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Hyperalgesic agents increase a tetrodotoxin-resistant Na^+ current in nociceptors

(capsaicin/hyperalgesia/pain/primary afferent/sensitization)

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ABSTRACT **Sensitization of primary afferent neurons underlies much of the pain and tenderness associated with tissue injury and inflammation. The increase in excitability is caused by chemical agents released at the site of injury. Because recent studies suggest that an increase in voltage-gated Na^+ currents may underlie increases in neuronal excitability associated with injury, we have tested the hypothesis that a tetrodotoxin-resistant voltage-gated Na^+ current (TTX-R I_{Na}), selectively expressed in a subpopulation of sensory neurons with properties of nociceptors, is a target for hyperalgesic agents. Our results indicate that three agents that produce tenderness or hyperalgesia *in vivo*, prostaglandin E_2, adenosine, and serotonin, modulate TTX-R I_{Na}. These agents increase the magnitude of the current, shift its conductance–voltage relationship in a hyperpolarized direction, and increase its rate of activation and inactivation. In contrast, thromboxane B_2, a cyclooxygenase product that does not produce hyperalgesia, did not affect TTX-R I_{Na}. These results suggest that modulation of TTX-R I_{Na} is a mechanism for sensitization of mammalian nociceptors.**

Nociceptors are primary afferent neurons that respond to noxious or potentially tissue-damaging stimuli and are unique among sensory neurons because they can be sensitized. The decrease in threshold and increase in the response to a constant stimulus that are characteristic of nociceptor sensitization are thought to underlie the hyperalgesia or tenderness associated with tissue injury. Agents released at the site of tissue injury sensitize nociceptors by initiating a cascade of events (1) that likely results in a change in ionic conductances of the nociceptor peripheral terminal.

Recent observations suggest that an increase in a voltage-gated Na^+ current might underlie the increased excitability of primary afferent neurons following injury. (*i*) The density of voltage-gated Na^+ channels increases in a locus of hyperexcitability after nerve injury (2). (*ii*) Nerve injury induces an increase in the mRNA encoding three distinct voltage-gated Na^+ channels (3). (*iii*) Nerve growth factor, an agent released at sites of injury (4), has been shown to increase expression of a number of voltage-gated Na^+ channels in sensory neurons. An increase in the expression of Na^+ channel mRNA would be expected to result in an increase in Na^+ channel density and consequently in Na^+ current. Thus, we hypothesized that hyperalgesic agents such as prostaglandin E_2 (PGE_2), serotonin (5-HT), and adenosine sensitize primary afferent nociceptors by increasing a voltage-gated Na^+ current. Specifically, we hypothesized that since a tetrodotoxin (TTX)-resistant voltage-gated Na^+ current (TTX-R I_{Na}) appears to be selectively expressed in nociceptive afferents (5), this current is a target for modulation by hyperalgesic agents.

We have demonstrated, using patch-clamp electrophysiological techniques to record from cultured adult rat dorsal root ganglion (DRG) neurons, that PGE_2, 5-HT, and adenosine modulate TTX-R I_{Na} in a manner consistent with nociceptor sensitization.

EXPERIMENTAL PROCEDURES

Cell Preparation. Neurons were obtained from the lumbar DRG of adult male Sprague–Dawley rats; ganglia were enzymatically treated and mechanically dispersed by methods similar to those described previously (6). Experiments were done on DRG neurons within 24 hr of plating on laminin/ornithine-coated glass coverslips. Neurons were incubated in minimal essential medium/10% fetal bovine serum/human recombinant nerve growth factor (20–50 ng/ml) at 37°C, 90% humidity, and 3% CO_2 before recording.

Electrophysiology. Voltage-clamp recordings were done with an Axopatch 1B amplifier (Axon Instruments, Foster City, CA). Data were low-pass filtered at 1–10 kHz with a 4-pole Bessel filter and digitally sampled at 2–20 kHz. Capacity transients were canceled and series resistance was compensated (>80%); a P/4 protocol was used for leak subtraction. For whole-cell recording, electrodes (0.7–3 MΩ) were filled with 140 mM CsCl/10 mM NaCl/0.1 mM $CaCl_2$/2 mM $MgCl_2$/11 mM EGTA/10 mM Hepes/2 mM Mg-ATP/1 mM Li-GTP; pH was adjusted to 7.2 with Tris-base and osmolality was adjusted to 310 mOsm. Bath solution used to record whole-cell Na^+ currents in isolation contained 35 mM NaCl/30 mM tetraethylammonium chloride/65 mM choline chloride/0.1 mM $CaCl_2$/5 mM $MgCl_2$/10 mM Hepes/10 mM glucose, pH adjusted to 7.4, and osmolality adjusted to 325 mOsm. TTX-resistant Na^+ currents were isolated from TTX-sensitive Na^+ currents in two ways: TTX (50 nM) was added to the bath solution or the membrane potential was clamped at −50 mV for at least 100 ms prior to depolarizing voltage-steps used to evoke I_{Na}. For cell-attached patch recording 7- to 10-MΩ electrodes were used with an electrode solution containing 250 mM NaCl/5 mM CsCl/0.1 mM $CaCl_2$/5 mM $MgCl_2$/10 mM Hepes/1 μM TTX, pH was adjusted to 7.4 with Tris-base, and osmolality was determined to be 500 mOsm. Normal bath solution was used for cell-attached patch recording and contained 130 mM NaCl/3 mM KCl/2.5 mM $CaCl_2$/0.6 mM $MgCl_2$/10 mM Hepes/1.2 mM $NaHCO_3$/10 mM glucose, pH was adjusted to 7.4 with NaOH, and osmolality was adjusted to 325 mOsm. For cell-attached patch recordings of TTX-R I_{Na} the membrane potential was estimated from the activation and inactivation curves obtained before PGE_2 application.

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Abbreviations: TTX, tetrodotoxin; DRG, dorsal root ganglion; PGE_2, prostaglandin E_2; 5-HT, serotonin; TTX-R I_{Na}, TTX-resistant voltage-gated Na^+ current; TTX-S I_{Na}, TTX-sensitive voltage-gated Na^+ current; TxB_2, thromboxane B_2.

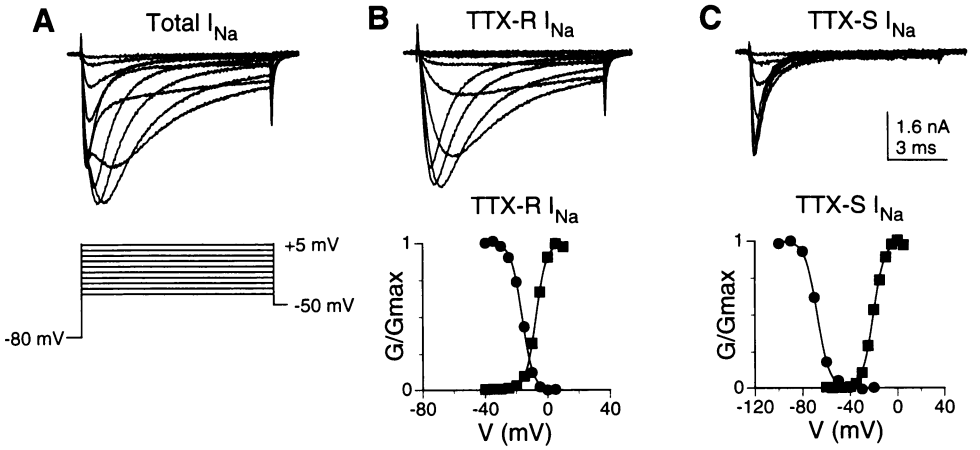

FIG. 1. Voltage-gated Na^+ currents (I_{Na}) present in DRG neurons are distinguished by sensitivity to TTX and gating properties. (*A*) Voltage-gated Na^+ current evoked from a 25-μm DRG neuron during 20-ms command potentials ranging between −40 mV and +5 mV from a holding potential of −80 mV; the voltage clamp protocol used to evoke the current is shown beneath the current traces. (*B*) TTX-R I_{Na} is isolated after bath application of 50 nM TTX; normalized conductance–voltage relationships for activation and steady-state inactivation of TTX-resistant I_{Na} are plotted beneath the current traces (a 500-ms conditioning pulse was used to generate the steady-state inactivation data). (*C*) TTX-S I_{Na} is isolated as the difference between the current evoked before and after TTX application (current evoked at −5, 0, and +5 mV has been omitted for clarity); normalized conductance–voltage relationships for activation and steady-state inactivation of TTX-sensitive I_{Na} are plotted beneath current traces (a 500-ms conditioning pulse was used to generate the steady-state inactivation data).

Stock solutions of PGE_2 (Sigma) and thromboxane B_2 (TxB_2; Cayman Chemicals, Ann Arbor, MI) were prepared in 100% ethanol and diluted in bath solution so that final concentration of ethanol never exceeded 1%; vehicle controls were performed. All agents except capsaicin were applied through the bath perfusion system. Capsaicin (500 nM) was puffed through a micropipette; a positive response consisted of a rapid inward current (see Fig. 2) evoked in normal bath solution. The recording chamber (500-μl vol) was continuously perfused (2 ml/min) with bath solution at room temperature (21–24°C).

Conductance–voltage (g–V) relationships were calculated from peak current-voltage (I–V) relationships according to $g = I/(V - V_r)$, where I is the peak current at voltage (V), and V_r is the reversal potential extrapolated from the linear portion of the I–V relationship. A Boltzmann function of the form $1/(1 + \exp[(V - V_{1/2})/k])$, where $V_{1/2}$ is the voltage of half-activation or inactivation and k is a slope factor, was used to fit g–V relationships and inactivation curves. Pooled data are reported as mean ± SEM.

RESULTS AND DISCUSSION

DRG neurons in culture expressed voltage-gated Na^+ currents distinguished by their sensitivity to TTX. TTX-sensitive current (TTX-S I_{Na}) was completely blocked by 50 nM TTX, whereas TTX-resistant current (TTX-R I_{Na}; Fig. 1) was unaffected by 10 μM TTX (data not shown). TTX-S and -R I_{Na} also were distinguished by differences in kinetic and steady-state properties (Fig. 1). TTX-R I_{Na} was determined to be a Na^+ current based on our observation that changing the extracellular Na^+ concentration from 35 to 17.5 mM shifted the reversal potential of the current from 27.9 ± 0.4 mV to 14.1 ± 0.6 mV (n = 7), similar to the values predicted by the Nernst equation.

The TTX-R I_{Na} was primarily restricted to a subpopulation of DRG neurons that expressed properties associated with nociceptors *in vivo*, including a small cell body diameter (7) and responsiveness to the algogenic agent capsaicin (8). In the present study, the mean cell body diameter of DRG neurons with TTX-R I_{Na} (28.6 ± 0.4 μm, n = 157) was significantly smaller than the mean cell body diameter of DRG neurons without TTX-R I_{Na} (41.4 ± 1.2 μm, n = 40, $P < 0.01$ Student's t test). TTX-S I_{Na} was present in all neurons studied (n = 197). The different distributions of TTX-R I_{Na} and TTX-S I_{Na} with respect to cell body size are illustrated in a plot of TTX-R/TTX-S I_{Na} vs. membrane capacitance where the relative magnitude of TTX-R I_{Na} is dramatically reduced in neurons with a membrane capacitance > 70 pF (Fig. 2*A*). Expression of TTX-R I_{Na} also correlated with capsaicin responsiveness: of 77 neurons studied for capsaicin responsiveness, 40 of 63 DRG neurons with TTX-R I_{Na} responded to capsaicin, whereas only 3 of 14 DRG neurons without TTX-R I_{Na} responded to capsaicin ($P < 0.01$, χ^2 test). Capsaicin responsiveness was also primarily restricted to small DRG neurons (Fig. 2*B*).

We had demonstrated (9) that DRG neurons with small cell-body diameters that are capsaicin-responsive are very likely to express other properties associated with nociceptors *in vivo*, including the neuropeptides substance P and/or calcitonin gene-related peptide (7) and an action potential

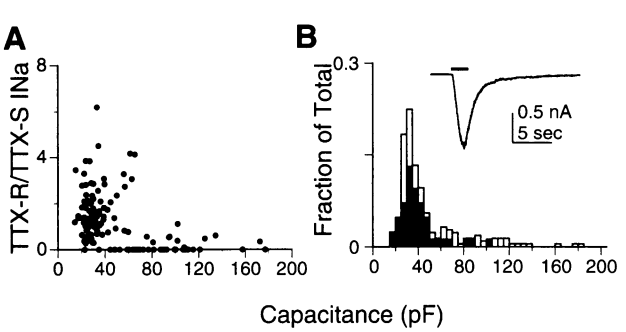

FIG. 2. TTX-R I_{Na} is selectively expressed in a subpopulation of putative nociceptors. (*A*) Expression of TTX-R I_{Na} is restricted to a population of DRG neurons with small cell bodies; the ratio of TTX-R I_{Na} to TTX-S I_{Na} with respect to membrane capacitance is plotted (n = 197). TTX-R I_{Na} was isolated from TTX-S I_{Na} as described. (*B*) Size distribution of DRG neurons responsive to puffer application of 500 nM capsaicin; filled bars represent the fraction of capsaicin-responsive neurons in each capacitance bin (a total of 48 neurons were capsaicin-responsive) relative to the number of neurons in which a capsaicin response was assessed (n = 84), and open bars represent the fraction of all neurons in each capacitance bin relative to the total number of neurons studied (n = 197). (*Inset*) Capsaicin response. From a holding potential of −70 mV, the inward current evoked by a 2-sec application of 500 nM capsaicin through a puffer pipette is typical of capsaicin-responsive DRG neurons; bar indicates the capsaicin-application period.

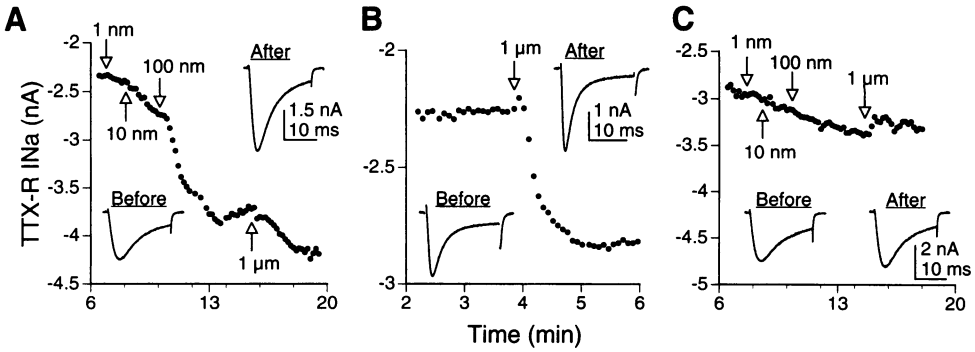

FIG. 3. Dose-dependence and time course of PGE_2-induced changes in TTX-R I_{Na} amplitude. (*A*) PGE_2 dose-dependently increased the magnitude of TTX-R I_{Na}: TTX-R I_{Na} was evoked with a 20-ms depolarizing voltage step to −10 mV from a holding potential of −80 mV every 10 sec. PGE_2 (1, 10, 100, and 1000 nM) was added through the bath perfusion system at the times indicated by arrows. The *Inset* traces were evoked immediately before (Before) application of 1 nM PGE_2, and 200 sec after application of 1 μM PGE_2 (After). (*B*) The same protocol used to study the neuron in *A* was used to study a different neuron, except that the depolarizing voltage step was to 0 mV. The stable baseline obtained prior to application of 1 μM PGE_2 was typical of neurons both responsive and unresponsive to PGE_2. (*C*) The same protocol used to study the neuron in *A* was used to study a different neuron unresponsive to application of PGE_2. There is little change in the current evoked immediately before (Before) application of 1 nM PGE_2 and 200 sec after the application of 1 μM PGE_2 (After), as shown in *Insets*.

"shoulder" (10). Importantly, changes in excitability associated with the hyperalgesic agent-induced sensitization of nociceptors *in vivo* (11) are also observed *in vitro* (9). Therefore, the results from the present study confirm that expression of TTX-R I_{Na} is largely restricted to a subpopulation of putative nociceptors (5).

The effects of hyperalgesic agents on TTX-R I_{Na} were determined by comparing properties of the current evoked before and after bath application of hyperalgesic agents; a stable baseline current was observed for at least 1 min before the application of any test agent. In some experiments the effects of hyperalgesic agents on both TTX-R I_{Na} and TTX-S I_{Na} were studied in the same neuron to determine whether these agents affect TTX-R I_{Na} selectively.

Under whole-cell voltage clamp, PGE_2 dose-dependently increased the magnitude of TTX-R I_{Na} (Fig. 3*A*). The threshold concentration for PGE_2-induced effects was between 1 and 10 nM (n = 5). There was a 10- to 15-sec delay before the changes in TTX-R I_{Na} were detected and the maximal increase in current was achieved 30–100 sec later (Fig. 3*B*). After wash of PGE_2, TTX-R I_{Na} began to return to preexposure amplitude, usually within 5–15 min. In 18 of 35 (51%) small-diameter DRG neurons PGE_2 (1 μM) increased peak TTX-R I_{Na} by 22.7 ± 2.4%; the change in TTX-R I_{Na} in neurons unaffected by PGE_2 was −3.6 ± 1.6%. All (18 of 18) neurons tested in which PGE_2 increased TTX-R I_{Na} were capsaicin-responsive. Of note, PGE_2 sensitizes a similar proportion (56%) of putative nociceptors *in vitro* with a similar dose dependence and time course (9). The proportion of nociceptors sensitized *in vivo* is also similar (8, 12). TTX-R I_{Na} in DRG neurons unresponsive to PGE_2 was relatively stable over many minutes of recording (Fig. 3*C*).

In 11 neurons in which PGE_2 increased the magnitude of TTX-R I_{Na}, there was no increase in the magnitude of TTX-S I_{Na} (data not shown).

TxB_2 is a chemical similar in structure to PGE_2 that is released at sites of injury and inflammation (13) but has no effect on nociceptive threshold when administered into peripheral tissue (14). Consistent with the idea that changes in TTX-R I_{Na} are a specific mechanism of nociceptor sensitization and hyperalgesia, bath application of 10 μM TxB_2 had no effect on peak TTX-R I_{Na} (−0.4 ± 2.2%, n = 9). TTX-R I_{Na} in five of these neurons was subsequently increased by 1 μM PGE_2 (Fig. 4*B*).

To determine whether TTX-R I_{Na} is a common target for different agents that produce hyperalgesia, we tested the effects of adenosine and 5-HT (15, 16). Adenosine (Fig. 4*C*) and 5-HT (Fig. 4*D*) dose-dependently induced changes in TTX-R I_{Na} that were very similar to those induced by PGE_2. Adenosine (1 μM) increased peak TTX-R I_{Na} by 14 ± 4.4% in 5 of 14 neurons; the change in TTX-R I_{Na} in neurons unaffected by adenosine was −2.0 ± 1.8%. In 7 of 11 neurons, 5-HT (10 nM) increased peak TTX-R I_{Na} 18.5 ± 3.3%; the change in TTX-R I_{Na} in neurons unaffected by 5-HT was −3.9 ± 2.4%.

Data obtained with PGE_2, adenosine, and 5-HT suggested the existence of two populations of cells with different re-

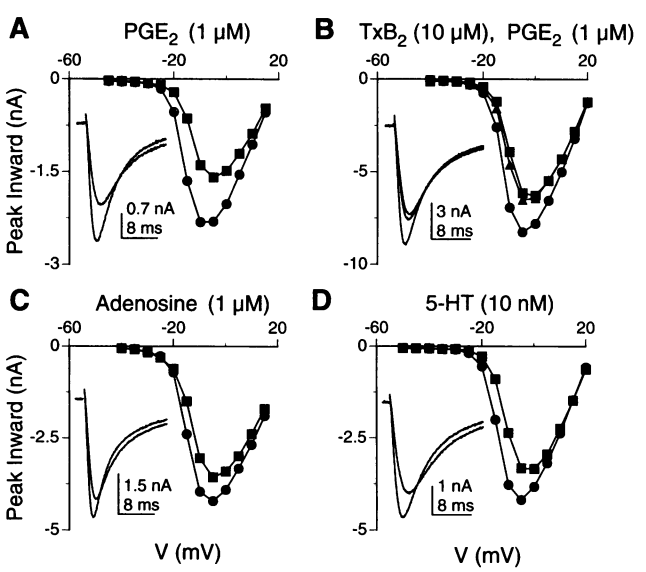

FIG. 4. Effects of PGE_2, TxB_2, adenosine, and serotonin (5-HT) on TTX-R I_{Na}. (*A*) PGE_2 (1 μM) increases TTX-R I_{Na}. Current–voltage relations of TTX-R I_{Na} were obtained before (■) and after (●) application of PGE_2. (*Inset*) TTX-R I_{Na} was evoked at 0 mV from a DRG neuron before and after bath application of 1 μM PGE_2. (*B*) TxB_2 failed to increase TTX-R I_{Na} in a DRG neuron in which the current was subsequently increased by PGE_2. Current–voltage relations of TTX-R I_{Na} were obtained in normal bath (■), in bath with TxB_2 (▲), and in bath with PGE_2 (●). (*Inset*) TTX-R I_{Na} was evoked at 0 mV before and after application of 10 μM TxB_2 and after application of 1 μM PGE_2. (*C*) Adenosine increases TTX-R I_{Na}. Current–voltage relations of TTX-R I_{Na} were obtained before (■) and after application of adenosine (●). (*Inset*) TTX-R I_{Na} was evoked at 0 mV before and after application of 1 μM adenosine. (*D*) 5-HT increases TTX-R I_{Na}. Current–voltage relations of TTX-R I_{Na} were obtained before (■) and after (●) 5-HT application. (*Inset*) TTX-R I_{Na} was evoked at 0 mV before and after application of 10 nM 5-HT.

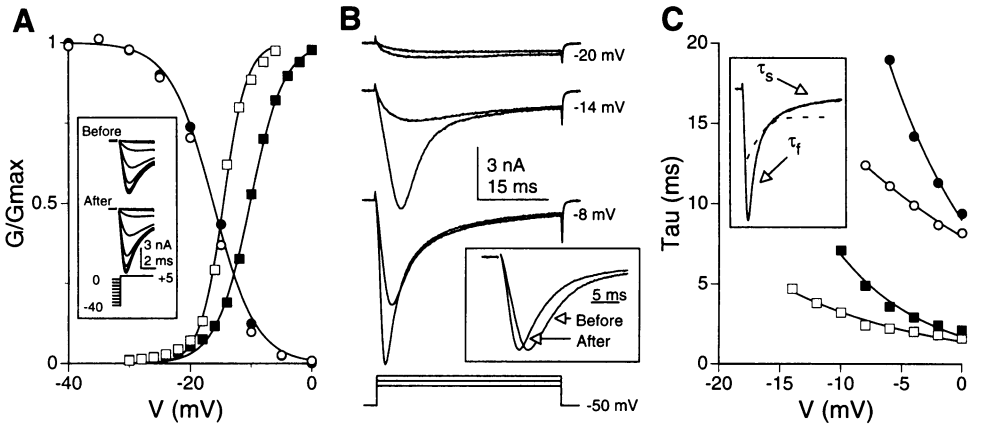

FIG. 5. PGE_2 affects steady-state and kinetic properties of TTX-R I_{Na}. (*A*) Bath application of 1 μM PGE_2 decreases activation threshold and increases the voltage-dependence of activation of TTX-R I_{Na} (squares) without affecting steady-state inactivation (circles). To facilitate comparison of data obtained before (filled symbols) and after (open symbols) PGE_2, data were normalized with respect to the maximal current evoked after PGE_2 application (Gmax). Data were fit with a Boltzmann function. (*Inset*) The steady-state inactivation traces used to plot data in *A*; currents were evoked at +5 mV following 100-ms prepulses to potentials ranging from −40 to 0 mV. (*B*) Representative current traces used to generate the data plotted in *A*, evoked before and after PGE_2 application (smaller and larger amplitude currents, respectively). (*Inset*) PGE_2 increases activation and inactivation rates. TTX-R I_{Na} evoked at 0 mV before and after PGE_2; the current trace evoked before PGE_2 application has been multiplied by a factor of 1.3 to facilitate comparison of the kinetics. (*C*) Voltage dependence of TTX-R I_{Na} inactivation rate (τ) before and after PGE_2. TTX-R I_{Na} evoked during 20-ms depolarizing voltage steps to between −14 mV and 0 mV from a holding potential of −80 mV. The rate of inactivation was estimated by fitting the decaying portion of the current trace with exponential functions. Inactivation of TTX-R I_{Na} was best-fit by a double exponential; the voltage dependence of both fast (squares) and slow (circles) components is faster after (open symbols) than before (filled symbols) PGE_2. (*Inset*) Current trace obtained at −8 mV in *B*, fit with two single exponential equations that can be used to describe fast (τ_f, 3.6 ms) and slow (τ_s, 19 ms) components of inactivation.

sponses to hyperalgesic agents: in one population of cells, the three agents increased the magnitude of TTX-R I_{Na} and shifted the voltage dependence of activation, whereas in the other population of cells, the agents had little effect on the current. Histograms of percentage change in peak TTX-R I_{Na} induced by PGE_2, adenosine, and 5-HT revealed bimodal distributions of the cell population with distinct peaks around 0 and 20% (data not shown). While a spontaneous drift in the magnitude and voltage dependence of TTX-R I_{Na} was observed in some neurons (Fig. 3*C*), such changes were small (<5% with respect to peak current) and progressed steadily over many minutes. In contrast, changes in TTX-R I_{Na} in response to hyperalgesic agents began to develop within 15 sec after application of these agents (Fig. 3*B*). Furthermore, a stable TTX-R I_{Na} was routinely recorded for at least 1 min before the application of any agent (Fig. 3*B*). Finally, we never observed spontaneous changes (>5%) in TTX-R I_{Na} induced by simply changing between bath solutions that did not contain PGE_2, adenosine, or 5-HT.

PGE_2 was used to more thoroughly characterize the effects of hyperalgesic agents on TTX-R I_{Na} because this dose produces a robust increase in the excitability of putative nociceptors *in vitro* (9). The increase in TTX-R I_{Na} induced by PGE_2 was associated with a leftward shift in the activation midpoint of TTX-R I_{Na} (Fig. 5*A*, 5.2 ± 0.8 mV, n = 7). This shift primarily reflects an increase in the voltage dependence of activation (i.e., the slope factor of the Boltzmann function, k, decreases by 28 ± 6.3%, n = 7); activation threshold generally shifted <5 mV. In addition, activation and inactivation rates of the current were increased after exposure to PGE_2 (Fig. 5 *B* and *C*). PGE_2 had no effect on the steady-state inactivation of the TTX-R I_{Na} (Fig. 5*A*) with either short (100 ms) or long (1 min) conditioning voltage steps. Furthermore, neither the voltage dependence nor time course of deactivation and recovery from inactivation was affected by PGE_2 (data not shown).

Our observations of the selective distribution of TTX-R I_{Na} among DRG neurons, the hyperalgesic agent-induced changes in the current, and the differential sensitivity of the current hyperalgesic versus nonhyperalgesic agents were all consistent with the hypothesis that modulation of the current is a mechanism of nociceptor sensitization. To investigate the possibility that the limited duration of PGE_2-induced modulation of TTX-R I_{Na} observed with whole-cell patch clamp recording reflected a "washout" of components necessary for maintaining the changes in the current properties, we tested the effects of hyperalgesic agents on TTX-R I_{Na} recorded from cell-attached patches. TTX-R I_{Na} channels appeared to be clustered in small regions of the membrane of acutely cultured DRG neurons; >90% of patches contained no TTX-R I_{Na}, whereas the remaining 10% of the patches contained 10–30 channels (estimated by nonstationary ANOVA). In 62% (8 of 11) of the neurons in which PGE_2 increased TTX-R I_{Na} (Fig. 6*A*), peak current was increased by an average of 225 ± 38%. While the PGE_2-induced changes seen with cell-attached patch recording were qualitatively similar to those obtained with whole-cell recordings, the changes differed quantitatively: in the patch, the mean increase in peak current was almost an order of magnitude larger, and the mean shift in the $V_{1/2}$ of

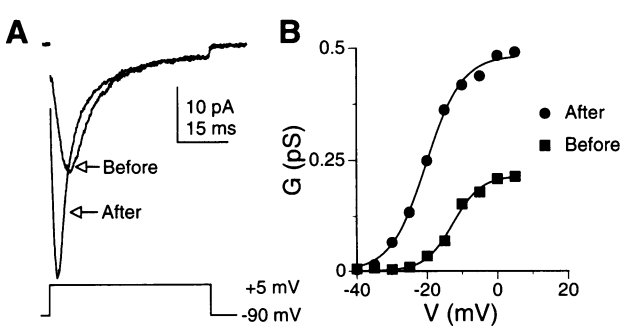

FIG. 6. Effects of PGE_2 on TTX-R I_{Na} recorded from a cell-attached patch. (*A*) TTX-R I_{Na} evoked with a step from −80 to 0 mV, before and after application of 1 μM PGE_2; each trace is the average of 15 consecutive depolarizations. Note the increase in activation and inactivation rates after PGE_2 application. (*B*) Conductance–voltage relations of TTX-R I_{Na} evoked before (■) and after (●) application of PGE_2; PGE_2 decreases threshold and increases maximal conductance. Each data point was determined from the average of five consecutive current traces. Data were fit with a Boltzmann function.

activation (14.5 ± 3.6 mV, $n = 4$) was more than 2-fold larger (Fig. 6 *A* and *B*) and was associated with a larger shift in the activation threshold. In addition, with cell-attached patch recording, the increase in TTX-R I_{Na} persisted for the duration of recording (≥30 min). The shift in the conductance–voltage relationship was not the result of a change in resting membrane potential because there was no change in the steady-state inactivation of the current (data not shown). Adenosine (1 μM) produced similar changes in TTX-R I_{Na} as those observed for PGE_2 in the cell-attached patch recording configuration (data not shown).

The results we obtained using cell-attached patch recording of TTX-R I_{Na} suggest that modulation of TTX-R I_{Na} involves a diffusible intracellular second messenger because it is unlikely that the hydrophilic agent adenosine could gain access to channels separated from the bath by a 5- to 10-GΩ seal. Such a second messenger-mediated process would also be consistent with the delayed onset of the hyperalgesic agent-induced changes in TTX-R I_{Na}. That the second messenger is diffusible also is suggested by the observation that hyperalgesic agent-induced changes in TTX-R I_{Na} recorded from cell-attached patches were larger and lasted longer than those observed with whole-cell configuration; a diffusible second messenger necessary for the hyperalgesic agent-induced effects may have been "washed out" of the cell during whole-cell recording. Results obtained with the less invasive cell-attached recording configuration are likely to reflect more closely the physiological effects of hyperalgesic agents on TTX-R I_{Na} in DRG neurons *in vivo*.

We suggest that the hyperalgesic agent-induced modulation of TTX-R I_{Na} may underlie nociceptor sensitization *in vivo*. The changes in TTX-R I_{Na} we observed could account for both the decrease in threshold and the increase in number of action potentials evoked in response to a constant stimulus associated with nociceptor sensitization *in vivo* (11). Changes in TTX-R I_{Na} could underlie the decrease in action-potential threshold associated with sensitization because the decreased activation threshold, increased activation rate, and increased magnitude of the current all contribute to a more rapid depolarization of the neuronal membrane. It is likely that TTX-R I_{Na} determines action potential threshold in nociceptors since this subpopulation of neurons has a resting potential that inactivates TTX-S I_{Na} (17). Hyperalgesic agent-induced changes in TTX-R I_{Na} could also underlie the increase in the number of action potentials in response to a constant stimulus because the increase in TTX-R I_{Na} inactivation rate would speed membrane repolarization and so decrease the interspike interval. Furthermore, data from a recent study indicate that the TTX-R I_{Na} is present in the central terminals of sensory neurons (18), and therefore it is likely that the current is also present in the peripheral terminals. Finally, since there is evidence to suggest that a hyperalgesic agent-induced decrease in a Ca^{2+}-dependent K^+ current is also a mechanism of nociceptor sensitization (19), changes in this current and TTX-R I_{Na} may act in concert to increase nociceptor excitability in the presence of inflammation and injury.

In summary, our data indicate that TTX-R I_{Na} is selectively expressed by putative nociceptors, is a common target for a number of hyperalgesic agents, and is selectively modulated by these agents in a manner consistent with nociceptor sensitization. These observations suggest that pharmacological therapies designed to specifically block TTX-R I_{Na} might be a potential treatment for pain syndromes characterized by nociceptor sensitization.

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