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Differential efficacy of intrathecal NMDA receptor antagonists on inflammatory mechanical and thermal hyperalgesia in rats

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Abstract

Spinal cord dorsal horn *N*-methyl-D-aspartate (NMDA) receptors have been implicated in central sensitization, enhanced responsiveness to peripheral stimuli following peripheral injury. Since hyperalgesia is a behavioral consequence of central sensitization, it should be attenuated at the level of the dorsal horn with NMDA receptor antagonists. However, responsiveness to thermal and mechanical hyperalgesia may be distinct, and have thus far not been directly compared in chronic inflammatory pain models. In the present study, inflammation was induced with complete Freund's adjuvant (CFA) injected into the rat hind paw and NMDA receptor antagonists dizocilpine (MK-801) or 2-amino-5-phosphonovaleric acid (AP-5) were intrathecally injected in rats to determine the effects on both mechanical and thermal hyperalgesia. Locomotor tests and reflexes were also conducted to evaluate potential motor side effects. The NMDA receptor antagonists dose-dependently ameliorated mechanical hyperalgesia, but had marginal effects on thermal hyperalgesia. In ranges near antihyperalgesic doses, significant disruption of motor coordination was observed for both antagonists. These results suggest that, depending on the stimulus, NMDA receptors may have variable significance for central sensitization-mediated hyperalgesia, and that NMDA receptor antagonists may have therapeutic potential for some, but not all components in the clinical manifestation of inflammatory pain.

Keywords: Spinal cord; Sensitization, central; NMDA (*N*-methyl-D-aspartate); Pain

1. Introduction

An acute peripheral injury may lead to long-lasting increased sensitivity to innocuous (allodynia) as well as noxious (hyperalgesia) stimuli. Following injury, inflammatory mediators are released that either sensitize or activate primary afferent nociceptors (Kidd and Urban, 2001). The sensitized and spontaneously firing nociceptors activate spinal dorsal horn nociceptive neurons or wide dynamic range neurons that receive both innocuous and noxious stimuli (Baranauskas and Nistri, 1998; Dubner and Ruda, 1992). This abnormal presynaptic activity can lead to sensitization of spinal dorsal horn neurons, which persists long after the initial injury.

It is believed that glutamate is one of the neurotransmitters that facilitates spinal dorsal horn neuron activity following injury. Application of the glutamate analogue *N*-methyl-D-aspartate (NMDA) onto rat dorsal horn neurons activates NMDA-sensitive glutamate receptors, which leads to increased neuronal excitation. In addition, NMDA-sensitive spinal neurons become highly responsive to succeeding peripheral stimulation, which is behaviorally manifested as hyperalgesia (Aanonsen et al., 1990; Hama et al., 1999; Malmberg and Yaksh, 1992; Sher and Mitchell, 1990). Inflammatory injury leads to elevated levels of glutamate in the spinal dorsal horn (Sluka and Westlund, 1992), and this is blocked by treatment with NMDA receptor antagonists (Sluka and Westlund, 1993). Studies have demonstrated that intrathecal (i.t.) NMDA receptor antagonists administered prior to peripheral nerve or inflammatory injury suppresses or delays the onset of hyperalgesia and when administered after injury can attenuate fully developed hyperalgesia (Davar et al., 1991; Mao et al., 1992; Ren et al., 1992b; Smith et al., 1994; Zhang et al., 1998). Overexpression of the NR2B subunit of the NMDA receptor leads to increased inflammatory mechanical allodynia,

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whereas knock down of spinal cord NMDA receptors with NMDA receptor antisense prevents the expression of chemically induced hyperalgesia (Garry et al., 2000; Wei et al., 2001). Thus, several lines of evidence suggest that spinal dorsal horn NMDA receptors are critical in the development and maintenance of the neural events that lead to hyperalgesia.

Although it is believed that similar mechanisms underlie injury-evoked hypersensitivities such as allodynia and hyperalgesia, recent data indicate that these behaviors are probably neurochemically distinct, particularly in the contribution of the NMDA receptor. Studies in rats with pain due to neuronal injury have suggested such a possibility (Bennett et al., 2000; Wegert et al., 1997). Since heat hyperalgesia and mechanical allodynia have distinct neural pathways, it should not be surprising that NMDA receptors differentially modulate each behavior (Ossipov et al., 2000). However, it is less certain if a differential involvement of the NMDA receptor exists between injury-evoked hyperalgesias, such as between mechanical and thermal hyperalgesia. The literature suggests that the NMDA receptor has an important role in either or both following nerve injury-induced hyperalgesia (Kim et al., 1997; Tal and Bennett, 1994). A differential involvement of the NMDA receptor may also exist in rats with hyperalgesia evoked by inflammation. Although i.t. NMDA receptor antagonists have been shown to attenuate complete Freund's adjuvant (CFA)-induced mechanical hyperalgesia and carrageenan-induced thermal hyperalgesia (Ren and Dubner, 1993; Ren et al., 1992b), the effect of i.t. NMDA receptor antagonists on both thermal and mechanical hyperalgesia in the same inflammatory model has not been evaluated.

A previous study in our laboratory indicated that a peptide NMDA receptor antagonist, [Ser^1]histogranin, attenuated CFA-evoked mechanical but not thermal hyperalgesia, suggestive of distinct mechanisms and sensitivity to NMDA receptor antagonists (Hama and Sagen, 2002). However, the lack of effect on thermal hyperalgesia may have been due in part to weak intrinsic efficacy of [Ser^1]histogranin on NMDA receptors. If NMDA receptors and central sensitization underlie mechanical and thermal hyperalgesia, it is possible that NMDA receptor antagonists with better efficacy than [Ser^1]histogranin would attenuate both mechanical and thermal hyperalgesia. In order to address this, the i.t. effects of the noncompetitive NMDA receptor antagonist MK-801 and competitive antagonist 2-amino-5-phosphonovaleric acid (AP-5) on CFA-evoked thermal and mechanical hyperalgesia were evaluated in rats.

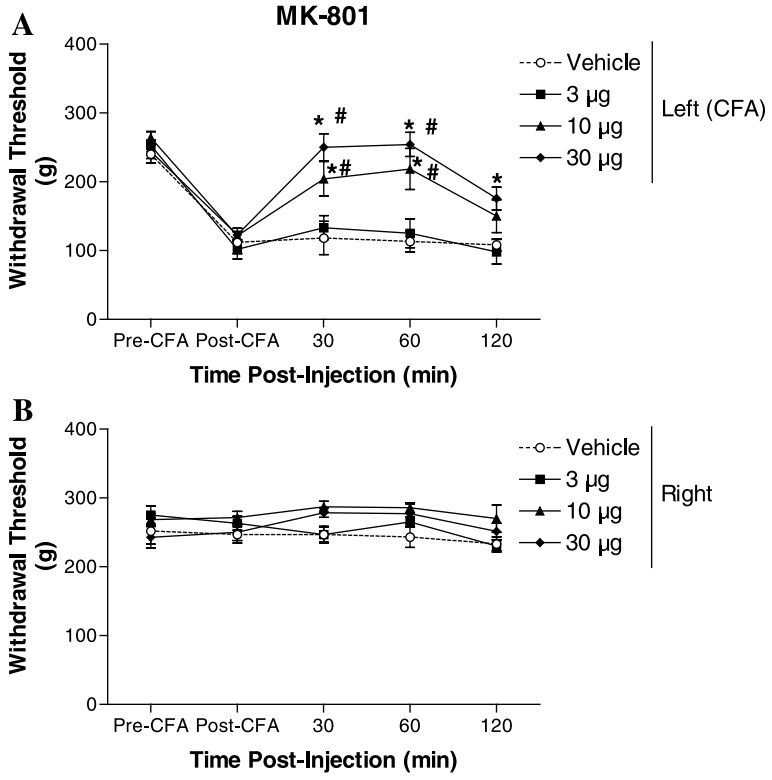

Fig. 1. Effect of intrathecal MK-801 on CFA-induced mechanical hyperalgesia. (A) Baseline thresholds (g) to increasing pressure applied to the hind paws were measured prior to injection of CFA into the left hind paw ('Pre-CFA'). Forty-eight hours after injection of CFA, thresholds were remeasured ('Post-CFA'). Rats were then intrathecally injected with either MK-801 or saline (vehicle) and tested 30, 60, and 120 min following intrathecal injection. (B) Thresholds of the non-inflamed paw. Values are expressed as mean ± S.E.M. $n = 6–7$/group. *$p < 0.05$ vs. vehicle; #$p < 0.05$ vs. Post-CFA.

2. Methods

2.1. Animals

Experimental procedures were reviewed and approved by the University of Miami School of Medicine Institutional Animal Care and Use Committee and followed the guidelines of the National Institutes of Health for the use of laboratory animals.

Male Sprague–Dawley rats (275–325 g; Charles River) were intrathecally cannulated using aseptic technique as previously described (Yaksh and Rudy, 1976). Rats were anesthetized with isoflurane in O_2 and the atlanto-occipital membrane was exposed. A PE-10 catheter was threaded down the intrathecal space, the tip resting in the region of the spinal lumbar enlargement. The catheters were flushed with saline and melted shut. Following surgery, rats were housed individually and allowed free access to food and water. Rats that exhibited abnormal motor coordination immediately following surgery were euthanized. Rats were allowed at least 3 days to recover from intrathecal surgery and used only once.

2.2. Behavioral testing

Baseline sensitivity to noxious stimuli was assessed using devices described by Randall and Selitto (1957) (noxious pressure) and Hargreaves et al. (1988) (noxious heat), both of which were obtained from Stoelting (Wood Dale, IL).

Following i.t. surgery, one group of rats ($n = 50$) was tested for sensitivity to a noxious mechanical stimulus. The rat was wrapped in a towel and the hind paw rested on a stage. A plinth was placed between the second and third metatarsals of the plantar hind paw. An increasing amount of force was applied to the paw and the rat's withdrawal from the stage-terminated application of the force. The amount of force (g) applied at the time of the withdrawal was recorded. The thresholds of the left and right paw were measured three times at each time point and the withdrawal threshold was the average of these measurements. To prevent tissue damage, a cut-off of 450 g was used.

A second group of rats ($n = 54$) was tested using the Hargreaves apparatus. Rats were placed in a plexi-glass enclosure that rested on a glass surface. An infra-red beam was shined on the plantar surface of the hind paw. The stimulus was discontinued when the rat lifted its paw away from the stimulus. The length of time between the onset of the stimulus and the hind paw withdrawal was recorded as the withdrawal latency (s). The latencies of the left and right paw were measured twice at each time point and the withdrawal latency was the average of these measurements. To prevent tissue injury, a cut-off of 20 s was used.

Following baseline determination of either withdrawal threshold or latency, 30 μl of 1 mg/ml complete Freund's

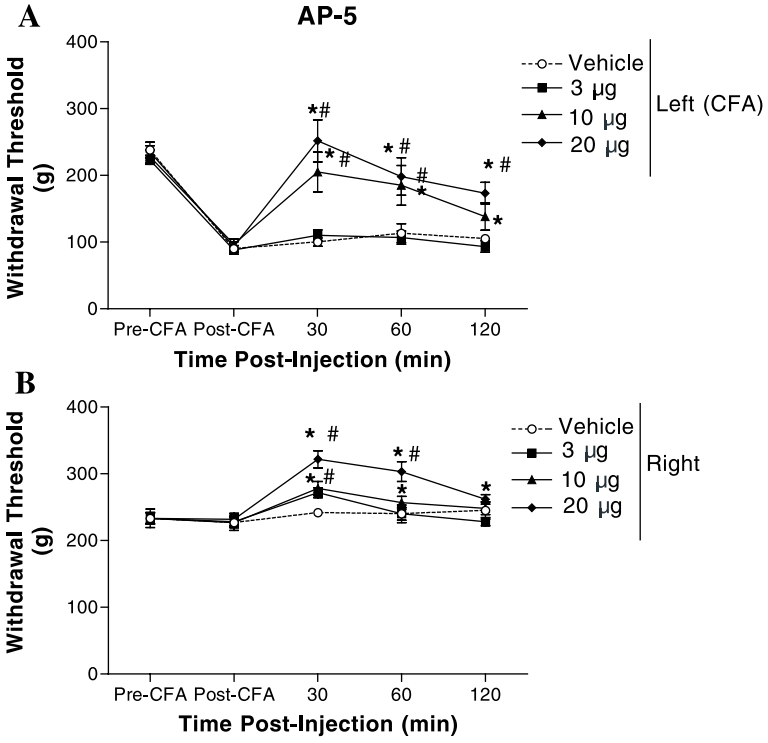

Fig. 2. Effect of intrathecal AP-5 on CFA-induced mechanical hyperalgesia. (A) Baseline thresholds (g) to increasing pressure applied to the hind paws were measured prior to injection of CFA into the left hind paw ('Pre-CFA'). Forty-eight hours after injection of CFA, thresholds were re-measured ('Post-CFA'). Rats were then intrathecally injected with either AP-5 or vehicle and tested 30, 60, and 120 min following intrathecal injection. (B) Thresholds of the non-inflamed paw. Values are expressed as mean ± S.E.M. $n = 6$/group. *$p < 0.05$ vs. vehicle; #$p < 0.05$ vs. Post-CFA.

adjuvant (CFA; Sigma, St. Louis, MO) was subcutaneously injected into the plantar left hind paw. Rats were re-tested for thermal and mechanical responses 48 h after CFA injection. Animals were then i.t. injected with either 3, 10, 20 μg of (D,L)-2-Amino-5-phosphonovaleric acid (AP-5), 3, 10, 30 μg of dizocilpine maleate ((+)-MK-801) or saline and again tested for thermal withdrawal latencies or mechanical thresholds at 30, 60 and 120 min following i.t. injection.

In order to determine whether efficacious doses of i.t. NMDA receptor antagonists resulted in potentially interfering motor side effects, a separate group of non-inflamed rats was evaluated for motor reflexes and motor coordination. Reflex function was assessed as described previously (Coderre and Van Empel, 1994; Hama and Sagen, 2002; Siegan and Sagen, 1998). Baseline responses, and responses 30 min following the i.t. injection of either MK-801 (7.5, 15, or 30 μg), AP-5 (3, 10, or 20 μg) or saline vehicle were determined in animals with i.t. catheters. NMDA receptor antagonist doses and saline were rotated such that all animals were tested using each dose, separated by at least 48 h between injections ($n = 6–9$/dose). The righting reflex was determined by placing the rat on its back on a flat surface and recording whether the rat immediately assumes the normal upright position. To assess the placing reflex, the animal was elevated slightly above a table and the dorsal surface of the hind paw gently brought into contact with the surface. A positive reflex occurs when the hind paw is extended and placed on the table surface. To assess the grasping reflex, the rat is placed on a wire grid, and observed for grasping of the hind paws to the contacted wire. Five trials of each reflex test were given (alternating between sides), and animals were scored based on the number of normal reflex responses obtained. Motor coordination was assessed in separate groups of rats using the accelerating rotorod. Drugs, doses, and scheduling were the same as described for the reflex testing ($n = 6–9$/dose). For 3 days prior to i.t. injections, rats underwent training trials on the rotorod apparatus to acclimate them to the test, until they were able to remain on the rotorod for at least 60 s with speed increasing from 5 to 10 rpm over a 60-s period. Baseline latencies to fall off the rotorod and latencies 30 min following i.t. injections at three accelerations (from 5 to 10, 5 to 20, and 5 to 30 rpm over 60 s; 40 s intertrial intervals) were recorded.

2.3. Drugs

(D,L)-2-amino-5-phosphonovaleric acid was obtained from Tocris (Ballwin, MO) and MK-801 was obtained from Sigma. Drugs were dissolved in saline and injected in a volume of 10 μl, followed by a 10-μl saline flush.

2.4. Statistical analysis

The data are presented as mean ± S.E.M. The effects of drug treatment and dose comparisons over time were analyzed using a two-way repeated measure ANOVA. A Student–Newman–Keuls test was used as a pos-hoc test. Statistical significance was at taken at $p < 0.05$. The A_{50} value (dose at which antinociception is 50% of maximal) was determined from the linear regression of the maximum possible effect (MPE) on hyperalgesia. To calculate MPE:

$$(\text{Response}_{\text{post-drug}} - \text{Response}_{\text{baseline}}) / (\text{Cut} - \text{off} - \text{Response}_{\text{baseline}}) \times 100,$$

where $\text{Response}_{\text{baseline}}$ is the pre-drug injection threshold or latency and $\text{Response}_{\text{post-drug}}$ is the threshold or latency following drug injection. The A_{50} value was calculated using a modified computer program (Tallarida and Murray, 1987).

3. Results

3.1. Effect of NMDA receptor antagonists on mechanical hyperalgesia

Prior to hind paw injection of CFA, mean (± S.E.M.) withdrawal thresholds to noxious pressure were 241.4 ± 4.5

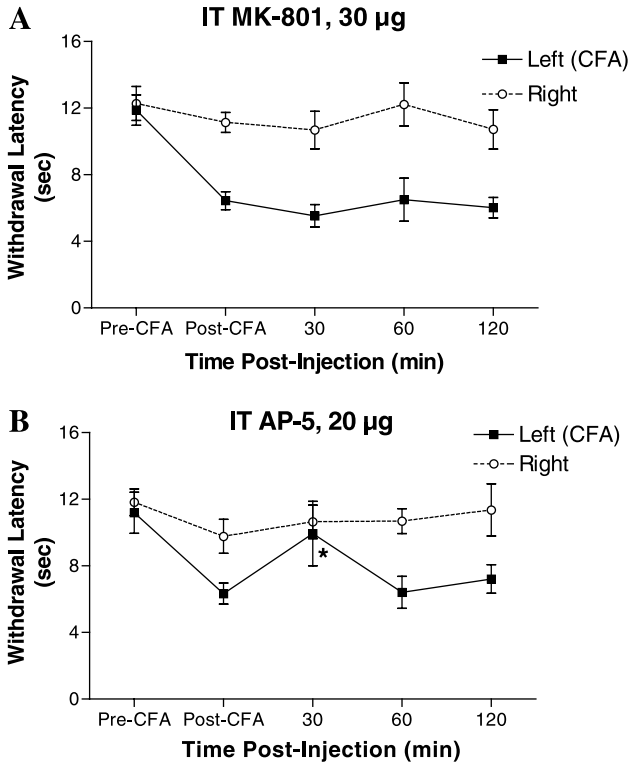

Fig. 3. Effect of intrathecal NMDA receptor antagonists on CFA-induced thermal hyperalgesia. Baseline latencies (s) to a thermal stimulus applied to the hind paws were measured prior to injection of CFA into the left hind paw ('Pre-CFA'). Forty-eight hours after injection of CFA, latencies were re-measured ('Post-CFA'). Following baseline measurement, rats were intrathecally injected with either 30 μg MK-801 (A) or 20 μg AP-5 (B) and tested 30, 60, and 120 min following intrathecal injection. Values are expressed as mean ± S.E.M. $n = 7–12$/group. * $p < 0.05$ vs. Post-CFA.

and 249.1 ± 4.6 g for the left and right paw, respectively. The mean withdrawal threshold of the left hind paw 48 h after injection of CFA was significantly decreased (104.0 ± 3.7 g) compared to the uninjected right paw (247.6 ± 4.6 g) and Pre-CFA threshold ($p < 0.05$; Fig. 1). Intrathecal injection of MK-801 dose-dependently increased withdrawal thresholds of the CFA-injected paw at 30, 60 and 120 min post-injection. In contrast, i.t. injection of saline did not significantly affect the withdrawal thresholds of either the left of right hind paw. MK-801 did not significantly increase thresholds of the uninflamed paw.

Intrathecal injection of AP-5 dose-dependently increased withdrawal thresholds of the CFA-injected paw at 30, 60 and 120 min post-injection (Fig. 2). In contrast, i.t. injection of saline did not significantly affect the withdrawal thresholds of either the inflamed or uninflamed hind paw. In contrast to MK-801, AP-5 significantly increase thresholds of the uninflamed paw, though not to the same extent as the CFA-injected paw ($p < 0.05$).

The A_{50} (95% confidence interval) of MK-801 and AP-5 30 min after i.t. injection for reducing mechanical hyperalgesia was 6.5 (4.5–9.5) μg and 7.8 (4.0–15.2) μg, respectively.

3.2. The effect of NMDA receptor antagonists on thermal hyperalgesia

Prior to CFA injection, mean withdrawal latencies of the left and right hind paw to a noxious thermal stimulus were 11.93 ± 0.6 and 12.36 ± 0.7 s, respectively (Fig. 3). Forty-eight hours after CFA injection, the latency of the CFA-injected paw was significantly decreased (6.1 ± 0.4 s) compared to the uninflamed paw (11.4 ± 0.6 s; $p < 0.05$). Intrathecal injection of MK-801 up to 30 μg (lower doses not shown) did not significantly alter withdrawal latencies of either the inflamed or uninflamed hind paw. Intrathecal injection of 20 μg of AP-5 significantly increased withdrawal latency of the inflamed paw, compared to Post-CFA

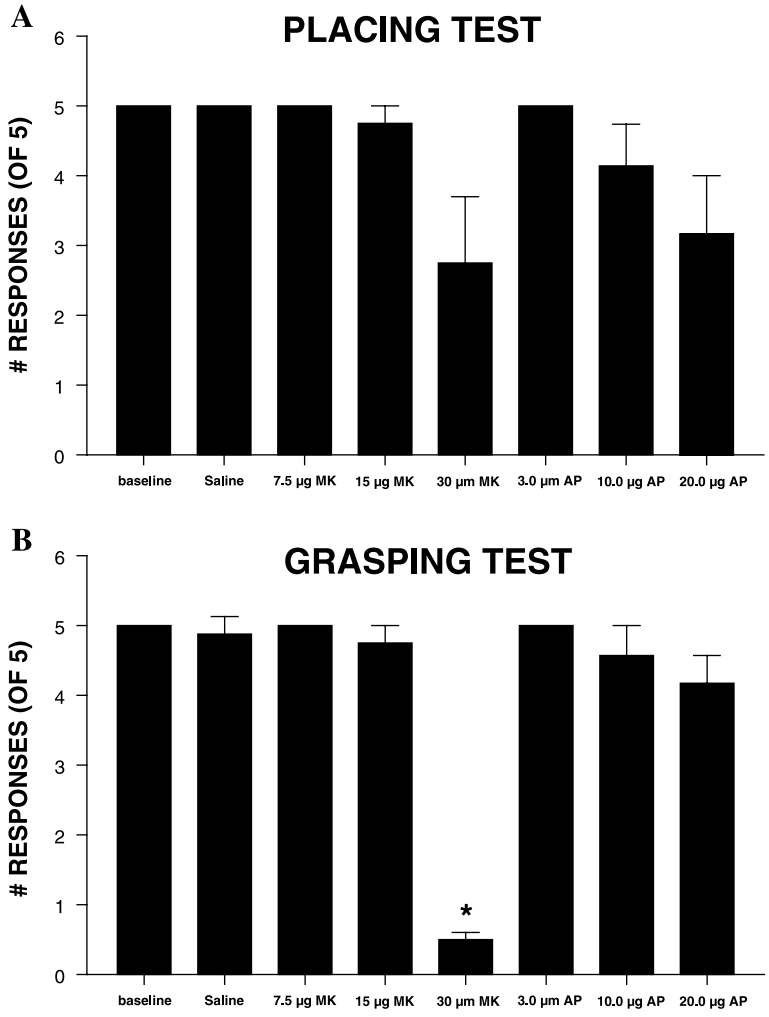

Fig. 4. Effect of intrathecal NMDA receptor antagonists on motor reflexes. The number of positive reflex responses (out of five trials) are shown for the placing test (A) and grasping test (B). Baseline responses and responses 30 min following intrathecal injections of saline vehicle, MK-801 (MK; 7.5, 15, and 30 μg), or AP-5 (AP; 3, 10, or 20 μg) were determined. Values expressed as mean ± S.E.M. $n = 6–9$/group. *$p < 0.05$ vs. saline.

and vehicle-treated rats, but only transiently at 30 min post-injection ($p < 0.05$). Lower doses of AP-5 had no effect (not shown). Intrathecal injection of vehicle did not alter withdrawal latencies (data not shown).

3.3. Side effect profile of NMDA receptor antagonists

Motor reflexes were largely unaltered by i.t. injections of NMDA receptor antagonists at the doses utilized, with the exception of the highest dose of MK-801 (Fig. 4). Righting reflexes were not disrupted in any of the animals following i.t. injections (data not shown). The 30 μg dose of MK-801 significantly disrupted grasping reflexes, and reduced positive placing responses, but the latter was not statistically significant. Motor coordination, as assessed by the accelerating rotorod, was significantly disrupted by 15 and 30 μg MK-801 (Fig. 5). The 30 μg dose severely disrupted rotorod performance at all three accelerations. Similarly, rotorod performance was significantly reduced by 10 and 20 μg AP-5, noted at all three accelerations, and even by 3 μg at the highest acceleration.

4. Discussion

The potential therapeutic usefulness of NMDA receptor antagonists in the management of persistent pain has been predicted by their anti-hyperalgesic effects in several animal models. However, findings are often variable and inconsistent, due to variations in drugs, routes of administration, timing of administration (preemptive vs. reversal), models employed (nerve injury vs. inflammation), and quality of sensory stimulus (thermal vs. mechanical). Few studies have attempted to directly compare NMDA receptor antagonist

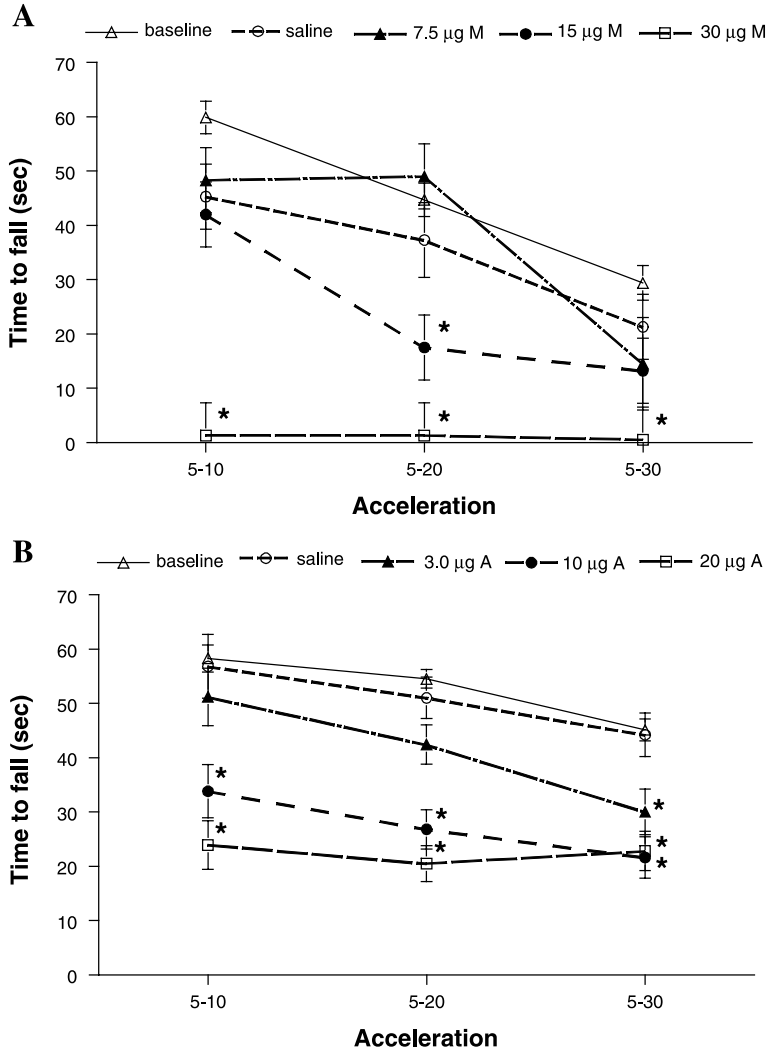

Fig. 5. Effect of intrathecal NMDA receptor antagonists on motor coordination as assessed by the accelerating rotorod. The latency to fall from the rotorod at three accelerations (5–10, 5–20, and 5–30 rpm over 60 s) was recorded electronically. (A) Baseline responses and responses 30 min following intrathecal injections of saline vehicle or MK-801 (M; 7.5, 15, and 30 μg) are shown. (B) In a separate group of animals, baseline responses and responses 30 min following intrathecal injections of saline vehicle or AP-5 (A; 3, 10, or 20 μg) are shown. Values expressed as mean ± S.E.M. $n = 6–9$/group. *$p < 0.05$ vs. saline.

effects on mechanical vs. thermal hyperalgesia in the same model. In a previous study in our laboratory, CFA-induced inflammatory mechanical hyperalgesia but not thermal hyperalgesia was sensitive to the peptide NMDA receptor antagonist [Ser[1]]histogranin (Hama and Sagen, 2002). It is possible that the lack of efficacy with [Ser[1]]histogranin was due in part due to its weak intrinsic efficacy and its noncompetitive antagonist properties. Thus, the goal of the present study was to directly compare mechanical and thermal antinociceptive effects in established inflammatory pain using NMDA receptor antagonists that have exhibited efficacy in other pain models.

Results showed that i.t. injection of NMDA receptor antagonists MK-801 or AP-5 reversed mechanical hyperalgesia in a dose-dependent manner. However, the highest dose of MK-801, which attenuated mechanical hyperalgesia, did not significantly affect thermal hyperalgesia. Intrathecal AP-5 reversed thermal hyperalgesia, but transiently, and only at the highest dose tested. These findings indicate, using the same post-inflammation timing, route of administration, and single dosing for comparisons, that NMDA receptor antagonists are more efficacious in reducing mechanical than thermal hyperalgesia in persistent inflammation, and suggest that inflammatory mechanical hyperalgesia is mediated through spinal dorsal horn NMDA receptors, but these may have less of a role in inflammatory thermal hyperalgesia. The data also suggest that thermal and mechanical inflammatory hyperalgesia may have distinct mechanisms and that attaining efficacy with NMDA receptor antagonists may depend on the stimulus.

The currently accepted hypothesis is that spinal cord NMDA receptors are critical in the maintenance of hyperalgesia, and would predict NMDA receptor antagonist efficacy irrespective of stimulus type or etiology. However, our data would suggest that such a hypothesis should be re-evaluated using various stimuli and pain etiologies, as the clinical usefulness of NMDA antagonists is also likely to vary depending on pain conditions. The reason for the lack of effect on thermal hyperalgesia observed in the current study and the varied responsiveness of NMDA receptor antagonists described in the literature is not clear. Possibilities include distinct pharmacologic mechanisms underlying inflammation-induced mechanical and thermal hyperalgesia, differing stimulus intensities produced by thermal and mechanical tests employed as experienced by the inflamed paw, or a combination of these.

The literature suggests different degrees of involvement of the spinal NMDA receptor in persistent hypersensitive states depending on the etiology of the injury. One of the goals of our study was to compare responses to differing noxious stimuli (thermal vs. mechanical) in the same model. Similar systematic comparisons have been made in other models. These have also reported that different stimuli are differentially sensitive to spinal NMDA receptor blockade (i.e. intrathecal delivery). Spraggins et al. (2001) observed that zymosan-induced thermal hyperalgesia but not mechanical hyperalgesia was sensitive to spinally applied AP-5. There is also an apparent differential pharmacology in models of neuropathic pain. Bennett et al. (2000) demonstrated that intrathecal AP-5 was not effective on thermal allodynia, but the same doses of AP-5 reversed mechanical allodynia in a rat model of spinal cord injury pain. On the other hand, Wegert et al. (1997) noted opposite effects in nerve-injured rats, that an intrathecal dose of MK-801 that fully reversed thermal hyperalgesia had no effect on mechanical allodynia. A similar lack of robust anti-allodynic effect of intrathecal MK-801 has also been reported (Chaplan et al., 1997). In other studies, following sciatic nerve ligation, thermal and mechanical hyperalgesia were both attenuated with an intrathecal NMDA receptor antagonist (Siegan et al., 1997; Sonoda and Omote, 1998; Yamamoto and Yaksh, 1992).

In particular, thermal hyperalgesia in the chronic constriction nerve injury model of neuropathic pain appears to be sensitive to most competitive and non-competitive NMDA receptor antagonists evaluated (Davar et al., 1991; Eisenberg et al., 1995; Mao et al., 1993; Tal and Bennett, 1993). In contrast, NMDA receptor antagonists can decrease mechanical hyperalgesia and mechanical allodynia in a tight ligation model of neuropathic pain (Carlton and Hargett, 1995; Chaplan et al., 1997; Qian et al., 1996). In tissue injury models of post-operative pain, i.t. NMDA receptor antagonists had no effect on mechanical hyperalgesia when injected either before or after the injury (Pogatzki et al., 2000; Zahn and Brennan, 1998). Clearly, the degree of involvement of the NMDA receptor in the various pain models is not the same.

This also appears to be the case in inflammatory pain models. Using the carrageenin model of acute inflammation, intrathecally administered NMDA receptor antagonists reduced thermal hyperalgesia (Ren et al., 1992a,b), while in the CFA model, mechanical hyperalgesia was attenuated by i.t. NMDA receptor antagonists (Ren and Dubner, 1993). Although the authors demonstrated that increasing doses of MK-801 administered systemically could reduce CFA-induced thermal hyperalgesia, intrathecal MK-801 was not evaluated on established (i.e. 24–48 h Post-CFA injection) thermal hyperalgesia in the CFA model. The effects of systemically administered MK-801 leaves open the possibility that these may be mediated via peripheral NMDA receptors or at higher CNS centers. In another related study, Zhang et al. (1998) utilized intrathecal MK-801 in the CFA model. However, in this case, MK-801 was administered preemptively the day preceding CFA injection and continuously infused intrathecally throughout the 24–72 h testing period. The authors observed reduced thermal hyperalgesia, but this effect was marginal (increased withdrawal latencies of the inflamed paw by about 1–2 s) and significant thermal hyperalgesia was still observed in these treated animals compared with thermal latencies of the non-inflamed hind paws, a finding which tends to support findings of weak effects observed in the present study. Similarly, intrathecal injection of cumulative doses of 0.3–92.2 nmol of MK-801

increased withdrawal latencies of the inflamed paw, but this effect was small (only about 1 s at 30 nmol), and did not return thermal withdrawal thresholds near baseline (Ren et al., 1994). Finally, in a recent study in our laboratory using the CFA model, mechanical hyperalgesia but not thermal hyperalgesia was reversed following intrathecal injection of the endogenous NMDA receptor antagonist histogranin (Hama and Sagen, 2002). In contrast, intrathecal morphine reversed both mechanical and thermal hyperalgesia (Hama and Sagen, 2002). Based on these previous findings and the present study, the data suggests that NMDA receptors have a minor role in the mechanisms that underlie CFA-induced thermal hyperalgesia.

In the present study, intrathecal injection of the potent noncompetitive antagonist MK-801 was not effective on thermal hyperalgesia, while the competitive antagonist AP-5 suppressed CFA-evoked thermal hyperalgesia, but only transiently and the effect was not dose-dependent. Nevertheless, this raises the possibility that the mechanism of receptor blockade of an antagonist underlies its efficacy on hyperalgesia. AP-5 was shown to be more potent than MK-801, and other noncompetitive NMDA receptor antagonists, on neuropathic mechanical allodynia (Chaplan et al., 1997). Similarly, a competitive NMDA receptor antagonist LY-235959 was about 10 times more potent than MK-801 in blocking i.t. NMDA-induced pain related behaviors in mice (Fairbanks et al., 2000).

Another possibility that may underlie the lack of effect of NMDA receptor antagonist on thermal hyperalgesia in this study is the stimulus intensity experienced by the inflamed paw. It is possible that sensitized primary afferents are more responsive to heat than mechanical stimuli, which would lead to a greater level of spinal neuron activity. Thus, increased spinal neuron activity due to a heat stimulus may be more difficult to treat with an NMDA receptor antagonist.

Other spinal cord neurotransmitter systems also show differential efficacy in the same model, depending on stimulus type. In the injured state, opiates (Bian et al., 1995), substance P (Kuraishi et al., 1991a; Zheng and Chen, 2001), galanin (Kuraishi et al., 1991b) and catecholamines (Esser and Sawynok, 1999) have been shown to have "preferential" effects depending on the stimulus. In rats with an inflammation, intrathecal lamotrigine attenuated tactile allodynia but not thermal hyperalgesia (Lee et al., 2002). Spinal NK1/2 receptors are involved in the thermal hyperalgesia but not mechanical hyperalgesia caused by hind paw bee venom injection (Zheng and Chen, 2001). The spinal or medullary dorsal horn pharmacology of uninjured animals also suggests a preference in stimulus type (Hughes and Barr, 1988; Salt and Hill, 1983). Thus, the finding that different stimuli may be differentially sensitive to a particular drug should not be surprising.

The present study also suggests the potential for significant motor dysfunction associated with therapeutic dose levels of i.t. NMDA receptor antagonists. Motor effects, including agitation, motor weakness or hind limb paralysis, disruption of righting, grasping, and placing reflexes have been previously reported by other laboratories using a variety of NMDA receptor antagonists and doses (Chaplan et al., 1997; Coderre and Van Empel, 1994; Ren et al., 1992b). Although dosages used in the present study did not substantially alter motor reflexes with the exception of the highest MK-801 dose, motor coordination as assessed by the rotorod, was significantly disrupted in the anti-hyperalgesic dose ranges of both antagonists. This contrasts with previous findings in our laboratory using the weak NMDA receptor antagonist peptide, [Ser^{1}]histogranin, which attenuated mechanical hyperalgesia in the absence of motor dysfunction (Hama and Sagen, 2002). These findings suggest that more potent NMDA antagonists may have a narrow therapeutic index, and unwanted side effects can occur in the same dosage ranges as anti-hyperalgesic effects.

In summary, the results of the present study suggest that, depending on the type of noxious stimulation, the injury-induced hyperalgesias are pharmacologically distinct. The currently accepted hypothesis that spinal cord NMDA receptors are critical in the maintenance of hyperalgesia, irrespective of stimulus type or etiology, is not supported by these findings and should be re-evaluated using various stimuli and pain etiologies. This presents the clinician with the potential problem of keeping an array of drugs to treat the various hyperalgesias, as clinical usefulness of NMDA antagonists is also likely to vary depending on pain conditions. Development of effective treatments for the various hyperesthesias that occur following injury will depend on better understanding of the neuronal events that occur following injury.

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