Prostaglandins Suppress an Outward Potassium Current in Embryonic Rat Sensory Neurons

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Nicol, G. D., M. R. Vasko, and A. R. Evans. Prostaglandins suppress an outward potassium current in embryonic rat sensory neurons. J. Neurophysiol. 77: 167–176, 1997. The cellular mechanisms giving rise to the enhanced excitability induced by prostaglandin E2 (PGE2) and carbo prostacyclin (CPGI2) in embryonic rat sensory neurons were investigated using the whole cell patch clamp recording technique. Exposing sensory neurons to 1 μM PGE2 produced a twofold increase in the number of action potentials elicited by a ramp of depolarizing current, but this eicosanoid had no effect on the resting membrane potential or the amplitude of the slow afterhyperpolarization. Characterization of the outward potassium currents in the embryonic sensory neurons indicated that the composition of the total current was variable among these neurons. A steady-state inactivation protocol was used to determine the extent of residual noninactivating current. A conditioning prepulse to +20 mV demonstrated that some of these neurons exhibited only a sustained potassium current with little steady-state inactivation whereas others exhibited some combination of a sustained as well as a rapidly inactivating Ia-type current. Treatment with 1 μM PGE2 or 1 μM CPGI2, but not 1 μM prostaglandin F2alpha (PGF2alpha), produced a time-dependent suppression of the total potassium current. After a 20-min exposure, PGE2 and CPGI2 inhibited the maximal current obtained at +60 mV by 48 and 40%, respectively. The prostaglandin-induced suppression of the potassium current was not associated with a shift in the voltage dependence for activation. Subtraction of the currents remaining after PGE2 or CPGI2 treatment from their respective control recordings revealed that the prostaglandin-sensitive current had characteristics that were consistent with a sustained-type of potassium current. This idea is supported by the following observation. The steady-state inactivation protocol revealed that for prepulse voltages activating both rapidly inactivating and sustained currents, the relaxation of the current was accelerated after treatment with PGE2 or CPGI2 suggesting the removal of a slower component. This effect was not observed in neurons exhibiting only the sustained type current. These results suggest that pro-inflammatory prostaglandins enhance the excitability of rat sensory neurons, in part, through the suppression of an outward potassium current that may modulate the firing threshold for generation of the action potential.

INTRODUCTION

Prostaglandins are known to enhance the sensitivity of sensory neurons to chemical mediators of inflammation and pain such as bradykinin (reviewed by Handwerker and Reeh 1991). For example, studies have demonstrated that the frequency of action potentials recorded from C fibers in response to bradykinin was increased after the application of prostaglandin E2 (Handwerker 1976) or prostaglandin E1 (Chahl and Iggo 1977). Similarly, prostaglandin F2alpha (prostacyclin) enhanced the sensitivity of afferent nerve fibers to either chemical or mechanical stimulation (Birrell et al. 1991; Mizumura et al. 1987; Schepelman et al. 1992). This sensitizing action of prostaglandins results from a direct action on the neuron because pretreatment with prostaglandin E2 (PGE2) increased the rate of action potential firing induced by elevated potassium (Baccaglini and Hogan 1983) or by the focal application of bradykinin (Nicol and Cui1994) in isolated sensory neurons grown in culture. In addition, exposure of these isolated sensory neurons grown in culture to the pro-inflammatory prostaglandins, PGE2 or carbo prostacyclin, produced a nearly twofold increase in the bradykinin- or capsaicin-evoked release of the neuroactive substances P and calcitonin gene-related peptide (Hingtgen and Vasko 1994; Vasko et al. 1994). Although the cellular mechanisms generating the prostaglandin-induced sensitization are unknown, the above results demonstrate that treatment with prostaglandins leads to an increase in membrane excitability and an increase in the release of neuropeptides from sensory neurons.

In Aplysia sensory neurons, a similar form of sensitization has been observed (Byrne and Kandel 1996; Byrne et al. 1993; Kandel and Schwartz 1982) in that exposure to serotonin produced an increase in the number of action potentials elicited by a depolarizing current (Baxter and Byrne 1990; Critz et al. 1991; Goldsmith and Abrams 1992; Klein et al. 1986; Walters et al. 1983). Treatment with serotonin also enhanced the release of neurotransmitter from the sensory neuron (Castellucci and Kandel 1976; Hochner et al. 1986; Klein and Kandel 1980; Klein et al. 1982). This serotonin-induced sensitization arises, in part, through the inhibition of multiple potassium currents (see Baxter and Byrne 1989; Goldsmith and Abrams 1992; Hochner and Kandel 1992; Klein and Kandel 1980; Siegelbaum et al. 1982).

Although it is well established that modulation of potassium currents leads to alterations in membrane excitability in mammalian neurons (Hille 1992; Rudy 1988), the question remains as to whether modulation of potassium currents mediates the prostaglandin-induced sensitization of sensory neurons. Therefore, as an initial step in determining the cellular mechanisms giving rise to the sensitization produced by prostaglandins, we investigated whether PGE2 and CPGI2 (a stable analogue of PGI2) altered membrane excitability through the inhibition of potassium currents as recorded from embryonic rat sensory neurons grown in culture. Both PGE2 and CPGI2, but not PGF2alpha, produced a time-dependent suppression of a sustained-type of potassium current. These findings are consistent with the notion that pro-inflammatory...
prostaglandins may, in part, enhance the sensitivity of sensory neurons to a variety of stimuli through inhibition of potassium currents. Portions of this work have been published previously in abstract form (Evans et al. 1995).

METHODS

Isolation and culture of embryonic rat sensory neurons

The procedures for isolation and culture of rat sensory neurons have been described previously (Vasko et al. 1994). Briefly, pregnant rats (days 15–17 of gestation) were rendered unconscious by placing them in a chamber filled with CO2; animals then were killed by cervical dislocation. The dorsal root ganglia (DRG) from the embryos were dissected free and placed in a dish containing sterile calcium-free, magnesium-free Hank’s balanced saline (HBSS) at 4°C. The DRGs then were incubated in HBSS containing 0.025% trypsin for 25 min at 37°C. The digestion was terminated with the addition of 0.25% trypsin inhibitor; ganglia were washed once with HBSS, centrifuged, and then resuspended in growth medium that consisted of Dulbecco’s minimum Eagle’s medium (GIBCO BRL, Grand Island, NY) supplemented with 2 mM glucose, 50 µg/ml penicillin and streptomycin, 10% (v/v) heat-inactivated fetal bovine serum, 50 µM 5-fluoro-2'-deoxyuridine, 150 µM uridine, and 250 ng/ml 7-nsere growth factor (Harlan Bioproducts for Science, Indianapolis, IN). Individual cells were obtained by mechanical agitation with a fire-polished pipette until a cloudy suspension was observed. Approximately 300,000 viable cells were plated in a collagen-coated culture dish containing small plastic cover slips. Cells were grown at 37°C in a 5% CO2 atmosphere and fed every 2 day. All procedures were approved by the Animal Care and Use Committee at Indiana University School of Medicine.

Electrophysiology

The procedures for whole cell patch-clamp recordings of rat sensory neurons have been described in detail previously (Nicol and Cui 1994). Briefly, a cover slip with the sensory neurons (typically after 4 day in culture) was placed in a recording chamber where the neurons were superfused with normal Ringer solution of the following composition (in mM) 140 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and 10 glucose, pH 7.4 with NaOH. Using the whole cell patch-clamp technique (Hamill et al. 1981) membrane voltages or currents were recorded with a List EPC-7 (List Electronic, Darmstadt, Germany) or an Axopatch 200 (Axon Instruments, Foster City, CA) patch-clamp amplifier. Recording pipettes were pulled from borosilicate disposable pipettes and typically had resistances of 2–4 MΩ when filled with the following solution (in mM) 140 KCl, 5 MgCl2, 4 ATP, 0.3 GTP, 2.5 CaCl2, 5 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (calculated free Ca2+ concentration of 100 nM), and 10 HEPES, at pH 7.3 with KOH.

The whole cell recording configuration was established in normal Ringer solution. The cell capacitance was compensated by the nulling circuitry of the recording amplifier. Typically, the series resistance was uncompensated, however, in some series of experiments the effects of series resistance compensation on the actions of PGE2 were ascertained. In these sensory neurons, the compensation ranged from 95 to 87% (average 89 ± 1.1%, n = 7) yielding values for the uncompensated series resistance between 0.14–0.35 MΩ. For the current-clamp recordings, the neuron was superfused with normal Ringer solution and the membrane potential was permitted to change freely. To assay the levels of excitability, a ramp of depolarizing current (150–300 pA/s for a duration of 1 s) was applied to the neuron and the resting potential, number of action potentials, and the amplitude of the slow afterhyperpolarization were measured before and after exposure to prostaglandins. To isolate potassium currents, the superfusate was changed to one containing 140 mM N-methyl-glutamine chloride Ringer (NMG, an equimolar substitution for NaCl), pH 7.4 with KOH. Different voltage-step protocols without leak subtraction were used to determine the activation and steady-state inactivation of the current. To determine the voltage-dependent activation, the membrane was held at −60 mV [which is close to the value of the normal resting potential of −55 mV (Nicol and Cui 1994)] and voltage steps of 175 ms were applied in +10 mV increments to a maximal value of +60 mV. To determine the steady-state inactivation, a conditioning prepulse was applied (−100 to +20 mV in +10-mV increments, 500 ms in duration), and upon termination of the prepulse, the voltage was then stepped to +60 mV (200 ms in duration). After obtaining the control response in current- or voltage-clamp mode, the superfusate was changed to normal Ringer or NMG-Ringer, respectively, containing either prostaglandin E2 (PGE2), carba prostacyclin (CPGI, a nonhydrolyzable analogue of prostaglandin I2), or prostaglandin F2α (PGF2α) and allowed to incubate for the appropriate time period. All experiments were done at room temperature (~23°C).

Only the results obtained from neurons that satisfied the following criteria are presented in this report. First, neurons had to maintain resting potentials or zero-current potentials more hyperpolarized than −45 mV for ±4–5 min after establishing the whole cell configuration. Second, neurons had to be sensitive to capsaicin. At the conclusion of every recording period, each neuron was exposed to Ringer solution containing 100 nM capsaicin. Neurons were judged to be capsaicin-sensitive if they exhibited either an inward small plastic cover slips. Cells were grown at 37°C in a 5% CO2 atmosphere and fed every 2 day. All procedures were approved by the Animal Care and Use Committee at Indiana University School of Medicine.

Analysis

All values are reported as the means ± SE. The voltage dependence for activation and inactivation of the currents were fitted with the Boltzmann relation (Akins and McCleskey 1993; McFarlane and Cooper 1991). For activation, the relation 

\[
G/G_{\text{max}} = \frac{1}{1 + \exp((V_{0.5} - V)/k)}
\]

was used where \(G\) is the conductance, \(G_{\text{max}}\) is the maximal conductance obtained at +60 mV, \(b\) is a factor to account for inhibition by prostaglandin, \(V_{0.5}\) is the voltage for half-maximal activation, \(V_m\) is the membrane voltage, and \(k\) is a steepness factor. The conductance, \(G\), was determined from the relation 

\[
G = I/(V_m - E_K)
\]

where \(I\) is the measured membrane current, \(V_m\) is the voltage step, and \(E_K\) is the potassium equilibrium potential. Under our experimental conditions (external [K+] was 5 mM, internal [K+] was 140 mM), \(E_K\) was calculated to be −84 mV. For inactivation the relation 

\[
G/G_{\text{max}} = c - c/[1 + \exp((V_{0.5} - V_0)/k)]
\]

was used where \(c\) is the amount of nonactivating current and the other parameters are as defined above. Fits were obtained using the curve fitting protocols in SigmaPlot 5.0 (Jandel Scientific, San Rafael, CA). Statistical differences between the control recordings and those obtained under various treatment conditions were determined by using either a one-tailed Student’s t-test or a one-way analysis of variance (ANOVA). When a significant difference was obtained using ANOVA, a Student-Newman-Keuls or Dunnett’s post hoc test was performed. Values of \(P < 0.05\) were judged to be statistically significant.

Chemicals

Prostaglandins were obtained from Cayman Chemical (Ann Arbor, MI); HEPES Ultrapure was obtained from Calbiochem.
average resting potential and amplitude of the afterhyperpolarization remained unaltered for all time points. In contrast, PGE₂ produced about a twofold increase in the number of APs elicited by the ramp. These results indicate that, even though PGE₂ does not cause a significant depolarization, this eicosanoid enhances the excitability of sensory neurons.

**Potassium currents in embryonic rat sensory neurons**

To investigate the possibility that prostaglandins alter neuronal excitability through modulation of potassium currents ($I_K$), we initially characterized $I_K$ existing in embryonic rat sensory neurons. As illustrated in Fig. 2, these neurons exhibited an outward $I_K$ that ranged from those that were characterized by a sustained current showing little time-dependent inactivation (Fig. 2A, left) to those that displayed greater amounts of time-dependent relaxation for large depolarizing voltage pulses (Fig. 2A, right). Examination of the steady-state inactivation further characterized the variety of $I_K$ observed in embryonic sensory neurons. Figure 2B (left) illustrates the steady-state inactivation obtained from a neuron (same neuron as in Fig. 2A, left) exhibiting the sustained type of $I_K$ where the fraction of residual noninactivating current (measured for a voltage step to +60 mV after a prepulse to +20 mV) was 0.77. In contrast, Fig. 2B, right, shows a neuron (same neuron as Fig. 2A, right) exhibiting both the sustained and the rapidly relaxing $I_K$ where the fraction of residual noninactivating current was 0.47. In control recordings from a total of 65 neurons, the average value of the fraction of residual non-inactivating current was 0.67 ± 0.01 (mean ± SE, range 0.40–0.99).

**Prostaglandin E₂ and carba prostacyclin, but not prostaglandin F₂α, suppress $I_K$ in embryonic sensory neurons**

To explore whether prostaglandins modulate $I_K$ in sensory neurons, cells were exposed to various prostaglandins and the activation and inactivation properties of $I_K$ were determined during a 20-min period. The application of 1 μM PGE₂ in NMG-Ringer solution produced a time-dependent suppression of $I_K$ in all 14 cells examined (see Fig. 3A). After an initial 2-min exposure to PGE₂, $G/G_{max}$ (measured at +60 mV) was reduced significantly from a control value of 1.0 to 0.76 ± 0.03. Over time, $G/G_{max}$ continued to decline, and after a 20-min exposure the maximal $I_K$ was reduced to 0.52 ± 0.04. The activation of $I_K$ could be described by the Boltzmann relation and the fitting parameters are summarized in Table 2. Under control conditions, the activation curve had a $V_0$ of $7 ± 1$ mV and a $k$ of $16 ± 1$ mV ($n = 14$). After a 20-min treatment with PGE₂, the values

**TABLE 1. Measures of neuronal excitability before and after PGE₂ treatment**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2 min</th>
<th>6 min</th>
<th>10 min</th>
<th>20 min</th>
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<tbody>
<tr>
<td>Resting potential, mV</td>
<td>$-51 ± 1$</td>
<td>$-49 ± 1$</td>
<td>$-48 ± 1$</td>
<td>$-48 ± 1$</td>
<td>$-51 ± 2$</td>
</tr>
<tr>
<td>Slow afterhyperpolarization, mV</td>
<td>$-6 ± 0.5$</td>
<td>$-8 ± 0.4$</td>
<td>$-8 ± 1$</td>
<td>$-8 ± 0.6$</td>
<td>$-8 ± 0.6$</td>
</tr>
<tr>
<td>Number of action potentials</td>
<td>$5.9 ± 0.9$</td>
<td>$10.9 ± 1.2^*$</td>
<td>$11.9 ± 1.1^*$</td>
<td>$11.5 ± 0.9^*$</td>
<td>$11.6 ± 0.9^*$</td>
</tr>
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</table>

Values represent means ± SE, n = 16 neurons. *P < 0.05, analysis of variance.
inactivation, the effects of PGE2 on the amplitudes of the change in overall shape of the steady-state inactivation curve of $V_{0.5}$ (5 ± 2 mV) and $k$ (18 ± 1 mV) were not altered significantly. These values were, however, significant decreases in $\delta$ (1.0 to 0.55 ± 0.04), which reflected the PGE2-induced inhibition of the maximal $I_k$ obtained at +60 mV.

In those sensory neurons in which the series resistance was compensated (89 ± 1.1%, n = 7), the extent of inhibition of $I_k$ by PGE2 was nearly identical to that observed in uncompensated neurons. After 2-, 6-, 10-, and 20-min exposures, the average value of the fraction of $I_k$ remaining after 1 μM PGE2 treatment was 0.85 ± 0.09, 0.74 ± 0.10, 0.67 ± 0.11, and 0.57 ± 0.10 of the control, respectively. Fitting these data with the Boltzmann relation (parameters are described in Table 2) indicated that the values of $V_{0.5}$ were significantly different from those obtained from the uncompensated neurons. This indicates that the absolute values for the voltage-dependent activation of $I_k$ were shifted to more hyperpolarized values in those neurons with series resistance compensation. In spite of this difference, treatment with PGE2 did not significantly alter the value of $V_{0.5}$ in the compensated neurons (see Table 2). The values for $k$ and $\delta$ also did not differ significantly between the uncompensated and compensated neurons. Taken together, these results suggest that PGE2 blocked ~50% of the total $I_k$ without causing significant changes in the voltage dependence of activation.

To determine whether this PGE2-induced inhibition of $I_k$ arose from an alteration in the properties of steady-state inactivation, the effects of PGE2 on the amplitudes of the maximal currents obtained at +60 mV after various conditioning prepulse voltages were examined. Under control conditions, the steady-state inactivation curves were fit by the Boltzmann relation (see Fig. 3B) where $V_{0.5}$, $k$, and $c$ had the following values of −36 ± 7 mV, 21 ± 2 mV, and 0.67 ± 0.05 (n = 14), respectively. In those neurons with series resistance compensation, the Boltzmann parameters were not significantly different (t-test) and had the following values of −48 ± 8 mV, 20 ± 3 mV, and 0.63 ± 0.06 (n = 7) for $V_{0.5}$, $k$, and $c$, respectively. In contrast to the effect of PGE2 on the maximal $I_k$, when the steady-state inactivation values obtained for each neuron treated with PGE2 were normalized to the −100 mV prepulse value, the shape of the inactivation curves was not altered (see Fig. 3B). These results indicate that the PGE2-induced reduction in $I_k$ did not result from alterations in the inactivation properties.

To ascertain if the PGE2-induced suppression of $I_k$ was specific to PGE2 or whether this effect could be extended to other pro-inflammatory prostaglandins, the capacity of CPGI1 to block $I_k$ was examined. As shown in Fig. 4A, 1 μM CPGI1 produced a time-dependent suppression of $I_k$ that was similar to that of PGE2. In five neurons, exposure to CPGI1 reduced $G/G_{\text{max}}$ (at +60 mV) from a control value of 1.0 to 0.67 ± 0.04 and 0.60 ± 0.05 after 10 and 20 min, respectively. The activation curves for $I_k$ under control conditions were fit by the Boltzmann relation and had values that were similar to control values obtained in the PGE2 studies (see Table 2). In contrast to PGE2 and CPGI1, the application of 1 μM PGF2α, which is a prostaglandin that does not sensitize sensory neurons (Hingsten and Vasko 1994; Nicol and Cui 1994), resulted in little time-dependent suppression of $I_k$ (Fig. 4B). The mean values obtained for $G/G_{\text{max}}$ under the control condition and after 20 min in PGF2α were not significantly different (using ANOVA). The Boltzmann parameters were unaffected by treatment with PGF2α. Neither CPGI1 nor PGF2α caused any significant change in overall shape of the steady-state inactivation curve obtained for $I_k$ (data not shown). These results clearly demonstrate that pro-inflammatory prostaglandins significantly inhibit the total $I_k$, whereas PGE2α (a nonsensitizing prostaglandin) has no significant effect.

Prostaglandin $E_2$ and carba prostacyclin increase membrane resistance

Because pro-inflammatory prostaglandins sensitize sensory neurons and suppress $I_k$, we examined whether these
eicosanoids have significant effects on membrane resistance and thus, potentially, alter the capacity of sensory neurons to generate APs. As illustrated in Fig. 5, exposure to CPGI₂ significantly increased membrane resistance, as measured between −60 and −30 mV, by 1.64-, 1.92-, and 2.4-fold after 6-, 10-, and 20-min exposures, respectively. Similarly, PGE₂ significantly increased resistance by 1.51- and 1.98-fold after 10- and 20-min exposures, respectively. In contrast, neither PGF₂α (1.21-fold increase) nor vehicle (1.15-fold increase, data not shown) had any significant effect on resistance over this 20-min period. These findings are consistent with the results presented above and further support the notion that the PGE₂- and CPGI₂-mediated suppression of I_K may lead to the enhanced excitability of sensory neurons.

**PGE₂-sensitive I_K is likely a sustained type of current**

As illustrated in Fig. 2, embryonic rat sensory neurons exhibit at least two different subtypes of I_K, nominally a sustained type and a rapidly inactivating type. To determine if a particular subtype of I_K was sensitive to PGE₂, the currents obtained for the various voltage steps after the 20-min exposure to PGE₂ were subtracted from their respective control recordings to yield the current that was sensitive to PGE₂. Figure 6A illustrates the PGE₂-sensitive I_K obtained from representative neurons that exhibited only the sustained type (left) or a combination of the rapidly inactivating and the sustained types (right). In all sensory neurons, the PGE₂-sensitive current appeared to be a delayed rectifier-like current that demonstrated a small amount of time-dependent inactivation for voltage steps to +40 and +60 mV (top 2 traces, each panel). Subtraction of the traces obtained with the steady-state inactivation protocol also demonstrated that the PGE₂-sensitive current exhibited little time-dependent inactivation (compare Figs. 6B and 2B, right). Figure 6B, left and right, illustrates the current obtained from the same neurons shown in Fig. 6 A, left and right, for conditioning prepulses to −90 and +20 mV. Note the lack of a rapidly inactivating component for the −90 mV prepulse, supporting the idea that the PGE₂-sensitive current is a delayed rectifier-like current. In addition, subtraction of the currents as described above in the five neurons treated with CPGI₂ yielded results that were analogous to those obtained with PGE₂. Based on the assumption that PGE₂ and CPGI₂ suppress the same I_K, the results obtained for PGE₂- and CPGI₂-treated neurons have been combined to determine the voltage-de-

![Figure 3](http://jn.physiology.org/)

**Figure 3.** PGE₂ suppresses activation of an outward potassium current but has no effect on steady-state inactivation. A: time-dependent suppression of I_K after application of 1 μM PGE₂. Data points represent means ± SE from 14 neurons. Lines through average values are drawn according to Boltzmann relation; fitting parameters are described in Table 2. B: effects of PGE₂ on steady-state inactivation for neurons, symbols are as in A. Lines through points are drawn according to Boltzmann relation. For those data points appearing to lack error bars, error bars are less than size of symbol.

### Table 2. Effects of PGE₂ and CPGI₂ on the Boltzmann parameters for activation

<table>
<thead>
<tr>
<th></th>
<th>Uncompensated Rₛ</th>
<th>Compensated Rₛ</th>
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<tbody>
<tr>
<td><strong>PGE₂, min</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7 ± 1</td>
<td>0.2 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>4 ± 1</td>
<td>0.3 ± 2</td>
</tr>
<tr>
<td>10</td>
<td>4 ± 1</td>
<td>0.3 ± 2</td>
</tr>
<tr>
<td>20</td>
<td>5 ± 1</td>
<td>0.4 ± 2</td>
</tr>
<tr>
<td><strong>CPGI₂, min</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8 ± 1</td>
<td>0.3 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>8 ± 1</td>
<td>0.3 ± 2</td>
</tr>
<tr>
<td>10</td>
<td>8 ± 1</td>
<td>0.3 ± 2</td>
</tr>
<tr>
<td>20</td>
<td>8 ± 1</td>
<td>0.3 ± 2</td>
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Values are means ± SE. *n = 14 for uncompensated Rₛ and n = 7 for compensated Rₛ. †P < 0.05. ‡n = 5.
dependent activation of the prostanoid-sensitive current (Fig. 6C). The activation curve was fit with the Boltzmann relation and had the following values. $V_{0.5}$ was 13 $\pm$ 2 mV and $k$ was 16 $\pm$ 1 mV ($n = 19$). The value of $V_{0.5}$ for the prostaglandin-sensitive $I_K$ was significantly different from that obtained under control conditions whereas $k$ remained unchanged (compare with Table 2), suggesting that the prostaglandin-sensitive current may be activated at slightly more depolarized membrane voltages.

To further corroborate the idea that the PGE$_2$-sensitive current is a sustained type of $I_K$, we examined the relaxation kinetics of $I_K$ at different levels of steady-state prepulse voltages before and after exposure to prostaglandins. The various prepulse voltages were used to selectively activate or inactivate different components of the total $I_K$. For example, a conditioning prepulse to $-90$ mV should remove most of the residual inactivation associated with the rapidly inactivating $I_K$ (Connor and Stevens 1971; Neher 1971), whereas, a prepulse to 0 mV will produce significant inactivation of $I_K$ yielding an outward current dominated by the delayed rectifier currents. As illustrated in Fig. 7A ($-90$ mV panel), a representative recording from a sensory neuron exhibiting the sustained type $I_K$ demonstrated that $I_K$ was reduced after a 20-min treatment with PGE$_2$ (trace labeled PGE$_2$) and that normalization of the current after PGE$_2$ exposure to the peak control current (labeled nPGE$_2$) revealed that the current relaxations were identical. Similarly, the relaxation kinetics were identical after prepulses to $-60$, $-30$, and 0 mV suggesting that in these neurons lacking a rapidly inactivating component, PGE$_2$ suppressed a sustained type(s) of $I_K$. Comparable effects of prepulse voltages on the response kinetics were observed in those sustained type neurons treated with CPGI$_2$.

In contrast to the sustained-type, in those neurons exhibiting a combination of sustained and rapidly inactivating $I_K$, PGE$_2$ produced a suppression of the total current, however, the effects of PGE$_2$ on inactivation kinetics were dependent on the prepulse voltage (see Fig. 7B). For a prepulse voltage to $-90$ mV, PGE$_2$ reduced the amplitude of the maximal current, but this current relaxed more quickly after PGE$_2$ treatment compared with the control response (contrast the nPGE$_2$ and control traces in $-90$ mV panel). Similar results were obtained for prepulse voltages to $-60$ and $-30$ mV, suggesting that at these voltages PGE$_2$ removed a more slowly inactivating component from the total current. However, after prepulses to 0 mV both the control and PGE$_2$-treated responses displayed identical relaxation kinetics. Similar results were obtained with either PGE$_2$ or CPGI$_2$ for all neurons exhibiting a combination of sustained and rapidly inactivating $I_K$. The significance of these results are twofold. First, the 0 mV prepulse sufficiently inactivated the rapidly decaying component so as to leave a total $I_K$ composed largely of the sustained component(s). Second, for prepulse voltages that permit a combination of currents, the relaxation was faster after PGE$_2$ treatment, whereas after removal of the rapidly inactivating component, the relaxation kinetics were the same, suggesting that PGE$_2$ somehow leads to the suppression of a slowly inactivating type(s) of $I_K$.

**FIG. 5.** PGE$_2$ and carba prostacyclin, but not PGE$_{iso}$, increase membrane resistance. Membrane resistance was calculated as change in voltage (from $-60$ to $-30$ mV) divided by change in peak current values. Values obtained during prostaglandin treatment then were divided by control value to yield fold-increase in resistance. Columns represent means $\pm$ SE obtained after different exposure times from 10, 5, and 5 neurons for PGE$_2$, carba prostacyclin, and PGE$_{iso}$ treatments, respectively. *Significant difference between control and experimental treatments using analysis of variance.

**FIG. 4.** Carba prostacyclin but not PGE$_{iso}$ blocks outward potassium current. A: time-dependent inhibition of $I_K$ by $1 \mu$M carba prostacyclin. Values represent means $\pm$ SE obtained from 5 neurons. B: lack of effect of $1 \mu$M PGE$_{iso}$ on $I_K$. Values represent means $\pm$ SE obtained from a different set of 5 neurons. Lines through points are drawn according to Boltzmann relation. For those data points appearing to lack error bars, error bars are less than size of symbol.

**DISCUSSION**

Previous studies have demonstrated that pro-inflammatory prostaglandins, like PGE$_2$ and prostacyclin, enhance the sensitivity of sensory neurons to most modalities of stimulation. Measurements of this enhanced sensitivity have ranged from behavioral assays of pain perception (Handwerker and Kobal 1993) to recordings from isolated sensory neurons (Baccaglini and Hogan 1983; Nicol and Cui 1994). In this study,
we have shown that treatment of embryonic sensory neurons with PGE$_2$ increased the number of evoked APs and that both PGE$_2$ and CPGL$_2$ suppressed $I_K$. These findings are consistent with the notion that inhibition of $I_K$ may, in part, account for this increased membrane excitability and thus the enhanced sensitivity of sensory neurons to either chemical or mechanical stimulation.

Prostaglandins, potassium currents, and neuronal excitability

Mammalian sensory neurons isolated from either neonatal or adult animals exhibit at least two different types of voltage-dependent $I_K$, a rapidly inactivating $I_K$-type and a delayed rectifier-type of $I_K$ (Kameyama 1983; Kostyuk et al. 1981; Mayer and Sugiyama 1988). However, the properties of the various types of $I_K$ have not been described previously in sensory neurons of embryonic rats. Considering the variety of recording conditions, the voltage-dependent activation of $I_K$ in the embryonic neurons was described by the Boltzmann relation where the fitting parameters were consistent with previous reports for the activation of $I_K$ in neonatal or adult sensory neurons (Akins and McClesky 1993; McFarlane and Cooper 1991). Therefore, based on our initial characterization, the types of $I_K$ in embryonic sensory neurons are similar to those observed in the more mature animals.

We used the number of APs evoked by a ramp of depolarizing current as an index of neuronal excitability and found that treatment with PGE$_2$ produced a twofold increase in the number of evoked APs. These observations are similar to the threefold increase in the number of APs elicited by the focal application of bradykinin after exposure to PGE$_2$ (Nicol and Cui 1994). Furthermore, our current findings are consistent with a previous report where the number of APs elicited by the application of elevated K$^+$ concentrations was increased after treatment with PGE$_2$ in cultured sensory neurons isolated from the DRG (Baccaglini and Hogan 1983). In contrast to the increased generation of APs, PGE$_2$ had little effect on the resting potential and is similar to our previous findings where PGE$_2$ at 1 and 10 $\mu$M had no effect on the resting potential (Nicol and Cui 1994). Thus these findings demonstrate that PGE$_2$ enhances the sensitivity of sensory neurons to both chemical and electrical stimulation.

We observed that treatment with the pro-inflammatory...
FIG. 7. PGE\(_2\) appears to block a sustained component of the total whole cell \(I_\text{K}\). These panels demonstrate current responses that were elicited by a voltage step to \(-60\) mV from indicated prepulse voltages before and after a 20-min exposure to 1 \(\mu\)M PGE\(_2\). A: effects shown of prepulse voltage on response kinetics obtained from a neuron exhibiting sustained \(I_\text{K}\). B: effects illustrated of prepulse voltage on response kinetics obtained from a neuron exhibiting a combination of sustained and rapidly relaxing \(I_\text{K}\). In each panel, control recording is shown as bold trace labelled control, recording obtained after PGE\(_2\) treatment is shown as thin trace labelled PGE\(_2\), and PGE\(_2\)-treated current normalized to peak amplitude of control is shown as thin trace labelled nPGE\(_2\).

prostaglandins, PGE\(_2\) or CPGI\(_2\), produced a time-dependent suppression of \(I_\text{K}\) so that after a 20-min exposure \sim 50\% of the total current had been blocked. The activation curves for the \(I_\text{K}\) remaining after exposure to PGE\(_2\) or CPGI\(_2\) were fit by the Boltzmann relation wherein neither prostaglandin had a significant effect on \(V_{0.5}\) or \(k\), however, both PGE\(_2\) and CPGI\(_2\) produced a significant reduction in the value of \(\delta\). In those neurons where series resistance was compensated, PGE\(_2\) produced an almost identical time-dependent suppression of \(I_\text{K}\). Although there were small differences in the absolute values for \(V_{0.5}\) between the uncompensated and compensated neurons, PGE\(_2\) did not cause a significant shift in the value of \(V_{0.5}\) for either of these two different sets of recordings. Taken together, these findings are consistent with the notion that PGE\(_2\) and CPGI\(_2\) suppress \(I_\text{K}\) in the absence of a significant change in the voltage dependence of activation and may account for the observed sensitization to both chemical or electrical stimulation.

Our findings suggest that the pro-inflammatory prostaglandins act on a sustained or delayed rectifier-like current. This notion is based upon the slow relaxation kinetics for the prostaglandin-sensitive \(I_\text{K}\) obtained with large depolarizing voltage steps (see Fig. 6). Furthermore, the steady-state inactivation protocol revealed that for prepulse voltages activating both \(I_\Lambda\) and sustained types of \(I_\text{K}\), the relaxation of the current was accelerated after treatment with PGE\(_2\) or CPGI\(_2\) suggesting that a slower component was removed from these current traces. Thus the results obtained from both the subtraction and inactivation protocols are consistent with the idea that these prostaglandins suppressed a sustained component of the total \(I_\text{K}\). However, it remains possible that pro-inflammatory prostaglandins also affect the rapidly inactivating component(s) of \(I_\text{K}\) because there was significant PGE\(_2\)-induced inhibition at prepulse voltages that should give rise to currents having significant amounts of the rapidly inactivating component (see Fig. 7B).

In the absence of a significant membrane depolarization, the question arises as to how pro-inflammatory prostaglandins enhance the excitability of these embryonic sensory neurons. It is possible a reduction in the firing threshold of the neuron may result from the PGE\(_2\)-mediated suppression of \(I_\text{K}\) and its accompanying increase in membrane resistance because the time course for the increased generation of APs and inhibition of \(I_\text{K}\) paralleled one another. This idea is supported by our previous observation that treatment with PGE\(_2\) lowered the AP firing threshold to brief steps of depolarizing current even though there were no significant changes in the resting potential (Nicol and Cui 1994). Our results imply that the K\(^+\) channels modulated by PGE\(_2\) might be active at membrane voltages near or slightly depolarized from the resting potential. It would appear that this \(I_\text{K}\) does not make a significant contribution to the overall resting potential but rather may play a critical role in setting the point at which small depolarizations generate the AP. In an analogous manner, the resting potential in Aplysia sensory neurons that had undergone sensitization training was not different from that recorded in control cells (Scholz and Byrne 1987). Furthermore, an enhancement of neuronal excitability in the absence of membrane depolarization has been described previously by Mayer et al. (1986). Sensory neurons isolated from the DRG of neonatal rats and exposed to herpes simplex virus underwent changes in neuronal excitability that were associated with altered membrane resistance, however, there were no direct correlations between the change in resistance and
the resting membrane potential. The studies presented above have not addressed the specific question concerning the resting conductance, however, this possibility is under investigation.

In Aplysia sensory neurons, serotonin produces a sensitization that is similar to that induced by PGE$_2$ and CGP12177 in mammalian sensory neurons. The serotonin-mediated sensitization is characterized by both an enhancement of membrane excitability in the sensory neuron (Baxter and Byrne 1990; Critz et al. 1991; Goldsmith and Abrams 1992; Klein et al. 1986; Walters et al. 1983) as well as an increase in neurotransmitter release (Castellucci and Kandel 1976; Hochner and Kandel 1992; Hochner et al. 1986; Klein and Kandel 1980; Klein et al. 1982, 1986). Likewise, prostaglandin treatment of mammalian sensory neurons enhances the excitability (Baccaglini and Hogan 1983; Nicol and Cui 1994; Weinrich and Wonderlin 1987) and increases the evoked release of neuropeptides (Hingtgen et al. 1995; Vasko et al. 1994). Sensitization in Aplysia sensory neurons results from the suppression of a $I_K$ (called the $S$ (serotonin)-current) that is voltage dependent and remains active at rest (Klein and Kandel 1980; Klein et al. 1982; Siegelbaum et al. 1982). Recent investigations, however, indicate that serotonin-induced sensitization may result from the inhibition of multiple potassium currents (Baxter and Byrne 1989; Goldsmith and Abrams 1992; Hochner and Kandel 1992). Similarly, pro-inflammatory prostaglandins appear to sensitize mammalian sensory neurons through the suppression of an as yet uncharacterized, potassium current(s). Taken together, all of these observations are consistent with the notion that there are generalized cellular mechanisms activated by sensitizing agents that consequently give rise to the enhanced sensitivity of sensory neurons to chemical and/or mechanical stimulation.

In conclusion, our results demonstrate that pro-inflammatory prostaglandins enhance the excitability of sensory neurons and that this sensitization may result from the suppression of a sustained or delayed rectifier type(s) of K$^+$ channel that modulates the threshold of AP firing. It is possible that inhibition of these K$^+$ channels accounts, in part, for the increased generation of APs in in vitro models as well as the hyperalgesia observed in behavioral models of pain and inflammation.

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