Nicotine Inhibits Voltage-Dependent Sodium Channels and Sensitizes Vanilloid Receptors


INTRODUCTION

On a daily basis, much of the world’s population uses nicotine and/or capsaicin. Nicotine is found in tobacco leaves and subsequently is a component of tobacco products. Capsaicin is found in chili peppers and used as a spice in food and also as an anti-nociceptive and antiinflammatory compound. Both these compounds exhibit a wide range of biological activity in nervous and non-nervous tissue. Nicotine usually elicits its actions through the activation of specific neuronal nicotinic acetylcholine receptors (nAChRs) (Lindstrom 1977; Role and Berg 1996), whereas capsaicin, typically activates TRPV1 receptors (Caterina et al. 1997; Gunthorpe et al. 2002).

In the peripheral terminals of trigeminal ganglion (TG) neurons, nicotine activates nAChRs and capsaicin activates TRPV1 receptors (Alimohammadi and Silver 1999; Carstens et al. 1998; Jansco et al. 1961; Liu and Simon 1996; Sekizawa and Tsubone 1994; Silver and Finger 1991; Steen and Reeh 1993; Tanellian 1991; Walker et al. 1996). When they are present in nociceptors, the activation of either of these receptors produces a burning sensation (Dressier et al. 1999; Duner-Engstrom et al. 1986; Green 1989; Jarvik and Assil 1988). Although both these compounds may excite nociceptors, they paradoxically also exhibit anesthetic or analgesic properties (Damaj and Flores 2002; Carstens et al. 2001; Jansco et al. 1961; Jinks and Carstens 1999; Lundberg 1995; Phan et al. 1973; Szallasi and Blumberg 1993). These characteristics could arise either through the direct activation of nAChRs or TRPV1 receptors and/or by nonselective mechanisms. The analgesic property of capsaicin is so well established that it is sold over the counter as a pain reliever (Sterner and Szallasi 1999; Szallasi and Blumberg 1993). The analgesic properties of nicotine could arise centrally (Tripathi et al. 1982; Yang et al. 1992) or peripherally (Bannon et al. 1998; Carstens et al. 2001; Damaj and Flores 2002; Phan et al. 1973). In regard to peripheral anesthetic mechanisms, in vagal nerve neurons, nicotine (0.6 mM) was shown to reduce the amplitude of action potentials (Arimett and Ritchie 1961) suggesting that it may block voltage-gated sodium channels. In nociceptors, however, the effect of nicotine on voltage-gated sodium channels and, in particular, the tetrodotoxin-resistant subtypes that are involved in neurogenic pain (Baker and Wood 2001), has not been explored. We consequently investigated whether nicotine can modulate voltage-gated sodium channels and, if so, whether the modulation requires the activation of nAChRs.

In light of the large literature involving both nicotine and capsaicin, we found surprisingly few studies of how capsaicin interacts with nAChRs or how nicotine interacts with TRPV1 receptors. Capsaicin (1–30 μM) was found to inhibit nAChRs in PC-12 cells (Nakazawa et al. 1994). This inhibition, however, may have been nonspecific as capsaicin was shown to inhibit many other channels, especially at higher concentrations (Docherty et al. 1991; Grissmer et al. 1994; Liu et al. 2001; Petersen and LaMotte 1991; Petersen et al. 1987, 1989; Su et al. 1999). In this regard, capsazepine, a TRPV1 antagonist, has also been shown to inhibit nAChRs (Liu and Simon 1997), suggesting that the inhibitory effects of capsaicin on nAChRs may occur, at least in part, through nonspecific mechanisms.

Similarly, in only a few systems has the effect of nicotine on
capsaicin-evoked responses have been investigated. In rat buccal mucosa, nicotine has been shown to increase peptide (CGRP) release induced by capsaicin (Dussor et al. 2003). Because nicotine by itself had no effect on peptide release, this effect reflects the sensitization of the capsaicin response. Because the peptide release was blocked by the nAChR antagonist mecamylamine and was activated by other nAChR agonists, the sensitization of capsaicin-induced peptide release involved the activation of nAChRs (Dussor et al. 1998, 2003). Such sensitizing effects of nicotine may reflect a more general phenomenon as chronic exposure to tobacco smoke increased airway sensitivity to capsaicin (Bergren 2001). To address some of the mechanisms underlying these physiological sensitizing responses of nicotine, we have explored nicotine’s ability to modulate vanilloid (TRPV1) receptors.

In this study, we have found that whereas nicotine inhibits voltage-gated sodium channels, it sensitizes the TRPV1 receptors response to capsaicin.

**METHOdS**

**Cell culture for TG neurons**

Care of animals conformed to standards established by the National Institutes of Health. All animal protocols were approved by the Duke University Institutional Animal Care and Use Committee. The methods for culturing TG neurons from adult Sprague-Dawley rats have been described previously (Liu and Simon 2003). Trigeminal ganglia were dissected and collected in modified Hank’s balanced salt solution (mHBSS). After washing in mHBSS, the ganglia were diced into small pieces and incubated in mHBSS for 30–50 min at 37°C in 0.1% collagenase (Type XI-S). Individual cells were dissociated by triturating the tissue through a fire-polished glass pipette, followed by a 10-min incubation at 37°C in 10 μg/ml DNase I (Type IV) in F-12 medium (Life Technologies, Gaithersburg, MD). After washing three times with F-12, the cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) that was supplemented with 10% fetal bovine serum (FBS). The cells were plated on poly-L-lysine-coated glass coverslips (15 mm diam) and cultured overnight at 37°C in a water-saturated atmosphere with 5% CO₂. Neurons without, or with only short processes, were used. All experiments were carried out at room temperature (22–24°C).

**Chinese hamster ovary (CHO) cells**

CELL CULTURE AND TRANSFECTION. CHO cells were prepared following the procedure in Kuzhilandathil et al. (2001). Cells were grown in Ham’s F-12 medium with 10% FBS and 100 U/ml penicillin and streptomycin. Cells were plated on glass coverslips coated with 40 μg/ml poly-L-lysine and transiently transfected with an expression plasmid encoding the TRPV1 receptor (kindly provided by Dr. David Julius, University of California, San Francisco, CA) and a reporter plasmid encoding enhanced green fluorescent protein (EGFP; Clontech, Palo Alto, CA) using the LipofectAMINE reagent as per manufacturer’s instructions (Life Technologies, Gaithersburg, NY). Expression efficiencies of 15–40%, assessed by (EGFP) fluorescence and inward currents were routinely achieved. CHO cells stably expressing the rat TRPV1 receptor were generated by clonal selection after LipofectAMINE (Life Technologies)-mediated transfection, and stable transfectants were maintained in 500 μg/ml geneticin (Life Technologies).

**HEK293 cells**

CELL CULTURE AND TRANSFECTION. The α subunit of the human cardiac sodium channel gene hH1 was cloned into the mammalian expression plasmids pHRE4 (Invitrogen, San Diego, CA) as previously described (Chandra et al. 1998). Transfection of HEK293 cells was performed with pHRE/hH1 using Lipofectamine (GIBCO-BRL, Gaithersburg, MD) in DMEM. Stable transfectants were selected by culturing the cells in media containing 500 μg/ml of hygromycin (Boehringer Mannheim, Indianapolis, IN). This stable cell line has been established for >2 yr and continues to express robust TTX-resistant sodium currents.

**Patch-clamp recordings**

GENERAL METHODS. Recordings were obtained using an Axopatch-200A or -B patch-clamp amplifier (Axon Instruments, Foster City, CA), and the output was digitized with a Digidata 1322A converter (Axon Instruments). For whole cell recording, we used glass pipettes (R-6 or N51A borosilicate, Drummond Scientific, Broomall, PA) with resistances of 1–2 MΩ. In voltage-clamp experiments, the capacitance and series resistance was compensated by 90%.

The chamber containing the cells had a volume of ~360 μl and was continuously perfused by external solution at a rate of 2–6 ml/min. Solutions were delivered using a glass or quartz micropipette placed ~20–50 μm from the cell. Event markers associated with the opening or closing of the valves obtained the onset and removal times of the stimuli.

All experiments were performed at room temperature (21–23°C).

CURRENT-CLAMP: MEASUREMENTS OF ACTION POTENTIALS. In current-clamp experiments, the external solution, named Krebs-Henseleit (KH), contained (in mM) 145 NaCl, 5 KCl, 2.0 CaCl₂, 1.0 MgCl₂, 10.0 HEPES, and 10.0 D-glucose, pH 7.4, and the internal solution contained in (mM) 116 K-aspartate, 20 KCl, 10.0 EGTA, 10.0 HEPES, 2.0 Tris-GTP, and 3.0 Tris-ATP, pH 7.4. The resting potential was adjusted to ~80 mV, and action potentials were evoked by step depolarization’s of 20 ms width and amplitudes ranging between ~0.1 to ~2 nA in 0.05-nA steps. As controls, we used the magnitude of the injected current when the shape of the action potentials (APs) became independent of the injected current. Under these conditions, 1 mM nicotine (in KH buffer) was applied, and after a 3-min incubation, the protocol was repeated. The sampling rate was 10 kHz.

APs were also generated by increasing the current in a linear manner from 0 to ~2 nA in 1.5 s before, during, and after application of nicotine. The effect of nicotine was determined by counting the number of APs per ramp.

MEASUREMENTS OF CURRENTS ACTIVATED BY NICOTINE, CAPSAICIN, ACETYLCHELON, OR ACETYL-B-METHYCHOLINE. In these experiments, neurons were held at ~80 mV while one or more of these compounds were applied: 1 mM nicotine, 1 mM acetylcholine chloride, 3 mM acetyl-β-methylcholine chloride, or 0.3 μM capsaicin. These compounds were dissolved in the extracellular buffer. Measurements were made from capsaicin-sensitive neurons as defined when 0.3–1 μM capsaicin evoked currents more than ~200 pA. The soma diameters of these cells were ≤29 μm (Liu and Simon 2000). In experiments in which the capsaicin evoked current-voltage relation was measured, the potential was ramped every second from +60 to ~40 mV before, during, and after application of the chemicals (see Liu et al. 1998). Then, at the times corresponding to a potential of ~40 mV, the currents were plotted to provide the temporal evolution of the capsaicin-evoked inward currents (see Fig. 4B). The sampling rate was 10 kHz.

VOLTAGE-CLAMP MEASUREMENTS OF VOLTAGE-GATED SODIUM CHANNELS (TGNs). In measurements of VGSCs, the external solution was (in mM) 30 NaCl, 110 TEACl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 D-glucose, 10 HEPES, 0.1 CuCl₂, and 3 4-aminopyridine (4-AP), pH 7.4. The pipette solution was (in mM) 130 CsCl, 10 NaCl, 1.0 CaCl₂, 2.0; MgCl₂, 10 EGTA, 10 HEPES, 3 Tris-ATP, and 2 Tris-GTP, pH 7.3. With the above-described pipette solution, ENa was calculated to be 27.9 mV.
The peak current–voltage (I_p-V) relationship was determined using 5-mV step depolarization increments from the holding potential of −80 to +40 mV. Peak currents were analyzed using pCLAMP 8 and plotted against the voltage to obtain the I_p-V relation of sodium current activation. The voltage dependence of inactivation was determined by measuring the peak of the remaining maximal sodium current (I_pmax) after the delivery of −100- to 20-mV prepulse voltage steps of 40-ms duration (see Fig. 1B).

THERMAL STUDIES IN TG NOCICEPTORS. The thermal responsiveness of TG neurons were measured as previously described (Liu and Simon 2000). The temperature of the extracellular KH buffer was controlled (ramped) using a modified in-line SH-27A solution heater and a TC-324B automatic temperature controller (Warner Instruments, Hamden, CT). The internal and external solutions were the same as used for the AP measurements. The threshold temperature was taken as the temperature where the current recorded (at a holding potential of −60 mV) exhibited a large change in slope (Liu and Simon 2000).

CHO CELLS. The cells were voltage-clamped at a holding potential of −60 mV, and the current responses to nicotine and mecamylamine were measured. The external solution contained (in mM) 145 NaCl, 5 KCl, 2 CaCl_2, 1 MgCl_2, 10 HEPES, and 10 glucose. The internal solution in the patch electrode contained (in mM): 130 K-aspartate, 20 KCl, 1 EGTA, 1 MgCl_2, 10 HEPES, and 10 glucose. All solutions were adjusted to a pH of 7.4 and osmolality of ~300 mosM. To test whether putative nAChRs in CHO cells could be influencing the response of capsaicin on TRPV1 receptors, we performed some experiments in the presence of 20 μM of the broad-spectrum nAChR antagonist, mecamylamine. At 20 μM, mecamylamine has been shown to block >80% α4β2, α3β4, α3β2, and α7 nAChRs (Papke et al. 2001) as well as all the nicotine-induced 86 Rb^+ flux in F-11 cells (Puttfarcken et al. 1997). In TG neurons, 20 μM mecamylamine inhibited virtually all nicotine-evoked currents (L. Liu and S. A. Simon, unpublished observations). All antagonists and agonists were dissolved in the extracellular buffer.

HEK293 CELLS. All recordings were done from isolated cells. The recording conditions were selected to isolate the sodium current from the endogenous currents. Cells were superfused with an external solution containing (in mM) 130 NaCl, 4 KCl, 1 CaCl_2, 5 MgCl_2, 5 HEPES, and 5 glucose (pH 7.4 with NaOH). Micropipettes were filled with a solution containing (in mM) 130 CsCl, 1 MgCl_2, 5 MgATP, 10 Cs-EGTA, and 10 HEPES (pH 7.2 with CsOH). The cesium ions were used to block endogenous potassium currents. Under these recording conditions, the sodium current was the only measurable ionic current. The cells were voltage-clamped at a holding potential of −100 mV, and the current responses to nicotine were measured. In none of the cells tested did nicotine evoke an inward current. The current-voltage relationship was determined with 40-ms pulses from −80 to +40 mV in an increment of 5-mV steps. The voltage dependence of inactivation was detected by the application of 500-ms prepulse from −130 to −15 mV. A 20-ms test pulse to −20 mV followed each prepulse (see Fig. 3). The prepulse potential was incremented in 5-mV steps.

FIG. 1. Nicotine decreases AP amplitude in capsaicin-sensitive neuron. A: current-clamp measurements. A 20-ms injection of −200 pA produced an action potential (AP) having a hump on the repolarization phase. Holding potential = −80 mV. In the presence of 1 mM nicotine, the amplitude of the AP decreased and the threshold potentials increased (arrows). After a 3-min wash these effects were partially reversible. Inset: in the same neuron the application of 1 μM capsaicin produced a depolarization that generated a burst of APs. B: the effect of 10 mM nicotine on the generation of APs was measured by current ramps 0 to −2 nA of 1.5-s duration. After a 3-min incubation, the number of AP triggered by the ramp was reduced from 24 to 14, and after 3-min wash, 25 APs were evoked. HP = −80 mV.
Statistics and curve fitting

Data were analyzed and fitted using Clampex7 or pClamp8 (Axon Instruments) and SigmaPlot (SPSS, Chicago, IL) software. Data are presented as means ± SE. Where appropriate, the data were fitted to the Boltzmann function $I/I_{\text{max}} = 1/[1 + \exp(V_{0.5} - V_m)/k]$ or $G/G_{\text{max}} = 1/[1 + \exp(V_{0.5} - V_m)/k]$ with $V_{0.5}$ being membrane potential ($V_m$) at which 50% of activation or inactivation of the current, $k$ is the slope of the function and $G = I/(V - E_{rev})$. Because there are several types of VGSCs in TG neurons, to ascertain the effects of nicotine, we averaged the individual responses. The activation and inactivation kinetics were fit to a single exponential. Data were analyzed for statistical significance using either paired or unpaired (as indicated in the text) Student’s t-test. The significance was indicated as $P < 0.05$.

RESULTS

Nicotine decreases APs in capsaicin-sensitive neurons

It has been shown that cultured capsaicin-sensitive (CS) neurons have many of the same properties as nociceptors (Baccaglini and Hogan 1983; Gold et al. 1996). In addition to being capsaicin sensitive, these neurons can be identified by the shape of their APs that are of relatively long duration and that have a characteristic hump on their repolarization phase. The application of 1 mM nicotine to a CS neuron decreases the amplitude of the AP (Fig. 1A). On average, 1 mM nicotine decreased the AP amplitude 5.3 mV from 130 ± 5 to 125 ± 5 mV ($n = 6$, paired $P < 0.01$) that recovered to 132 ± 4 mV ($n = 6$) after a 3-min wash. We also tested the effect of nicotine using a current-ramp protocol (Fig. 1B). After a 3-min incubation period, the number of evoked APs was reduced by nicotine from 25 ± 14 to 12 ± 7 ($n = 5$, $P < 0.05$ paired), and after a 3-min wash, 21 ± 8 APs were evoked. Both of the reduction in AP amplitude and decrease in the number of evoked responses are consistent with nicotine inhibiting voltage-gated sodium channels. Therefore we directly tested the effects of nicotine on these channels.

Nicotine inhibits voltage-gated sodium channels (VGSC)

Figure 2 shows an example of the inhibitory effect of 1 mM nicotine on the total VGSCs ($I_{\text{Na-Total}}$). Here it is seen that...
Nicotine inhibited the maximum peak inward current ($I_{\text{max}}$) 50% (Fig. 2A) and that after a 3-min wash, the current partially recovered. Although the activation rate constants were unchanged by nicotine, the inactivation time constants were slightly but significantly ($P < 0.01$ paired t-test) slowed being 3.0 ± 0.3 and 3.6 ± 0.3 ms ($n = 15$), respectively. Also shown in the figure is the peak current ($I_p$)–voltage plot. On average, 1 mM nicotine inhibited $I_{\text{max}}$ 42 ± 3% ($n = 15$). For these experiments, the reversal potentials were 28 ± 1 mV in the absence of nicotine and 26 ± 2 mV ($n = 15$) in its presence. Experiments were also performed using 0.001, 0.01, 0.1, 0.3, and 10 mM nicotine. At these concentrations, nicotine inhibited $I_{\text{max}}$ 0.2 ± 10% ($n = 6$), 11 ± 13% ($n = 8$), 31 ± 15% ($n = 6$), 35 ± 13% ($n = 6$), and 51 ± 18% ($n = 6$), respectively (data not shown). Thus nicotine inhibited VGSCs even at quite low concentrations. Nicotine (1 mM) did not, however, produce a significant ($P = 0.93$) shift in the G-V plot (control: $V_{0.5} = -15 \pm 2$ mV, $k = 10 \pm 0.9$; 1 mM nicotine: $V_{0.5} = -15 \pm 2$ mV, $k = 10 \pm 1$; $n = 15$). (These values, as are subsequent values, the result of fits to the data of the individual experiments. These values will slightly differ from those given in the figure legend in which the averages were calculated at each voltage and the data were fit to a Boltzmann function.)

We then tested whether the inhibition of VGSCs by nicotine could be accounted, at least in part, for by a hyperpolarizing shift in the inactivation-voltage relationship (Fig. 2B). Nicotine produced a statistically significant ($P < 0.01$ paired t-test), 5 mV hyperpolarizing shift in the inactivation-voltage relation (control: $V_{0.5} = -37 \pm 5$ mV, $k = -9 \pm 1$; 1 mM nicotine: $V_{0.5} = -42 \pm 5$ mV, $k = -10 \pm 1$; $n = 9$).

Is nicotine’s inhibition of VGSCs dependent on the activation of nAChRs?

To determine whether nicotine’s inhibition of VGSCs depended on the activation of nAChRs, we performed experiments in the presence of 20 μM mecamylamine. Although at this mecamylamine concentration most of the nicotine-induced currents are markedly inhibited, small inward currents are often observed (Liu and Simon, unpublished observation). Therefore to eliminate the possibility that the activation of nAChRs is responsible for the inhibition of VGSCs, two control experiments were performed in capsaicin-sensitive neurons. In the first experiment, we tested whether 1 mM acetylcholine chloride or acetyl-β-methylcholine chloride inhibited VGSCs. We found that neither of these compounds evoked an inward current, and because the differences between them were not significant (4 neurons were tested for each compound), the results were combined. On average, 1 mM of these compounds inhibited the peak sodium current by 17 ± 1% ($n = 8$), a number much less than the 42% inhibition produced by 1 mM nicotine.

The second test involved measuring the effect of 1 mM nicotine on TTX-resistant cardiac hH1 VGSCs that were expressed in HEK293 cells. One reason to use this channel to test the effect of nicotine is that H1 message is present in rat TG neurons (L. Liu and S. A. Simon, unpublished observation). A more important reason, however, is that TTX-r channels are important in pain, inflammation, and the normal functioning of the heart. In these cells, 1 mM nicotine inhibited the peak sodium current 17 ± 2% ($n = 8$; Fig. 3). However, it did not activate an inward current, and thus these cells are unlikely to have many endogenous nAChRs. Nicotine induced statistically significant −4-mV hyperpolarizing shifts of $V_{0.5}$ in the conductance-voltage relation (control: $V_{0.5} = -37.1 \pm 1.2$ mV, $k = 7.0 \pm 0.2$; 1 mM nicotine: $V_{0.5} = -40.2 \pm 1.0$ mV, $k = 6.9 \pm 0.2$; $n = 8$, $P < 0.01$, paired) and in the inactivation-voltage relation (control: $V_{0.5} = -87.6 \pm 0.8$ mV, $k = -5.7 \pm 0.1$; 1 mM nicotine: $V_{0.5} = -92.5 \pm 1.1$ mV, $k = -5.8 \pm 0.1$; $n = 8$, $P < 0.01$, paired). Consistent with the lack of Na⁺ in the pipette solution, we did not observe a true reversal of the sodium current. The extrapolated reversal potential, however, was 59 ± 3 mV during control, and 59 ± 2 mV during exposure to nicotine ($P > 0.05$).

**Nicotine sensitizes capsaicin-induced currents in TG neurons**

We found that in 1 mM nicotine markedly increased the capsaicin-activated inward currents sensory TG neurons. In the example shown in Fig. 4A, the first application of capsaicin evoked a slowly desensitizing inward current. After a 3-min wash, 1 mM nicotine was applied and activated a small inward current that desensitized and eventually became outward (see inset). After a 3-min nicotine incubation period, the co-application of 0.3 μM capsaicin with 1 mM nicotine produced a large increase in the capsaicin-activated inward current (from −7.3 to −11.9 nA). It is evident that the final current is much larger than the sum of the magnitude of the peak current activated by 1 mM nicotine (−0.11 nA) and the original capsaicin-evoked current (−7.3 nA). The nicotine-induced increase in the current was reduced after a 3-min wash, although a part of that decrease could be due to the desensitization that occurs on repeated applications (Dressier et al. 1998a,b; Liu and Simon 1996). In summary, 1 mM nicotine increased the peak capsaicin-activated current 1.9 ± 0.3-fold ($n = 10$) and after 3-min wash, the current (relative to 1st application) was 0.9 ± 0.1 (see Fig. 4B).

To determine whether this nicotine-induced increase in current is through capsaicin-sensitive channels, current-voltage (I-V) relations were obtained in the presence of 0.3 μM capsaicin alone and in the presence of 0.3 μM capsaicin and 1 mM nicotine. If nicotine were increasing current through vanilloid receptors, we would expect that the reversal potential to remain unchanged and the current to remain outwardly rectifying (Caterina et al. 1997). Figure 4C, top, refer to a subset of the family of currents that were obtained before and during the application of the two compounds and that correspond to a voltage of −40 mV. The responses to capsaicin in the presence and absence of 1 mM nicotine, taken at the times indicated by the arrows in the I-V plots are outwardly rectifying and have reversal potentials 0.4 ± 1 mV ($n = 5$) and 1 ± 1 mV ($n = 5$), respectively. The preceding information suggests, but does not prove, that the nicotine-induced current increase occurs by sensitizing vanilloid receptors without the necessity of activating nAChRs.

**Nicotine sensitizes capsaicin-induced currents from TRPV1 receptors**

To confirm that the nicotine-induced sensitization seen in TG neurons is not the result of its indirectly activating nAChRs, we repeated these experiments in CHÖ cells with
expressed TRPV1 receptors. In these cells, nicotine did not evoke a current, but also sensitized capsaicin-induced currents (Fig. 5A). To essentially eliminate the possibility that the activation of nAChRs could produce this sensitization, the cells were preincubated with 20 μM mecamylamine. Under this condition, nicotine also sensitized the capsaicin-induced currents, albeit to the same extent as it did in the absence of mecamylamine. Figure 5B shows that relative to control, nicotine (0.1–1 mM) produced a fourfold increase in the capsaicin-induced current.

Effects of nicotine on thermal stimuli

It was previously shown that ethanol, like nicotine, sensitizes responses to capsaicin and, in addition, increases the thermal sensitivity of hTRPV1 receptors (Trevisani et al. 2002). We consequently tested whether nicotine will also sensitize thermal responses in TG neurons. Figure 6A shows a plot of the changes in inward current versus temperature in a sensory neuron that was held at a potential of −60 mV. It is seen that the current remained relatively constant until ~42°C, whereupon it increased rapidly. We defined the threshold temperature as the temperature where the current exhibited an abrupt increase or change in slope (Liu and Simon 2000). We found that the threshold temperature is unaltered by 1 mM nicotine [control: 40 ± 1°C (n = 8); 1 mM nicotine: 41 ± 0.7°C (n = 8)]. At the maximum temperature recorded before the receptors desensitized (not shown), the currents were −4 ± 1 nA (n = 8) and −6 ± 1 nA (n = 8) in absence and presence of nicotine.
DISCUSSION

We explored the effects of nicotine on two channels that are commonly found in nociceptors. Pursuant to its local anesthetic properties we have shown, for the first time, that nicotine inhibits TTX-resistant sodium channels. These channels have been shown to be important in modulating pain and inflammation as well as in maintaining the cardiac AP (Baker and Wood 2001). In addition, we have also shown, for the first time, that nicotine sensitizes responses to TRPV1 receptors. This sensitization could be relevant in capsaicin-evoked peptide release (Dussor et al. 1998, 2003) or in gustatory physiology by increasing the burning sensation of ingested capsaicin. Both these effects may occur at modest nicotine concentrations, and both can occur without the necessity of activating nAChRs.

Anesthetizing effects of nicotine

We have found that the application of nicotine to capsaicin-sensitive nociceptors can reduce the frequency and amplitude of APs and inhibit VGSCs (Figs. 1 and 2). Because capsaicin-sensitive sensory neurons contain a high percentage of TTX-resistant currents (Kim et al. 1999; Petruska et al. 2000; Stucky and Lewin 1999), most of the total sodium current reflects the TTX-resistant population. The reduction in the AP amplitude produced by nicotine that we found in TG neurons (Fig. 1) is consistent with the magnitude of the reduction found on vagal nerve C fibers (Arnett and Ritchie 1961). However, in a voltage-clamp study from squid giant axons, it was found that the (extracellular) addition of 1 mM nicotine did not markedly alter the response of these VGSCs (Frazier et al. 1973). It is unclear why nicotine did not affect squid sodium channels but markedly decreased sodium channels in nociceptors as well as in HEK293 cells (Figs. 2 and 3). However, the experiments with squid axons were performed at different temperatures and with very different ionic conditions.

That nicotine can inhibit VGSCs in a nonspecific manner (without activating nAChRs) suggests that nicotine, like many hydrophobic molecules that are anesthetics, can interact with the channels to decrease its activity. In this regard, molecules as diverse as ethanol, oleamide, halothane, digoxin, phenytoin,
carbamazepine, and tricyclic antidepressants have been found to decrease sodium channel activity (Henkel 2003; Hille 1993; Stadnicka et al. 1999). The inhibitory effects of nicotine arise, at least in part, from the hyperpolarizing shifts in the conductance and/or inactivation-voltage curves (Figs. 2 and 3). These shifts are more evident in the HEK293 cells that expressed only a single α subunit of hH1VGSC than in TG neurons that contain many different types of VGSC subunits (Liu et al. 2001).

The molecular basis of the shifts in the voltage-dependent parameters can have many origins. In a recent commentary on the location of the gates in voltage-gated channels, it was written that “the location of S4—not embedded in the protein core but loose in the membrane—explains an old puzzle.

![Figure 5](image1)

**FIG. 5.** Nicotine increases capsaicin-activated currents in CHO cells. A: voltage-clamp traces of current responses to 50 nM capsaicin performed in the absence and presence of nicotine. Each trace is from a different cell. In the 1st cell, the 1st 2 applications of capsaicin produced small reproducible inward currents, whereas a 3-min pretreatment with 1 mM nicotine dramatically potentiated the capsaicin response. Note that nicotine by itself did not evoke an inward current. The 2nd cell shows a control pair of responses and the 3rd cell demonstrates potentiation by 0.1 mM nicotine. B: this figure shows that the nicotine-induced potentiation is unaffected by the presence of mecamylamine. C: cumulative data (n values above bars) on potentiation under the indicated conditions shown as relative response to 50 nM capsaicin (mean ± SE compared with control). *P < 0.05. Holding potential = −60 mV.

![Figure 6](image2)

**FIG. 6.** Nicotine does not alter sensitivity of heat-activated currents in TG neurons. Currents evoked by temperature ramps from neurons in the absence (A) and presence (B) of nicotine. The threshold temperature is defined by the intersection of the dotted lines. Holding potential = −80 mV.
namely that small lipid-soluble molecules somehow have ready access to voltage sensors” (Sigworth 2003). Moreover the data suggest that nicotine does not inhibit these channels by preferentially blocking their open states. There is, however, another mechanism that can contribute to nicotine’s inhibitory effect, namely that on binding to the plasma membrane, it can alter its mechanical and structural properties (e.g., bending and compressibility moduli, and membrane thickness). Indeed, it has been shown that alterations in these parameters can affect channel activity (Lundbaek and Andersen 1994, 1999).

Nicotine sensitizes capsaicin-activated currents

We have shown that capsaicin-activated (TRPV1) currents are sensitized by nicotine (Figs. 4 and 5). Moreover, we have shown that this sensitizing effect does not require the activation of nAChRs because the sensitization was seen in CHO cells in which nicotine did not activate currents (Fig. 5) and because the sensitizing effect was unchanged in the presence of the broad-spectrum nAChR antagonist, mecamylamine (Fig. 5B). In buccal mucosa, however, it was found that nicotine sensitized responses to capsaicin-induced peptide release, but in that case, it appeared to occur through the activation of nAChRs (Dussor et al. 1998, 2003). It follows therefore that nicotine can increase the responses to capsaicin both through the activation of nAChRs and in a nonspecific manner. As in the preceding text, there are several mechanisms that can rationalize the nonspecific nicotine-induced sensitization of TRPV1 receptors (Figs. 4 and 5). First, it could simply increase the number of functional channels. However, given the rapid reversibility of the response, we do not think this is the most parsimonious explanation (Figs. 4A and 5A). Second, it could affect the channel conformation by making it more sensitive to capsaicin. In this case (for a given capsaicin concentration), there would be an increase in the probability that the TRPV1 channels is in one of its open states (Hui et al. 2003; Kwak et al. 2000). Third, as noted in the preceding text, nicotine could alter the membrane’s mechanical properties, and to this point, TRPV1’s sensitivity can be modulated by it binding to the lipid, PIP2 (Prescott and Julius 2003).

We also tested whether nicotine alters the thermal responses in TG neurons that are activated at temperatures that correspond to the activation of TRPV1 receptors (~43°C). The rationale behind these experiments was to test whether nicotine could mimic the effect of ethanol (Trevisini et al. 2002) or release the channel from PIP2 inhibition (Prescott and Julius 2003) because both of these procedures (by unknown mechanisms) lower the threshold temperature. We found that 1 mM nicotine did not markedly alter the threshold temperature (Fig. 6). Moreover, the thermally induced currents were not significantly increased by 1 mM nicotine as they are when TRPV1 channels are released from PIP2 inhibition (Prescott and Julius 2003). Our data suggest that nicotine does not simply act as a more hydrophobic version of ethanol because its effect is more selective in that it only increased the sensitivity to capsaicin. Moreover, nicotine is not likely cause PIP2 disassociation from its binding site, although this must be tested experimentally. These data also suggest that nicotine can be used to dissociate the capsaicin binding/gating portions of the channel from those activated by thermal stimuli (Jung et al. 2003).

What are the possible physiological consequences of nicotine sensitizing vanilloid receptors? This sensitization should result in a greater influx of calcium and therefore the release of neurotransmitters (Kiyosawa et al. 2001) and peptides (Dussor et al. 1998, 2003) that could result in edema, allodynia or hyperalgesia. From a psychophysical perspective, these results predict that smokers or persons chewing tobacco would be expected to perceive chili peppers “hotter” than nonsmokers or chewers, not only because nicotine by itself may produce a burning sensation (that would be diminished to some extent by capsaicin), but also because it sensitizes vanilloid receptors.

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