Spinal NMDA Receptor Involvement in Expansion of Dorsal Horn Neuronal Receptive Field Area Produced by Intracutaneous Histamine

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Jinks, Steven L. and E. Carstens. Spinal NMDA receptor involvement in expansion of dorsal horn neuronal receptive field area produced by intracutaneous histamine. J. Neurophysiol. 79: 1613±1618, 1998. Histamine elicits the sensation of itch at the site of skin application as well as alloknesis (itch elicited by innocuous mechanical stimuli) in a surrounding area in humans and expansion of the low-threshold mechanosensitive receptive field area of spinal wide dynamic range (WDR)-type dorsal horn neurons in rats. We presently tested if the histamine-evoked expansion of neuronal receptive field area depends on a spinal N-methyl-D-aspartate (NMDA) receptor-mediated process. In pentobarbital sodium±anesthetized rats, mechanical receptive field areas of single WDR-type dorsal horn neurons were mapped with graded von Frey filaments before and 10 min after intracutaneous (ic) microinjection of histamine (1 μl; 1, 3, or 10%) at a low-threshold site within the receptive field. Intracutaneous microinjection of histamine evoked dose-related increases in firing rate, as well as a dose-dependent expansion in mean receptive field area 10 min after 3 and 10%, but not 1%, histamine doses. When a noncompetitive or competitive NMDA receptor antagonist dizocilpine [MK-801; d(-)-2-amino-5-phosphonovalerate (APV), respectively; 1 μM] was first applied topically to the surface of the spinal cord, there was no significant change in mean receptive field area after ic microinjection of 10% histamine. The mean neuronal response to histamine in the presence of spinal MK-801 or APV was not significantly different from spinal NMDA receptors are involved in histamine-induced expansion of mechanical receptive field area, a neural event possibly involved in the development of alloknesis.

INTRODUCTION

In humans histamine elicits a sensation of itch at the site of intracutaneous (ic) injection (Simone et al. 1987, 1991; Yosipovitch et al. 1996) or iontophoretic application (Handwerker et al. 1991; Magerl and Handwerker 1988; Ward et al. 1996), a flare, and alloknesis (itchy skin) in a surrounding area in which innocuous mechanical stimuli evoke or intensify itch sensation (Simone et al. 1987, 1991). Alloknesis is thought to be mediated, at least partly, through activity-dependent central sensitization similar to the development of secondary hyperalgesia and allodynia in response to pain-producing stimuli (LaMotte 1992; Simone et al. 1991). Many studies have shown that wide dynamic range (WDR) and nociceptive-specific dorsal horn neurons become sensitized after noxious stimulation or injury. Sensitization is characterized by a decrease in threshold and an increase in response duration and magnitude to noxious stimuli and an expansion of the mechanosensitive receptive field of dorsal horn neurons (for reviews, see Dubner and Melzack 1987; Coderre et al. 1993; Dickenson et al. 1997; Dubner 1991; Woolf 1995, 1996). These events are thought to be mediated through activation of the N-methyl-D-aspartate (NMDA) receptor-channel complex by excitatory amino acids (EAA) (Coderre and Melzack 1992). Intrathecal application of NMDA decreases nociceptive withdrawal latencies (Kolhekar et al. 1993) and flexor reflex thresholds (Ma and Woolf 1995). Furthermore, competitive and noncompetitive NMDA receptor antagonists prevent or diminish behavioral hyperalgesia (Chapman et al. 1997; Coderre and Melzack 1992; Eisenberg et al. 1994; Ren et al. 1992, 1996; Vaccarino et al. 1993) and sensitization of dorsal horn neurons (Chapman and Dickinson 1994; Dickinson and Sullivan 1990; Dougherty et al. 1992; Ren et al. 1992) and prevent and reverse the expansion of dorsal horn neuron receptive fields during inflammation (Ren et al. 1992).

Recent studies indicate that a large proportion of primate spinothalamic tract (Li et al. 1995) and WDR neurons (Carstens 1997) responds to ic injection of histamine as well as algic chemicals such as capsaicin. After ic histamine, there was a significant increase in the area of low-threshold mechanosensitive receptive fields of WDR neurons (Carstens 1997). In the present study, we tested the hypothesis that spinal NMDA receptors are involved in the expansion of dorsal horn neuronal mechanosensitive receptive field area evoked by ic histamine, analogous to the role of spinal NMDA receptors in central sensitization of spinal neurons by noxious peripheral stimuli. This was accomplished by measuring changes in dorsal horn neuronal receptive field area after three different doses of histamine and by determining if receptive field expansion was prevented by prior spinal application of competitive [d(-)-2-amino-5-phosphonovalerate (APV)] and noncompetitive dizocilpine (MK-801) NMDA receptor antagonists.

METHODS

Surgical and recording methods

Electrophysiological experiments were conducted using 32 adult male Sprague-Dawley rats (371 to 550 g). Anesthesia was induced with pentobarbital sodium (65 mg/kg ip) and maintained by constant infusion of pentobarbital (9–14 mg·kg⁻¹·h⁻¹ iv). Body temperature was maintained between 36 and 38°C with a lamp and a heating pad. The lumbar enlargement of the spinal cord was exposed by laminectomy and fixed rigidly with vertebral clamps placed rostral and caudal to the exposed area. The dura was retracted, and agar was used to form a pool over the recording area, which was filled with warmed saline. A Teflon-coated tungsten microelectrode (Frederick Haer, 10 MΩ) was then advanced into the dorsal horn to record extracellular single-unit activity, which

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was amplified and displayed by conventional means. Unit activity was digitized and fed to a computer for storage, display (in stimulus-time histogram format) and analysis using the Spike software (Forster and Handwerker 1990).

Unit selection and receptive field mapping

Single units were isolated using innocuous mechanical stimulation of the plantar surface of the ipsilateral hind paw. Only those that additionally responded to noxious heat (50°C, 5 s) were studied further; these correspond to the multireceptive or WDR type. Mechanosensitive receptive fields were mapped onto scale drawings of the ventral hind paw as isothreshold contours (Carstens 1997). Three von Frey filaments (0.7, 1.5, and 4 g bending force), categorized as low, medium, and high threshold, were used to map the receptive field of each cell. The rationale was to test if there was a relationship between bending force of the stimulus and degree of histamine-evoked receptive field expansion. These bending forces activate low-threshold mechanoreceptors but not mechanical nociceptors; forces >4 g were not used presently because our earlier study (Carstens 1997) indicated that ic histamine did not cause expansion of receptive fields mapped with von Frey hairs of higher bending force. With each filament, the area within which the stimulus always elicited a response was mapped; at the perimeter of this area stimuli elicited a response on approximately one-half of trials. Most units exhibited little or no (0–3 Hz) spontaneous activity, so that it was straightforward to map receptive field areas in this manner. Mechanical receptive field mapping was performed during a 3–5 min period.

Intracutaneous microinjection

Histamine (1, 3, or 10% in 0.9% NaCl; Sigma) or vehicle (0.9% NaCl) was injected ic in a 1 μl volume through a 30.5-gauge needle inserted 1–2 mm into the skin, parallel to the surface and just beneath the epidermis (Carstens 1997). Because the needle insertion evoked an increase in unit firing rate, all microinjections were made well (>10 min) after unit activity had returned to the level before needle insertion. Spontaneous activity for each unit was recorded for ≥30 s before a microinjection and ≥90 s after the injection. The microinjection period lasted ~1 s.

NMDA antagonists

The saline was aspirated from the agar pool, and either APV (Sigma Chemical) or MK-801 hydrogen maleate (Dizocilpine; Research Biochemicals International) (both 1 μM in 0.9% NaCl) was applied to the exposed spinal cord and left on for 7–10 min, after which it was aspirated and a fresh solution was reapplied 5–8 min before the ic histamine and left on the cord during ic microinjections to ensure that a sufficient amount of drug had diffused into the spinal cord.

Experimental design and analysis

In early experiments, the unit’s mechanical receptive field was mapped initially and then 1 μl of vehicle (0.9% NaCl) was injected into the low-threshold area of the receptive field. The receptive field was mapped again 10 min after the vehicle injection. A 1 μl volume of histamine then was injected in the same area of the receptive field (within <1 mm of the saline injection site), and the unit’s receptive field was mapped once again 10 min posthistamine injection. The 10-min waiting period was chosen based on physiological studies showing that the area of allokwness peaked 10 min after ic histamine injection (Simone et al. 1991). In later experiments, mechanical receptive fields were mapped before and after ic histamine injection without the vehicle control. The above procedure was followed under the following conditions: 10% histamine ic with saline on the cord; 10% histamine ic with MK-801 (1 μM) applied to the cord; 10% histamine ic with APV (1 μM) applied to the cord; 3% histamine ic with saline on the cord; 3% histamine with APV applied to the cord; and 1% histamine ic with saline on the cord.

In 13 animals, two units were studied (1 on either side of the cord), whereas in the remainder only one cell was studied per animal. Only one NMDA antagonist was applied once per animal.

Data analysis

For each drug treatment group, mean pre- and posthistamine ic receptive field areas were compared using a paired t-test, with P < 0.05 considered to be statistically significant. Mean unit responses to ic histamine (spikes/60 s) also were determined and compared between groups with an unpaired t-test with P < 0.05 considered to be statistically significant.

RESULTS

Unit sample

A total of 45 WDR-type units was studied. High-threshold mechanical receptive fields located on the ipsilateral hind paw and distal hind limb ranged in area from 85.2 to 549 mm2. Most (53%) covered approximately three-quarters to all of the ventral hind paw, including one or more digits, and an additional 11% also included a portion of the distal hind limb. The remainder had smaller receptive fields covering about one-half of the hind paw (29%) or even smaller areas restricted to 1–3 digits (7%; see Fig. 3, top, for examples). Spontaneous activity ranged from 0 to 32.8 Hz (4.1 ± 5.6 Hz, mean ± SD), with 58% of the units exhibiting no or low (0–3 Hz) spontaneous activity, 31% firing at 3−8 Hz, and 11% firing at >8 Hz. Recording sites ranged from depths of 50–1.126 μm (598 μm ± 232.3) below the spinal cord surface.

Response to histamine

Forty of 45 units (89%) responded to ic histamine (1, 3, or 10%) with a >200% increase in firing rate relative to spontaneous activity. The other five units showed smaller ic histamine-induced increases in firing (117–190%); two of these received 1% histamine. Mean responses to histamine increased in a dose-dependent manner; the response to 10% was significantly larger compared with responses to 1% histamine, NaCl or spontaneous firing (Fig. 1). Mean responses to ic histamine with MK-801 or APV present on the spinal cord were somewhat lower than the mean responses without the blockers present (Fig. 1), but these differences were not statistically significant. Responses to histamine were not attributable to some artifact of the microinjection because equivalent vehicle (0.9% saline) microinjections did not significantly increase the response compared with spontaneous firing (n = 16, P = 0.088; Fig. 1).

Expansion of receptive field area after ic histamine

In confirmation of a previous study (Carstens 1997), mechanical receptive fields were significantly larger after ic microinjection of 10% histamine (Figs. 2 and 3A). Figure
ic microinjection of vehicle (0.9% saline) did not cause compared with prehistamine control, range 33 ± 263% ) ver-
low threshold: mean 4% decrease, range -24 to +66%; middle threshold: mean 4% decrease, range -22 to +38%; high-threshold: mean 2% increase, range -13 to +26% ), in contrast to the significant expansion in receptive field area after 10% histamine with saline on the cord (Fig. 3A, □). Two units in which MK-801 prevented any histamine-induced change in receptive field areas were studied further. More than 1 h after the cord had been rinsed repeatedly with saline to wash out the MK-801, the receptive field areas were remapped. Then ic histamine (10%) was microinjected, and in both cases, there was a marked expansion in receptive field area 10 min later.

Effects of MK-801 and APV on receptive field expansion

With 1 μM MK-801 present on the spinal cord, there was no difference in the mean prehistamine receptive field area compared with receptive field areas mapped with saline on the cord (Fig. 3, A and B, □). There was also no significant difference in mean spontaneous firing rates (before histamine) before and after the application of MK-801 or APV to the spinal cord. After ic microinjection of the highest histamine dose (10%), there was no significant change in receptive field area with MK-801 present on the cord (Fig. 3R, □; n = 8) (low threshold: mean 14% increase, range -24 to +66%; middle threshold: mean 4% decrease, range -22 to +38%; high-threshold: mean 2% increase, range -13 to +26% ), in contrast to the significant expansion in receptive field area after 10% histamine with saline on the cord (Fig. 3A, □). Two units in which MK-801 prevented any histamine-induced change in receptive field areas were studied further. More than 1 h after the cord had been rinsed repeatedly with saline to wash out the MK-801, the receptive field areas were remapped. Then ic histamine (10%) was microinjected, and in both cases, there was a marked expansion in receptive field area 10 min later.

Similar to results with MK-801, when 1 μM APV was present on the spinal cord, there was no significant difference in mean prehistamine receptive areas compared with units in which saline was on the cord (Fig. 3, A and C, □). After ic histamine (10%), there was no significant change in receptive field area with APV present on the spinal cord (low threshold: mean 4% decrease, range -22% to +13%; middle threshold: mean 7% decrease, range -25 to +12%; high threshold: mean 4% decrease, range -23 to +27%). In two units, 3% histamine was microinjected ic after spinal application of 1 μm APV, and in both cases, there was no

Receptive field expansion after higher doses of histamine was due to mechanical stimulation at the injection site.

2 shows that the percent increase in receptive field area was relatively larger for low threshold (mean 48% increase compared with prehistamine control, range 33–263%) versus medium threshold (mean 19%, range -11 to +188%) or high threshold (mean 11%, range -6 to +147%) subareas for 12 units when mapped 10 min after histamine injection (Fig. 2, □). An individual example is shown in Fig. 3A, top. The relative increase in area was inversely proportional to the stimulus force used to map the receptive field subarea. Figure 2 additionally shows that the magnitude of mechanical receptive field expansion increased as a function of histamine dose. Thus mean receptive field areas expanded after 3% histamine injections in eight units; this was significant for low threshold (mean 27% increase, range -16 to +197%) and middle (mean 13% increase, range 4–125%) but not for high threshold (mean 3% increase, range 0–14%) receptive field subareas (Fig. 2, □). In eight other units, a 1% histamine injection did not cause significant expansion of any mean areas (low threshold: mean 6% increase, range -16 to +20%; middle threshold: mean 3% increase, range -9 to +15%; high threshold: mean 2% increase, range -4 to +8%) (Fig. 2, □). For 16 units, control ic microinjection of vehicle (0.9% saline) did not cause any significant change in mean receptive field area (low threshold: mean 4% decrease, range -39 to +77%; middle threshold: mean 4% decrease, range -18 to +51%; high-threshold: mean 2% decrease, range -20 to +32%), confirming our earlier report (Carstens 1997). That neither saline nor 1% histamine microinjections significantly affected receptive field areas argues against the possibility that the

FIG. 2. Dose-dependent expansion of receptive field area after ic histamine. Bar graph plots the mean percent expansion in dorsal horn neuron mechanical receptive field subareas mapped with von Frey filaments having low, medium and high bending forces, 10 min after ic microinjection of 1% (□), 3% (□), or 10% (□) histamine. *Significantly larger compared with prehistamine area (P < 0.05, paired t-test).
FIG. 3. Prevention of ic histamine-evoked expansion of mechanical receptive fields by prior spinal application of NMDA antagonists. A, top left: figure of rat hind paw shows an individual example of 1 dorsal horn neuron’s mechanical receptive field subareas mapped using 3 graded von Frey filaments at the indicated bending forces. Top right: figure shows the expanded receptive field of the same neuron mapped 10 min after ic microinjection of 10% histamine into the lowest-threshold area on the middle toe. Bar graph below plots mean receptive field subareas for low-, medium-, and high-threshold stimuli before (pre, □) and 10 min after ic microinjection of 10% histamine (posthistamine, ■). Error bars: SE. * Mean area significantly different compared with prehistamine area (P < 0.05, paired t-test; n = 12). B, top: figures as in A, showing example in which the size of receptive field subareas did not change markedly after ic microinjection of 10% with 1 μM MK-801 present on the spinal cord. Graph below shows lack of change in mean receptive field subareas for the population (n = 8; format as in A). C, top: figures show lack of marked change in receptive field subareas after ic microinjection of 10% histamine with 1 μM APV present on the spinal cord (format as in A and B). Bottom: graph shows lack of change in mean receptive field areas for the neuronal population (n = 9) after ic histamine with APV present on the spinal cord (format as in A and B).

change in receptive field area (low-threshold areas: 96 and 103% of prehistamine controls, respectively).

DISCUSSION
Response to ic histamine and itch

These results confirm an earlier report (Carstens 1997) that a large majority of WDR-type dorsal horn neurons responds to ic microinjection of histamine in a dose-related manner, with a subsequent expansion in area of the low-threshold mechanical receptive field. The present data show that the degree of receptive field expansion is dose related, with a threshold concentration between 1 and 3%. After the highest histamine dose (10%), we presently observed a significant increase in both low- and high-threshold receptive field areas, although the relative increase was greater for the lower-intensity stimulus (Fig. 2). This differs from our previous study (Carstens 1997) in which only the low-threshold, but not high-threshold, receptive field area expanded posthistamine. However, in the former study (Carstens 1997), the high-threshold area was mapped with a much stronger mechanical stimulus (76 g) compared with that used presently (4 g), likely explaining the difference.

The threshold dose of histamine sufficient to elicit itch sensation in humans (~10⁻³ μg in 10 μl = 0.00001%) (Simone et al. 1991) was lower than the doses employed presently. In humans, the area of allknesis increased with histamine dose, reaching a peak ~10 min after the histamine injection (Kuriashi et al. 1995). In this latter study, the number of scratches elicited by 3% histamine was higher than that elicited by ic injection of algesic substances (capsaicin, formalin) or vehicle; higher concentrations of histamine were not tested. The behavioral data (Kuriashi et al. 1995) suggest that the threshold for scratching behavior in mice may be near 3% histamine; the threshold for scratching after
ocular application of histamine appears to be substantially lower (0.05%) in hairless guinea pigs (Woodward et al. 1995). The present results indicate that the threshold for ic histamine-induced increases in neuronal response and expansion of receptive field area on hind paw skin in rats is between 1 and 3%.

**Role of spinal NMDA receptors in mechanical receptive field expansion**

The present data, showing that receptive field expansion was prevented by prior topical spinal application of the selective NMDA receptor antagonists MK-801 or APV, implicate spinal NMDA receptors in the neural process underlying ic histamine-evoked receptive field expansion. It has been proposed that activation of chemosensitive cutaneous primary afferent C fibers (particularly a postulated subpopulation that is insensitive to noxious thermal or mechanical stimuli) by histamine leads to a central sensitization of second-order dorsal horn neurons (LaMotte 1992), similar to NMDA-mediated mechanisms of central sensitization proposed to underlie the development of secondary hyperalgesia and allodynia after noxious stimulation of the skin (LaMotte 1992; LaMotte et al. 1991, 1992). NMDA receptors have been localized on spinal dorsal horn neurons (Croul et al. 1995; Mitchell and Anderson 1991), and EAAs have been localized to the spinal cord, including primary afferent terminals (DeBiasi and Rustioni 1988). There is evidence that cutaneous application of histamine or other pruritogens excites subpopulations of C-fiber polymodal nociceptors (Handwerker et al. 1991; Tuckett and Wei 1987) and thermally and mechanically insensitive C fibers (Schmelz et al. 1997). The C fibers activated by histamine are proposed to excite dorsal horn neurons by releasing EAAs to activate postsynaptic glutamate receptors; sufficient depolarization activates NMDA receptors to open Ca\(^{2+}\) channels. Influx of Ca\(^{2+}\) in turn activates intracellular second-messenger pathways leading ultimately to an increase in neuronal excitability. Expansion of mechanical receptive fields can occur because mechanoreceptors at the "subliminal" fringe of the receptive field (Woolf and King 1990), which are normally incapable of depolarizing the neuron sufficiently to fire action potentials, now can depolarize the hyperexcitable neuron sufficiently.

The present data are consistent with the proposed sequence of events described above. We observed that both competitive (APV) and noncompetitive (MK-801) NMDA antagonists prevented ic histamine-induced expansion of mechanical receptive fields. This observation was not due to direct effects of MK-801 or APV themselves on receptive field area (Fig. 3). Furthermore, neuronal responses to histamine in the presence of these blockers were not significantly reduced (Fig. 1), arguing against the possibility that receptive field expansion was simply due to inadequate depolarization of the dorsal horn neurons by ic histamine. We conclude that ic histamine-evoked expansion of mechanical receptive fields is mediated by spinal NMDA receptors.

**Alloknesis**

Injection of a pruritogen such as histamine into the skin produces a local wheal, surrounding flare, and an even larger surrounding area of spreading alloknesis (Simone et al. 1987, 1991). The distal spread of alloknesis away from the site of pruritic application was blocked by creating a thin barrier of local anesthesia in the skin, prompting the authors to propose that the spreading alloknesis is dependent on peripheral neural activity (Simone et al. 1991). The nerve fibers involved were proposed to have widespread branches in the skin, with a primary afferent fiber projecting into the spinal cord capable of sensitizing dorsal horn neurons involved in signaling itch (LaMotte 1992; Simone et al. 1991). That is, alloknesis depends both on peripheral and central neural processes. Mild stimulation of the expanded mechanical receptive field of such hyperexcitable "itch" neurons would evoke activity that would be interpreted as itch.

Although we did not presently investigate peripheral mechanisms involved in ic histamine-induced expansion of dorsal horn neuronal receptive fields, we did show that such expansion is prevented by prior spinal application of NMDA antagonists. These results support the idea that part of the development of alloknesis involves a NMDA-dependent sensitization of spinal neurons. However, the relation of the present findings to alloknesis presupposes that dorsal horn neurons responsive to ic histamine are involved in signaling itch (rather than pain) sensation. As discussed in detail elsewhere (Carstens 1997), there are several theories concerning the neural mechanisms of itch, and a role for itch cannot be ascribed to spinal neurons based solely on their responsiveness to ic pruritogens. A large percentage of histamine-responsive WDR-type dorsal neurons also responded to algesic chemicals such as capsaicin (Carstens 1997). Conceivably, WDR neurons responsive to both pruritic and algesic chemicals might signal itch at a low firing rate and pain at a higher rate. However, there is considerable evidence that itch sensation elicited by cutaneous (Tuckett 1982) or intraneural electrical microstimulation (Schmidt et al. 1993) does not become painful at higher stimulus frequencies nor does painful intraneural stimulation of nociceptors become itch at lower stimulus frequencies (Handwerker et al. 1991; Ochoa and Torebjork 1989), arguing that itch and pain sensations are conveyed by separate neural pathways. Alternatively, it has been suggested that pruritogens excite a subpopulation of polymodal nociceptors, which in turn excite nociceptive spinal neurons to signal itch; more intense stimuli recruit a larger population of nociceptive neurons to elicit pain sensation and simultaneously occlude itch (Handwerker 1992). It is also conceivable that itch is signaled by a hitherto unstudied population of neurons receiving input from specific itch receptors (e.g., thermally and mechanically insensitive chemoreceptors) (Schmelz et al. 1997). A final possibility is that the expansion in WDR neuronal receptive field area reflects a noxious, rather than pruritic, effect of the ic histamine and therefore has more relevance to mechanisms of secondary hyperalgesia and allodynia that also are mediated through spinal NMDA receptors. Three percent histamine, which resulted in significant receptive field expansion, produced itch but not pain when self-injected ic (unpublished observation); however, we cannot rule out the possibility that the same stimulus might be painful in the rat. In conclusion, our data provide evidence for involvement of...
spinal NMDA receptors in the expansion of WDR neuronal mechanical receptive field areas after ic histamine.

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