Activation of Spinal Wide Dynamic Range Neurons by Intracutaneous Microinjection of Nicotine

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Jinks, Steven L. and E. Carstens. Activation of spinal wide dynamic range neurons by intracutaneous microinjection of nicotine. J. Neurophysiol. 82: 3046–3055, 1999. Nicotine evokes pain in the skin and oral mucosa and excites a subpopulation of cutaneous nociceptors, but little is known about the central transmission of chemogenic pain. We have investigated the responses of lumbar spinal wide dynamic range (WDR)-type dorsal horn neurons to intracutaneous (ic) microinjection of nicotine in pentobarbital-anesthetized rats. Nearly all (97%) units responded to nicotine microinjected ic (1 μl) into the low-threshold region of the hind-paw mechanosensitive receptive field in a concentration-related manner (0.01–10%). Responses to repeated injections of 10% nicotine exhibited tachyphylaxis at 5-, 10-, and 15-min inter-stimulus intervals. Significant tachyphylaxis was not seen with 1% nicotine. All nicotine-responsive units tested (n = 30) also responded to ic histamine (1 μl, 3%) and did not exhibit tachyphylaxis to repeated histamine. However, there was significant cross-tachyphylaxis of nicotine to histamine. Thus 5 min after ic nicotine, histamine-evoked responses were attenuated significantly compared with the initial histamine-evoked response prior to nicotine, with partial recovery over the ensuing 15 min. Neuronal excitation by ic nicotine was not mediated by histamine H1 receptors because ic injection of the H1 receptor antagonist, cetirizine, had no effect on ic nicotine-evoked responses, whereas if significantly attenuated ic histamine-evoked responses in the same neurons. The lowest-threshold portion of cutaneous receptive fields showed a significant expansion in area at 20 min after ic nicotine 10%, indicative of sensitization. Responses to 1% nicotine were significantly reduced after ic injection of the nicotinic receptor antagonist, mecamylamine (0.1% ic), with no recovery over the ensuing 40–60 min. These data indicate that nicotine ic excites spinal WDR neurons, partly via neuronal nicotinic acetylcholine receptors that are presumably expressed in cutaneous nociceptor terminals. Repeated injections of high concentrations of nicotine led to tachyphylaxis and cross-tachyphylaxis with histamine, possibly relevant to peripheral analgesic effects of nicotine.

INTRODUCTION

Nicotine and other cholinergic agonists evoke sensations of irritation or pain when delivered to skin or oral mucosa (Desirier et al. 1997, 1998; Jansco et al. 1961; Jarvik and Assil 1988; Keele and Armstrong 1964; Mageer et al. 1990). Cholinergic agonists excite unmyelinated sensory fibers, including identified nociceptors (Douglas and Ritchie 1960; Fjallebrant and Igg 1961; MacIver and Tanelian 1993; Steen and Reeh 1993; Tanelian 1991; Wang et al. 1993). Little is known about central nociceptive processes triggered by peripheral nicotine. It recently was reported that nicotine, as well as a variety of other irritant chemicals, excites neurons in the dorsomedial or ventrolateral aspects of trigeminal subnucleus caudalis when applied topically to the surface of the tongue or cornea, respectively (Carstens et al. 1995, 1998). Trigeminal neuronal responses to nicotine increased in a concentration-dependent manner and exhibited tachyphylaxis to repeated application (Carstens et al. 1998).

Although nicotine is associated with cutaneous pain and irritation, systemic administration of nicotine produces analgesia (e.g., Cooley et al. 1990; Yang et al. 1992). Peripheral administration of ABT-594, a derivative of the powerful cholinergic agonist epibatidine, is also analgesic (Bannon et al. 1998). In the present study, we have investigated further nicotinic excitation and possible sensitization of spinal neurons associated with nociception and determined if subsequent neuronal responses show tachyphylaxis possibly consistent with a peripheral analgesic effect. We also investigated the role of neuronal nicotinic acetylcholine receptors (nAChRs) in the nicotinic excitation of spinal neurons. An abstract of part of this work has appeared previously (Jinks and Carstens 1998b).

METHODS

Preparation, recording, and unit characterization

Experiments were conducted with approval from the local animal care and use advisory committee. The methods were the same as in our previous studies (Carstens 1997; Jinks and Carstens 1998a) and are summarized here. Single-unit recordings were made in 44 adult male Sprague-Dawley rats anesthetized with pentobarbital sodium (induction: 65 mg/kg iv, maintenance: 10–20 mg · kg⁻¹ · h⁻¹ iv via the jugular vein). Anesthesia was sufficient if animals were areflexic and did not exhibit tachycardia (monitored by electrocardiogram) in response to noxious stimulation. The lumbar spinal cord was exposed by laminectomy and stabilized in a frame, and the dura was opened to allow extracellular single-unit recordings with a tungsten microelectrode. Units were searched using innocuous mechanical stimulation of the ipsilateral hind paw, and only those that additionally responded to noxious skin heating (48 or 50°C for 5 s) were tested with chemical stimulation. Unit receptive fields were mapped using von Frey monofilaments with three different bending forces (0.7, 1.5, and 4 g) (see Jinks and Carstens 1998).

Chemical stimulation

A 30.5-gauge needle attached to PE 50 tubing filled with chemical solution was inserted just beneath the epidermis in the lowest-threshold portion of the mechanical receptive field (see Carstens 1997). Separate needles were used for different chemicals or concentrations. There was no evidence of inflammatory response to needle placements over the <1-h recording period. Chemicals were injected in a 1 μl volume over ~1 s. The following chemicals were used: nicotine (0.01–10% = 6 × 10⁻⁴ – 6 × 10⁻¹ M in 0.9% NaCl; free base,
RESULTS

Unit sample

A total of 71 lumbar WDR-type dorsal horn units responded to low-threshold mechanical as well as noxious thermal stimulation of skin within the cutaneous receptive field on the ipsilateral hind paw. Receptive fields varied in size, spanning as little as one to two toes or as much as the entire ventral hind paw surface (Figs. 1–7). Most units were localized to the mid-dorsal horn [mean depth: 518.8 ± 246.9 (SD) μm].

Ninety-seven percent of the units responded to ic nicotine, and each of 30 nicotine-responsive units tested also responded to ic histamine. Responses of a typical WDR unit to noxious heat and irritant chemicals are shown in Fig. 1. The majority (60%) of units showed no or little (1–3 Hz) spontaneous firing, whereas a minority (40%) showed spontaneous firing rates from 3 to 20 Hz.

Concentration-response relationship

All units tested exhibited a concentration-dependent increase in firing after ic nicotine. Figure 2A shows an example of a unit that gave progressively larger and longer-lasting responses to increasing concentrations of ic nicotine. In Fig. 2B, individual (thin lines) and mean (thick line) responses of units are plotted against the concentration of ic nicotine. Responses increased significantly with concentration (ANOVA, $P = 0.006$), with mean responses to 0.01 and 0.1% being significantly less compared with 10%.

Tachyphylaxis

At the highest concentration used (10%), the initial injection of nicotine evoked a large response and subsequent injections elicited progressively smaller responses. An example is shown in Fig. 3A. Figure 3B plots individual (thin lines) and mean (thick lines) neuronal responses to repeated injections of 10% nicotine at a 5-min interstimulus interval, showing that responses to both the second and third application of nicotine were reduced significantly compared with the first injection. When the interstimulus interval was increased to 10 (Fig. 3C) or 15 min (Fig. 3D), only the mean response to the third, but not the second, injection of nicotine was reduced significantly. Figure 3, E and F, shows individual and mean responses to a lower (1%) concentration of nicotine delivered at 5- and 10-

![Image](http://jn.physiology.org/ by 10.220.32.3 on October 28, 2016)
FIG. 2. Nicotine concentration-response relationship. A: individual example. PSTHs (binwidth: 1 s) show increasing responses of individual WDR unit to increasing concentrations of nicotine (1 μl) microinjected intracutaneously (ic). Top inset: cutaneous receptive field (black) and site (arrow) of nicotine injection. Bottom inset: example of recorded action potential. B: graph plots individual (thin lines) and mean (thick line with error bars) neuronal responses as a function of nicotine concentration (n = 12 units). Error bars = SE. Responses to 1 and 10% nicotine were significantly different from each other and from 0.1% (P < 0.05, ANOVA).
min interstimulus intervals, respectively, which did not exhibit significant tachyphylaxis.

Cross-tachyphylaxis to histamine

Because repeated application of the higher concentration of nicotine produced tachyphylaxis, we tested if there was cross-tachyphylaxis to histamine. An individual example is shown in Fig. 4, A–E, and mean responses of all units tested are plotted in Fig. 4F. After nicotine, the mean response to the next application of histamine (His-1) was significantly lower compared with the initial histamine-evoked response (His) before nicotine. Responses to subsequent histamine (His-2, His-3 in Fig. 4F) showed a partial recovery and, while still reduced, were not significantly different compared with the initial trial with histamine. In separate units we verified our prior report (Carstens 1997) of a lack of tachyphylaxis to repeated injection of histamine (mean response to 1st injection: 1,995 ± 1,536 impulses/min; mean response to 2nd: 1,790 ± 1,395; $P > 0.05$; $n = 7$).

Histamine H1 receptor antagonist

Because nicotine exhibited cross-tachyphylaxis to histamine, we addressed the possibility that nicotine might act via cutaneous histamine H1 receptors by determining if a selective histamine H1 receptor antagonist, cetirizine, affected neuronal responses evoked by ic nicotine. We first verified that the cetirizine reduced ic histamine-evoked responses in the same units. In the example shown in Fig. 5, the response to ic histamine (Fig. 5A) was attenuated significantly after ic cetirizine (Fig. 5B) with recovery (Fig. 5C), confirming an earlier
report (Carstens 1997). Responses of the same unit to ic nicotine before and after ic cetirizine are shown in Fig. 5, D and E, respectively, and demonstrate that the histamine antag-
onist had no effect on the nicotine-evoked response. Figure 5 F plots mean responses to histamine and nicotine for six units and shows that the mean response evoked by histamine, but not nicotine, was reduced significantly by ic cetirizine, with sub-
sequent recovery.

Receptive field expansion

Although 10% nicotine induced tachyphylaxis, we also tested if it increased receptive field area consistent with sensi-
tization. We measured receptive field areas before and again 20 min after ic microinjection of 10% nicotine. Figure 6A shows mechanosensitive receptive field areas of a unit mapped using three different von Frey filaments, before (left) and 20 min after, ic nicotine (right). After nicotine there was expansion of the lowest-threshold portion (Fig. 6A, left), as well as a reduction in mechanical sensitivity in a small area surrounding the injection site (†). Figure 6B plots mean receptive field areas determined with the three von Frey filaments before and 20 min after nicotine. There was a significant expansion only in the lowest-threshold area (Fig. 6B, left). However, when the area around the injection site showing reduced tactile sensitivity was subtracted from the low-threshold area, the postnicotine change in low-threshold receptive area was no longer statistically significant. Thus 10% nicotine triggers a limited expansion at the fringe of the low-threshold mechanical receptive field suggestive of sensitization but also reduces mechanical sensitivity at the injection site.

Mecamylamine

We addressed the possible role of nAChRs in mediating nicotinic excitation of spinal dorsal horn neurons by testing if responses to nicotine were reduced after local microinjection of its antagonist, mecamylamine. Figure 7A shows an example. Before mecamylamine, ic nicotine (1%) evoked a robust re-
sponse (Fig. 7A, left). Mecamylamine then was injected ic as close as possible to the site of nicotine microinjection (Fig. 7A, middle) and elicited a small response as seen in 2/10 other units. Nicotine then was injected 1 min later and evoked a response that was reduced markedly (Fig. 7A, middle), followed by partial recovery 40 min later (right). Averaged nicotine-evoked responses for 10 units are plotted in Fig. 7B versus time. Mean responses were significantly lower at 10, 30, and 40–60 min postmecamylamine compared with the preme-
camylamine level.

DISCUSSION

Nicotine ic excites spinal WDR dorsal horn neurons in a concentration-dependent manner. Excitation by a high concen-
tration of nicotine is associated with expansion at the fringe of the lowest-threshold cutaneous receptive field region. At the same time, the 10% (but not 1%) concentration of nicotine triggered tachyphylaxis to subsequent nicotine, as well as cross-tachyphylaxis to histamine. Nicotine-evoked responses were unaffected by the selective H1 histamine receptor antag-
onist, cetirizine, indicating that nicotine did not act via cuta-
neous H1 receptors. Neuronal responses to nicotine were at-
tenuated by local ic injection of mecamylamine, indicating
involvement of nAChRs in nicotinic excitation of cutaneous nociceptors that, in turn, excite spinal WDR neurons.

**Tachyphylaxis and cross-tachyphylaxis**

Repeated application of 10% nicotine led to significant tachyphylaxis, which may be mediated by a reduction in sensitivity of peripheral nociceptors and/or a central mechanism. Nicotine excites nociceptors presumably via nAChRs expressed in their peripheral fiber terminals in the skin (see following text); the nociceptor afferent fibers in turn excite dorsal horn neurons. A peripheral desensitization of nociceptor endings by nicotine might be mediated via influx of Ca$^{2+}$ through nAChRs such as $\alpha_4\beta_2$, $\alpha 7$ and $\alpha 3\beta 2$ (Flores et al.

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**FIG. 5.** Selective reduction by H1 receptor antagonist, cetirizine, of neuronal responses to ic histamine but not nicotine. A–C: individual example shows PSTHs of WDR unit responses to ic histamine (3%), before (A), shortly after ic cetirizine (B), and 5 min after cetirizine (C). *Top inset:* receptive field with ic injection site denoted (→). *Bottom inset:* action potential waveform. D and E: responses of same unit to ic nicotine (1%) before (D) and after cetirizine (E). F: bar graph plots mean responses of 6 WDR units to ic histamine (■) and ic nicotine (□) vs. time relative to ic microinjection of cetirizine. Error bars: SE. *, significant difference ($P < 0.05$, paired t-test).

**FIG. 6.** Expansion of cutaneous receptive field after nicotine. A: shaded areas show extent of individual WDR unit’s low (■), medium (□), and high-threshold (○) mechanical receptive field areas mapped with graded von Frey filaments. *Left:* receptive fields before nicotine; *right:* figurine shows expanded low-threshold receptive field (■) 20 min after injection of 10% nicotine (1 µl). →, site of nicotine injection. B: graph plots mean receptive field areas before (Pre-Nic) and 20 min after (Post-Nic 20 min) a 10% nicotine injection ($n = 13$ units). Area of reduced mechanosensitivity at nicotine injection site not subtracted from low-threshold area. Error bars: SE. *, significantly larger ($P < 0.05$, t-test) compared with prenicotine.
1996; Galzi and Changeaux 1995; Gotti et al. 1997; Holladay et al. 1997; Lindstrom et al. 1996; McGehee and Role 1995; Puttfarcken et al. 1997) or by gating of voltage-sensitive Ca$^{2+}$ channels. Ca$^{2+}$ influx then might trigger intracellular events leading to a reduction in excitability of the nociceptor terminal, possibly analogous to the desensitizing effect of capsaicin on dorsal root ganglion neurons (Caterina et al. 1997; Chard et al. 1995; Cholewinski et al. 1993; Liu and Simon 1996b; Malmberg et al. 1998). A local anesthetic effect of the high nicotine concentration also may have contributed to the tachyphylaxis. This possibility is supported by our observation of increased mechanical threshold at the site of 10% nicotine injection (Fig. 6A).

Dorsal horn neurons do not exhibit significant tachyphylaxis to repeated application of histamine at 5- or 10-min interstimulus intervals (Carstens 1997; Carstens et al. 1998). It has been reported (Stahle-Backdahl et al. 1988) that human itch sensation and cutaneous flare exhibit tachyphylaxis to histamine, which, however, was repeated at much longer interstimulus intervals (90 min or 24 h). Dorsal horn neuronal responses to ic histamine were reduced significantly by prior injection of nicotine (Fig. 4) or capsaicin (Carstens 1997) at the same skin locus. Nicotine cross-desensitization to histamine might involve a peripheral mechanism by which nicotine reduces the sensitivity of nociceptive endings to subsequent histamine, e.g., via nAChRs or a local anesthetic effect. Failure of cetirizine to affect nicotine-evoked excitation of dorsal horn neurons indicates that nicotine cross-desensitization to histamine is not mediated by intracutaneous histamine H1 receptors. Alternatively, cross-desensitization could be mediated centrally. Nicotine might trigger a centrally mediated depression in dorsal horn neuronal excitability, although this is inconsistent with our observation of receptive field expansion postnicotine. Subsequently delivered histamine would activate a
subset of chemonociceptive cutaneous afferent fibers; if such fibers converged onto the same dorsal horn neurons that were conditioned by prior nicotine, they might evoke a lesser response. One practical implication of the present findings is that peripheral application of cholinergic agonists might have value in reducing itch in addition to any analgesic effect they may have.

It recently was reported that a synthetic derivative of epibatidine, ABT-594, has powerful nonopioid analgesic effects that are partly mediated by the CNS (Bannon et al. 1998). Interestingly, however, ic injection of ABT-594 also reduced responses of spinal dorsal horn neurons to noxious stimulation in a manner that was antagonized by local ic mecamylamine (Bannon et al. 1998). This might conceivably be mediated by desensitization of peripheral nociceptor fibers. In this regard, nicotine also has analgesic properties via a central action (e.g., Cooley et al. 1990; Iwamoto 1991; Yang et al. 1992). Nicotine injected into the skin might be taken up into the circulation and distributed to the brain to exert an antinociceptive effect on dorsal horn neurons. However, we injected 10% nicotine in a 1 μl volume (=0.1 mg dose of nicotine); even if all of the nicotine was taken up into the circulation, it would still be several-fold lower than dosages of nicotine that produce analgesia in rats (e.g., 0.8 mg/kg) (Cooley et al. 1990).

Receptive field expansion

After 10% ic nicotine there was an expansion at the fringe of the lowest-threshold area of the cutaneous mechanical receptive field (Fig. 6). This was accompanied by a reduction in mechanical sensitivity at the injection site. This decreased sensitivity might be attributable to a reduction in sensitivity of local mechanoreceptors by nicotine, although an earlier study reported that the cholinergic agonist, carbachol, increased mechanical thresholds of C-fiber polymodal nociceptors but not myelinated mechanoreceptors in rat skin (Stein and Reeh 1993). No such increase in mechanical threshold was observed after control ic saline injections (Carstens 1997; Jinks and Carstens 1998a), indicating that it was not due to volumetric or other nonspecific effects.

The increase in receptive field area may reflect a peripheral and/or central sensitization. Cutaneous receptive fields of WDR and nociceptive-specific dorsal horn neurons expand after inflammation (Ren et al. 1992) or ic injection of capsaicin (Simone et al. 1991b) or histamine (Carstens 1997; Jinks and Carstens 1998a). Receptive field expansion after inflammation (Ren et al. 1992) or ic histamine (Jinks and Carstens 1998a) was prevented by intrathecal pretreatment with N-methyl-D-aspartate (NMDA) receptor antagonists, consistent with the idea that the receptive field expansion reflects an NMDA-receptor-dependent central sensitization of dorsal horn neurons (see Jinks and Carstens 1998a). Alternatively, however, nicotine might act peripherally to sensitize intracutaneous fiber terminals of nociceptors within the receptive field fringe area, which also might manifest as an increase in the WDR neuronal receptive field area. In either case, an increase in the area of mechanical receptive fields of pain-signaling neurons may be associated with allodynia (pain elicited by a normally nonpainful mechanical stimulus) that normally ensues after peripheral tissue injury. Although ic application of nicotine is painful, it is not presently known if this is accompanied by hyperalgesia and allodynia.

Role of nAChRs in nociception

nAChRs consisting of heteromeric α4β2 or α3β4 subunit combinations, or an α7 homooligomer, are distributed widely in the CNS (Galzi and Changeux 1995; Gotti et al. 1997; Holladay et al. 1997; Lindstrom et al. 1996; McGehee and Role 1995) as well as in dorsal root and trigeminal ganglion neurons (Boyd et al. 1991; Flores et al. 1996; Liu et al. 1998; Swanson et al. 1987; Wada et al. 1989). A fraction of dorsal root (Sucher et al. 1990) or trigeminal ganglion neurons (Liu and Simon 1996a; Liu et al. 1993) exhibited inward, depolarizing currents in the presence of nicotine and other cholinergic agonists. nAChR antagonists hexamethonium and neuronal (κ-) bungarotoxin, but not the muscarinic antagonist atropine, blocked the currents (Sucher et al. 1990), suggesting involvement of the α4β2 nAChR. Another study (Liu et al. 1993) reported that nAChR antagonists mecamylamine and hexamethonium, and in some cases also atropine and α-bungarotoxin (consistent with an α7 nAChR), blocked the inward currents. Our present results are also consistent with a partial role for nAChRs because nicotine-evoked responses of dorsal horn neurons were significantly reduced after ic mecamylamine. However, we cannot rule out the possibility that other cholinergic receptors also participate in nociceptive mechanisms. Cutaneous nociceptors with afferent C-fibers were excited by carbachol applied to the skin, and some were excited additionally by nicotine, muscarine, or both (Stein and Reeh 1993). Nicotine- and muscarine-evoked excitations were antagonized, respectively, by hexamethonium and atropine, suggesting involvement of both nAChRs and muscarinic cholinergic receptors in chemonociceptive transduction (Stein and Reeh 1993). Together with these previous studies, our present data are consistent with a major role for nAChRs in mediating nicotine-evoked excitation of dorsal horn neurons and pain, whereas a role for muscarinic receptors is less certain.

Pain and itch

Histamine produces itch sensation in humans (Keele and Armstrong 1964; Simone et al. 1987, 1991a). Some cholinergic agonists are reported to produce sensations of itch or itch mixed with pain (Magerl et al. 1990). It is not clear if nicotine evokes itch, rather than or in addition to pain. It recently was reported that acetylcholine excited histamine-sensitive, mechanically insensitive C-fiber nociceptors in skin (Schmelz et al. 1998). These latter histamine-sensitive fibers have been suggested to be “itch” receptors (Schmelz et al. 1997). Therefore it is possible that some of the present WDR units responsive to both ic histamine and nicotine signal itch. However, the same neurons also responded to noxious heat. Although the present results do not resolve mechanisms by which pain and itch are signaled by ascending sensory pathways (see Carstens 1997; Jinks and Carstens 1998a), they emphasize the potentially important role of cholinergic mechanisms in pain and possibly itch and their interactions.

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