Capsaicin sensitivity and voltage-gated sodium currents in colon sensory neurons from rat dorsal root ganglia

XIN SU,1 RUTH E. WACHTEL,2,3 AND G. F. GEBHART1

Su, Xin, Ruth E. Wachtel, and G. F. Gebhart. Capsaicin sensitivity and voltage-gated sodium currents in colon sensory neurons from rat dorsal root ganglia. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G1180–G1188, 1999.—Dil-labeled colon sensory neurons were acutely dissociated from S1 rat dorsal root ganglia (DRG) and studied using perforated whole cell patch-clamp techniques. Forty-six percent (54/116) of labeled sensory neurons responded to capsaicin (10−8–10−5 M) with an increase in inward current, which was a nonspecific cation conductance. Responses to capsaicin applied by puff ejection were dependent on dose, with a half-maximal response at 4.9 ± 10−9 M; bath application was characterized by marked desensitization. Voltage-gated Na+ currents in 23 of 30 DRG cells exhibited both TTX-sensitive and TTX-resistant components. In these cells, capsaicin induced an inward current in 11 of 17 cells tested. Of the cells containing only a TTX-sensitive component, none of six cells tested was sensitive to capsaicin. In all cells that responded to capsaicin with an increase in inward current, capsaicin abolished voltage-gated Na+ currents (n = 21). Capsazepine (10−6 M) significantly attenuated both the increase in inward current and the reduction in Na+ currents. Na+ currents were not significantly altered by adenosine, bradykinin, histamine, PGE2, or serotonin at 10−8 M and 10−5 M. These findings may have important implications for understanding both the irritant and analgesic properties of capsaicin.

visceral pain; bradykinin; prostaglandin E2; serotonin; tetrodotoxin

NOCICEPTIVE NEURONS ARE CHARACTERIZED by a sensitivity to capsaicin, the active ingredient in hot red peppers. Capsaicin binds to specific vanilloid receptors on the membrane of sensory neurons, where it opens nonspecific cation channels. Capsaicin produces an initial excitation of peripheral sensory nerve endings, with depolarization and generation of action potentials. The response is not blocked by specific Na+ channel or Ca2+ channel blockers but is antagonized by the vanilloid receptor antagonist capsazepine and by ruthenium red. Stimulation is followed by desensitization and subsequent conduction block, which may account for the analgesic properties of capsaicin. High concentrations of capsaicin or systemic administration can produce degeneration of primary afferent fibers, resulting in permanent sensory deficits. Degeneration of fibers probably results from Ca2+ and NaCl entry and accumulation, accompanied by cell swelling (see Refs. 4, 8, 10, 11, 19, 41).

Although the cellular effects of capsaicin have been widely studied, little is known about the effects of capsaicin on nociceptive afferents innervating the rat colon. In the colon, noxious chemical, mechanical, or thermal stimuli activate afferents that appear to function as polymodal nociceptors (37). Their axons are contained in the pelvic nerve and are either unmyelinated or thinly myelinated C or Aβ fibers (36), whereas the cell bodies of colon sensory neurons are found in the dorsal root ganglion (DRG). Studies of capsaicin responses at the cellular level using isolated DRG neurons have differentiated between cell types on the basis of cell size or action potential characteristics but not on the basis of presumed cell function. This report examines capsaicin responses in a select population of nociceptive neurons that innervate the colon.

We also characterized these colon sensory neurons in terms of other properties commonly associated with nociceptive neurons, including expression of voltage-gated Na+ currents that are resistant to block by TTX and modulation of Na+ currents by hyperalgesic agents. In contrast to other studies, we report that cell size does not correlate with nociceptive properties and that hyperalgesic agents do not appear to enhance Na+ current amplitude in colon sensory neurons. We did find, however, that capsaicin markedly attenuated voltage-gated Na+ currents in those cells that exhibited a direct agonist response to capsaicin.

METHODS

Labeling of colon sensory neurons. Male adult Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 250–300 g were anesthetized with pentobarbital sodium (45 mg/kg ip, Nembutal, Abbott Laboratories, North Chicago, IL). The descending colon was exposed under sterile conditions, and 70 μl of the di-2,4,6-trinitrophenylacetoxymethyl-substituted hydroxamic acid tetraethylammonium dye (DTX; 25 mg in 0.5 ml methanol) was injected into the smooth muscle of the colon through a 30-gauge needle. The surgical incision was closed, and the animal was allowed to recover for 1–2 wk to permit DTX to be transported back to the cell soma of colon sensory neurons. Care and use of animals conformed to standards established by the US Department of Agriculture and by the National Institute of Health. All protocols were approved by the University of Iowa Institutional Animal Care and Use Committee.

Cell dissociation and culture. Rats were anesthetized with pentobarbital sodium (Nembutal). The S1 DRG were removed, stripped of their connective tissue capsules, transferred into ice-cold culture medium, and minced with microscissors. The tissue was digested in modified L-15 culture medium containing collagenase (type Ia, 1 mg/ml), trypsin (type III, 1 mg/ml), and DNAase (type IV, 0.1 mg/ml) at 37°C for 50 min. Chemical digestion was terminated by addition of soybean trypsin inhibitor (2 mg/ml) and BSA (1 mg/ml). The
tissue fragments were then gently triturated with a siliconized sterile Pasteur pipette and centrifuged at 800 g for 5 min. The neurons were resuspended in the modified L-15 medium supplemented with 5% rat serum and 2% chick embryo extract and plated onto polyl-L-lysine-coated glass coverslips. Cultured neurons were kept at 37°C in an incubator containing 5% CO₂ saturated with water vapor and were studied within 24 h.

Whole cell current recordings. Dil-labeled neurons were studied using perforated whole cell patch-clamp techniques. Cells plated onto coverslips were transferred into a 1-mL recording chamber with medium of the following composition (in mM): 20 NaCl, 30 tetrathylammonium (TEA) chloride, 75 choline chloride, 0.1 CaCl₂, 5 MgCl₂, 5 CoCl₂, 10 HEPES, and 10 D-glucose. The pH was adjusted to 7.35 with CsOH, and the osmolarity was adjusted to 320 mosmol/L with sucrose. The tips of patch electrodes were filled with a solution containing (in mM) 100 CsCl, 5 sodium glutamate, 30 TEA chloride, 0.1 CaCl₂, 2 MgCl₂, 11 EGTA, and 10 HEPES. Electrodes were then backfilled with nystatin (stock solution of 50 mg/mL in DMSO diluted 1:300 in electrode solution immediately before use). The pH was adjusted to 7.25, and the osmolarity was adjusted to 310 mosmol/L. Cell diameter was measured using a calibrated reticle. For some experiments, CoCl₂ was replaced with an equal concentration of MgCl₂. Cells were superfused with bathing solution at a rate of 1.8 mL/min.

Enzymatically dissociated DRG cells were viewed under Hoffman contrast optics in regular light and in fluorescent light with a rhodamine filter (excitation wavelength of ~546 nm and barrier filter at 580 nm). Only Dil-labeled neurons exhibiting a red-orange color under fluorescent light were selected for study. After establishment of a whole-cell recording, whole-cell capacitance was minimized using the analog compensation facility available on the recording amplifier (Axopatch 1C). Series resistance compensation was fixed at a level of 88% in all experiments, resulting in a final series resistance averaging 4 MΩ. Signals were low-pass filtered at 5 kHz, digitized at 200 µs per point, and stored on a computer for later analysis. Voltage protocols were generated and data acquisition and analysis were performed using pCLAMP software (version 5.5.1, Axon Instruments). Experiments were performed at room temperature.

To determine the ionic basis of the capsaicin current, responses were recorded in solutions of varying ionic composition (Table 1). The reversal potential of the capsaicin response was determined by subjecting the cells to a series of 360-ms stepwise depolarizations to test potentials of −60 mV, −30 mV, 0 mV, and +30 mV. The steady-state current was determined at each potential both before and during application of capsaicin, and the net capsaicin-induced current was plotted as a function of the step potential. The interpolated potential at which the current was zero was the reversal potential. The relative permeabilities of the ions were estimated from the measured reversal potentials using the Goldman-Hodgkin-Katz equation, with relative permeabilities of K⁺ to Cs⁺ to Na⁺ to choline⁺ to TEA⁺ at 1:0.9:0.5:0.3:0.1. TEA, tetraethylammonium. High K⁺ did not contain TEA.

Table 1. Solutions used to determine the ionic basis of the response to capsaicin

<table>
<thead>
<tr>
<th>Electrode solution, mM</th>
<th>Standard</th>
<th>High K⁺</th>
<th>High Na⁺</th>
<th>High K⁺</th>
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<td>CsCl 100</td>
<td>80</td>
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<td>5</td>
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<td>100</td>
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<tr>
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<tr>
<td>EGTA 11</td>
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<tr>
<td>Sucrose yes</td>
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</table>

Measured Eₚ is the reversal potential obtained from stepwise depolarization experiments as shown in Fig. 3. Predicted Eₚ is the calculated reversal potential determined from the Goldman-Hodgkin-Katz equation, with relative permeabilities of K⁺ to Cs⁺ to Na⁺ to choline⁺ to TEA⁺ at 1:0.9:0.5:0.3:0.1. TEA, tetraethylammonium. High K⁺ did not contain TEA.

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diluted in the bath solution to achieve final concentrations of 10⁻⁶ to 10⁻⁸ M. Capsazepine was dissolved in 10% methanol to give a 10⁻⁴ M stock solution and then further diluted in bath solution to yield a final concentration of 10⁻⁶ M. Capsaicin and capsazepine were applied by addition to the bathing solution. In some experiments, multiple test doses of capsaicin were applied by pressure ejection (puffer ejection) from a blunt electrode positioned near the cell.

Data analysis. All data are expressed as means ± SE. A positive response to capsaicin was defined as an inward current >100 pA. The concentration at which capsaicin generated a half-maximal response was calculated using GraphPad Prism (version 2.01). Results were analyzed using Student’s t-test or ANOVA for repeated measures. A value of P < 0.05 was considered statistically significant.

RESULTS

Only Dil-labeled DRG cells that exhibited a red-orange color under fluorescence were selected for study; these cells were considered to be colon sensory neurons. Roughly 1% of dissociated cells were labeled, consistent with our earlier report (38).

Figure 1A shows a typical inward current produced by bath application of capsaicin. Capsaicin produced an inward current in 54 of 116 (46%) colon sensory neurons. At 10⁻⁶ M capsaicin, the peak response recorded in standard electrode and bath solutions averaged 1923 ± 585 pA (n = 9; range of 293–5,400 pA). At 10⁻⁶ M capsaicin, the peak response averaged 1,714 ± 316 pA (n = 9; range of 303–3,700 pA).
Fig. 1. Typical inward currents and desensitization produced by bath application of capsaicin to rat sensory neurons. A: application of vehicle (arrow) produced no detectable change in current, whereas \(10^{-8}\) M capsaicin produced an inward current with a peak amplitude of \(-3,200\) pA. Current decayed spontaneously over a period of \(-1\) min, even though capsaicin was not washed out of the bath. Similar responses to capsaicin were seen in 54 of 116 cells tested (46%). B: in another cell, application of a test dose of \(10^{-8}\) M capsaicin (arrow) initially produced a large inward current. A second application of the same concentration following 5–10 min of wash generated only a small inward current. C: summary data showing that the response to a second dose of capsaicin (caps) was significantly less than the initial response. Initial responses to \(10^{-8}\) M capsaicin averaged \(4,040 \pm 1,472\) pA (mean \(\pm\) SE from \(n = 5\) cells), whereas a second application of the same concentration 5–10 min later produced an inward current of only \(464 \pm 250\) pA (\(P < 0.05\)).

Responses to bath application of capsaicin were characterized by marked desensitization and gradually diminished with time, even though capsaicin was not washed out of the bath. The time required for responses to decay to one-half of peak values averaged \(48\) \(\pm\) 6 M (mean \(\pm\) SE from \(n = 5\) cells), whereas 10–15 min later produced an inward current of only \(464 \pm 250\) pA (\(P < 0.05\)).

To determine the ionic basis for the inward current, membrane potential was changed in a stepwise fashion during the peak of the capsaicin response (Fig. 3). A plot of current amplitude as a function of membrane potential was then used to determine the reversal potential for the capsaicin-evoked current. Reversal potentials were measured in several different solutions, and relative ionic permeabilities were calculated using the Goldman-Hodgkin-Katz equation. Table 1 shows the results of these ion substitution experiments, which yielded a permeability sequence of \(K^+\) to \(Cs^+\) to \(Na^+\) to choline\(^+\) to TEA\(^-\) of 1:0:9:0:5:0:3:0:3. The capsaicin-activated channel in these identified S1 DRG cells is a nonspecific cation channel that allows most positive ions, including relatively large ones, to pass.

Fig. 2. Concentration-response relationship of inward currents produced by pressure ejection of various concentrations of capsaicin. Inward currents are negative. Response was half-maximal at a capsaicin concentration of \(4.9 \times 10^{-7}\) M (\(n = 5\)).
Additional experiments were performed to characterize voltage-gated Na\(^+\) currents in colon sensory neurons. Na\(^+\) currents were evoked by a series of 48-ms depolarizing step pulses to test potentials of -40 to +45 mV. Figure 5 illustrates results from a typical cell in which currents were only partially blocked by addition of 10\(^{-6}\) M TTX to the bathing solution, indicating the presence of both a TTX-sensitive (TTX-S) and a TTX-resistant (TTX-R) component to the Na\(^+\) current.

The TTX-R component that was still present after addition of TTX (Fig. 5B) exhibited a relatively slower time course and activated only at more depolarized potentials. The TTX-S current, obtained by subtracting the TTX-R component from the total current (Fig. 5C), activated more rapidly in response to depolarization, activated at less depolarized potentials, and rapidly inactivated. Differences in the voltage dependence of activation are illustrated more clearly in the current-voltage relationships shown in Fig. 5D. TTX (10\(^{-6}\) M) completely blocked the Na\(^+\) currents in 7 of 30 cells tested and partially blocked the currents in 23 of 30 cells.

There was no correlation between sensitivity to TTX and cell diameter. Cells exhibiting both TTX-S and TTX-R components had a median diameter of 28.6 µm (n = 23), whereas cells with only a TTX-S component also had a median diameter of 28.6 µm (n = 7).

TTX sensitivity did correlate, however, with responsiveness to capsaicin. Of cells exhibiting both TTX-S and TTX-R components, 11 of 17 responded to capsaicin with an increase in inward current. In contrast, 0 of 6 cells with only a TTX-S component responded to capsaicin. All of the cells that were sensitive to capsaicin contained a TTX-R component to the Na\(^+\) current.

We also noted another relationship between voltage-gated Na\(^+\) currents and sensitivity to capsaicin. As shown in Fig. 6, a series of depolarizing pulses applied during the peak of the capsaicin response failed to evoke voltage-gated Na\(^+\) currents. Both 10\(^{-6}\) M and 10\(^{-8}\) M capsaicin virtually abolished Na\(^+\) currents in capsaicin-sensitive neurons (n = 21). Na\(^+\) currents remained depressed and did not recover spontaneously on decay of the capsaicin response. In contrast, capsaicin had no significant effect on voltage-gated Na\(^+\) currents in capsaicin-insensitive neurons that did not respond with an increase in holding current (n = 14).

Summary data are shown in Figure 7. This effect of capsaicin was significantly attenuated by pretreatment with capsazepine. In five cells in which capsaicin was applied by puffer ejection, capsaicin decreased the peak Na\(^+\) current from 1,102 ± 124 to 63 ± 35 pA. After bath
application of capsazepine (10⁻⁶ M), the reduction in Na⁺ current produced by capsaicin was much less, from 629 ± 115 to 346 ± 97 pA (n = 5).

In contrast to capsaicin, other compounds that have been reported to alter voltage-gated Na⁺ currents in DRG cells had little effect in these experiments. Currents were not significantly altered by adenosine, bradykinin, histamine, PGE₂, or serotonin at concentrations of 10⁻² M and 10⁻³ M (Fig. 8).

It is conceivable that our results may have been affected by the presence of Co²⁺ in the bathing solution. The decrease in Na⁺ current amplitude produced by capsaicin may have been caused by Co²⁺ entering the cell through capsaicin-activated channels. Experiments were therefore repeated in the absence of external Co²⁺. Results were unchanged, however, with capsaicin (10⁻⁵ M) reducing Na⁺ currents to 0.18 ± 0.06% of control (n = 4). Similarly, the lack of effect of modulators may have been related to the presence of Co²⁺. Because Co²⁺ may decrease the amplitude of TTX-R Na⁺ current (21, 22, 30), some component of the Na⁺ currents that is altered by these modulators may have already been attenuated by Co²⁺ in the bathing solution. Experiments with PGE₂ were also repeated in the absence of external Co²⁺ with similar results. In capsaicin-sensitive cells, PGE₂ had no effect on Na⁺ current amplitude, which was 101 ± 9% of control at 10⁻² M (n = 4) and 107 ± 14% of control at 10⁻³ M (n = 4).

DISCUSSION

This study characterized responses to capsaicin in a select subset of sensory neurons from S1 DRG of the rat. All cells studied were identified as afferent neurons innervating the colon, a population of cells in which we previously studied high-voltage-activated Ca²⁺ currents and documented drug effects different from those reported in unidentified DRG cells (38). These sensory neurons are associated with C and Aδ fibers that convey nociceptive information from the colon to the spinal cord (36, 37) and thus are important in visceral pain mechanisms.

Although binding studies using the potent capsaicin receptor agonist resiniferatoxin have demonstrated the
presence of capsaicin receptors in the colon (16), no previous study has examined capsaicin-induced currents in identified colon sensory neurons. Our finding that capsaicin-induced inward currents were significantly attenuated by the competitive vanilloid receptor antagonist capsazepine provides direct evidence for the existence of capsaicin receptors on colon sensory neurons. This work also demonstrates that these receptors are expressed on the cell bodies of acutely dissociated neurons, not only on the nerve terminals.

In these experiments, 54 of 116 labeled sensory neurons responded to capsaicin with an increase in inward current, although bath application of capsaicin was characterized by desensitization, as reported by others (19, 25). The percentage of cells responding to capsaicin (46%) is consistent with studies of identified rat DRG cells, which found 21% (18), 32% (2), 50% (43), 64% (14), or 73% (25) of cells responsive. Hu-Tsai et al. (20) reported regional differences in sensitivity to capsaicin, with 20–30% of skin afferents, 40% of muscle afferents, and 60% of bladder afferents being sensitive to capsaicin. Because the present study determined whether cell somata were sensitive to capsaicin, it is possible that receptors may have been present on nerve endings but not expressed on cell bodies, yielding an underestimate of the fraction of cells sensitive to capsaicin.

The capsaicin-induced current is a nonspecific cation conductance with relative permeabilities of $K^+ > Cs^+ > Na^+$ > choline = TEA$. Although we did not specifically measure the permeability of divalent cations because they were present at such low concentrations, our results are consistent with those of Bevan and Docherty (3). They also noted that the conductance pathway discriminates poorly among cations and allows bulky ions to cross the membrane, with a permeability sequence for capsaicin-activated currents of $Ca^{2+} > Mg^{2+} >$ guanidinium $> K^+ > Cs^+ > Na^+$ > choline.

We did not find any relationship between cell size and capsaicin sensitivity. All cell sizes were similarly responsive to capsaicin, and the magnitude of the inward current correlated poorly with cell diameter. The DRG cells studied here fell within a relatively narrow range of diameters (19–40 µm), likely because we restricted cells studied to those labeled from the colon. Several studies, however, have suggested that smaller cells may be more sensitive to capsaicin (14, 40, 42). In intact DRG, C fibers, which tend to be associated with cells that are smaller in diameter, are more sensitive to capsaicin than A fibers (18, 26). Liu and Simon (24) found capsaicin-activated currents in 60% of rat trigeminal ganglion cells and reported that cells <25 µm in diameter were more likely to be activated by capsaicin. Cells with the smallest soma diameters also had the largest current densities. In unidentified rat DRG cells, the mean diameter of capsaicin-sensitive cells was reported to be less than the mean diameter of capsaicin-insensitive cells (14). However, Petersen and LaMotte (31) reported that 100% of DRG cells in the range of 32–36 µm in diameter were sensitive to capsaicin, whereas only 28–33% of cells that were <32 µm or >36 µm were capsaicin sensitive. They also reported that the current density was the same for all cells, regardless of size. Vlachova and Vyklíčky (39) reported that neonatal rat DRG cells 30 µm in diameter were insensitive to capsaicin; only smaller cells that were 10–20 µm in diameter showed a response. We also did not find any
correlation between cell size and the presence of a 
TTX-R component to the Na\(^+\) current, although others 
have reported that cells containing both TTX-R and 
TTX-S currents tended to be smaller in diameter than 
cells with only a TTX-S component (15, 27, 28, 33, 34).

The functional significance of the two types of voltage-gated Na\(^+\) currents is not known, although their presence 
may be related to transmission of different types of 
sensory information. A shoulder or inflection in the 
falling phase of the action potential is considered 
characteristic of small, thinly myelinated A\(\alpha\) fibers and 
unmyelinated C fibers. The shape of the action potential 
may be due to the presence of TTX-R channels, with 
their slower activation and inactivation kinetics and 
higher threshold for activation (27). The TTX-R current 
may also be related to long-term adaptation to sus-
tained stimuli. TTX-R channels recover from inactiva-
tion faster than TTX-S channels, suggesting that TTX-R 
channels may be more slowly adapting to depolarizing 
stimuli (12, 30). In contrast, Ogata and Tatebayashi 
(29) reported an extremely slow component to inactiva-
tion that might be related to enhanced adaptation over 
much longer time periods. TTX-R channels may also 
exclude significant physiological and pharmacological 
properties, suggesting the existence of multiple 
channel subtypes. Caffrey et al. (6) described three 
types of Na\(^+\) channels in adult rat DRG cells, including 
a channel with intermediate kinetics that seemed to 
predominate in the larger cells (>58 \(\mu\)m).

The presence of TTX-R currents in DRG neurons 
appears to be associated with other nociceptive prop-
ties, including a sensitivity to capsaicin. In the present 
experiments, all capsaicin-sensitive colon sensory cells 
had both TTX-R and TTX-S components. Arbuckle and 
Docherty (2) also found that capsaicin sensitivity was 
associated with the presence of both TTX-R and TTX-S 
currents and that TTX-R currents were larger in capsa-
icin-sensitive cells than in capsaicin-insensitive cells. Gold 
et al. (15) also reported that sensitivity to capsaicin was 
more common in cells with TTX-R currents and sug-
gested that TTX-R was selectively expressed in a 
subpopulation of putative nociceptive neurons. A TTX-R 
Na\(^+\) channel expressed selectively in smaller-diameter 
DRG cells, including capsaicin-sensitive neurons, has 
been cloned (1, 35).

In those colon DRG cells that responded to capsaicin 
with an inward current, capsaicin virtually abolished 
voltage-gated Na\(^+\) currents. This effect was prevented 
by capsazepine. Capsaicin had no effect on Na\(^+\) cur-
cents in the cells that did not show an inward current. 
Petersen et al. (32) reported that capsaicin decreased 
the amplitude of Na\(^+\) currents in guinea pig and chick 
neurons but did not correlate the decrease in Na\(^+\) 
current with any agonist effects of capsaicin. Grosskreutz 
et al. (17) found that capsaicin blocked 
TTX-R Na\(^+\) action potentials in human sural nerve C 
fibers and that the reduction in C spike amplitude was 
accompanied by membrane depolarization. Other stud-
ies have demonstrated a decrease in Ca\(^{2+}\) current in 
those cells that showed a direct response to capsaicin 
(5, 9, 17). However, the decrease in Ca\(^{2+}\) current is 
thought to be secondary to the rise in intracellular Ca\(^{2+}\) 
rather than a direct effect on the Ca\(^{2+}\) channel.

Gold et al. (14) proposed that nervous nociceptor 
properties were coexpressed together, including small 
cell body diameter, the presence of substance P and 
calcitonin gene-related peptide, an inflection or shoul-
der on the falling phase of the action potential, respon-
siveness to capsaicin, and sensitization in response to 
PGE\(_2\). Gold et al. (15) subsequently reported that the 
putative sensitizing agents adenosine, PGE\(_3\), and 
serotin increased the magnitude of TTX-R Na\(^+\) currents 
in a subgroup of DRG neurons (however, see Ref. 23 
regarding endogenous sensitizing mediators). PGE\(_2\) 
decreased the firing threshold and increased the num-
ber of action potentials in adult rat DRG cells, leading 
them to suggest that modulation of TTX-R may be a 
mechanism for sensitization of mammalian nocicep-
tors. England et al. (13) also reported that PGE\(_2\) 
increased the excitability of neonatal DRG cells. Peak 
TTX-R Na\(^+\) currents were larger, and both activation 
and inactivation curves were shifted slightly in a 
hyperpolarizing direction due to cAMP-protein kinase 
A-dependent modulation of the TTX-R Na\(^+\) channel, 
sensitive with sensitization of primary afferents. Carden-
as et al. (7) found that serotonin increased TTX-R 
Na\(^+\) currents in a subpopulation of cells previously 
identified as being sensitive to capsaicin, whereas 
PGE\(_2\) increased both TTX-R and TTX-S Na\(^+\) currents 
in capsaicin-sensitive and capsaicin-insensitive neurons. 
Our study reports different findings: voltage-gated Na\(^+\) 
currents in capsaicin-sensitive cells were not significa-
cantly altered by adenosine, bradykinin, histamine, 
PGE\(_2\), or serotonin. Cardenas et al. (7) also reported 
that an adenosine receptor agonist had no effect, simi-
lar to our results. Furthermore, Kress and Reeh (23) 
reported that neither serotonin nor PGE\(_2\) altered Na\(^+\) 
currents in dissociated DRG cultures of capsaicin-
sensitive neurons from 1-day-old rats, also consistent 
with the present results. Differences in susceptibility to 
putative sensitizing agents may possibly reflect varia-
tions in cell culture conditions, including the presence 
of nerve growth factors.

When studying rapid currents such as voltage-gated 
Na\(^+\) currents, series resistance is often a problem. 
Inadequate series resistance compensation may result 
in voltage errors or poor space clamping during applica-
tion of voltage-clamp command pulses, especially when 
membrane currents are large. In the present 
experiments, external Na\(^+\) was lowered to 20 mM to reduce 
the peak amplitude of Na\(^+\) currents to an average 
of ~4–6 nA and series resistance compensation on the 
patch-clamp amplifier was adjusted to 88%. Voltage 
errors were apparently still a problem, however, as 
evidenced by the rapid activation of Na\(^+\) currents as 
the magnitude of the depolarizing pulse was increased 
and by the high degree of voltage dependence exhibited 
in the current-voltage relationship curves. Problems with 
series resistance would not have altered the results, 
however. The peak amplitude of Na\(^+\) currents was 
always measured at the potential at which current was
the greatest so that small voltage errors would not have affected measurement of peak current amplitude. In addition, we were not attempting to measure extremely rapid events, such as Na\(^+\) current kinetics or activation/inactivation rates.

The present findings suggest that capsaicin may depress action potential conduction rather than cause sensitization of colon sensory afferents. This suppression of voltage-gated Na\(^+\) currents in DRG cells may account for the transient blockade of nerve conduction seen with capsaicin and may explain some of its analgesic properties as opposed to its irritant effects.

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