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Chelation of Zinc in the Extracellular Area of the Spinal Cord, Using Ethylenediaminetetraacetic Acid Disodium-Calcium Salt or Dipicolinic Acid, Inhibits the Antinociceptive Effect of Capsaicin in Adult Mice[1]

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ABSTRACT

Capsaicin depolarizes primary afferent C-fibers releasing substance P (SP) whose N-terminal metabolites appear to play a role in the development of antinociception. Because some effects of SP(1–7) are similar to those of zinc, we tested the hypothesis that zinc in the extracellular area plays a role in capsaicin-induced antinociception, as measured using the abdominal stretch (writhing) assay. Decreases in zinc were achieved by intrathecal (i.t.) injection of membrane-impermeable compounds: ethylenediaminetetraacetic acid disodium-calcium salt (Ca^{++} EDTA), a calcium-saturated chelator of divalent cations, or dipicolinic acid, a zinc chelator. Ten nanomoles of Ca^{++} EDTA had no effect on writhing at either 90 min or 24 h after injection, yet pretreatment with Ca^{++} EDTA prevented the development of antinociception 24 h after i.t. injection of either 2.8 nmol of capsaicin or 10 nmol of SP(1–7). One nanomole of dipicolinic acid injected i.t. also blocked capsaicin- and SP(1–7)-induced antinociception. When injected 24 h after SP(1–7), Ca^{++} EDTA failed to reverse antinociception. Acute antinociception produced 30 min after injection of SP(1–7) was also blocked when Ca^{++} EDTA was injected 24 h, but not 60 min, before SP(1–7). Thus, the optimal time of Ca^{++} EDTA-induced hyperalgesia (90 min), described previously, did not correspond to that of its inhibitory effect on antinociception (24 h). In contrast, we found that the previously described antinociception after an i.t. injection of zinc (90 min) is greatly attenuated by 24 h. Thus, zinc appears to be necessary, but may not be sufficient, for the long-term antinociceptive effect of capsaicin, acting downstream from the action of substance P N-terminal metabolites.

Capsaicin selectively excites polymodal nociceptive primary afferent fibers in adult animals and all types of C-fibers in neonates (reviewed by Buck and Burks, 1986). Although capsaicin produces antinociception that lasts 2 to 7 days (Gamse, 1982), the mechanism is not well understood. Substance P (SP), which is found in primary afferent neurons (De Biasi and Rustioni, 1988) and released in response to treatment with capsaicin (Gamse et al., 1981; Go and Yaksh, 1987), appears to play an essential role. Although capsaicin decreases SP release for several weeks (Yaksh et al., 1979; Gamse, 1982), the antinociceptive effect, observed as early as 24 h after injection, precedes inhibition of SP release (Goettl et al., 1997). Thus, there is a lack of correlation between decreases in SP release and antinociception in rats (Gamse, 1982; Bittner and Lahann, 1984), guinea pigs (Miller et al., 1982), and mice (Goettl et al., 1997).

Although inhibition of SP release is not responsible for the antinociceptive effect of capsaicin, SP metabolites that accumulate after the initial depolarizing effect of capsaicin appear to be necessary for desensitization and antinociception. Like capsaicin, intrathecal (i.t.) injection of SP N-terminal metabolites produces antinociception 24 h later in the hot-plate and abdominal stretch assays (Kreeger et al., 1994; Mousseau et al., 1994; Goettl et al., 1997). In contrast, the C-terminal metabolite SP(5–11), which contains the tachykinin sequence active at neurokinin receptors, is without effect on nociception when similarly tested (Mousseau et al., 1994). The D-isomer of SP(1–7), D-SP(1–7) {[D-Pro^2,D-Phe^7]SP(1–7)}, which inhibits [^{3}H]SP(1–7) binding (Igwe et al., 1990), blocks the antinociceptive effects of both capsaicin and SP(1–7) (Larson and Sun, 1993; Kreeger et al., 1994; Mousseau et al., 1994), whereas the neurokinin antagonist DPDT-SP ([D-Pro^2,D-$Trp^{7,9}$]SP) does not (Larson and Sun, 1993; Mousseau et al., 1994). Thus, the ability of capsaicin to induce antinociception may depend on a stereoselective action of SP N-terminal fragments.

Examination of the spectrum of effects produced by SP

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ABBREVIATIONS: Ca^{++} EDTA, ethylenediaminetetraacetic acid disodium-calcium salt; CNS, central nervous system; NMDA, *N*-methyl-D-aspartate; SP, substance P.

N-terminal fragments reveals that these peptides produce some effects similar to those produced by zinc. SP N-terminal fragments injected i.t. inhibit the behavioral response to excitatory amino acids acting at *N*-methyl-D-aspartic acid (NMDA) (Hornfeldt et al., 1994), whereas it potentiates that at kainic acid-sensitive receptors (Larson and Sun, 1992). Zinc has a similar spectrum of activities, potentiating kainic acid-induced activity and inhibiting NMDA-induced activity (Peters et al., 1987; Frederickson, 1989; reviewed by Smart et al., 1994). These effects may be important in the regulation of pain because excitatory amino acids are thought to mediate nociception based on the pharmacological effects of agonists and antagonists at these sites (Aanonsen et al., 1990; Coderre and Melzack, 1992; Näsström et al., 1992).

Using histological methods of defining zinc-containing systems in the central nervous system (CNS) (Frederickson, 1989), neurons capable of sequestering and releasing zinc have been shown to project to many areas within the spinal cord. For example, zinc-selenite stain is densely localized in the neuropil of the dorsal spinal cord (Danscher, 1982) and dorsal root ganglia (Velázquez et al., 1997), areas important in sensory processing. In addition, metallothionein(III), which is found only in areas containing neurons whose processes sequester zinc in synaptic vesicles (Masters et al., 1994) and postulated to play a role in the availability of zinc (Bremner, 1987; reviewed by Ebadi et al., 1995) in histologically reactive pools (Palmiter et al., 1992; Erickson et al., 1995), is found in the spinal cord and dorsal root ganglia (Velázquez et al., 1997). Consistent with a role for zinc in pain processing, injection of zinc i.t. in mice produces a transient antinociception when tested using the writhing assay, whereas injection of chelators of divalent cations or of zinc produces hyperalgesia in the tail-flick assay (Larson and Kitto, 1997). Together, these data strongly support the possibility that zinc serves as a neuromodulator in the spinal cord area.

Like the well characterized population of zinc-containing neurons in the hippocampus, primary afferent C-fibers contain excitatory amino acids, like aspartate and glutamate (Wanaka et al., 1987; De Biasi and Rustioni, 1988; Tracy et al., 1991), which are released in response to noxious stimulation (Skilling et al., 1988; Sorkin et al., 1992) and capsaicin-induced depolarization (Jeftinija et al., 1991; Ueda et al., 1994). Zinc has been postulated to be coreleased with glutamate at central synapses in response to potassium, kainic acid, electrical stimulation, or seizure activity (Assaf and Chung, 1984; Howell et al., 1984; Sloviter, 1985; Aniksztejn et al., 1987; Frederickson et al., 1988). NMDA and kainic acid receptors, which are sensitive to zinc availability, have been localized on primary afferent fibers (Sato et al., 1993; Liu et al., 1994). These ionotropic receptors allow influx of zinc (Yin and Weiss, 1995), an arrangement that may permit the accumulation of zinc in afferent neurons. Thus, the distribution of zinc along pain-relevant systems may contribute to long-term changes in pain transmission.

Whether capsaicin-induced antinociception involves changes in the availability of zinc is not known. To test the hypothesis that zinc is important in capsaicin-induced antinociception, we examined its dependence on the availability of zinc in the extracellular area of the spinal cord in mice. Because the antinociceptive effect of capsaicin appears to be mediated by SP N-terminal metabolites, we also examined the role of zinc in the antinociceptive effect of SP(1–7). The blood-brain barrier was bypassed by injecting all compounds i.t. Zinc in the extracellular area was selectively decreased by injection of either of two membrane-impermeable compounds: ethylenediaminetetraacetic acid disodium-calcium salt (Ca^{++} EDTA), a membrane-impermeable chelator of divalent cations, or dipicolinic acid, a selective chelator of zinc. Nociception was tested using the writhing assay, which involves the measurement of abdominal contractions induced by acetic acid injected i.p.

Materials and Methods

Animals. Male Crl:CFW (SW) BR mice (20–25 g; Charles River Lab, Portage, MI) were housed four per cage and allowed to acclimate for at least 24 h before use. Mice were allowed free access to food and water. Animals were used strictly in accordance with the Guidelines of the University of Minnesota Animal Care and Use Committee and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council [DHEW publication (NIH) no. 78-23, revised 1978].

Drug Administration. Except where indicated, all injections were made intrathecally (i.t.) in mice at approximately the L5-6 intervertebral space using a 30-gauge, 0.5-inch disposable needle on a 50-μl Luer-tip Hamilton syringe. A volume of 5 μl was used for all i.t. injections. Throughout the studies, zinc chloride and Ca^{++} EDTA were each dissolved in saline and administered i.t. Control groups were injected with an equivalent volume of vehicle. Capsaicin used for i.t. injection was dissolved (2.8 nmol/5 μl) first in dimethyl sulfoxide and diluted with saline to a final concentration of 5% dimethyl sulfoxide by volume. Dipicolinic acid and SP(1–7) were each dissolved in acidified saline and compared with controls injected with the same vehicle.

Antinociceptive Testing. The abdominal stretch, or writhing assay, was performed by injecting 0.3 ml of 1.0% acetic acid in manually restrained mice. Immediately after injection, animals were placed in a large glass cylinder containing approximately 2 cm of bedding. The number of abdominal stretches occurring in a 5-min interval was counted beginning 5 min after acetic acid. Treatments that produced a significant decrease in the number of abdominal stretches were considered to be antinociceptive. Mice were euthanized immediately after testing.

Drugs. Ca^{++} EDTA, zinc chloride, and capsaicin (8-methyl-*N*-vanillyl-6-noneamide) were purchased from Sigma Chemical Co. (St. Louis, MO). Dipicolinic acid was purchased from Molecular Probes (Eugene, OR).

Ca^{++} EDTA was chosen as an appropriate chelator of zinc for the following two reasons. First, the membrane-impermeable nature of Ca^{++} EDTA ensures that it chelates only divalent cations in the extracellular space rather than protein-bound zinc, which is necessary for structural purposes or enzymatic activity. Second, EDTA saturated with calcium is used widely as a chelator of zinc (Frederickson et al., 1989; Wang and Quastel, 1990; Westergaard et al., 1995; Koh et al., 1996). Although a variety of trace elements are found in the body, most are associated with protein complexes and located intracellularly. Of all the divalent cations found endogenously, zinc is the only one present in relatively high concentrations both intracellularly and extracellularly. Thus, zinc is the only divalent cation in the extracellular fluid with an affinity that would compete with calcium for the EDTA divalent cation binding site. Although Ca^{++} EDTA is less selective for zinc than dipicolinic acid, it has the advantage of having been previously used to chelate zinc and is active at physiological pH. In contrast, dipicolinic acid is relatively new and only active as a chelator in an acidified medium. Because of their different advantages and disadvantages, both compounds were used to ensure that two structurally distinct compounds

produced identical results based on their common ability to chelate zinc.

Data Analysis. Mean differences (±S.E.M.) are presented in the figures. Throughout the experiments, each group represents at least six mice. Statistical analysis of the results was performed using ANOVA followed by the Schéffe *F* test for multiple comparisons. The *p* values less than .05 were used to indicate a significant difference for all tests. Mean values of the test groups were routinely compared with control values collected the same day.

Results

Injection of 2.8 nmol of capsaicin i.t. in mice produced a reproducible antinociceptive effect in the acetic acid-induced writhing assay when tested 24 h later (Fig. 1), as previously described (Kreeger et al., 1994). Doses of 1 to 100 nmol of Ca^{++} EDTA were coadministered with capsaicin, and antinociception was measured 24 h later. A dose as low as 10 nmol of Ca^{++} EDTA was sufficient to prevent the antinociceptive effect of capsaicin. The administration of Ca^{++} EDTA alone has been previously shown to have no effect 90 min later on the number of writhes induced by the injection of acetic acid (Larson and Kitto, 1997). Doses of 0.1, 10, and 100 nmol of Ca^{++} EDTA were also without effect on the number of writhing behaviors when tested 24 h after their injection (Fig. 2A).

To determine whether the antinociceptive effect of capsaicin is not only prevented but also reversed by Ca^{++} EDTA, we injected the chelator 90 min before testing in mice that were pretreated 24 h previously with vehicle or capsaicin. The mean (±S.E.M.) number of writhes in mice injected with 2.8 nmol of capsaicin (24 h) plus 10 nmol of Ca^{++} EDTA (90 min) was 6.7 ± 0.9, which did not differ from the group injected with capsaicin (24 h) plus vehicle (90 min), whose mean was 7.6 ± 1.5 writhes. Both capsaicin-pretreated groups had mean values that were significantly less ($p < .05$) than the group injected at 24 h and at 90 min with vehicle only, whose mean was 15.2 ± 0.9 writhes.

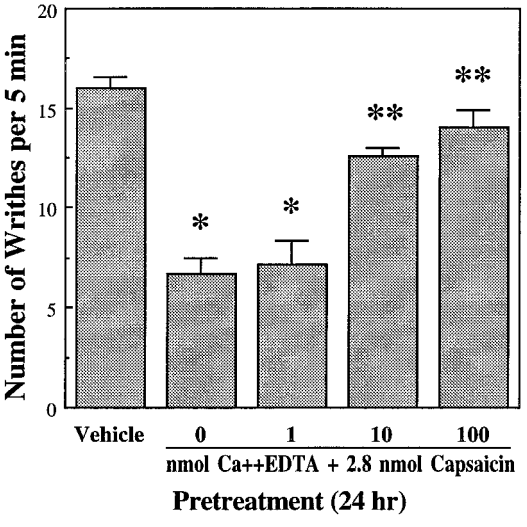

Fig. 1. Influence of Ca^{++} EDTA on the antinociceptive effect of capsaicin. Values throughout represent the mean (±S.E.M.) number of writhing behaviors for the 5-min interval beginning 5 min after injection of acetic acid i.p. where each value represents a different group of at least six mice. Mice were injected i.t. with either 2.8 nmol of capsaicin or vehicle 24 h before nociceptive testing. Doses of Ca^{++} EDTA indicated (1, 10, and 100 nmol) were coadministered i.t. with capsaicin. *Significant difference ($P < .05$) between the group indicated and i.t. vehicle-injected control mice tested on the same day. **Significant difference ($P < .05$) from the group pretreated with 2.8 nmol of capsaicin only. See *Materials and Methods* for details.

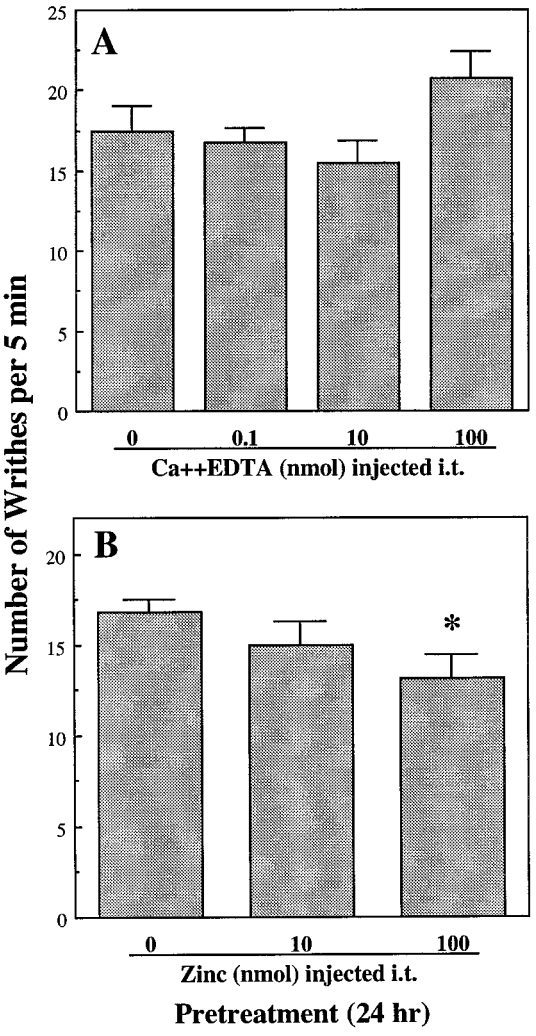

Fig. 2. Influence of pretreatment (24 h) with either Ca^{++} EDTA or zinc on acetic acid-induced writhing. A, Groups were injected i.t. with 0.1, 10, and 100 nmol of Ca^{++} EDTA alone 24 h before testing in the acetic acid-induced writhing assay. B, Groups were injected i.t. with 10 or 100 nmol of zinc 24 h before testing in the writhing assay. *Significant difference ($P < .05$) between the group indicated and i.t. vehicle-injected control mice tested on the same day.

The ability of Ca^{++} EDTA to attenuate capsaicin-induced antinociception suggests that a noncalcium divalent cation is necessary for the production of capsaicin-induced antinociception. To determine whether an enhanced availability of zinc in the spinal cord would be sufficient to induce antinociception at this time, we injected 10 and 100 nmol of zinc chloride i.t. and monitored the number of writhing behaviors 24 h later (Fig. 2B). Although a dose as low as 1 ng of zinc has been previously reported to inhibit acetic acid-induced writhing behaviors 90 min after zinc injected by this same route, followed by recovery by 2 h, we found that a dose as large as 100 nmol was necessary to produce even a small inhibitory effect on this nociceptive activity at 24 h.

We have previously shown that SP N-terminal fragments may mediate the chemical antinociceptive effect of capsaicin in adult mice. To determine whether the effect of Ca^{++} EDTA is upstream or downstream from the action of these metabolites, we assessed the ability of Ca^{++} EDTA to pre-

vent SP(1–7)-induced antinociception. Ten nanomoles of SP(1–7) was used as this dose was found to inhibit the number of writhes to approximately the same degree as the 2.8-nmol dose of capsaicin (Kreeger et al., 1994). Coadministration of 1 to 100 nmol of Ca^{++} EDTA with 10 nmol of SP(1–7) inhibited SP(1–7)-induced antinociception (Fig. 3) in a fashion identical with the effect of Ca^{++} EDTA on capsaicin.

To determine whether chelation of zinc, rather than another divalent cation, is necessary for the inhibition of antinociceptive effects produced by capsaicin and SP(1–7), we also used dipicolinic acid, a selective chelator of zinc. One nanomole of dipicolinic acid, injected 30 min before capsaicin or coadministered with SP(1–7), was sufficient to completely prevent their antinociceptive effects typically observed 24 h later. Injection of this dose of dipicolinic acid alone had no effect on the number of writhes measured 24 h later compared with the response after the injection of either vehicle used for injections of capsaicin or SP(1–7) (Fig. 4). As an additional test that dipicolinic acid inhibits the development of antinociception by virtue of its ability to chelate zinc, which requires an acidic environment, rather than a pharmacological action unrelated to chelation, an equivalent dose of dipicolinic acid dissolved in a vehicle at a neutral pH was tested. Dipicolinic acid delivered at a neutral pH failed to prevent the development of antinociception when injected together with capsaicin (mean ± S.E.M. = 7.1 ± 1.1 writhes) compared with when capsaicin was injected alone (4.3 ± 0.7) as both values were significantly different ($p < .05$) from that of the vehicle-injected control group (17.3 ± 1.2) and were not significantly different from each other.

In addition to its delayed and prolonged antinociceptive effect from 24 to 48 h after injection, SP(1–7) has also been shown to induce antinociception at just 30 min after injection of relatively small doses of the peptide with full recovery by 90 min (Goettl and Larson, 1994). To determine whether this short-term antinociceptive effect is also dependent on a divalent cation in the extracellular area, we examined its sensitivity to pretreatment with Ca^{++} EDTA injected at two different times before SP(1–7). A dose of 100 nmol of Ca^{++} EDTA was selected as this dose prevented the antinociceptive effect of 10 nmol of SP(1–7) measured 24 h after injection. When 25 pmol of SP(1–7) was injected 60 min after this dose of Ca^{++} EDTA, the acute (30 min) antinociceptive effect of 25 pmol of SP(1–7) was not attenuated (Fig. 5A). However, when this same dose of Ca^{++} EDTA was administered 24 h before injection of 25 pmol of SP(1–7), the antinociceptive effect produced 30 min after injection of 25 pmol of SP(1–7) was completely prevented (Fig. 5B), indicating that the time of optimal Ca^{++} EDTA-induced hyperalgesia (90 min) described previously (Larson and Kitto, 1997) is not coincident with the onset of its inhibitory effect on the antinociceptive effect of SP(1–7) (24 h).

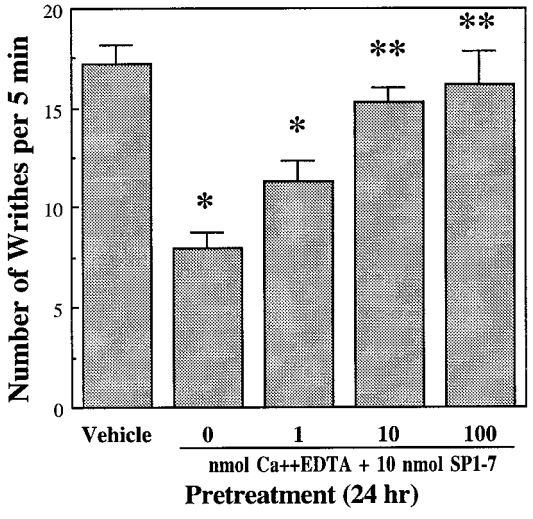

Fig. 3. Influence of Ca^{++} EDTA on the antinociceptive effect of SP(1–7). Values throughout represent the mean (±S.E.M.) number of writhing behaviors for the 5-min interval beginning 5 min after injection of acetic acid i.p. where each value represents a different group of at least six mice. Mice were injected i.t. with either 10 nmol of SP(1–7) or its vehicle 24 h before nociceptive testing. Doses of Ca^{++} EDTA (1, 10, and 100 nmol) indicated were coadministered i.t. with SP(1–7). *Significant difference ($P < .05$) between the group indicated and i.t. vehicle-injected control mice tested on the same day. **Significant difference ($P < .05$) from the group pretreated with 10 nmol of SP(1–7) only.

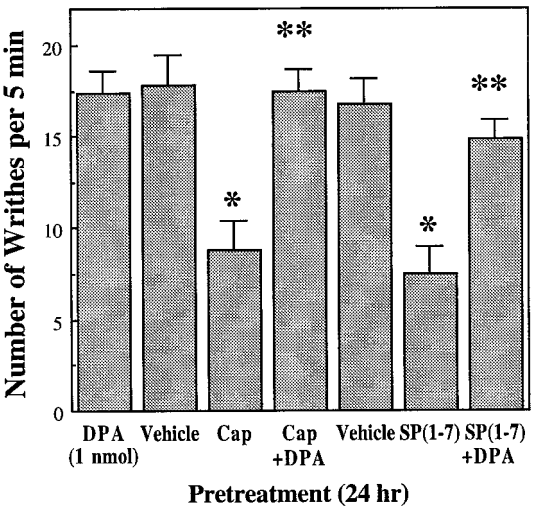

Fig. 4. Prevention of capsaicin- and SP(1–7)-induced antinociception by pretreatment with dipicolinic acid. One nanomole of dipicolinic acid (DPA) was injected i.t. 24 h before the writhing assay either alone or 30 min before injection of 2.8 nmol of capsaicin (Cap) or coadministered with 10 nmol of SP(1–7). Asterisks indicate differences between the bar indicated and the adjacent bar immediately to the left. *Significant difference ($P < .05$) between the group indicated and i.t. vehicle-injected control mice tested on the same day. **Significant difference ($P < .05$) from the group pretreated with 2.8 nmol of capsaicin or 10 nmol of SP(1–7) only.

Discussion

Histologically reactive zinc in the dorsal horn of the spinal cord may represent a releasable pool (Frederickson, 1989) that is strategically localized to play a role in sensory transmission (Danscher, 1982; Velázquez et al., 1997). The present study tested the hypothesis that zinc, localized in the extracellular area of the spinal cord, plays a role in the development of capsaicin-induced antinociception. Our results support a role for zinc in the production of capsaicin-induced antinociception because Ca^{++} EDTA, a chelator of divalent cations, and dipicolinic acid, a selective chelator of zinc, each prevented this classic effect of capsaicin.

Although the majority of zinc in the CNS is localized intracellularly where it serves a biochemical and/or structural function, these stores would not be available for chelation by Ca^{++} EDTA or dipicolinic acid because both compounds are membrane impermeable. Histochemically reactive zinc re-

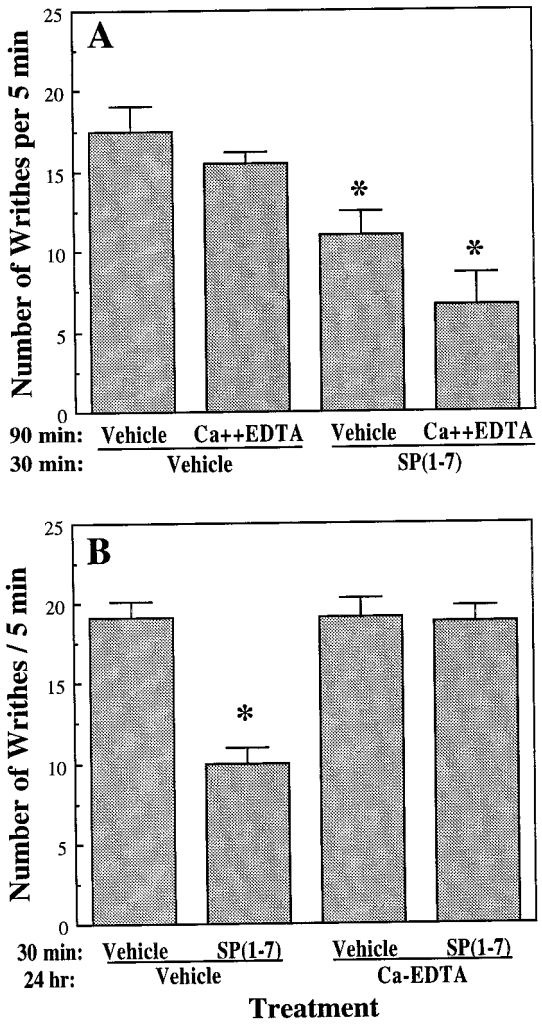

Fig. 5. Influence of pretreatment with Ca^{++} EDTA on the acute (30 min) antinociceptive effect of SP(1–7) injected i.t. in mice. Mice were pretreated i.t. with 100 nmol of Ca^{++} EDTA or vehicle, as indicated, 90 min (A) or 24 h (B) before testing in the writhing assay. Mice were also injected with either 25 pmol of SP(1–7) or vehicle i.t. 30 min before testing. *Significant difference between the bar indicated and the group injected with vehicle only.

leased from zinc-containing neurons and zinc associated with cell-surface metalloenzymes are the only pools of divalent cations that would be predicted to be found in the extracellular area.

The chelators used, Ca^{++} EDTA and dipicolinic acid, are two structurally distinct compounds. Although sodium EDTA has a high affinity for calcium and magnesium, once saturated with calcium, the only cations with which it would be predicted to bind would be cobalt, cesium, copper, nickel, lead, and zinc. Of these, only zinc is found in abundance in the CNS. The ability of Ca^{++} EDTA to chelate zinc when injected in vivo has been previously demonstrated by the ability of 500 nmol of Ca^{++} EDTA, injected i.c.v. in rats, to protect against zinc translocation and neuronal death associated with transient global ischemia (Koh et al., 1996). Ca^{++} EDTA has also been used to elucidate the influence of zinc on receptor activity (Westergaard et al., 1995), on transmitter release (Wang and Quastel, 1990), and during excitotoxicity induced by excitatory amino acids (Frederickson et al., 1989). The ability of dipicolinic acid to produce effects identical with those of Ca^{++} EDTA supports the conclusion that their actions result from a common ability to chelate zinc. In addition, the inability of dipicolinic acid to prevent the antinociceptive effect of capsaicin when these drugs were delivered at a neutral pH, rather than an acidified pH, further suggests that the inhibitory effect of dipicolinic acid on capsaicin is due to chelation of zinc, an action of dipicolinic acid that requires acidification.

Mobilization of zinc by capsaicin appears to take place downstream from the action of SP N-terminal metabolites as the antinociceptive effect of SP(1–7) was inhibited by Ca^{++} EDTA and dipicolinic acid in a fashion identical with their inhibitory effects on capsaicin. Acute antinociception, observed 30 min after injection of a relatively low dose of SP(1–7) (Goettl and Larson, 1994), was inhibited only when Ca^{++} EDTA was injected 24 h before SP(1–7). These data indicate that the onset of action for Ca^{++} EDTA to inhibit SP(1–7)-induced antinociception (24 h) is longer that that for its ability to induce hyperalgesia in the tail-flick assay (60–90 min) (Larson and Kitto, 1997). The absence of hyperalgesia 24 h after Ca^{++} EDTA indicates that this chelator does not antagonize the antinociceptive effect of SP(1–7) merely by an opposing hyperalgesic action. One might speculate that the hyperalgesia immediately after the injection of these chelators results from the sequestration of zinc in the extracellular area. Inhibition of the long-term (24 h) antinociceptive effect of SP(1–7) may result from the gradual depletion or leaching of zinc from its intracellular stores by interfering with the recycling of released zinc. The latter is a process that would likely require a more protracted time interval. Because of the multiple sites at which zinc has been reported to act in the CNS, these possibilities require further study.

An i.t. injection of zinc produces an acute (90 min) antinociception in the acetic acid-induced writhing assay in mice (Larson and Kitto, 1997). The role of zinc appears to depend on the nociceptive modality because sequestration of zinc in the extracellular area by an i.t. injection of either Ca^{++} EDTA or dipicolinic acid produces thermal hyperalgesia in the tail-flick assay, whereas identical treatment of mice is without effect in the writhing assay that reflects chemical nociception. Based on the hypersensitivity of primary afferent C-fibers during conditions of zinc deficiency in the rat (Izumi et al., 1995), antinociception has been proposed to result from a general ability of zinc to stabilize primary afferent C-fibers. Consistent with this, patients whose plasma zinc is lowered by repeated hemodialysis often experience spontaneous pruritus, a sensation transmitted by primary afferent C-fibers (Gilchrest et al., 1982; Stahle-Backdahl et al., 1988). The mechanism underlying the effect of zinc on C-fiber activity is not clear.

Although the injection of 10 nmol of Ca^{++} EDTA i.t. prevented the development of capsaicin-induced antinociception measured 24 h later, it is of interest that the immediate biting and scratching behavioral response of mice to capsaicin injected i.t. is unaffected by pretreatment with this same dose of Ca^{++} EDTA (Larson and Kitto, 1997). Although two pools of zinc may be influenced by these chelators when administered at two different time intervals, as discussed above, it is also possible that the behavioral response associated with depolarization of C-fibers and the delayed antinociception may result from two different mechanisms: an im-

mediate response that does not involve zinc and another that does. The dose of capsaicin used to produce an immediate behavioral response is lower than that required for desensitization and antinociception. Lower concentrations of capsaicin are necessary to induce glutamate than SP release (Ueda et al., 1994), and these amino acids have been proposed to mediate the biting and scratching behavior (Okano et al., 1994). Based on this, one might speculate that zinc is not necessary for the capsaicin-induced release or action of these amino acids but rather essential for the release, formation, or activity of SP N-terminal metabolites that lead to antinociception after the injection of higher, desensitizing doses of capsaicin.

Although zinc is necessary, it may not be sufficient to induce a long-term antinociception because only a modest inhibition of writhing resulted from the i.t. injection of even a large dose of zinc. However, these studies are not definitive because the release of zinc from synaptic vesicles, where its concentration is estimated to be as high as 200 to 300 μM (Frederickson, 1989), may achieve a concentration in the synaptic cleft that is higher than that after an i.t. injection. Thus, it is possible that the concentration at the target area may not be mimicked by an i.t. injection of zinc due to the rapid and efficient action of zinc transport proteins (Palmiter et al., 1996a,b) that would rapidly sequester the ion. Similar pharmacodynamic effects prevent the replication of neurotransmitter effects after their injection or the injection of agonists at their receptors.

Together, these data suggest that zinc, localized in the extracellular area of the adult mouse, is necessary for the long-term antinociceptive effects of capsaicin and SP N-terminal metabolites administered i.t. However, zinc is not necessary for the behavioral response produced immediately after an injection of capsaicin. These data are consistent with two different mechanisms for the immediate aversive and delayed antinociceptive effects of capsaicin.

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