The cAMP transduction cascade mediates the PGE_2-induced inhibition of potassium currents in rat sensory neurones

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1. The role of the cyclic AMP (cAMP) transduction cascade in mediating the prostaglandin E_2 (PGE_2)-induced decrease in potassium current (I_K) was investigated in isolated embryonic rat sensory neurones using the whole-cell patch-clamp recording technique.

2. Exposure to 100 μM chlorophenylthio-adenosine cyclic 3′,5′-monophosphate (cpt-cAMP) or 1 μM PGE_2 caused a slow suppression of the whole-cell I_K by 34 and 36%, respectively (measured after 20 min), without a shift in the voltage dependence of activation for this current. Neither of these agents altered the shape of the voltage-dependent inactivation curve indicating that the suppression of I_K did not result from alterations in the inactivation properties.

3. To determine whether the PGE_2-mediated suppression of I_K depended on activation of the cAMP pathway, cells were exposed to this prostanoid in the presence of the protein kinase A (PKA) inhibitor, PKI. The PGE_2-induced suppression of I_K was prevented by PKI. In the absence of PGE_2, PKI had no significant effect on the magnitude of I_K.

4. Results obtained from protocols using different conditioning prepulse voltages indicated that the extent of cpt-cAMP- and PGE_2-mediated suppression of I_K was independent of the prepulse voltage. The subtraction of control and treated currents revealed that the cpt-cAMP- and PGE_2-sensitive currents exhibited little time-dependent inactivation. Taken together, these results suggest that the modulated currents may be delayed rectifier-like I_K.

5. Exposure to the inhibitors of I_K, tetraethylammonium (TEA) or 4-aminopyridine (4-AP), reduced the control current elicited by a voltage step to +60 mV by 40–50%. In the presence of 10 mM TEA, treatment with cpt-cAMP did not result in any further inhibition of I_K. In contrast, cpt-cAMP reduced I_K by an additional 25–30% in the presence of 1 mM 4-AP. This effect was independent of the conditioning prepulse voltage.

6. These results establish that PGE_2 inhibits an outward I_K in sensory neurones via activation of PKA and are consistent with the idea that the PGE_2-mediated sensitization of sensory neurones results, in part, from an inhibition of delayed rectifier-like I_K.

Enhanced sensitivity to noxious stimuli is one of the characteristics of the inflammatory response and may result from an increase in the excitability of small-diameter sensory neurones that conduct nociceptive signals. Indeed, a number of pro-inflammatory agents that are synthesized and released in response to trauma can stimulate directly or augment the activation of sensory neurones (Treede *et al.* 1992). In this regard, prostaglandins represent an important group of agents that sensitize sensory neurones. Exposing various preparations of sensory neurones to prostaglandin E_2 (PGE_2) or prostaglandin I_2 (PGI_2) increases the number of action potentials elicited by noxious thermal, chemical and/or mechanical stimuli (Handwerker, 1976; Mense, 1981; Baccaglini & Hogan, 1983; Martin *et al.* 1987; Nicol & Cui, 1994; Nicol *et al.* 1997). These prostanoids also facilitate neurotransmitter release evoked by exposing sensory neurones to bradykinin, capsaicin or high extracellular potassium (Franco-Cereceda, 1989; Andreeva & Rang, 1993; Hingtgen & Vasko, 1994; Vasko *et al.* 1994).

Although the cellular mechanisms underlying the actions of these prostaglandins are not well understood, recent evidence suggests that these eicosanoids modulate ion channel activity that could enhance the excitability of sensory neurones. For example, PGE_2 increases the amplitude of a tetrodotoxin (TTX)-resistant sodium current in adult and neonatal rat dorsal root ganglion (DRG) cells (Gold *et al.* 1996*a*; England

et al. 1996). This prostanoid also suppresses a calcium-dependent slow after-hyperpolarization in adult rat nodose ganglia cells and DRG neurones (Fowler *et al.* 1985; Gold *et al.* 1996*b*). Furthermore, we demonstrated recently that PGI_2 and PGE_2 attenuate whole-cell potassium currents (I_K), whereas the non-sensitizing prostanoid $PGF_{2\alpha}$ is ineffective in sensory neurones (Nicol *et al.* 1997).

The question remains as to whether the prostaglandin-induced modulation of ion channels results in sensitization of sensory neurones. To initially address this issue, it is important to assess whether prostaglandin-induced sensitization and alterations in ion channels are mediated by the same transduction mechanisms. Because the sensitizing actions of PGE_2 or PGI_2 on sensory neurones are mediated by the cyclic AMP (cAMP) transduction cascade (Ferreira & Nakamura, 1979; Taiwo *et al.* 1989; Hingtgen *et al.* 1995; Cui & Nicol, 1995), modulatory effects of prostanoids on ion channels regulating membrane excitability should also be dependent on the cAMP pathway. We hypothesize that the sensitizing actions of PGE_2 are also mediated by the suppression of I_K. This notion is based, in part, on observations wherein cAMP modulates voltage-dependent I_K in a variety of cell types. In the GH_4C_1 pituitary cell line, dibutyryl cAMP suppresses a delayed rectifier I_K (Chung & Kaczmarek, 1995). Similarly, the current that arises from the expression of Kv3.2 (delayed rectifier potassium) channels in Chinese hamster ovary cells is inhibited by a membrane-permeant analogue of cAMP (Moreno *et al.* 1995). Activation of the cAMP pathway in mouse neurones isolated from the colliculus leads to a long-term (2–4 h) enhancement of excitability as exhibited by an increased duration of the action potential and a greater number of action potentials evoked by a depolarizing current pulse (Ansanay *et al.* 1995). These authors found that stimulation of protein kinase A (PKA) inhibits a delayed rectifier-like I_K. Lastly, PGE_2, through activation of the cAMP–PKA cascade, may play a role in the regulation of vascular smooth muscle tone. In smooth muscle cells isolated from the tail artery of the rat, PGE_2 suppresses a non-inactivating I_K (Ren *et al.* 1996). Therefore, taken together, the sensitivity of excitable cells and its modulation by different mediators will play an important role in the regulation of the physiological function of the cell.

To ascertain whether suppression of I_K could also be a mechanism for prostaglandin-induced sensitization of sensory neurones, we examined the hypothesis that the cAMP pathway mediates the PGE_2-induced decrease in I_K in isolated rat embryonic sensory neurones. Our results demonstrate that the cAMP analogue, chlorophenylthio-adenosine cyclic 3′,5′-monophosphate (cpt-cAMP) inhibits a delayed rectifier-like I_K in sensory neurones in a manner analogous to PGE_2 and that the inhibitory effects of PGE_2 on the whole-cell I_K are blocked by the inhibition of PKA. Preliminary findings from this study have been reported in abstract form (Evans *et al.* 1996*b*).

METHODS

Isolation and culture of embryonic rat sensory neurones

The procedures for isolation and culture of rat sensory neurones have been described previously (Vasko *et al.* 1994). All procedures were approved by the Animal Care and Use Committee at Indiana University School of Medicine. Briefly, timed-pregnant rats were rendered unconscious with CO_2, and killed by cervical dislocation. Embryos (embryonic day (E) 15–E17) were removed from the uterus and placed in a dish containing calcium- and magnesium-free Hanks' balanced salt solution (Life Technologies, Grand Island, NY, USA). The dorsal root ganglia were dissected from each embryo and sensory neurones were dissociated from the ganglia with 0·025% trypsin (37 °C, 25 min) and mechanical agitation. The cells were grown in Dulbecco's modified Eagle's medium (Life Technologies, Grand Island, NY, USA) supplemented with 2 mM glutamine, 50 μg ml^{-1} penicillin, 50 μg ml^{-1} streptomycin, 10% (v/v) heat-inactivated fetal bovine serum, 50 μM 5-fluoro-2′-deoxyuridine, 150 μM uridine and 250 ng ml^{-1} 7S-nerve growth factor (Harlan Bioproducts for Science, Indianapolis, IN, USA). Approximately 150 000 cells ml^{-1} were plated in a collagen-coated culture dish containing small plastic coverslips. Cultures were maintained at 37 °C in a 5% CO_2 atmosphere and the medium was changed every second day.

Recording procedures

Recordings were made using the whole-cell patch-clamp technique as described previously (Hamill *et al.* 1981; Nicol *et al.* 1997). Briefly, a coverslip with the sensory neurones (4–6 days in culture) was placed in a recording chamber where the neurones were bathed in normal Ringer solution of the following composition (mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Hepes and 10 glucose; pH 7·4, adjusted with NaOH. Recording pipettes were pulled from disposable borosilicate glass tubing and typically had resistances of 2–5 MΩ when filled with the following solution (mM): 140 KCl, 5 $MgCl_2$, 4 ATP, 0·3 GTP, 2·5 $CaCl_2$, 5 EGTA (calculated free Ca^{2+} concentration of ~100 nM) and 10 Hepes; pH 7·3, adjusted with KOH. For these solutions, a junction potential of 3·7 mV was calculated using the approach described by Barry (1994). We have not corrected for this potential and expect that the actual membrane potentials are 3–4 mV more negative than those listed.

Whole-cell currents were recorded from sensory neurones with either an Axopatch 200 (Axon Instruments, Foster City, CA, USA) or an EPC-7 (List Electronic, Darmstadt, Germany) patch-clamp amplifier; the data were acquired and analysed using pCLAMP 6 (Axon Instruments). The whole-cell recording configuration was established in normal Ringer solution. Both capacitance and series resistance compensation were used; however, no compensation was made for leak currents. The remaining uncompensated series resistance was 1·9 ± 0·3 MΩ (mean ± S.E.M.; range, 0·16–5·4 MΩ; $n = 21$). The maximum voltage error resulting from the uncompensated series resistance was calculated for each cell and averaged 9·8 ± 1·8 mV ($n = 21$).

After establishing the whole-cell configuration, I_K was isolated by superfusing the cells with 140 mM *N*-methyl-glucamine chloride (NMG)-Ringer solution (equimolar substitution of NMG for NaCl), adjusted to pH 7·4 with KOH. The membrane voltage was held at −60 mV and two voltage-step protocols were then used to examine the activation and inactivation of the current. To determine the voltage dependence of activation, voltage steps of 175 ms were applied at 5 s intervals in +10 mV increments to a maximum of +60 mV. To determine the voltage dependence of inactivation, conditioning prepulses ranging from −100 to +20 mV were applied

at 6 s intervals in 10 mV increments for 500 ms followed by a voltage step to +60 mV (200 ms duration). Another series of experiments examined the effects of a conditioning prepulse on the activation of the outward I_K. Prepulses to either −100 or −30 mV were applied for 1 s; from this level incremental voltage steps (450 ms duration) depolarized the neurone to a maximum of +60 mV. After obtaining the control response, the superfusate was changed to NMG-Ringer solution containing either PGE_2 or cpt-cAMP and cells were superfused continuously for the appropriate time. In experiments investigating the role of PKA in the PGE_2-mediated decrease in I_K, 20 μM of the PKA inhibitor, peptide protein kinase inhibitor (PKI_{14-24}), was added to the whole-cell patch pipette and allowed to diffuse intracellularly for 10 min prior to recording potassium currents. In those experiments using 10 mM tetraethylammonium (TEA), the extracellular NaCl concentration in the Ringer solution was reduced to 130 mM, whereas 1 mM 4-aminopyridine (4-AP) was added directly to the Ringer solution. These agents were bath applied for 3–4 min prior to the acquisition of additional current recordings. All experiments were performed at room temperature (~23 °C).

Only the results obtained from neurones that satisfied the following criteria are presented in this report. First, after establishing the whole-cell configuration, neurones had to maintain zero-current potentials more hyperpolarized than −45 mV for at least 4–5 min, otherwise the recording was terminated. Second, over the initial 10 min period of the control recording, the peak amplitude of I_K could not change by greater than 10% (compared with the recording at time zero) or the recording was terminated. Third, neurones had to be sensitive to capsaicin. At the conclusion of each recording period, each neurone was exposed to normal Ringer solution containing 100 nM capsaicin. Neurones responsive to capsaicin exhibited an inward current that reversed following washout of capsaicin with normal Ringer solution. This neurotoxin was used to distinguish small-diameter sensory neurones as these neurones are believed to transmit nociceptive information (Holzer, 1991).

Data analysis

All values represent the mean ± standard error of the mean (S.E.M.). The voltage dependence of activation and inactivation of the outward I_K was fitted with the Boltzmann relation (McFarlane & Cooper, 1991; Akins & McCleskey, 1993). For activation, the relation: $G/G_{max} = \delta/[1 + \exp(V_{0.5} - V_m)/k]$ was used, where G is the conductance, G_{max} is the maximal conductance obtained at +60 mV, δ is a factor to account for the inhibition by PGE_2 and cpt-cAMP, $V_{0.5}$ is the voltage for half-maximal activation, V_m is the membrane voltage and k is a factor which describes the steepness of the voltage–conductance relation. Conductance 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 (calculated to be −84 mV). For inactivation, the relation: $G/G_{max} = c + \{(1 - c)/[1 + \exp(V_{0.5} - V_m)/k]\}$ was used, where c is the fraction of non-inactivating current (defined as the peak current obtained at +60 mV for the +20 mV prepulse) 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, USA). Statistical differences between the control recordings and those obtained under various treatment conditions were determined by using either Student's paired t test or a one-way analysis of variance (ANOVA) with repeated measures (whenever appropriate). When a significant difference was obtained with an ANOVA, *post hoc* analyses were performed using Student–Newman–Keuls test. Values of $P < 0.05$ were judged to be statistically significant.

Chemicals

PGE_2 was obtained from Cayman Chemical Co. (Ann Arbor, MI, USA); cpt-cAMP was purchased from Boehringer Mannheim Corp. (Indianapolis, IN, USA); PKI was purchased from Peninsula Laboratories, Inc. (Belmont, CA, USA). All other chemicals were obtained from Sigma Chemical Corp. Prostaglandins and capsaicin were dissolved in 1-methyl-2-pyrrolidinone (HPLC grade, Aldrich Chemical Co., Milwaukee, WI, USA) to obtain concentrated stock solutions. These stock solutions were then diluted with Ringer solution to yield the appropriate concentration. We have demonstrated previously that the vehicle, 1-methyl-2-pyrrolidinone, had no effect on either the activation or steady-state inactivation curves obtained for potassium currents (Nicol *et al.* 1997).

RESULTS

cpt-cAMP suppresses an outward potassium current

We demonstrated previously that 1 μM PGE_2 decreased whole-cell I_K in isolated sensory neurones (Nicol *et al.* 1997). To determine whether the cAMP transduction cascade mediates this action, we first examined whether an analogue of cAMP could suppress I_K in a manner analogous to PGE_2. The peak amplitudes of I_K for a series of voltage steps were measured in the absence and presence of 100 μM cpt-cAMP, a membrane-permeant analogue of cAMP. Consistent with our previous work, we found that the outward I_K in these neurones ranged from a sustained type of current to current that exhibited varying degrees of time-dependent inactivation (Nicol *et al.* 1997). As can be seen in a representative neurone (Fig. 1, top left panel) depolarization of the membrane, in 20 mV steps, from a holding potential of −60 mV elicited outward currents that exhibited a small degree of time-dependent inactivation. An inactivation protocol further illustrated the degree of inactivation (Fig. 1, bottom left panel). This same neurone was then exposed to 100 μM cpt-cAMP. After a 20 min exposure, the outward current at all voltages was reduced (Fig. 1, top right panel); the maximum current (+60 mV) was lowered from 6·6 to 4·4 nA (23% inhibition). Similar reductions in the test currents were observed using the inactivation protocol (Fig. 1, bottom right panel).

The capacity of cpt-cAMP to suppress the outward I_K in sensory neurones is summarized in Fig. 2. Under control conditions, the peak amplitude of I_K (at +60 mV) was 4·3 ± 0·6 nA ($n = 10$). After a 20 min exposure to 100 μM cpt-cAMP, the current was reduced to 2·9 ± 0·4 nA. Normalization of the current values to those obtained for the control recordings at +60 mV indicated that only 66 ± 2% of the total current remained after 20 min (data not shown). Treatment with cpt-cAMP significantly reduced all the current values compared with the controls except for those currents obtained after the 2 min exposure for voltages between −50 and −20 mV (ANOVA with repeated measures). In another series of experiments (sensory neurones from other harvests), a 20 min treatment with 1 μM PGE_2 significantly suppressed I_K by ~36% (see

Table 1. Effects of cpt-cAMP, PGE_2 and PKI on the voltage dependence of I_K activation

		$V_{0.5}$ (mV)	k (mV)	G/G_{max}
Control †		4 ± 2	17 ± 1	1·0
cpt-cAMP	2 min	7 ± 2	18 ± 1	0·92 ± 0·02*
	6 min	7 ± 3	18 ± 1	0·85 ± 0·04*
	10 min	6 ± 2	17 ± 1	0·76 ± 0·03*
	20 min	5 ± 2	17 ± 1	0·69 ± 0·02*
Control †		1 ± 2	14 ± 1	1·0
PGE_2	2 min	1 ± 2	15 ± 1	0·90 ± 0·02*
	6 min	1 ± 3	15 ± 1	0·81 ± 0·03*
	10 min	2 ± 3	15 ± 1	0·73 ± 0·03*
	20 min	−1 ± 3	16 ± 1	0·64 ± 0·05*
Control + PKI ‡		5 ± 2	15 ± 1	1·0
PGE_2 + PKI	20 min	5 ± 2	17 ± 1	0·97 ± 0·05

Values are means ± S.E.M. * Significant difference from respective controls; † $n = 10$, ‡ $n = 7$.

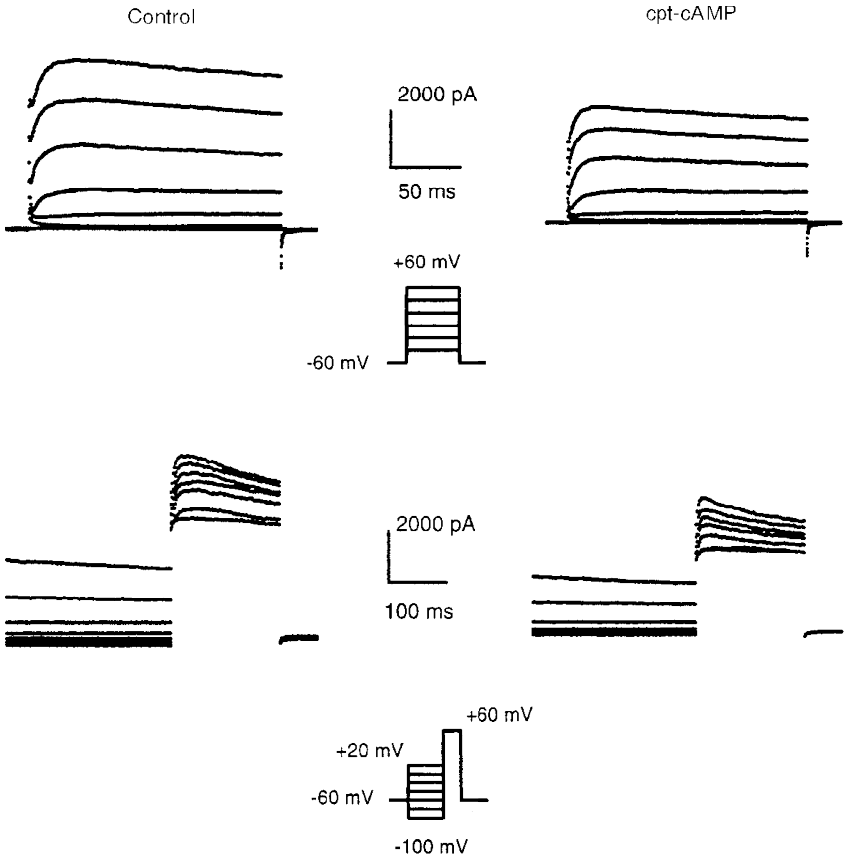

Figure 1. cpt-cAMP inhibits an outward potassium current in sensory neurones

Outward potassium currents are shown from a representative sensory neurone in the absence (left panels) and presence (right panels) of 100 μM cpt-cAMP. In the top panels, I_K was activated by incremental 20 mV steps from a holding potential of −60 mV (see inset). The bottom panels represent the currents obtained from this neurone using the steady-state inactivation protocol (see inset) before (left) and 20 min after (right) application of cpt-cAMP. In the bottom panels, the beginnings of the traces are the last 300 ms of the conditioning prepulses.

below). These results indicate that both cpt-cAMP and PGE_2 reduced I_K by very similar amounts.

To ascertain the effects of cpt-cAMP on the voltage dependence for the activation of I_K, the conductances were calculated and fitted with the Boltzmann equation to determine the voltage of half-maximal activation ($V_{0.5}$), the steepness of the voltage–conductance relation (k) and G/G_{max} (see Fig. 2*B* and Table 1). The value of G/G_{max} (obtained at +60 mV) was reduced significantly from a control value of 1·0 to 0·69 ± 0·02 after a 20 min exposure to 100 μM cpt-cAMP ($n = 10$, ANOVA with repeated measures). Neither $V_{0.5}$ nor k were altered significantly after exposure to cpt-cAMP (see Table 1). This lack of change in $V_{0.5}$ and k indicates that cpt-cAMP does not cause a shift in the voltage dependence of activation for I_K in these neurones. There was, however, a significant decrease in the value of the Boltzmann fitting parameter, δ, at all time points following exposure to cpt-cAMP and this is reflected in the decreased G/G_{max} values obtained at +60 mV (ANOVA with repeated measures).

The potassium currents inhibited by cpt-cAMP and PGE_2 are similar

Increasing intracellular cAMP levels appeared to suppress I_K in a manner analogous to that observed with PGE_2. These findings suggest that cpt-cAMP and PGE_2 may inhibit similar types of I_K. To address this idea, we used a subtraction protocol to obtain the currents inhibited by cpt-cAMP (the cpt-cAMP-sensitive current) for comparison with the PGE_2-sensitive current. The currents recorded for the different voltage steps after a 20 min exposure to these agents were subtracted from their respective control recordings. As illustrated in Fig. 3*A* and *B*, the cpt-cAMP- and PGE_2-sensitive currents in representative neurones displayed little time-dependent inactivation. Similar results were found in 20 neurones exposed to either cpt-cAMP ($n = 10$) or PGE_2 ($n = 10$), suggesting that the subtracted currents were a sustained type of I_K rather than a fast-inactivating type (e.g. an I_A-like potassium current). Comparison of the current–voltage relations for the cpt-cAMP- and PGE_2-sensitive currents revealed no significant differences (see Fig. 3*C*). Also, Boltzmann fits to individual cpt-cAMP- or PGE_2-sensitive conductances demonstrated no significant differences between either $V_{0.5}$ (6 ± 3 and 9 ± 2 mV, respectively) or k (18 ± 1 and 12 ± 1 mV, respectively).

cpt-cAMP and PGE_2 increase the resting input resistance

We examined the resting membrane resistance by assessing the amplitudes of the currents just prior to the termination of 500 ms conditioning prepulses (−100 to −40 mV) before and after treatment with cpt-cAMP or PGE_2. The slope of the linear regression line was used to calculate the input

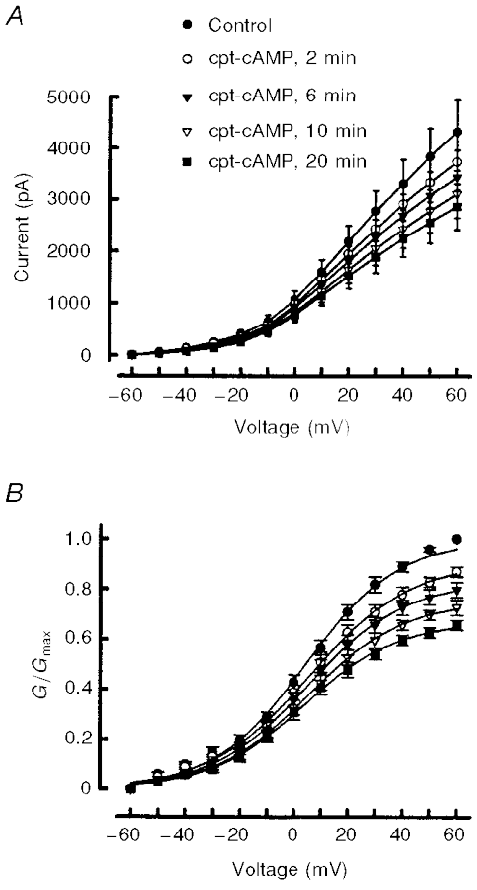

Figure 2. cpt-cAMP reduces the activation of an outward potassium current

A, time-dependent suppression of I_K after exposure to 100 μM cpt-cAMP. Currents were obtained in response to incremental 10 mV steps from a holding potential of −60 mV. *B* illustrates the currents shown in *A* after they were converted to conductance (G) values and fitted by the Boltzmann relation as described in the Methods section. In *A* and *B*, each point represents the mean ± S.E.M. from ten neurones. For those points appearing to lack error bars, the size of the bar is smaller than the symbol.

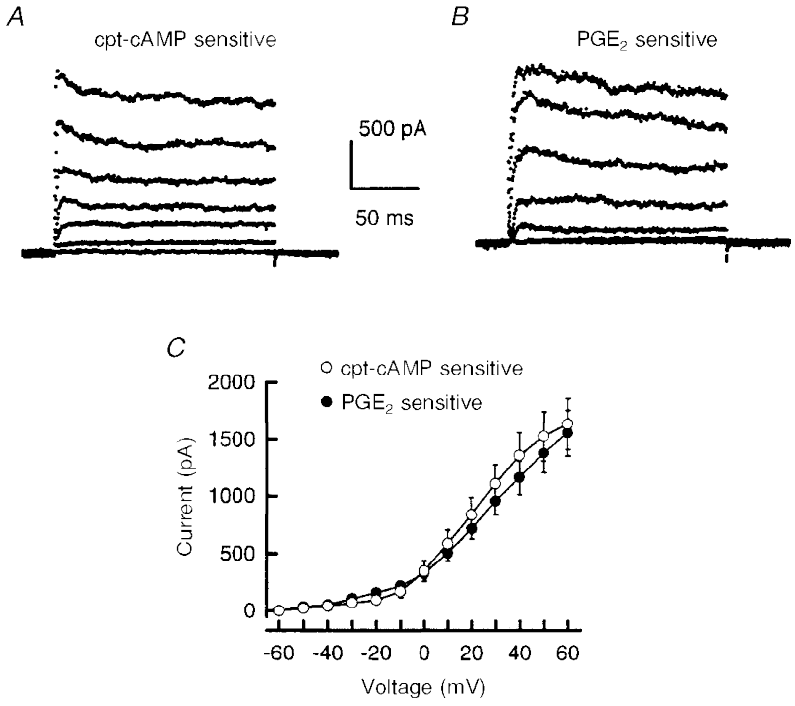

Figure 3. The cpt-cAMP- and PGE_2-sensitive currents are similar

The currents inhibited by 100 μM cpt-cAMP (*A*) or 1 μM PGE_2 (*B*) were obtained from representative neurones by subtracting the currents remaining after a 20 min exposure to these agents from those currents recorded under control conditions. The currents were obtained from incremental 20 mV steps from a holding potential of −60 mV. *C* shows the current–voltage curves for the cpt-cAMP- and PGE_2-sensitive currents. Each data point represents the mean ± S.E.M. from ten neurones.

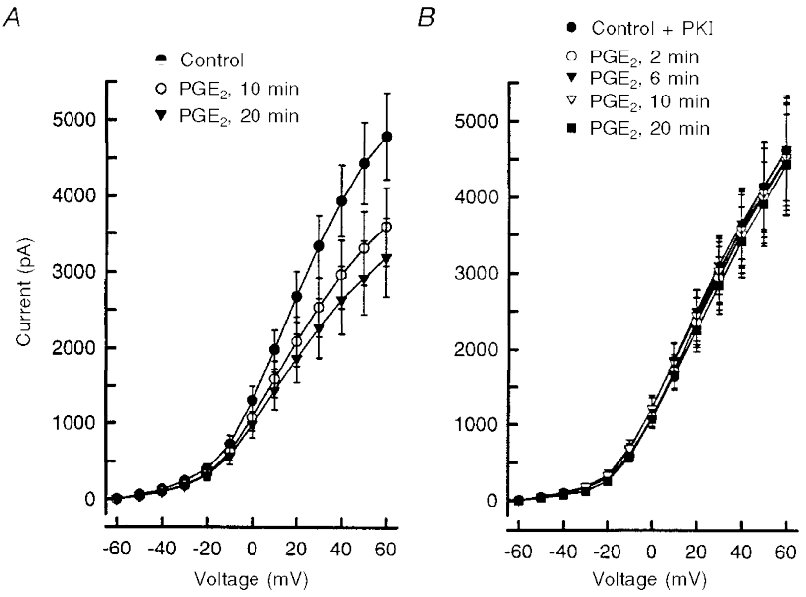

Figure 4. PKA mediates the PGE_2-induced suppression of I_K

A, treatment with 1 μM PGE_2 elicited a time-dependent suppression of I_K. Currents were elicited by incremental 10 mV steps from a holding potential of −60 mV. Each point represents the mean ± S.E.M. from ten neurones. *B* shows that 20 μM PKI abolished the inhibition of I_K produced by PGE_2. Data points represent means ± S.E.M. from seven neurones. For those data points appearing to lack error bars, the size of the bars is smaller than the symbol.

resistance between −100 and −40 mV. The resting input resistance was increased significantly after a 20 min exposure to 100 μM cpt-cAMP (paired t test, data not shown). Under control conditions, the resistance was 180 ± 26 MΩ whereas after treatment with cpt-cAMP the resistance increased to 324 ± 33 MΩ ($n = 10$). Similar results were observed for PGE_2. Treatment with 1 μM PGE_2 (20 min exposure) increased the resistance from a control value of 158 ± 13 to 258 ± 38 MΩ ($n = 10$). These results show that the activation of the cAMP signalling pathway increases the resting input resistance of sensory neurones. This modulation may, in part, give rise to the enhanced excitability observed after treatment with PGE_2 (Cui & Nicol, 1995; Nicol *et al.* 1997).

Inhibition of protein kinase A blocks PGE_2-mediated suppression of outward potassium currents

To establish a causal relationship between activation of the cAMP transduction cascade and the PGE_2-induced suppression of potassium currents, we examined the effects of PKI, a peptide inhibitor of PKA (Cheng *et al.* 1986), on the PGE_2-mediated decrease in potassium current. As shown in Fig. 4*A*, 1 μM PGE_2 elicited a time-dependent suppression of the total outward current. Under control conditions, the mean peak I_K obtained at +60 mV was 4·8 ± 0·6 nA ($n = 10$). After exposure times to PGE_2 of 10 and 20 min, the peak current was reduced to 3·6 ± 0·5 and 3·2 ± 0·5 nA, respectively. The current values after PGE_2 treatment for 10 and 20 min were significantly different from the controls at all voltage steps (ANOVA with repeated measures). Activation curves for I_K were fitted with the Boltzmann relation; the fitting parameters are shown in Table 1. After exposure to PGE_2, there was a significant decrease in the value of the Boltzmann fitting parameter, δ, which reflects the decrease in G/G_{max} obtained at +60 mV (ANOVA with repeated measures). Neither $V_{0.5}$ nor k were altered significantly following exposure to this prostanoid suggesting that PGE_2 did not alter the voltage-dependent activation profile of the potassium current.

To determine whether the PGE_2-mediated suppression of I_K depends on activation of the cAMP pathway, cells were exposed to this prostanoid in the presence of PKI. The recording pipette solution contained 20 μM PKI, which was allowed to diffuse into the cell for at least 10 min prior to obtaining the control I_K. As illustrated in Fig. 4*B*, PKI completely abolished the PGE_2-induced suppression of I_K; after a 20 min exposure to 1 μM PGE_2 the peak current at +60 mV (4·4 ± 0·6 nA, $n = 7$) was not changed from the mean control value of 4·6 ± 0·7 nA. For this I_K, a G/G_{max} value of 0·97 ± 0·05 was calculated; this was significantly different from the G/G_{max} value (0·64 ± 0·05) obtained for neurones exposed to PGE_2 in the absence of PKI, but was not significantly different from control values (ANOVA, compare Fig. 4*A* with *B*). Elimination of the PGE_2-mediated decrease in I_K by PKI was not a direct effect of PKI on the current because, in the absence of PGE_2, PKI had no significant effect on the magnitude of the outward currents. The mean amplitude of the peak current (obtained at +60 mV) in the presence of PKI was 4·6 ± 0·7 nA ($n = 7$), whereas, in those neurones that did not receive PKI, the peak current was 4·8 ± 0·5 nA ($n = 10$). Furthermore, the values of the Boltzmann parameters obtained from neurones exposed to PKI alone were not different from the values obtained under control conditions in either the PGE_2 or the cpt-cAMP experiments (Table 1). These results establish that PGE_2 inhibits an outward potassium current(s) in sensory neurones via activation of PKA.

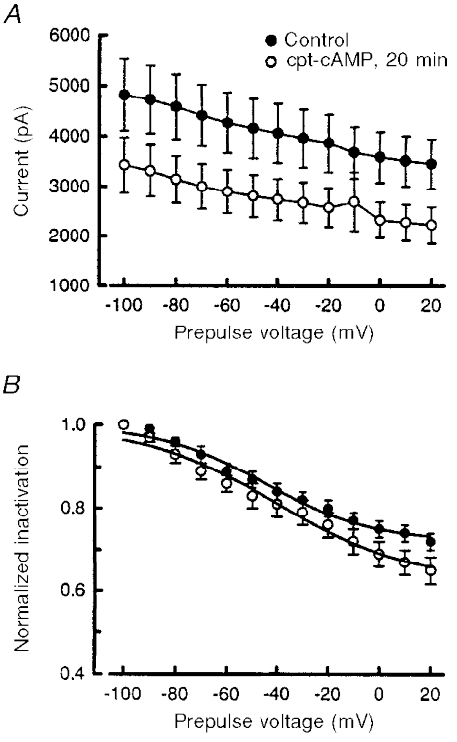

Figure 5. cpt-cAMP reduces I_K but does not alter the shape of the steady-state inactivation profile

A, the peak I_K obtained for the +60 mV step in the steady-state inactivation protocol was suppressed after 20 min treatment with 100 μM cpt-cAMP. The current values obtained after treatment with cpt-cAMP were significantly different from the controls at all voltage steps (paired t test). These values for I_K were then used to determine the Boltzmann parameters that described the inactivation of this current and are shown in *B*. The lines drawn through the data points represent the Boltzmann fits to the control data and and data obtained after 20 min treatment with cpt-cAMP. In *A* and *B*, data points represent means ± S.E.M. from ten neurones.

The nature of I_K suppressed by cpt-cAMP

The effects of cpt-cAMP on the voltage dependence of inactivation of I_K were examined by measuring the amplitudes of the peak currents obtained at +60 mV following the conditioning prepulse voltage step before and after the application of cpt-cAMP (see Fig. 5). The peak amplitude of I_K was reduced significantly by cpt-cAMP treatment at all prepulse voltages (see Fig. 5*A*; $n = 10$, paired t test). These currents were then normalized to the value obtained for the −100 mV conditioning prepulse step and fitted with the Boltzmann equation (Fig. 5*B*). Under control conditions, $V_{0.5}$, k and the fraction of non-inactivating current (c) were -39 ± 6 mV, 21 ± 2 mV and $0{\cdot}70 \pm 0{\cdot}03$, respectively. These values were not altered significantly following 20 min exposure to 100 μM cpt-cAMP, where $V_{0.5}$, k and c were -39 ± 6 mV, 24 ± 2 mV and $0{\cdot}62 \pm 0{\cdot}02$, respectively. Although the peak current was reduced by cpt-cAMP at all prepulse voltages, there was little change in the shape of the inactivation curves indicating that the cpt-cAMP-mediated suppression did not result from consequential alterations in the inactivation properties of I_K. Taken together, these results suggest that cpt-cAMP reduced the total amount of I_K capable of activation and that the extent of this reduction did not depend on the conditioning prepulse.

The notion that cpt-cAMP led to the suppression of I_K without having a significant effect on the inactivation of this current was examined in further detail. The results obtained with the inactivation protocol were used to determine the extent of inhibition by cpt-cAMP on the peak amplitude (I_{peak}) compared with the current just prior to the end of the +60 mV step (steady-state current; I_{ss}) for different prepulse voltages. These different conditioning prepulses could be used to activate/inactivate different populations of potassium currents (e.g. fast-inactivating I_A *vs.* delayed rectifier types). Subtraction of I_{ss} from I_{peak} for different prepulse voltages should reveal any selective inhibition produced by 100 μM cpt-cAMP. As shown in Fig. 6*A*, the difference ($I_{peak} - I_{ss}$) became smaller for more depolarizing prepulse voltages and was consistent with the loss of an I_A-like potassium current. However, after a 20 min exposure to cpt-cAMP the extent of ($I_{peak} - I_{ss}$) was not significantly different from the control values. Similarly, a comparison of the percentage of inhibition produced by cpt-cAMP for either I_{peak} or I_{ss} as a function of the prepulse voltage demonstrated that, regardless of the amplitude of the conditioning prepulse, cpt-cAMP produced an approximately 35% suppression of I_K (data not shown). Our results for cpt-cAMP are in contrast to those obtained by Chung & Kaczmarek (1995) in GH_4C_1 cells. They observed that dibutyryl cAMP reduced I_K to a greater extent at the end of the pulse compared with the beginning, suggesting that cAMP increased the rate of inactivation. Analysis of the effects of 1 μM PGE_2 and their dependence on the prepulse voltage demonstrated that PGE_2, like cpt-cAMP, had no significant action on the value of ($I_{peak} - I_{ss}$) compared with the control condition (see Fig. 6*B*) and that the inhibition of both I_{peak} and I_{ss} ranged between 35 and 40%. This observation supports the notion that cpt-cAMP and PGE_2 led to the suppression of the same I_K by reducing the number of channels capable of activation rather than by affecting their rates of inactivation.

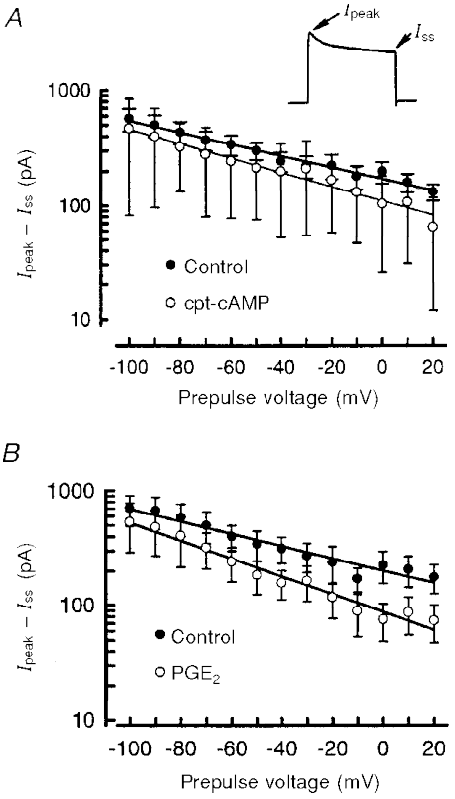

Figure 6. Inhibition of I_K by cpt-cAMP or PGE_2 does not depend on the conditioning prepulse voltage

The current ($I_{peak} - I_{ss}$) was measured as the difference between the peak (I_{peak}) and steady-state (I_{ss}) currents for the voltage step to +60 mV using the steady-state inactivation protocol and is shown in the inset in *A*. *A*, the value of ($I_{peak} - I_{ss}$) was determined in the absence and presence of 100 μM cpt-cAMP (20 min treatment) as a function of the conditioning prepulse voltage. The points represent means ± S.E.M. from ten neurones. Note that the ordinate is a logarithmic scale. The lines drawn through the data points are linear regression lines wherein the correlation coefficients (r^2) for the control and cpt-cAMP treatments were 0·98 and 0·99, respectively. *B* shows the value of ($I_{peak} - I_{ss}$) in the absence and presence of 1 μM PGE_2 (20 min treatment) as a function of the conditioning prepulse voltage. The points represent means ± S.E.M. from ten neurones. The lines drawn through the data points are linear regression lines; the correlation coefficients (r^2) for the control and PGE_2 treatments were 0·94 and 0·97, respectively.

Different holding potentials and their effects on the capacity of cpt-cAMP to reduce I_K

Analogous to the inactivation protocol, two specific conditioning prepulses of longer duration were used to manipulate selectively various components of the total whole-cell I_K. A prepulse voltage step to −100 mV (1 s duration) was used to remove most of the inactivation associated with the rapidly inactivating potassium currents, such as I_A, thereby revealing currents that are a combination of non-inactivating and inactivating currents (Connor & Stevens, 1971; Neher, 1971). Conversely, a prepulse voltage step to −30 mV (1 s duration) was used to inactivate rapidly inactivating currents and should result in the generation of currents dominated by sustained or delayed rectifier-type currents. From these long conditioning prepulses, an incremental series of depolarizing voltage steps (450 ms duration) was used to establish the current– voltage relation for both the peak current and the steady-state current.

The current–voltage relations for these two different holding potentials represent the compilation of all control studies for the experiments involving the effects of either the different prepulses alone or treatments with TEA or 4-AP (the pharmacology is shown below). For the 1 s prepulse to −100 mV, the mean peak current obtained at +60 mV was 5·6 ± 0·6 nA whereas for the prepulse to −30 mV the mean peak current was reduced significantly to 3·8 ± 0·4 nA ($n = 15$, data not shown). For all voltage steps, the peak I_K was smaller for the −30 mV prepulse compared with the −100 mV prepulse. Similarly, the mean steady-state currents obtained for all voltage steps were reduced significantly for the −30 mV prepulse compared with those for the −100 mV prepulse (e.g. at +60 mV, I_K was 3·1 ± 0·3 *vs.* 4·1 ± 0·4 nA, respectively). When the current values were normalized to their respective values obtained at +60 mV, all current–voltage relations exhibited a 'half-maximal' activation at 20 mV (data not shown).

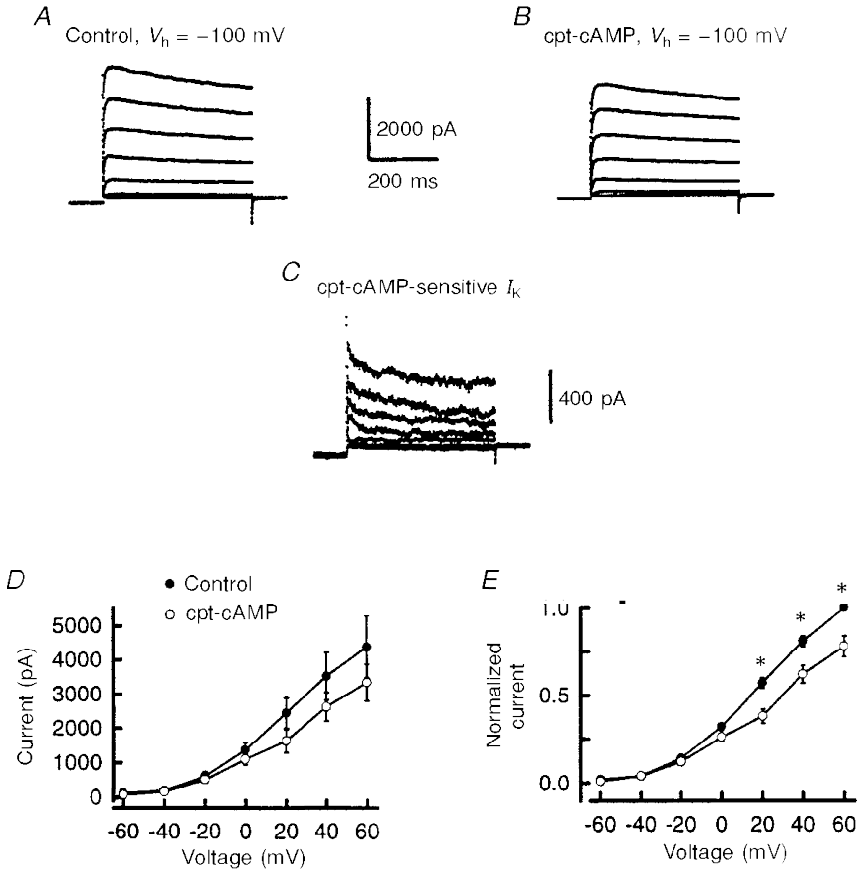

Figure 7. The cpt-cAMP-sensitive current obtained for a −100 mV conditioning prepulse

A illustrates representative currents obtained in response to 20 mV incremental steps from a prepulse voltage of −100 mV (V_h, holding potential). *B* shows these currents after a 20 min exposure to 100 μM cpt-cAMP. Subtraction of the respective traces in *B* from those in *A* yield the cpt-cAMP-sensitive currents for these different voltage steps (*C*). The calibration bar for time is the same for all three panels. *D* shows the effect of 100 μM cpt-cAMP on the current–voltage relation of I_K for the prepulse voltage of −100 mV. In *E*, the currents have been normalized to their respective control values obtained for the step to +60 mV. In *D* and *E*, the data points represent means ± S.E.M. obtained from four neurones. For those points appearing to lack error bars, the size of the bar is smaller than the symbol. * $P < 0·05$ *vs.* cpt-cAMP.

As described above, treatment with cpt-cAMP for 20 min produced a suppression of I_K that was similar for each conditioning prepulse. The representative currents obtained in the absence and presence of 100 μM cpt-cAMP for the conditioning prepulse to −100 mV are illustrated in Fig. 7. Under control conditions, the currents elicited by the depolarizing steps (450 ms duration) exhibited some time-dependent inactivation (Fig. 7*A*) and were similar to the traces shown in Fig. 1. Treatment with cpt-cAMP (Fig. 7*B*) reduced the peak current from 4·6 to 3·5 nA and the steady-state current from 3·8 to 3·2 nA, inhibitions of 23 and 20%, respectively. Subtraction of these traces yielded the cpt-cAMP-sensitive current exhibited by this sensory neurone (Fig. 7*C*). This current demonstrated a small amount of time-dependent inactivation wherein the peak current at +60 mV was 850 pA compared with a steady-state current of 580 pA. The cpt-cAMP-mediated inhibition of I_K for the −100 mV prepulse is summarized in Fig. 7*D*. When the effects of cpt-cAMP on the absolute values of I_K were examined, cpt-cAMP did not produce a significant suppression of I_K. This probably occurred because of the variability in the different amplitudes of I_K. This idea was corroborated when the currents obtained after application of cpt-cAMP were normalized to their respective control values obtained at +60 mV. As shown in Fig. 7*E*, cpt-cAMP caused a significant inhibition of I_K (paired t test). For a prepulse of −100 mV, a mean suppression of 22 ± 6% ($n = 4$) was observed for the voltage step to +60 mV.

The results obtained from a representative neurone before and after exposure to cpt-cAMP at a prepulse voltage of −30 mV are shown in Fig. 8*A* and *B*, respectively. Exposure to cpt-cAMP produced a similar extent of suppression of I_K for both the peak and steady-state currents, 27 and 23%, respectively, as observed at −100 mV. The cpt-cAMP-sensitive current obtained for the −30 mV prepulse is shown in Fig. 8*C*. The peak and the steady-state currents at +60 mV were 800 and 480 pA, respectively. The inhibition of I_K produced by cpt-cAMP for the −30 mV prepulse is

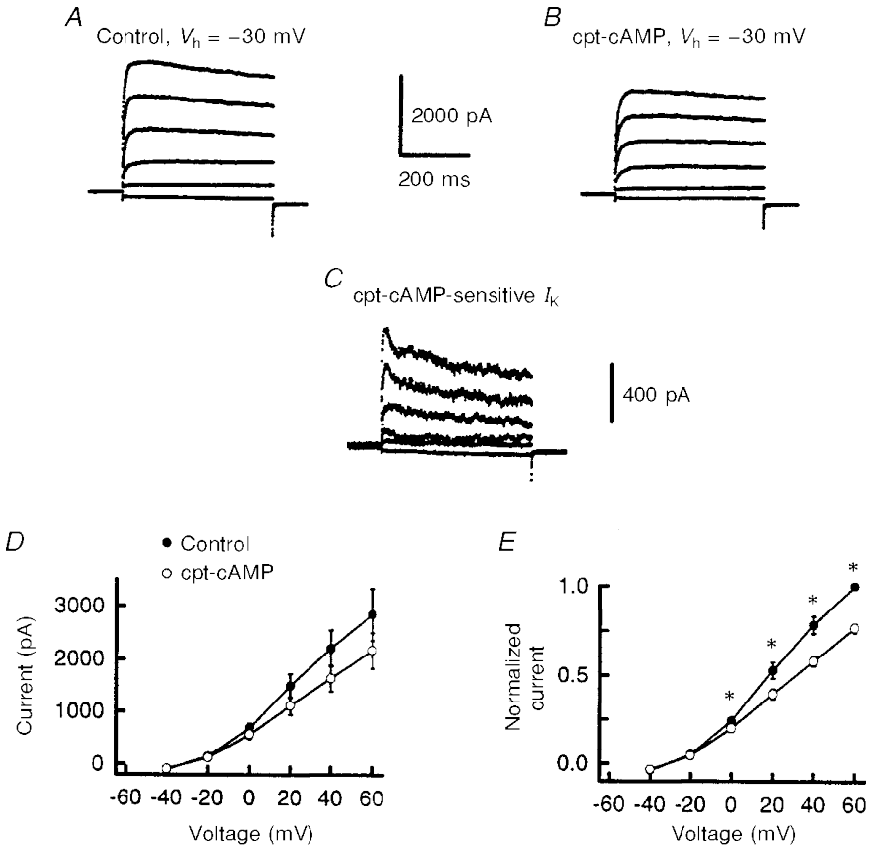

Figure 8. The cpt-cAMP-sensitive current obtained for a −30 mV conditioning prepulse

A illustrates representative currents elicited from the −30 mV prepulse voltage. These currents were obtained from the same neurone shown in Fig. 7. *B* shows those currents resulting after a 20 min exposure to 100 μM cpt-cAMP. The cpt-cAMP-sensitive current (*C*) was then determined by subtracting I_K remaining after a 20 min exposure to 100 μM cpt-cAMP (*B*) from its respective control I_K (*A*). The calibration bar for time is the same for all three panels. *D* shows the effect of 100 μM cpt-cAMP on the current–voltage relation of I_K for the prepulse voltage of −100 mV. In *E*, the currents have been normalized to their respective control values obtained for the step to +60 mV. In *D* and *E*, the data points represent means ± S.E.M. obtained from four neurones. For those points appearing to lack error bars, the size of the bar is smaller than the symbol. * $P < 0{\cdot}05$ *vs.* cpt-cAMP.

summarized in Fig. 8*D*. When the effects of cpt-cAMP on the absolute values of I_K were examined, cpt-cAMP did not produce a significant suppression of I_K. As shown in Fig. 8*E*, when the cpt-cAMP currents were normalized to their control values obtained at +60 mV, cpt-cAMP caused a significant inhibition of I_K (paired t test). For a prepulse of −30 mV, a mean suppression of $23 \pm 3\%$ ($n = 4$) was observed for the voltage step to +60 mV. Therefore, the results obtained for the cpt-cAMP-sensitive currents at both −100 and −30 mV were similar to those illustrated in Fig. 3 for a holding potential of −60 mV.

To determine whether the inhibition produced by cpt-cAMP was reversible, neurones were then superfused with the NMG-Ringer solution for 20 min and I_K was determined for the two prepulse voltages. For the −100 mV prepulse, neither the peak nor the steady-state I_K recovered to control values (data not shown). For example, the peak I_K (at +60 mV) after a 20 min washout was $3{\cdot}5 \pm 0{\cdot}8$ nA ($n = 3$) compared with $3{\cdot}3 \pm 0{\cdot}5$ nA ($n = 4$) for the cpt-cAMP treatment. Similar results for the lack of reversibility were obtained for the peak and steady-state I_K at the −30 mV prepulse (data not shown). Akins & McCleskey (1993) also reported that the inhibitory actions of membrane-permeant analogues of cAMP were irreversible. These results suggest that the extent of cpt-cAMP-induced suppression of I_K was long-lived (at least for 20 min). Thus, our findings suggest that the I_K suppressed by cpt-cAMP is analogous to a delayed rectifier-like current rather than a rapidly inactivating-like current such as that described as I_A (or that current exhibited by the expression of Kv1.4).

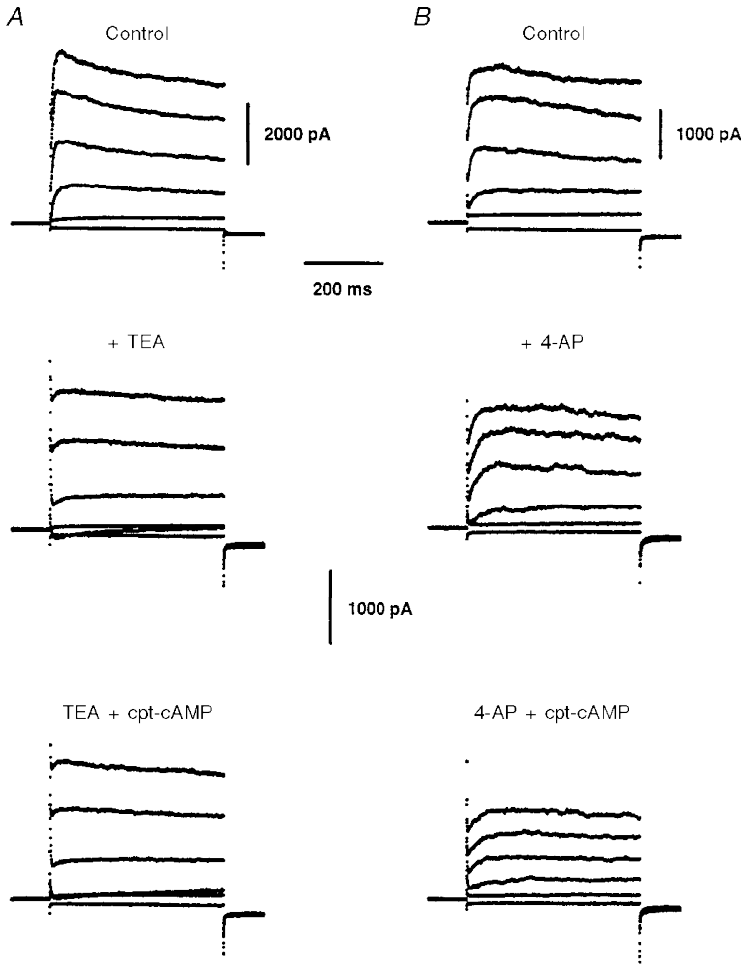

Figure 9. The effects of TEA or 4-AP and cpt-cAMP on I_K at the −30 mV conditioning prepulse voltage

These representative current traces illustrate the effects of either TEA or 4-AP and 100 μM cpt-cAMP in the presence of TEA or 4-AP. *A* shows currents obtained under control conditions from a prepulse of −30 mV (top). The middle panel represents the suppression of these currents by 10 mM TEA. The bottom panel shows the currents remaining after a 20 min exposure to cpt-cAMP in the presence of 10 mM TEA. *B* illustrates the effects of 4-AP, where the top panel shows the currents obtained under control conditions from a prepulse of −30 mV. The middle panel represents the suppression of these currents by 1 mM 4-AP. The bottom panel shows the currents remaining after a 20 min exposure to cpt-cAMP in the presence of 1 mM 4-AP. The calibration bar for time is the same for all six panels.

Pharmacological characterization of I_K inhibited by cpt-cAMP

The inhibitors of I_K, TEA and 4-AP, were used in an attempt to distinguish further the nature of the current reduced by cpt-cAMP (Thompson, 1977). Conditioning prepulses to −100 and −30 mV were used in combination with the pharmacological intervention to isolate delayed rectifier-like currents from rapidly inactivating currents. The effects of 10 mM TEA on I_K elicited from a prepulse of −30 mV in a representative sensory neurone are shown in Fig. 9*A*. Because it was established that the prepulse voltage did not influence the extent of I_K suppression by either PGE_2 or cpt-cAMP, only the results obtained for −30 mV will be represented. Under control conditions, a voltage step to +60 mV from the −30 mV prepulse evoked a peak I_K of 6·2 nA whereas the steady-state I_K was 5·3 nA (Fig. 9*A*, top panel). For the −100 mV prepulse, the peak and steady-state I_K were 8·2 and 6·3 nA, respectively (data not shown). Treatment with 10 mM TEA-Ringer solution caused an approximately 60% reduction in the peak and steady-state I_K for both conditioning prepulses (Fig. 9*A*, middle panel). After exposure to TEA, the time-dependent inactivation exhibited by I_K was still apparent for the −100 mV prepulse whereas it was reduced for the −30 mV prepulse. This sensory neurone was then treated with 100 μM cpt-cAMP and the results obtained after 20 min are illustrated in the bottom panel of Fig. 9*A*. Interestingly, after TEA, cpt-cAMP did not produce a further inhibition of the peak or the steady-state I_K for either prepulse voltage.

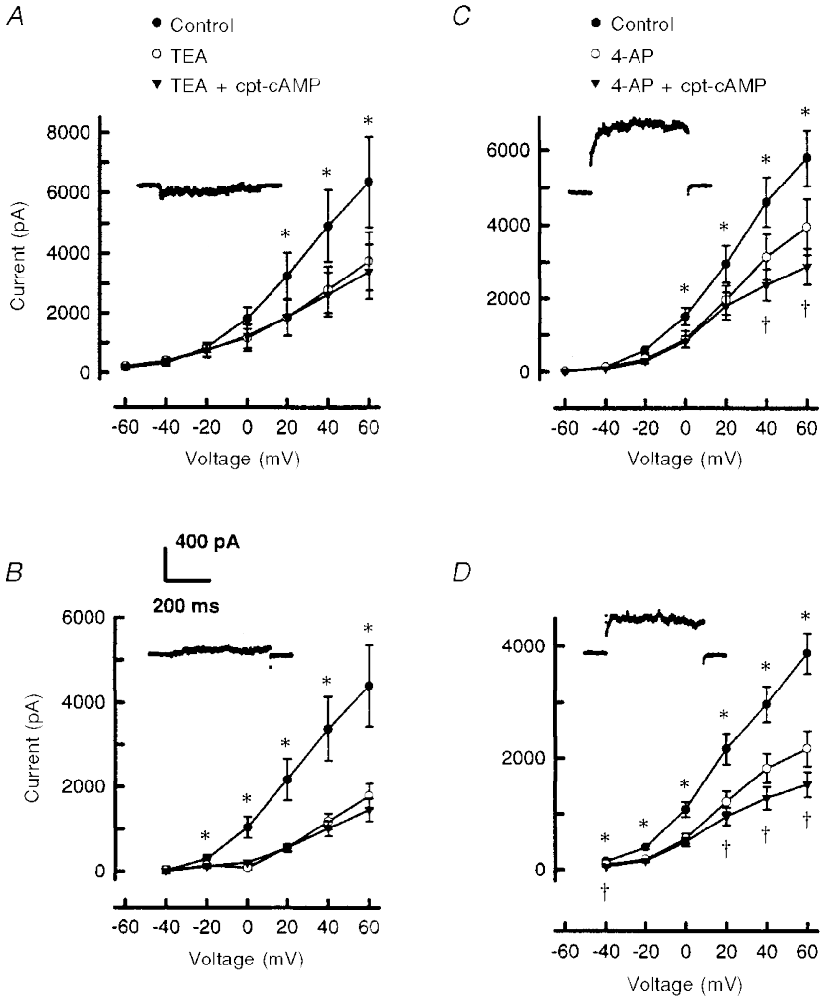

Figure 10. The effects of TEA or 4-AP on the inhibition of I_K by cpt-cAMP

A and *B* summarize the inhibition produced by 10 mM TEA and 100 μM cpt-cAMP in the presence of TEA for the peak I_K recorded for the −100 mV (*A*) and the −30 mV (*B*) prepulses. Values represent means ± S.E.M. from five neurones. *C* and *D* summarize the inhibition produced by 1 mM 4-AP and 100 μM cpt-cAMP in the presence of 4-AP for the peak I_K recorded for the −100 mV (*C*) and the −30 mV (*D*) prepulses. Values represent means ± S.E.M. from six neurones. The cpt-cAMP-sensitive currents (voltage step to +60 mV) obtained under these different conditions are illustrated in the insets in each respective panel. The calibration bars apply to each trace. * Significant difference (ANOVA with repeated measures, $P < 0{\cdot}05$) between the control and TEA or 4-AP treatments; † significant difference between the 4-AP and 4-AP + cpt-cAMP treatments.

Different classes of potassium currents have varying affinities for particular inhibitors. Therefore, we examined the sensitivity of the cpt-cAMP-modulated I_K to 4-AP. The representative effects of 1 mM 4-AP on I_K obtained for the −30 mV prepulse are illustrated in Fig. 9*B*. For a −30 mV prepulse, the application of 4-AP reduced the peak and steady-state I_K obtained at +60 mV by 50% (compare top and middle panels in Fig. 9*B*). The results obtained for the −100 mV prepulse were similar to those for −30 mV wherein 4-AP reduced the peak and steady-state I_K by 43 and 44%, respectively (data not shown). After treatment with 4-AP, I_K exhibited a time-dependent inactivation that was similar to that observed in the control recordings for the −100 mV prepulse. This observation, in combination with the similar reductions in peak and steady-state currents, suggests that the I_K comprising the component(s) that regulates the time-dependent inactivation is not sensitive to this concentration of 4-AP. In contrast to TEA, treatment with 100 μM cpt-cAMP in the presence of 1 mM 4-AP produced additional inhibition of I_K. In this representative neurone, the peak I_K in the presence of 4-AP (−30 mV prepulse) was reduced from 1·8 to 1·4 nA (+60 mV step) after a 20 min exposure to cpt-cAMP; steady-state I_K was decreased from 1·7 to 1·3 nA. At the more depolarized voltage steps, the current traces exhibited some time-dependent inactivation. For the −100 mV prepulse voltage, peak and steady-state I_K were suppressed by 25 and 31%, respectively, after a 20 min treatment with cpt-cAMP (data not shown).

The inhibitory effects of TEA, 4-AP and cpt-cAMP in the presence of either blocker are summarized in Fig. 10. For the prepulse to −100 mV, the mean peak I_K obtained at +60 mV was 6·4 ± 1·5 nA ($n = 5$, see Fig. 10*A*). Exposure to TEA reduced this peak current to 3·7 ± 0·9 nA. The inhibition of I_K by TEA was significantly different from the control for only the +20, +40 and +60 mV steps (ANOVA with repeated measures). Similar results were obtained for the current values normalized to their respective control values for the +60 mV step and indicated that TEA caused a significant decrease in I_K for the steps to +20 mV (control, 0·52 ± 0·03 *vs.* TEA, 0·31 ± 0·08), +40 mV (0·78 ± 0·01 *vs.* 0·45 ± 0·08) and +60 mV (1·0 *vs.* 0·61 ± 0·09). The addition of 100 μM cpt-cAMP in the presence of TEA did not significantly alter the peak currents (or the normalized values) obtained for any voltage step (e.g. for +60 mV, I_K was 3·4 ± 0·9 nA; ANOVA with repeated measures). The cpt-cAMP-sensitive current in the presence of TEA was determined in order to examine the inactivation kinetics of these traces. For a given prepulse, the currents obtained in TEA + cpt-cAMP were subtracted from the currents in TEA alone and are shown in the inset. The cpt-cAMP-sensitive currents shown in the insets to Fig. 10*A* and *B* were obtained for the voltage step to +60 mV from the neurone shown in Fig. 9*A*. These current traces indicate that in the presence of TEA there was very little, if any, cpt-cAMP-sensitive current. Figure 10*B* illustrates the effects of TEA and cpt-cAMP for the −30 mV prepulse. Here, the mean peak I_K obtained at +60 mV was 4·4 ± 0·9 nA ($n = 5$). TEA significantly inhibited I_K (both absolute and normalized current values) for the steps −20 to +60 mV. Similar to the results obtained with the −100 mV prepulse, normalization indicated that TEA significantly reduced I_K to 0·45 ± 0·06 of the control value at +60 mV (1·0). Treatment with 100 μM cpt-cAMP did not produce any additional inhibition of I_K for any voltage step. The cpt-cAMP-sensitive current is shown in the inset. Similar results were obtained for the effects of TEA and cpt-cAMP on the steady-state I_K (data not shown). After exposure to TEA, the remaining steady-state I_K was only 0·45 ± 0·03 and 0·44 ± 0·03 relative to the control values at +60 mV for prepulses to −100 and −30 mV, respectively. However, cpt-cAMP had no significant effect on steady-state I_K in the presence of TEA. Taken together, these results demonstrate that TEA inhibits both the peak and steady-state I_K by approximately 50% for either prepulse voltage. In the presence of TEA, cpt-cAMP was incapable of causing further inhibition of I_K, suggesting that the current modulated by cpt-cAMP is a potassium current sensitive to TEA.

The inhibitory effects of both 4-AP and cpt-cAMP are also summarized in Fig. 10. The peak I_K obtained for both the −100 (Fig. 10*C*) and −30 mV (Fig. 10*D*) prepulses was decreased significantly by 1 mM 4-AP (ANOVA with repeated measures). For the voltage step to +60 mV, these reductions corresponded to inhibitions of 35 ± 4 and 45 ± 4% ($n = 6$) for the −100 and −30 mV prepulses, respectively. The insets to Fig. 10*C* and *D* represent the cpt-cAMP-sensitive current obtained in the presence of 4-AP (same neurone as shown in Fig. 9*B*). Under these conditions, the cpt-cAMP-sensitive current exhibited little time-dependent inactivation. Suppression of the steady-state I_K by 4-AP was identical to that observed for the peak current wherein the extent of inhibition for the +60 mV step was 33 ± 6 and 41 ± 5% ($n = 6$) for the −100 and −30 mV prepulses, respectively (data not shown). In the presence of 4-AP, 20 min treatment with 100 μM cpt-cAMP further decreased the peak and steady-state I_K (ANOVA with repeated measures) by values that ranged from 26 to 30% for the more depolarized voltage steps (20–60 mV) obtained in 4-AP for either prepulse voltage. This amount of cpt-cAMP-induced inhibition was similar to that observed under control conditions (compare Figs 2, 7 and 8). Taken together, these results suggest that the I_K modulated by cAMP was insensitive to the actions of 4-AP.

DISCUSSION

Our results demonstrate that cpt-cAMP and PGE_2 suppress, in an analogous manner, potassium currents in embryonic rat sensory neurones. This suppression of I_K is dependent on activation of the cAMP–PKA transduction cascade since pretreatment with the selective PKA inhibitor, PKI, completely abolished the suppressive effects of PGE_2. Thus, these results support the growing evidence that the cAMP signalling pathway mediates the sensitizing actions of PGE_2. This evidence includes findings wherein PGE_2

elevates intracellular levels of cAMP in rat sensory neurones (Hingtgen *et al.* 1995). In addition, the PGE_2-induced facilitation of both neuropeptide release and the number of action potentials evoked by bradykinin are attenuated by blocking the production of cAMP or inhibiting the activation of PKA (Hingtgen *et al.* 1995; Cui & Nicol, 1995). In behavioural assays measuring the effects of noxious stimulation in rats, the application of membrane-permeant analogues of cAMP produce a sensitization similar to the hyperalgesia evoked by PGE_2 (Ferreira & Nakamura, 1979; Taiwo *et al.* 1989).

The question remains as to the specific mechanism of action whereby pro-inflammatory prostaglandins give rise to this sensitization in sensory neurones. These prostaglandins are known to modulate multiple types of membrane currents in various model systems. Examples of this mediation are as follows: a non-selective inward current activated by hyperpolarization termed I_h (Ingram & Williams, 1994), a calcium-dependent current that gives rise to a slow after-hyperpolarization (AHP_{slow}) (Fowler *et al.* 1985; Gold *et al.* 1996*b*), calcium currents (Miwa *et al.* 1988; Alloatti *et al.* 1991; Mochizuki-Oda *et al.* 1991; Nicol *et al.* 1992), a TTX-resistant sodium current (Gold *et al.* 1996*a*; England *et al.* 1996), and outward potassium current (Ren *et al.* 1996; Nicol *et al.* 1997). However, several lines of evidence suggest that alterations of some of these currents are not the mechanism(s) by which PGE_2 enhances the excitability of small-diameter sensory neurones. First, I_h is expressed mainly in medium to large cells, but not in small-diameter Aδ and C-type sensory neurones (Tokimasa *et al.* 1990). Second, the inhibitory effects of PGE_2 on AHP_{slow} were observed in only about half of the neurones sensitized by this prostanoid (Gold *et al.* 1996*b*). Also, PGE_2 increased the excitability of neurones not expressing AHP_{slow}, thus modulation of this current was not critical for sensitization. Finally, we reported recently that blockade of N-, L- or P-type voltage-dependent calcium channels did not attenuate the PGE_2-mediated facilitation of peptide release from rat sensory neurones, indicating that these channels were not involved in PGE_2-induced sensitization (Evans *et al.* 1996*a*).

Our findings are consistent with and expand on previous work that has shown that increasing levels of intracellular cAMP led to the suppression of voltage-dependent potassium currents in adult and neonatal DRG cells. Indeed, previous studies indicated a role for the cAMP signalling cascade in the enhancement of membrane excitability. In both chick (Dunlap, 1985) and mouse (Grega & Macdonald, 1987) sensory neurones grown in culture, activation of the cAMP pathway increased the duration of the action potential and this lengthening of the action potential resulted from the inhibition of I_K. In sensory neurones isolated from adult rats and grown in culture, the application of membrane-permeant analogues of cAMP produced an inhibition (~15%) of the outward I_K (Akins & McCleskey, 1993). The modulation of potassium currents as a means of controlling excitability in sensory neurones may be a general scheme. In *Aplysia* sensory neurones, the serotonin-induced sensitization is analogous to the effects of PGE_2 on mammalian sensory neurones and results, in part, from the cAMP-mediated suppression of multiple potassium currents (Klein *et al.* 1982; Baxter & Byrne, 1989; Goldsmith & Abrams, 1992). Furthermore, in another sensory system, increased levels of cAMP led to the suppression of a TEA-sensitive I_K in fungiform taste bud cells (Cummings *et al.* 1996).

The capacity of cAMP–PKA to decrease I_K has also been observed in other neuronal preparations. For example, the activity of the type 2 BK (high conductance calcium-sensitive) potassium channel in recordings from planar bilayers is reduced greatly after exposure to PKA (Reinhart *et al.* 1991). In addition, PKA reduced the open probability of the human BK channel (hSlo) when it was co-expressed with the β subunit (hSloβ) and exhibited properties that were similar to the native type 2 channel (Dworetzky *et al.* 1996). In mouse anterior pituitary cells (AtT20 cells), cpt-cAMP reduced an I_K believed to be carried by BK potassium channels by about 30% (Shipston *et al.* 1996). In the presence of TEA, cpt-cAMP was ineffective at reducing I_K. Thus, activation of the cAMP–PKA transduction cascade may be an important signalling mechanism in the regulation of membrane excitability in a variety of neurones.

The exact nature of the I_K modulated by activation of PKA is difficult to ascertain. At least two different types of voltage-dependent I_K have been described consistently in adult, neonatal and embryonic sensory neurones (Kostyuk *et al.* 1981; Kameyama, 1983; Akins & McCleskey, 1993; Nicol *et al.* 1997): an inactivating I_A-type and a delayed rectifier-type current. In addition, recent work of Gold and co-workers suggests that additional types of I_K exist (Gold *et al.* 1996*c*). Although a more careful characterization of the I_K modulated by PGE_2 and cAMP remains to be determined, our results suggest that the PGE_2-/cAMP-modulated I_K in rat sensory neurones may be a delayed rectifier-like current. This conclusion is based upon several lines of evidence. First, the current–voltage relations for both the PGE_2- and cpt-cAMP-sensitive currents were quite similar, both began to exhibit activation at approximately −20 mV (Fig. 3). Second, the cpt-cAMP-sensitive current observed under various experimental conditions exhibited slow inactivation kinetics, which are characteristic of delayed rectifier currents. Third, the extent of inhibition by either PGE_2 or cpt-cAMP did not depend on the level of the conditioning prepulse voltage and was approximately the same for either the peak or steady-state values. Lastly, cpt-cAMP effectively suppressed I_K in the presence of 4-AP, whereas no further inhibition of I_K was observed in the presence of TEA, an established inhibitor of delayed rectifier-like currents.

Our present work and that of Gold *et al.* (1996*a*) and England *et al.* (1996) demonstrate that PGE_2 may enhance neuronal excitability by suppressing potassium currents and/or augmenting a TTX-resistant sodium current. Additionally,

both the sensitizing actions of PGE_2 and the effects of this prostanoid on potassium and TTX-resistant sodium currents are dependent on the activation of the cAMP transduction cascade (Cui & Nicol, 1995; Hingtgen *et al.* 1995; England *et al.* 1996). Therefore, taken together, activation of the cAMP signalling pathway gives rise to PKA-mediated modulation of the activity of multiple ion channels, which, in turn, results in the enhanced excitability of sensory neurones. This prostaglandin-induced modification of ion channel activity may then account for the heightened sensitivity to noxious stimulation exhibited in behavioural observations.

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