyperalgesic response

Fig. 1 shows that pretreatment with morphine (5, 10 and 15 mg/kg) 3.5 or 48 h prior to the test reduced the hyperalgesic response. Morphine effects were statistically significant with respect to the events observed in saline-injected rats ($P < 0.01$).

Riluzole (2, 4 and 8 mg/kg) was effective in all doses assayed in controls 3.5 and 48 h after its administration (Fig. 2).

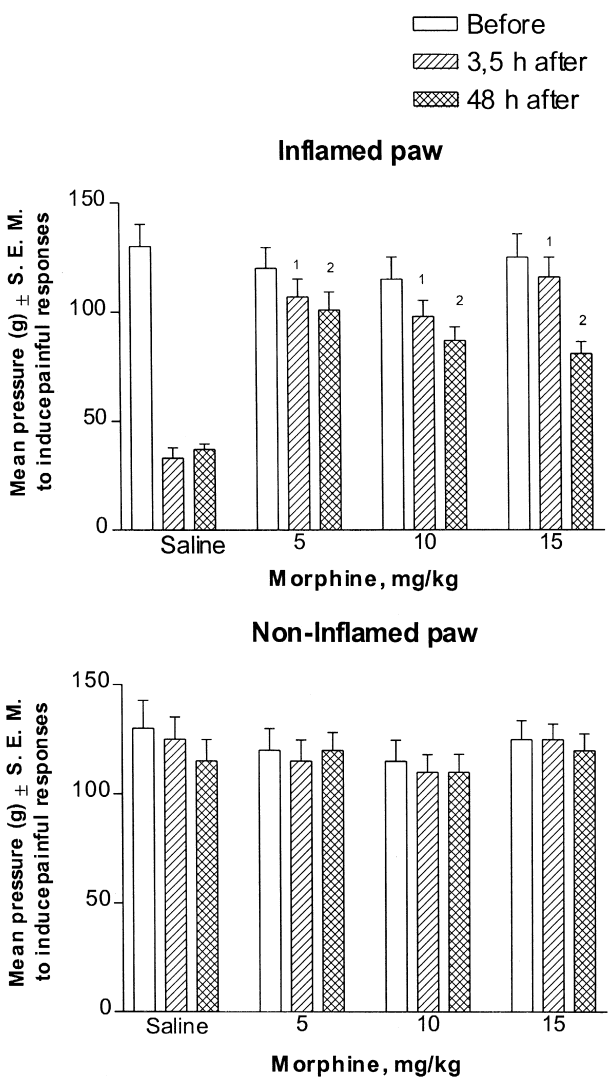

Fig. 1. Effects of morphine (s.c., 30 min before carrageenan) on mechanical pain threshold in the hind paw of rats. Results are expressed as the mean pressures (g) necessary to induce a painful response before and 3.5 or 48 h after saline or morphine administration. $n = 6$ rats per group. [1] Significantly higher than values for saline-injected rats ($F = 6.95$), $P < 0.002$. [2] Significantly higher than values for saline-injected rats ($F = 9.48$), $P < 0.001$ (ANOVA followed by the Student–Newman–Keuls multiple comparison test).

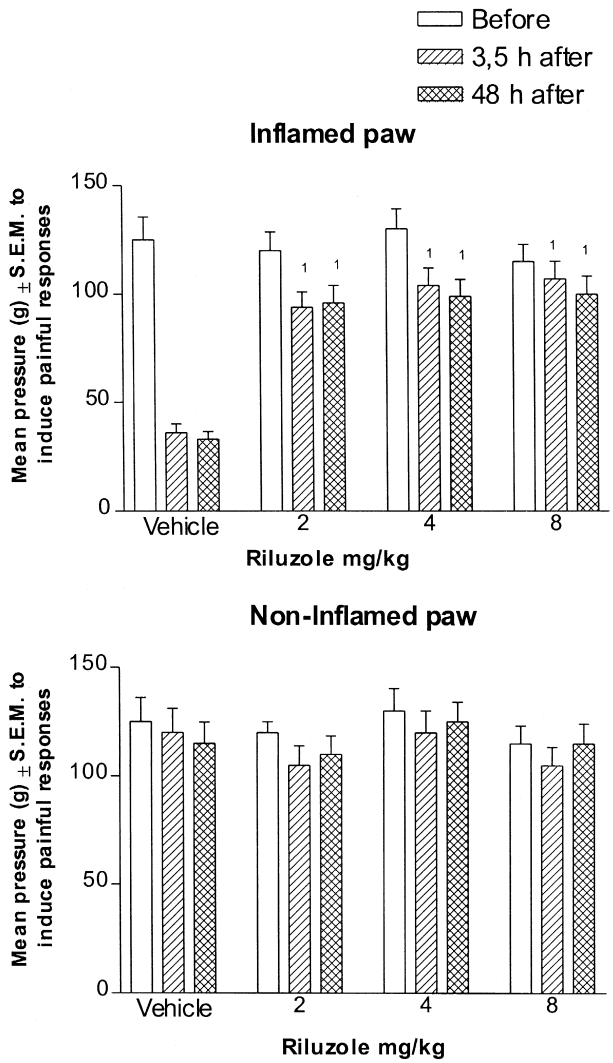

Fig. 2. Effects of riluzole (s.c., 30 min before carrageenan) on mechanical pain threshold in the hind paw of rats. Results are expressed as the mean pressures (g) necessary to induce a painful response before and 3.5 or 48 h after vehicle or riluzole administration. $n = 6$ rats per group. [1] Significantly higher than values for vehicle-injected rats (F values: 3.5 h = 3.75; 48 h = 3.98), $P < 0.001$ (ANOVA followed by the Student–Newman–Keuls multiple comparison test).

The effects of dizocilpine (0.01, 0.02, 0.04 and 0.08 mg/kg) are shown in Fig. 3. The drug was effective in controls 3.5 and 48 h after administration.

The effects observed in the inflamed paws contrasted with the absence of statistical differences in the pressure values obtained for the non-inflamed paws, indicating that the actions of the drugs under study depend on the existence of hyperalgesia.

3.2. Effects of morphine and riluzole on glutamate, aspartate, citrulline and arginine concentrations in dialysates of the ventral posterolateral nucleus of rats in the presence of hyperalgesia

Each value for the amino acids under study was calculated as percentage of the mean of 10 measurements

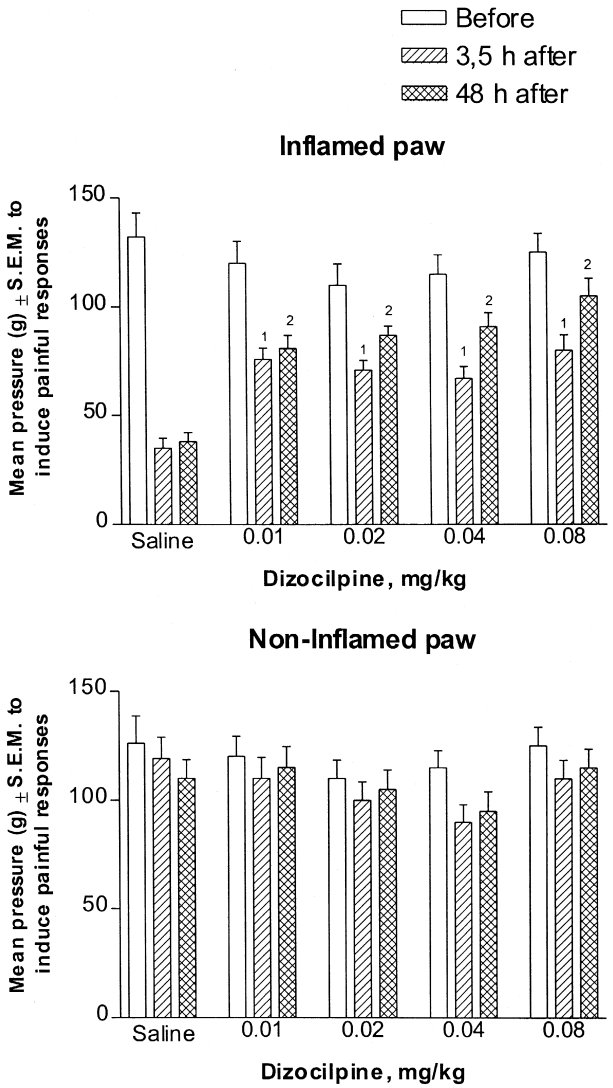

Fig. 3. Effects of dizocilpine (s.c., 30 min before carrageenan) on mechanical pain threshold in the hind paw of rats. Results are expressed as the mean pressures (g) necessary to induce a painful response before and 3.5 or 48 h after saline or dizocilpine administration. $n = 6$ rats per group. [1]Significantly higher than values for saline-injected rats (F value: 2.82), $P < 0.02$. [2]Significantly higher than values for saline-injected rats (F value: 4.68), $P < 0.001$ (ANOVA followed by the Student–Newman–Keuls multiple comparison test).

performed before treatment was started; these values are presented as micromolar concentrations of amino acids ± S.E.M. Ten samples were obtained in the course of 5 min, starting 3.5 h after morphine or riluzole administration.

To test the neural source of glutamate and aspartate, the ventral posterolateral nucleus was perfused with calcium-free Ringer's solution. Perfusion with the calcium-free solution decreased the basal concentrations of glutamate (0.48 ± 0.003 μM) and aspartate (0.46 ± 0.013 μM) by 87% and 88.9%, respectively (for glutamate, $F = 43.02$; $P < 0.0001$; and for aspartate, $F = 17.86$; $P < 0.0001$, in both cases $n = 3$).

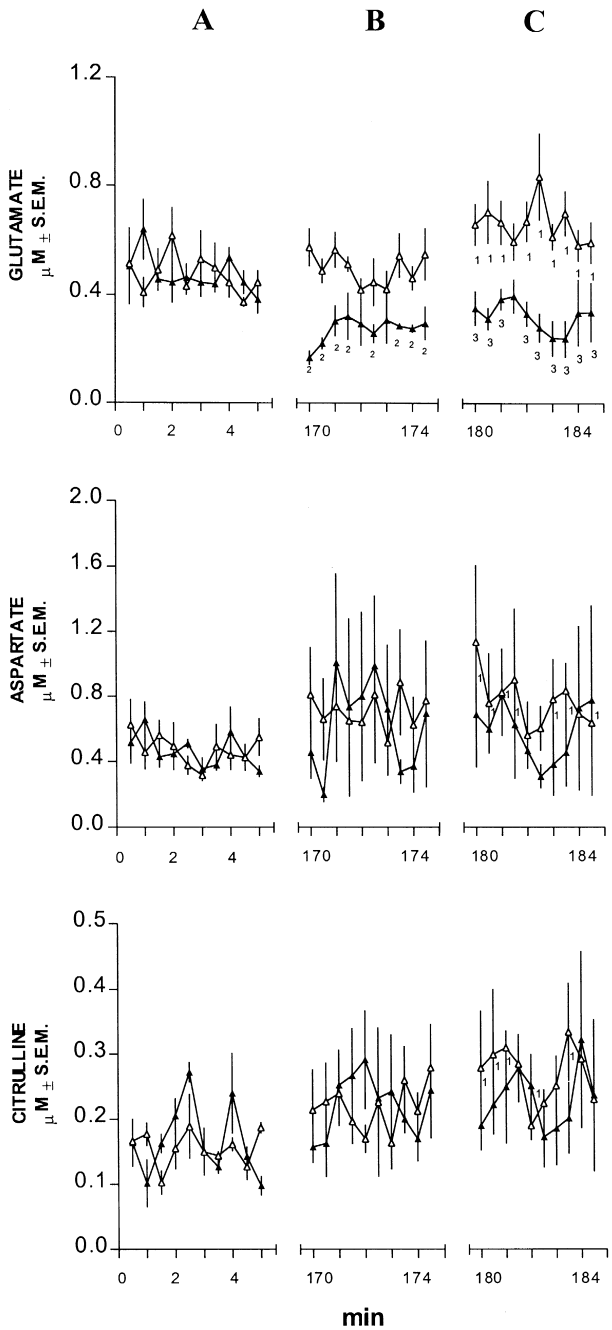

Fig. 4. Effects of morphine administration (15 mg/kg) on glutamate (top), aspartate (middle) and citrulline (bottom) concentrations in ventral posterolateral thalamic nuclei dialysates of rats. The results are expressed as micromolar concentrations ± S.E.M. Results from samples obtained during the 5-min period preceding treatments are shown in section A. Carrageenan was injected in one paw 0.5 h after morphine (filled triangles) or saline (open triangles) administration. The effects induced by carrageenan on the amino acid concentrations are shown in B and correspond to dialysates collected every 30 s (10 samples) starting 170 min after its administration. Section C corresponds to results observed during the application of painful pressure in the inflamed paw. [1]Significantly higher than the values observed in saline-injected rats in the absence of painful stimulation ($F = 24.32$), $P < 0.001$. [2]Significantly lower than the values observed in saline-injected rats ($F = 65.91$), $P < 0.001$. [3]Significantly lower than the effects observed in saline-injected rats ($F = 72.54$), $P < 0.001$ (two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor).

Application of pressure to the rat paw increased glutamate, aspartate and citrulline concentrations in dialysates of the ventral posterolateral nucleus (Figs. 4 and 5), whereas non-significant changes were observed for arginine (data not shown).

Increased glutamate and aspartate concentrations were also observed in the absence of painful stimulation as compared with the values measured in control rats.

Morphine significantly reduced the concentration of glutamate, but non-significant changes were observed in the concentrations of the other amino acids (Fig. 4). The morphine-induced decrease of glutamate release occurred both in the presence and in the absence of mechanical stimulation of the inflamed paw. Naloxone (4 mg/kg), administered immediately after morphine, antagonized the effect of the opiate on the glutamate concentration (Fig. 6).

As shown in Fig. 5, riluzole significantly reduced aspartate and glutamate concentrations in dialysates of the ventral posterolateral thalamic nucleus. Riluzole also decreased the concentrations of citrulline in the presence of mechanical stimulation of the inflamed paw (Fig. 5) and arginine. The concentration of arginine in vehicle-injected rats was 0.232 ± 0.008 μM in contrast with 0.169 ± 0.008

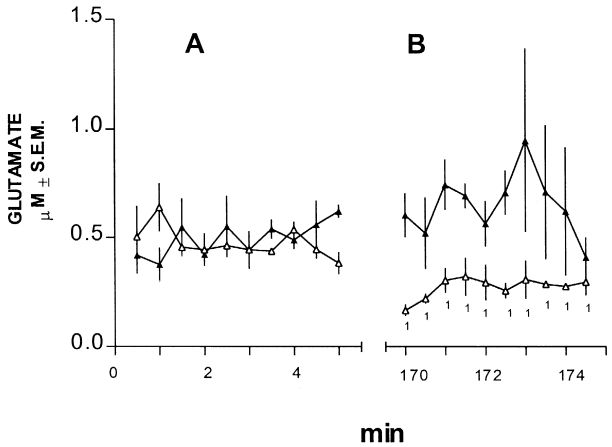

Fig. 6. Effects of morphine administration (15 mg/kg, open triangles) or morphine plus naloxone (4 mg/kg, filled triangles) on glutamate concentrations in ventral posterolateral thalamic nuclei dialysates of rats. The results are expressed as micromolar concentrations ± S.E.M. Results from samples obtained during the 5-min period preceding treatments are shown in section A. The effects induced by carrageenan injected in one paw 0.5 h after morphine or morphine plus naloxone on the amino acid concentrations are shown in B and correspond to dialysates collected every 30 s (10 samples) starting 170 min after its administration. [1]Significantly lower than the values observed in rats injected with morphine plus naloxone ($F = 60.35$), $P < 0.001$ (two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor).

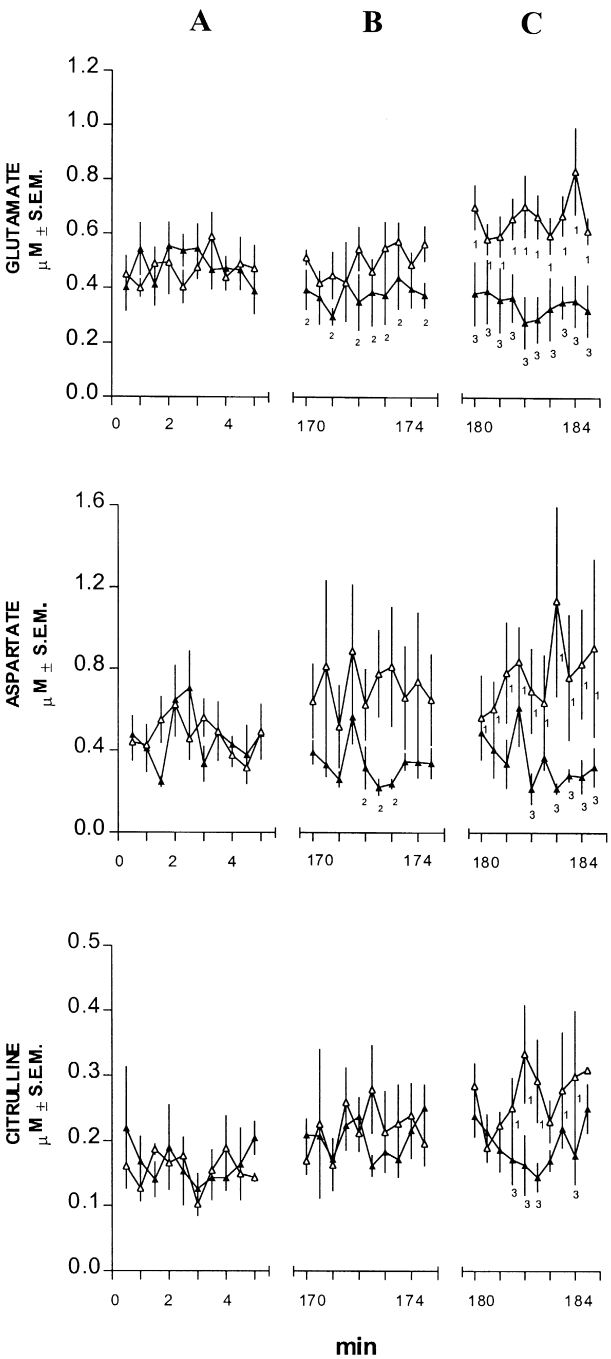

Fig. 5. Effects of riluzole administration (2 mg/kg) on glutamate (top), aspartate (middle) and citrulline (bottom) concentrations in ventral posterolateral thalamic nuclei dialysates of rats. The results are expressed as micromolar concentrations ± S.E.M. Results from samples obtained during the 5-min period preceding treatments are shown in section A. Carrageenan was injected in one paw 0.5 h after riluzole (filled triangles) or saline (open triangles) administration. The effects induced by carrageenan on the amino acid concentrations are shown in B and correspond to dialysates collected every 30 s (10 samples) starting 170 min after its administration. Section C corresponds to results observed during the application of a painful pressure in the inflamed paw. [1]Significantly higher than the values observed in vehicle-injected rats in the absence of painful stimulation ($F = 20.9$), $P < 0.001$. [2]Significantly lower than the values observed in vehicle-injected rats ($F = 51.56$), $P < 0.001$. [3]Significantly lower than the effects observed in vehicle-injected rats ($F = 63.7$), $P < 0.001$ (two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor).

μM ($F = 12.03$; $P < 0.001$) after carrageenan and 0.170 ± 0.081 after painful stimulation ($F = 16.25$; $P < 0.001$), $n = 4$.

4. Discussion

Measurements of morphine effects on chemically induced hyperalgesia were performed after a lapse of time, such that usual antinociceptive effects could not be detected, as may be observed from the results found for the non-inflamed rat paw. Furthermore, riluzole and dizocilpine did not affect pain perception by rats with non-inflamed paws. Therefore, the decrease of pain perception observed for the inflamed paw after drug treatments is thought to be induced by the reduction of central mechanisms involved in central sensitization (Woolf, 1994).

Drug effects were present in the early control, as well as 48 h after the induction of inflammation. In both controls, dose–response relationships were absent. In the case of morphine, the doses assayed were similar to those usually tested in nociceptive experiments; the other drugs were assayed in a wide range of doses. These results may indicate that the procedure for testing chemically induced hyperalgesia is not sensitive enough to accurately reflect neuronal processes involved in central sensitization. However, the possibility of having reached a ceiling effect for morphine responses cannot be discarded.

Our results indicate that, in rats, previous administration of drugs, which affects glutamate release or antagonizes the effects of the amino acid on NMDA receptors, may reduce carrageenan-induced hyperalgesia. Morphine or riluzole effects are probably due to decreased glutamate release at nerve terminals. Dizocilpine also reduced the hyperalgesic response; this effect may be attributed to its non-competitive antagonism of glutamate activation of NMDA receptors (Reynolds and Miller, 1988).

The induction of pain by mechanical stimulation of the inflamed paw revealed the hyperalgesic effect of carrageenan. The animal's responses were paralleled by neurochemical events in the ventral posterolateral thalamic nucleus: the concentrations of glutamate and aspartate are in line with their known effects in pain transmission at different levels of the central nervous system. Citrulline levels also increased, a finding that may reflect enhanced formation of nitric oxide (NO), a neurotransmitter also thought to be involved in pain transmission (Coderre and Yashpal, 1994). Kangrga and Randic (1991) reported that morphine reduces glutamate and aspartate release in the rat spinal dorsal horn in vitro. Malmberg and Yaksh (1995), using microdialysis in the spinal cord of conscious rats, have reported similar results. Both presynaptic and postsynaptic sites are involved in the opiate inhibition of amino acid release. Presynaptically, opiates inhibit calcium entry (Hori et al., 1992) and substance P release (Go and Yaksh, 1987). Opiates also inhibit postsynaptic events by activating K^+ channels, thereby leading to hyperpolarization of sensitive neurons (North et al., 1987).

It has been found that i.c.v. administration of arginine induces long-lasting antinociception in carrageenan-induced hyperalgesia (Kawabata et al., 1992). Involvement in the NO pathway has been proposed to explain the antinociceptive effect of this amino acid. Therefore, it was thought to be of interest to investigate the changes in the concentrations of arginine and citrulline as possible indicators of NO formation in the ventral posterolateral thalamic nucleus. Whereas the arginine concentration in dialysates did not change during painful stimulation of the rat paw, the concentration of citrulline was increased. In relation to citrulline, Malmberg and Yaksh (1995) observed similar results in the subarachnoid space of conscious rats after formalin injection in one hind paw.

Morphine reduced the concentrations of glutamate but not those of aspartate and other amino acids, suggesting a prevalence of the effects of the former transmitter in the painful response over the other substances investigated. The effects of morphine on glutamate release were sensitive to naloxone, indicating that this inhibition is induced through opiate receptors.

The increased amino acid release in hyperalgesia and the reduction induced by morphine and riluzole in dialysates of the ventral posterolateral nucleus of the thalamus suggest the involvement of this structure in neurogenic hyperalgesia.

The effects of riluzole differed from those of morphine in that the decrease of amino acid levels also affected aspartate and citrulline, confirming the more generalized effect of this drug on the release processes of central nervous system neurons (Chéramy et al., 1992; Martin et al., 1993). The decrease of amino acid levels was observed in the absence and in the presence of painful stimulation, which suggests that the drugs act throughout the duration of the process of chemical injury.

The results of the present investigation are in accordance with the participation of the ventral posterolateral nucleus in pain perception. In a recent paper, Sherman et al. (1997) demonstrated the role of the ventral posterolateral nucleus in the processing of somatosensory impulses in central nervous system structures, which are expressed in this nucleus by physiological changes in nociceptive and non-nociceptive neurons. In addition, Kolhekar et al. (1997) found that intrathalamic injections in rats of the NMDA receptor antagonist, D-2-amino-5-phosphonovaleric acid, reduced acute thermal and mechanical hyperalgesia. This may reflect the involvement of thalamic NMDA receptors in the hyperalgesia resulting from intraplantar injection of carrageenan in rats. Our findings confirm the participation of glutamate and other amino acids in these events.

Taken together, our results support the possibility that the ventral posterolateral nucleus plays an important role in the processing of painful stimuli and in the induction of hyperalgesia. These observations also suggest that gluta-

mate, acting through NMDA receptors, is involved in mechanical hyperalgesia. Interestingly, levels of citrulline, a possible indicator of NO production, were increased during painful stimulation of the rat paw and were reduced by riluzole administration. These effects may indicate that NO interferes in thalamic processes related to pain and hyperalgesia.

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