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Neurokinin-1 Receptors Are Involved in Behavioral Responses to High-intensity Heat Stimuli and Capsaicin-induced Hyperalgesia in Mice

Heikki Mansikka, M.D., Ph.D., Masako Shiotani, M.D.,† Richard Winchurch, Ph.D.,‡ Srinivasa N. Raja, M.D.§*

Background: **The neurokinin-1 (NK-1) receptor and its ligand, substance P, are thought to play important roles in nociception and hyperalgesia. This study evaluated the role of the NK-1 receptor in processing noxious stimuli in normal and inflammatory states.**

Methods: **Behavioral responses to heat and mechanical and chemical stimuli were studied in NK-1 receptor knockout mice and wild-type control mice. Thermal nociception was evaluated by measuring paw lick or jump latencies to hot plate (52, 55, and 58°C) and paw withdrawal latencies to radiant heat applied to the hind paws. Mechanical nociception was measured by von Frey monofilament applications to the hind paws. Intraplantar capsaicin-induced (10 μg/20 μl) paw licking and mechanical and heat hyperalgesia were compared in NK-1 knockout and wild-type mice.**

Results: **Withdrawal responses to radiant heat (4.3 ± 0.18 s for knockout and 4.4 ± 0.8 s for wild-type mice) and von Frey monofilaments were similar in knockout and wild-type mice. In the hot plate test, increasing the hot plate temperature from 52°C to 58°C resulted in a decrease in the response latency in the wild-type mice (30.4 ± 17.5 s to 15.2 ± 6.8 s, $P < 0.05$), whereas in the knockout mice the response latencies remained constant (28.2 ± 19.8 s to 29 ± 15.1 s, not significant). Capsaicin-induced paw licking (14.5 ± 12.8 s for knockout and 41.3 ± 37.3 s for wild-type mice, $P < 0.05$) and mechanical and heat hyperalgesia were attenuated in the knockout mice.**

Conclusion: **NK-1 receptors contribute to the withdrawal responses to high-intensity heat stimuli and to capsaicin-induced mechanical and heat hyperalgesia. (Key words: Neurogenic inflammation; substance P; pain.)**

THE neurokinin-1 (NK-1) receptor and its ligand, substance P, are thought to play important roles in coding noxious stimuli and in the mechanisms of hyperalgesia.[1–4] Substance P is synthesized and released after noxious stimulation from the small-diameter primary afferents to the spinal cord dorsal horn.[5–8] Neurokinin-1 receptors are located in the superficial laminae of the dorsal horn of the spinal cord, a region thought to be important in the processing of nociceptive stimuli.[9] Although substance P can excite spinal nociceptive neurons,[10] its primary role is believed to be modulation of the responses of excitatory amino acids.[11] Recent studies in mice with deletion of the gene encoding for substance P–neurokinin A (NKA) or the NK-1 receptor show alterations in pain sensitivity. A reduction in nociceptive responses to certain somatic and visceral noxious stimuli has been shown in substance P–NKA and NK-1 knockout mice.[12–14] In particular, the intensity coding of suprathreshold stimuli is impaired in the NK-1 knockout mice. Inflammation-induced mechanical hyperalgesia was similar between mutant and wild-type in substance P–NKA and NK-1 knockout mice. Mantyh *et al.*[15] showed in rats that destroying the lamina I spinal cord neurons expressing the substance P receptor attenuates neurogenic inflammation-induced hyperalgesia. However, neurons expressing the substance P receptor also might express some other neurotransmitter receptors that play a role in the mechanisms of hyperalgesia. To characterize further the role of NK-1 receptors in nociception, we compared the behavioral responses to thermal, mechanical, and chemical stimuli applied to the normal and inflamed paws of NK-1 knockout and wild-type control mice.

* Research Fellow, Department of Neurosurgery.

† Research Fellow, Department of Anesthesiology and Critical Care Medicine.

‡ Professor, Department of Surgery.

§ Professor, Department of Anesthesiology and Critical Care Medicine.

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Address reprint requests to Dr. Mansikka: Department of Neurosurgery, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Meyer 5-109, Baltimore, Maryland 21287. Address electronic mail to: mansikka@welchlink.welch.jhu.edu

Materials and Methods

The experiments were performed using NK-1 knockout (n = 22) and wild-type balb C (n = 21) adult mice (8–16 weeks old in both groups). Mice deficient in the NK-1 receptor were generated by gene targeting as described in another publication.[16] Breeding stocks of NK-1 knockout mice and wild-type controls were provided by Dr. Norma Gerard (Perlmutter Laboratory, Children's Hospital, Boston, Massachusetts). Colonies were established in our laboratories and maintained in microisolator cages. The genomic status was monitored periodically by polymerase chain reaction.[17] The animals were maintained in a 12-h light–dark cycle and were provided food and water *ad libitum*. The NK-1 receptor knockout animals were grossly normal developmentally, were fertile, and appeared to be healthy. Knockout animals could not be separated from the wild-type mice by their appearance. Locomotor activity has been shown to be normal in NK-1 knockout mice.[14] For all the behavioral experiments, the animals were allowed to habituate for 5 days to the testing environment and for 15 min each day before the actual testing was started. All the testing was performed blinded to the genotype of the mice. The research protocol was approved by the Johns Hopkins Animal Care and Use Committee.

Hot Plate Test

In the hot plate test (wild-type, n = 12; NK-1 knockout, n = 10), mice were placed on a metal plate surrounded by a clear plastic chamber. The latency to lick one of the hind paws or to jump off the plate was measured. Paw lick or jump latencies were measured at three different temperatures. The sequence of hot plate temperatures was 55, 58, and 52°C (wild-type, n = 10; NK-1 knockout, n = 6). For the remaining mice (wild-type, n = 2; NK-1 knockout, n = 4), the sequence was 58, 55, and 52°C. In each animal, the response to only one temperature was tested on a given day. The tests were separated by at least 24 h. A cutoff time of 60 s was used.

Radiant Heat

To measure withdrawal latency to radiant heat, the mice (wild-type, n = 10; NK-1 knockout, n = 10) were placed on a glass plate preheated to a constant temperature surrounded by a clear plastic chamber (model 336 Analgesia Meter; IITC Inc./Life Science Instruments, Woodland Hills, CA). Before withdrawal latencies were measured, plantar surfaces of both hind paws were painted black with waterproof ink (Eberhard Faber, Lewisburg, TN). Radiant heat was applied from below to the plantar surface of each hind paw, and the withdrawal latency was measured using an electronic timer.[18] Three measurements were taken in each hind paw spaced at least 1 min apart to determine the mean withdrawal latency.

Withdrawal Frequencies to Mechanical Stimuli

Mechanical withdrawal frequencies were measured by calibrated von Frey monofilaments (Stoelting Co., Wood Dale, IL). Mice (wild-type, n = 10; NK-1 knockout, n = 10) were placed in a clear plastic chamber on an elevated mesh screen. The von Frey monofilaments were applied to the plantar surface of each hind paw in ascending series. The monofilaments used in this study produced forces of 0.06, 0.20, 0.52, 1.15, 2.86, and 4.43 g. Every monofilament was applied to the hind paw for approximately 1 s. Each hind paw was studied with ascending series of monofilaments five times. The frequency percentage of paw withdrawal was calculated to correspond to each monofilament.

Capsaicin-induced Pain Behavior

To study the responses to chemical stimulus in wild-type and NK-1 knockout mice, we injected 10 μg/20 μl capsaicin to the intraplantar surface of the hind paw (wild-type, n = 14; NK-1 knockout, n = 13). Capsaicin was dissolved in 7.5% dimethylsulfoxide in saline. Vehicle injections (20 μl) served as control for capsaicin injections (wild-type, n = 6; NK-1 knockout, n = 7). The licking time of the injected hind paw was measured for 10 min after injection. After capsaicin or vehicle injections, withdrawal latencies to radiant heat and mechanical withdrawal frequencies were determined in the same mice as described previously at various time points. Latencies to radiant heat were tested before (baseline) and 10, 40, and 60 min after injections. Mechanical testing was performed before and 10, 25, 45, and 65 min after injections.

Experimental Protocol

Animals were tested on the hot plate test set at three different temperatures on separate days. Baseline mechanical and radiant heat paw withdrawal measurements were taken and were followed by the capsaicin or vehicle injections. After capsaicin or vehicle injections, mechanical and thermal testing were performed at various times for as long as 65 min after injection.

Statistical Analyses

Data were analyzed using a two- or three-way repeated measures analysis of variance. *Post hoc* comparisons were performed using the Tukey significant difference test. All the statistical analyses were performed using the statistical software package Statistica (StatSoft, Tulsa, OK). $P < 0.05$ was considered significant.

Results

Responses to Thermal and Mechanical Stimulation

The response latencies to the hot place test at 52°C were similar in the wild-type and NK-1 knockout mice. Significant interaction existed between the group and the hot plate temperature ($F2,40 = 4.79$, $P < 0.05$). Increasing the hot plate temperature from 52°C to 58°C resulted in a decrease in the response latency in the wild-type mice (30.4 ± 17.5 s to 15.2 ± 6.8 s, $P < 0.05$; fig. 1A). In the NK-1 knockout mice, the response latencies remained constant over the increased temperature (28.2 ± 19.8 s to 29 ± 15.1 s, not significant; fig. 1A).

Withdrawal latencies to radiant heat were not different in the NK-1 knockout and wild-type mice in the uninflamed paw. The latencies to withdrawal were measured in both hind paws, and the latencies were averaged in each animal. In NK-1 mice, the withdrawal latency was 4.3 ± 0.8 s; in wild-type mice, the latency was 4.4 ± 0.8 s (fig. 1B).

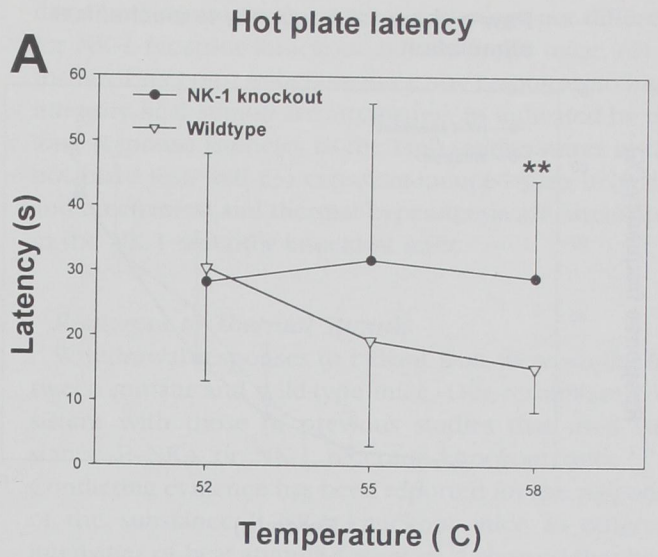
The baseline withdrawal frequencies to mechanical stimulation of NK-1 knockout and wild-type mice were not significantly different in the uninflamed paw ($F7,348 = 1.3$, $P > 0.05$; fig. 2).

Capsaicin-induced Pain Behavior

Paw licking time after capsaicin injection in wild-type mice was longer than in the NK-1 knockout mice (41.3 ± 37.3 s *vs.* 14.5 ± 12.8 s, $P < 0.05$; fig. 3). The licking time after vehicle or capsaicin injections in the NK-1 knockout mice were not significantly different (10.5 ± 10.4 s *vs.* 14.5 ± 12.8 s, not significant; fig. 3).

There was a significant interaction between group and time ($F21,168 = 1.84$, $P < 0.05$) in the withdrawal latencies to radiant heat after capsaicin or vehicle injections. In the wild-type mice, 10 min after capsaicin injection, withdrawal latencies in the injected paw decreased (pre- *vs.* postinjection latencies: 4.4 ± 0.8 s *vs.* 3.2 ± 1.2 s, $P < 0.01$; fig. 4) and returned to baseline values 40 min after capsaicin injection (3.8 ± 1.2 s, not significant; fig. 4). In the NK-1 knockout mice, capsaicin and vehicle injections did not decrease withdrawal latencies at any time (fig. 4). None of the injections had significant effects on the withdrawal latencies of the contralateral paw in wild-type or NK-1 knockout mice ($F3,28 = 1.16$, $P > 0.05$; data not shown).

After injection of capsaicin or vehicle, significant differences occurred in the mechanical withdrawal fre-



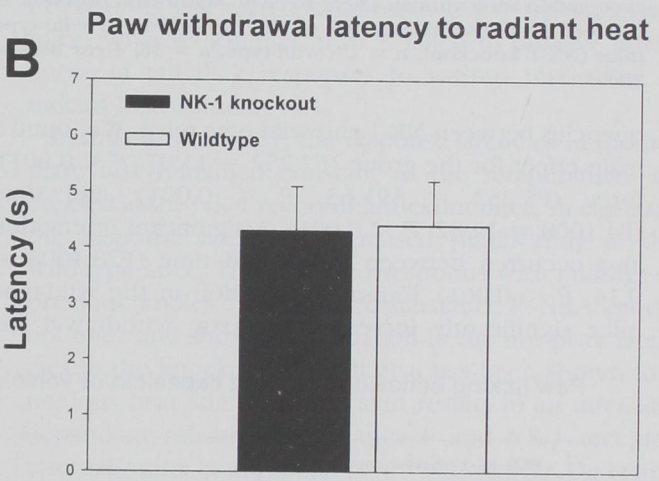


Fig. 1. (*A*) Response latencies in the hot plate test at three different temperatures. Increasing the hot plate temperature decreased the response latency in wild-type mice but not in NK-1 knockout mice (NK-1 knockout, n = 10; wild-type, n = 12; **$P < 0.01$, NK-1 compared with wild-type control; error bars = SD). (*B*) The paw withdrawal latency to radiant heat. The latencies to withdrawal were measured in both hind paws, and the latencies were averaged in each animal. There were no significant differences between response latencies in NK-1 knockout or wild-type mice (NK-1 knockout, n = 17; wild-type, n = 16; error bars = SD).

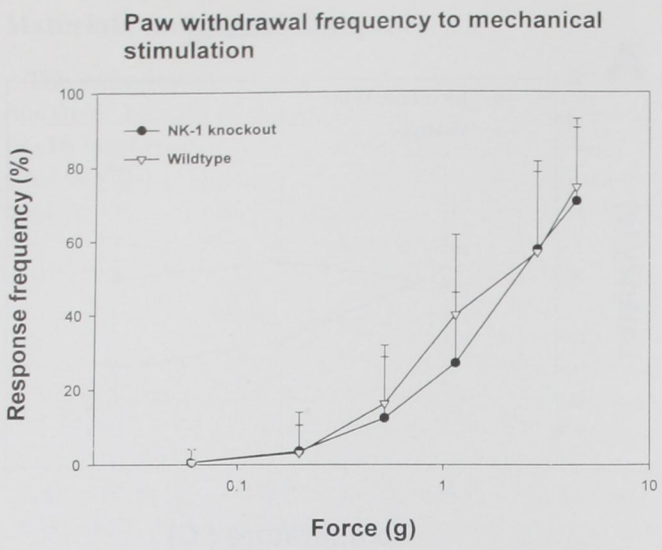


Fig. 2. Paw withdrawal frequency to mechanical stimulation. The frequency of withdrawal in the left and the right paws were averaged in each animal. There were no significant differences between response frequencies of NK-1 knockout or wild-type mice (NK-1 knockout, n = 17; wild-type, n = 16; error bars = SD).

quencies between NK-1 and wild-type mice. We found a main effect for the group ($F_{7,252} = 11.07$, $P < 0.001$), force ($F_{5,252} = 391.63$, $P < 0.001$), and time ($F_{4,1008} = 14.82$, $P < 0.001$). A significant interaction also occurred between group and time ($F_{28,1008} = 9.14$, $P < 0.001$). Capsaicin injection in the wild-type mice significantly increased the paw withdrawal frequencies compared with preinjection frequencies (figs. 5A and B). The increased responsiveness seen 10 min after capsaicin injection returned to baseline 25 min after injection (fig. 5C). In the NK-1 knockout mice, capsaicin-induced mechanical hyperalgesia was attenuated. Vehicle injections did not alter withdrawal frequencies in NK-1 knockout or wild-type mice (figs. 5A–C). In addition, neither the capsaicin nor the vehicle injections to hind paws affected the withdrawal frequencies of the contralateral hind paw of wild-type or NK-1 knockout mice ($F_{3,126} = 2.5$, $P > 0.05$; data not shown).

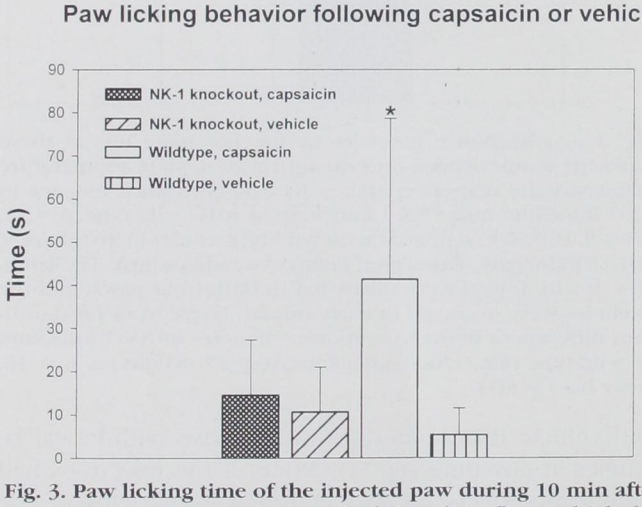


Fig. 3. Paw licking time of the injected paw during 10 min after intraplantar injection of capsaicin (10 μg/20 μl) or vehicle (20 μl). The licking times in NK-1 knockout mice with capsaicin (n = 13) or vehicle control (n = 7) were not significantly different. However, in the wild-type mouse, the licking time after capsaicin (n = 14) was significantly longer than after vehicle (n = 6; *$P < 0.05$, error bars = SD).

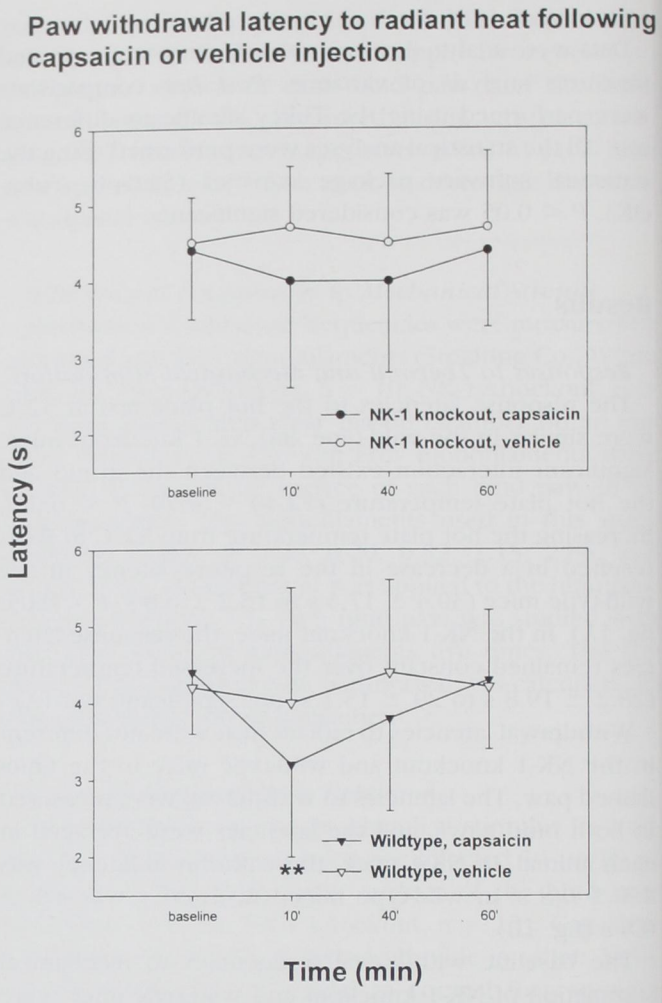


Fig. 4. Paw withdrawal latency to radiant heat after capsaicin (10 μg/20 μl) or vehicle (20 μl) injections. Preinjection withdrawal latencies were similar in NK-1 knockout (n = 10) and wild-type mice (n = 10). Capsaicin injection had no effect on the withdrawal latencies in the NK-1 knockout mice, whereas in the wild-type mice the withdrawal latencies were decreased ($P < 0.01$ compared with the preinjection values; error bars = SD). Vehicle injections did not have an effect on the withdrawal latencies in NK-1 knockout (n = 7) or wild-type (n = 6) mice.**

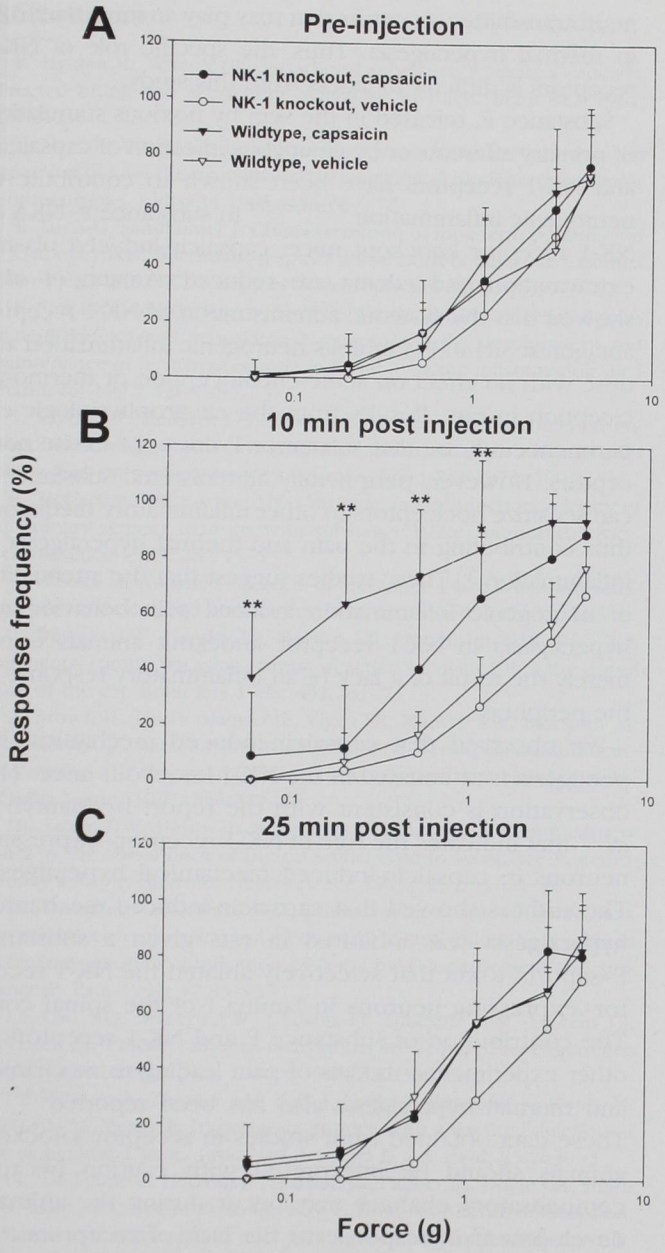


Fig. 5. Paw withdrawal frequencies for different forces of mechanical stimuli at various time points: (*A*) Before injection, (*B*) 10 min after capsaicin or vehicle, and (*C*) 25 min after capsaicin or vehicle. (Wild-type capsaicin, n = 10; wild-type vehicle, n = 6; NK-1 knockout capsaicin, n = 10; NK-1 knockout vehicle, n = 7; * $P < 0.05$ and ** $P < 0.01$ compared with the preinjection values, error bars = SD). Data for 45 and 65 min after injections were not different from the data for the 25-min time point and are not presented.

Discussion

We made three primary observations in the current study: (1) Nociceptive responses to mechanical and radiant heat stimuli in the normal paw were not different for NK-1 receptor knockout and wild-type mice; (2) in the NK-1 receptor knockout mice, the responses to high-intensity heat stimuli are attenuated, as indicated by the long response latencies to the high temperatures in the hot plate test; and (3) capsaicin-induced pain behavior and mechanical and thermal hyperalgesia are attenuated in the NK-1 receptor knockout mice.

Responses to Thermal Stimuli

Withdrawal responses to radiant heat were similar between mutant and wild-type mice. Our results are consistent with those of previous studies that used substance P–NKA or NK-1 receptor knockout mice.[12–14] Conflicting evidence has been reported for the response of the substance P–NKA knockout mice to different intensities of heat stimuli. Cao *et al.*[12] showed that with moderate-intensity radiant heat stimuli to the paw, pain behavior is attenuated in substance P–NKA knockout mice, whereas Zimmer *et al.*[13] failed to observe differences in tail flick responses to various intensities of radiant heat stimuli.

In the current study, the response latencies in the hot plate test remained constant as the temperatures increased in the NK-1 receptor knockout mice. In contrast, the response latencies decreased significantly in the wild-type mice. This result corresponds with findings of previous studies[12,13] that used substance P–NKA knockout mice and showed attenuation in the hot plate latencies in the knockout mice. It also has been shown that noxious heat stimuli to the skin results in an intensity-dependent release of substance P and NK-1 receptor internalization in the spinal cord.[8,19] However, De Felipe *et al.*[14] did not find any differences between NK-1 knockout and wild-type mice in hot plate latencies at 52°C. In the current study, response latencies at 52°C also were not different between NK-1 knockout and wild-type mice. Results from current and previous studies indicate that substance P–NK-1 receptors contribute to the responses to high-intensity heat stimuli. Previous electrophysiologic and behavioral studies have shown that nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different classes of nociceptors. Rapid heating of the skin preferentially activates Aδ nociceptors, whereas a slower rate of heating preferentially activates C-fiber nociceptors.[20,21] It is possible that the NK-1 receptor is preferentially involved in the Aδ-fiber–mediated nociception during physiologic conditions.

Responses to Mechanical Stimuli

Withdrawal responses to mechanical stimuli were similar in NK-1 and wild-type mice. This is in line with previous studies using substance P–NKA[12] or NK-1 receptor[14] knockout mice. Cao *et al.*[12] showed, however, that responses to more intense mechanical stimuli were attenuated in mutant mice, indicating that tachykinins, substance P, and NKA play an important role in coding suprathreshold stimuli. Further evidence to support this view is derived from studies showing that high-frequency primary afferent stimulation or suprathreshold mechanical stimuli are needed to evoke a release of substance P.[22,23] In addition, De Felipe *et al.*[14] did not see any intensity encoding to suprathreshold mechanical stimuli in NK-1 knockout mice. In the current study, withdrawal responses to suprathreshold von Frey monofilaments were not different between NK-1 knockout and wild-type mice. The mechanical stimuli used in these studies were more intense (for example, hemostat applied to the hind paw or a clip applied to the tail). Therefore, the intensity of mechanical stimuli in the current study might have been too weak to produce differences in responses to suprathreshold stimuli. Punctate stimuli delivered by von Frey monofilaments are short lasting and confined to a small area. In addition to the intensity of mechanical stimuli, temporal and spatial summation also may have contributed to the differences in responses to different mechanical stimuli.

Responses to Capsaicin

Capsaicin has been shown to evoke spontaneous pain and thermal and mechanical hyperalgesia in humans and in animals.[24–26] We observed that capsaicin-induced pain behavior and mechanical and thermal stimuli-evoked hyperalgesia were attenuated in the NK-1 knockout mice.

The finding that capsaicin-induced paw licking was abolished in the NK-1 knockout mice is in line with previous studies, which showed that (1) capsaicin evokes a release of substance P in the spinal cord,[27] (2) intrathecal substance P elicits pain behavior,[1] (3) capsaicin-induced pain behavior can be attenuated by intrathecal administration of NK-1 or tachykinin antagonists,[26,28] and (4) capsaicin-induced pain behavior also was reduced in substance P–NKA knockout mice.[12]

Capsaicin-induced heat hyperalgesia was abolished in the NK-1 knockout mice. Mantyh *et al.*[15] also reported similar attenuation of capsaicin-induced thermal hyperalgesia in rats with cytotoxic injury to lamina I NK-1 receptor–expressing neurons. These neurons also may express other neurotransmitter receptors that may play an important role in thermal hyperalgesia. Thus, the specific role of NK-1 receptors is difficult to assess from this study.

Substance P, released in the skin by noxious stimulation of primary afferents or by cutaneous injection of capsaicin, and NK-1 receptors have been shown to contribute to neurogenic inflammation.[12,14,29,30] In substance P–NKA or NK-1 receptor knockout mice, capsaicin-induced plasma extravasation and edema are reduced. Amann *et al.*[31] showed that the systemic administration of NK-1 receptor antagonist SR140333 inhibits neurogenic inflammation at a dose with no effect on acute chemoception or thermonociception in rats. Results from the electrophysiologic experiments indicate that substance P does not excite nociceptors. However, peripherally administered substance P can sensitize nociceptors to other inflammatory mediators, thus contributing to the pain and thermal hyperalgesia in inflammation.[32] These studies suggest that the attenuation of neurogenic inflammation induced pain behavior and hyperalgesia in NK-1 receptor knockout animals is not merely the result of a lack of an inflammatory response in the periphery.

We observed that capsaicin-induced mechanical hyperalgesia is attenuated in the NK-1 knockout mice. This observation is consistent with the report by Mantyh *et al.*[15] that indicates the role of NK-1 receptor–expressing neurons in capsaicin-induced mechanical hyperalgesia. The authors showed that capsaicin-induced mechanical hyperalgesia was inhibited in rats given a substance P–saporin toxin that selectively ablated the NK-1 receptor–expressing neurons in lamina I of the spinal cord. The contribution of substance P and NK-1 receptors in other experimental models of pain leading to mechanical and thermal hyperalgesia also has been reported[2,4,33,34] These data obtained from studies in receptor knockout animals should be interpreted with caution because compensatory changes may occur during the animal's development in response to the lack of receptors that are normally present.

The data from the current study indicate that NK-1 receptors contribute to the responses to high-intensity heat and chemical stimuli. NK-1 receptors do not play a major role in responses to noxious stimuli near the nociceptive threshold. However, in neurogenic inflammation, NK-1 receptors contribute to heat and mechanical hyperalgesia.

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References

1. Hylden JL, Wilcox GL: Intrathecal substance P elicits a caudally-directed biting and scratching behaviour in mice. Brain Res 1981; 271:212–5

2. Ma QP, Woolf CJ: Involvement of neurokinin receptors in the induction but not the maintenance of mechanical allodynia in rat flexor motoneurones. J Physiol 1995; 486:769–77

3. Liu X-G, Sandkuhler J: Characterization of long-term potentiation of C-fiber-evoked potentials in spinal dorsal horn of adult rat: Essential role of NK1 and NK2 receptors. J Neurophysiol 1997; 78:1973–82

4. Sluka KA, Milton MA, Willis WD, Westlund KN: Differential roles of neurokinin 1 and neurokinin 2 receptors in the development and maintenance of heat hyperalgesia induced by acute inflammation. Br J Pharmacol 1997; 120:1263–73

5. Hökfelt T, Kellerth J-O, Nilsson G, Pernow B: Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons. Brain Res 1975; 100:235–52

6. McCarthy PW, Lawson SN: Cell type and conduction velocity of rat primary sensory neurons with substance P-like immunoreactivity. Neuroscience 1989: 28:745–53

7. Otsuka M, Konishi S: Release of substance P-like immunoreactivity from isolated spinal cord of newborn rat. Nature 1976; 264:83–4

8. Duggan AW, Hendry IA, Morton CR, Hutchison WD, Zhao ZQ: Cutaneous stimuli releasing immunoreactive substance P in the dorsal horn of the cat. Brain Res 1988; 451:261–73

9. Brown JL, Liu H, Maggio JE, Vigna SR, Mantyh PW, Basbaum AI: Morphological characterization of substance P receptor-immunoreactive neurons in the rat spinal cord and trigeminal nucleus caudalis. J Comp Neurol 1995; 356:327–44

10. Salter MW, Henry JL: Responses of functionally identified neurones in the dorsal horn of the cat spinal cord to substance P, neurokinin A and physalaemin. Neuroscience 1991; 43(2-3):601–10

11. Dougherty PM, Willis WD: Enhancement of spinothalamic neuron responses to chemical and mechanical stimuli following combined micro-iontophoretic application of N-methyl-D-aspartic acid and substance P. Pain 1991; 47:85–93

12. Cao YQ, Mantyh PW, Carlson EJ, Gillespie A-M, Epstein CJ, Basbaum AI: Primary afferent tachykinins are required to experience moderate to intense pain. Nature 1998; 392:390–4

13. Zimmer A, Zimmer AM, Baffi J, Usdin T, Reynolds K, König M, Palkovits M, Mezey E: Hypoalgesia in mice with a targeted deletion of the tachykinin 1 gene. Proc Natl Acad Sci U S A 1998; 95:2630–5

14. De Felipe C, Herrero JF, O'Brien JA, Palmer JA, Doyle CA, Smith AJH, Laird JMA, Belmonte C, Cervero F, Hunt SP: Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature 1998; 392:394–7

15. Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA: Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. Science 1997; 278:275–9

16. Bozic CR, Lu B, Höpken UE, Gerard C, Gerard NP: Neurogenic amplification of immune complex inflammation. Science 1996; 273: 1722–5

17. Irwin MH, Moffatt RJ, Pinkert CA: Identification of transgenic mice by PCR analysis of saliva. Nat Biotechnol 1996; 14:1146–8

18. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A New and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988; 32:77–88

19. Allen BJ, Rogers SD, Ghilardi JR, Menning PM, Kuskowski MA, Basbaum AI, Simone DA, Mantyh PW: Noxious cutaneous thermal stimuli induce a graded release of endogenous substance P in the spinal cord: Imaging peptide action in vivo. J Neurosci 1997; 17(15):5921–7

20. Yeomans DC, Pirec V, Proudfit HK: Nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different nociceptors in the rat: Behavioral evidence. Pain 1996; 68: 133–40

21. Yeomans DC, Proudfit HK: Nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different nociceptors in the rat: Electrophysiological evidence. Pain 1996; 68: 141–50

22. Marvizon JCG, Martinez V, Grady EF, Bunnett NW, Mayer EA: Neurokinin 1 receptor internalization in spinal cord slices induced by dorsal root stimulation is mediated by NMDA receptors. J Neurosci 1997; 17:8129–36

23. Mantyh PW, DeMaster E, Malhotra A, Ghilardi JR, Rogers SD, Mantyh CR, Liu H, Basbaum AI, Vigna SR, Maggio JE, Simone DA: Receptor endocytosis and dendrite reshaping in spinal neurons after somatosensory stimulation. Science 1995; 268:1629–32

24. LaMotte RH, Shain CN, Simone DA, Tsai EP: Neurogenic hyperalgesia: Psychophysical studies of underlying mechanisms. J Neurophysiol 1991; 65:190–211

25. Gilchrist HD, Allard BL, Simone DA: Enhanced withdrawal responses to heat and mechanical stimuli following intraplantar injection of capsaicin in rats. Pain 1996; 67:179–88

26. Sakurada T, Katsumata K, Tan-No K, Sakurada S, Kisara K: The Capsaicin test in mice for evaluating tachykinin antagonist in the spinal cord. Neuropharmacology 1992; 31:1279–85

27. Yaksh TL, Jessell TM, Gamse R, Mudge AW, Leeman SE: Intrathecal morphine inhibits substance P release from mammalian spinal cord in vivo. Nature 1980; 286:155–7

28. Sakurada T, Katsumata K, Yogo H, Tan-No K, Sakurada S, Kisara K: Antinociception induced by CP 96,345, a non-peptide NK-1 receptor antagonist, in the mouse formalin and capsaicin test. Neurosci Lett 1993; 151:142–5

29. Lembeck F, Holzer P: Substance P as a neurogenic mediator of antidromic vasodilatation and neurogenic plasma extravasation. Nauyn Schmiedebergs Arch Pharmacol 1979; 310:175–83

30. Helme RD, Koschorke GM, Zimmermann M: Immunoreactive substance P release from skin nerves in the rat by noxious thermal stmulation. Neurosci Lett 1986; 63:295–9

31. Amann R, Schuligoi R, Holzer P, Donnerer J: The non-peptide NK1 receptor antagonist SR140333 produces long-lasting inhibition of neurogenic inflammation, but does not influence acute chemo- or thermonociception in rats. Nauyn Schmiedebergs Arch Pharmacol 1995; 352:201–5

32. Kress M, Reeh PW: Chemical excitation and sensitization in nociceptors, Neurobiology of Nociceptors. Edited by Belmonte C, Cervero F. New York, Oxford University Press, 1996, pp 258–97

33. Schaible HG, Jarrott B, Hope PJ, Duggan AW: Release of immunoreactive substance P in the spinal cord during development of acute arthritis in the knee joint of the cat: A study with antibody microprobes. Brain Res 1990; 529:214–23

34. Abbadie C, Brown JL, Mantyh PW, Basbaum AI: Spinal cord substance P receptor immunoreactivity increases in both inflammatory and nerve injury models of persistent pain. Neuroscience 1996; 70: 201–9