$5HT_4$ Receptors Couple Positively to Tetrodotoxin-Insensitive Sodium Channels in a Subpopulation of Capsaicin-Sensitive Rat Sensory Neurons

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The distribution of tetrodotoxin (TTX)-sensitive and -insensitive Na^+ currents and their modulation by serotonin (5HT) and prostaglandin E_2 (PGE_2) was studied in four different types of dorsal root ganglion (DRG) cell bodies (types 1, 2, 3, and 4), which were previously identified on the basis of differences in membrane properties (Cardenas et al., 1995). Types 1 and 2 DRG cells expressed TTX-insensitive Na^+ currents, whereas types 3 and 4 DRG cells exclusively expressed TTX-sensitive Na^+ currents.

Application of 5HT (1–10 μM) increased TTX-insensitive Na^+ currents in type 2 DRG cells but did not affect Na^+ currents in type 1, 3, or 4 DRG cells. The 5HT receptor involved resembled the $5HT_4$ subtype. It was activated by 5-methoxy-*N,N*-dimethyltryptamine (10 μM) but not by 5-carboxyamidotryptamine (1 μM), (+)-8-hydroxydipropylaminotetralin (10 μM), or 2-methyl-5HT (10 μM), and was blocked by ICS 205-930 with an EC_{50} of ~2 μM but not by ketanserin (1 μM). PGE_2 (4 or 10 μM) also increased Na^+ currents in varying portions of cells in all four groups.

The effect of 5HT and PGE_2 on Na^+ currents was delayed for 20–30 sec after exposure to 5HT, suggesting the involvement of a cytosolic diffusible component in the signaling pathway. The agonist-mediated increase in Na^+ current, however, was not mimicked by 8-chlorophenylthio-cAMP (200 μM), suggesting the possibility that cAMP was not involved.

The data suggest that the 5HT- and PGE_2-mediated increase in Na^+ current may be involved in hyperesthesia in different but overlapping subpopulations of nociceptors.

Key words: serotonin; PGE_2; capsaicin; nociceptor; tetrodotoxin; cAMP; dorsal root ganglion; $5HT_4$ receptor

Primary hyperesthesia is thought to be a consequence of the release of proinflammatory mediators in the vicinity of nociceptor endings. Proinflammatory agents such as serotonin (5HT), prostaglandins, and adenosine are derived from a number of sources (Cooper and Sessle, 1992). Interactions between such agents and endings of thinly myelinated and unmyelinated nociceptive afferents induce activity, decrease threshold, and increase suprathreshold mechanothermal reactivity (Handwerker, 1976; Kumazawa and Mizumura, 1980; Mense, 1981; Martin et al., 1987; Schaible and Schmidt, 1988; Lang et al., 1990; Grubb et al., 1991; Herbert and Schmidt, 1992).

Contributions to hyperesthesia by proinflammatory agents are possibly achieved by modulation of membrane currents involved in the initiation and repolarization of action potentials in nociceptive endings, as well as induction of edema in the surrounding matrix (Cooper, 1993). The study of acutely isolated dorsal root ganglion (DRG) cell bodies may be useful in determining the roles of various ion channels in the actions of proinflammatory agents on nociceptor function. A number of studies have shown that afferent cell bodies exhibit properties of nociceptor endings *in vitro*. These include expression of currents sensitive to proinflammatory mediators as well as features of peripheral transduction mechanisms (Baccaglini and Hogan, 1983; Fowler et al., 1985; Nicol and Cui, 1994; Weinreich et al., 1995; Cesare and McNaughton, 1996; Gold et al., 1996a,b).

In a recent study, Gold et al. (1996b) observed that 5HT, prostaglandin E_2 (PGE_2), and adenosine produced an increase in TTX-resistant Na^+ currents in a portion (51, 36, and 64%, respectively) of cultured rat DRG cells. To follow up on this initial observation, we have investigated the distribution of TTX-sensitive and -insensitive Na^+ currents and their modulation by 5HT, PGE_2, and adenosine in four subpopulations (types 1, 2, 3, and 4) of acutely isolated, small- and medium-diameter cells of the adult rat DRG. We have previously differentiated these subgroups on the basis of capsaicin sensitivity, I_H, I_A, and T-type Ca^{2+} current amplitude (Cardenas et al., 1995). Soma size and other characteristics mentioned above suggest that these DRG cell subpopulations represent subclasses of Aδ- and C-type (groups III and IV) afferents, and that types 1 and 2 DRG cell bodies likely represent nociceptors (Cardenas et al., 1995). The results of this study suggest the idea that in distinct subpopulations of nociceptors, an increase in Na^+ current produced by 5HT and PGE_2 may contribute to hyperesthesia.

MATERIALS AND METHODS

Male rats (75–150 gm; Sprague Dawley purchased from Harlan) were rendered unconscious with methoxyflurane and decapitated, and DRG cell bodies from thoracic and lumbar regions were dissected out. The ganglia were incubated at 36°C for 1 hr in Tyrode's solution (composition below) containing 2 mg/ml collagenase (Type 1, Sigma, St. Louis, MO) and 5 mg/ml Dipase II (Boehringer Mannheim, Indianapolis, IN). Individual DRG cell bodies were isolated by trituration, adhered to a poly-L-lysine-coated coverslip stuck to the bottom of a 35 mm petri dish, and superfused with Tyrode's solution containing (in mM): 140 NaCl, 4 KCl,

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2 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES, adjusted to pH 7.4 with NaOH. Currents were recorded in the whole-cell patch configuration using an Axopatch 200A (Axon Instruments, Foster City, CA). Voltage and current steps, holding potential, and data acquisition and analysis were controlled by an on-line IBM PC/AT clone computer programmed with Axobasic 1.0 (Axon Instruments).

Electrodes were fabricated from soda lime capillary glass (B4416-1; Scientific Products) using a Narishige two-stage vertical puller coated with Sylgard to ~200 μm from the tip and fire-polished to a final resistance of 0.8–2.0 MΩ using a Narishige microforge. For voltage-clamp experiments, series resistance was estimated from capacity transients, and compensated for as described previously (Scroggs and Fox, 1992). No data were included where series resistance resulted in >10 mV error in voltage commands.

Solutions were changed using the "sewer pipe" system, which consisted of a glass capillary tube mounted on a micromanipulator. The end of the glass capillary tube was placed near the cell under study, and the flow from it completely isolated the cell from the background flow of Tyrode's solution, which flowed continuously over the cells via another port. Different solutions were directed out of the capillary tube by means of a small manifold to which various 10 ml aliquots of drug or control solutions were connected. Changes in appropriate ion currents produced by switching from Tyrode's solution to solutions containing TEA, Ba^{2+}, Cd^{2+}, or elevated K^+ indicate that the solution surrounding the cell under study is changed in <4 sec by this system. All experiments were performed at room temperature (23°C).

Capsaicin and PGE_2 (Sigma) were dissolved in 100% ethanol at a concentration of 10 mM and diluted in Tyrode's solution for experiments. Previous control experiments determined that the ethanol vehicle had no effect by itself on holding current or membrane conductance in capsaicin-sensitive DRG cells (Del Mar et al., 1994). (+)-8-hydroxydipropylaminotetralin (8-OH-DPAT), 5HT, 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT), 5-carboxyamidotryptamine (5-CT), 2-methyl-5HT (2-me-5HT), and ketanserin (Research Biochemicals Inc., Natick, MA) were first dissolved in water as 1 mM or 10 mM stock solutions and then diluted to appropriate concentrations in the external solution. All drugs were made fresh daily.

The study was restricted to small- and medium-diameter neurons (defined by the average of the distance along their longest and shortest axis) with smooth outer membrane surfaces. DRG cells were categorized as type 1, 2, 3, or 4 on the basis of the expression of several membrane properties (capsaicin sensitivity, I_H, I_A, and T-type Ca^{2+} channel current amplitude) as described previously (Cardenas et al., 1995). Each cell was initially tested for I_H (a slowly activating nonselective cation current activated by hyperpolarization) using a 787 msec hyperpolarizing test pulse to −110 mV from a holding potential of −60 mV. A subtype of I_A (a group of transient, 4-aminopyridine-sensitive, outward K^+ currents) (Gold et al., 1996c) was subsequently tested for on repolarization of the cell back to the holding potential (−60 mV) from the −110 mV test pulse used to test for I_H. Next, cells were tested for capsaicin sensitivity by superfusion with 1 μM capsaicin, which produced an inward shift in holding current and an increase in membrane conductance in sensitive cells. Capsaicin-insensitive cells were subsequently tested regarding T-type Ca^{2+} channel current amplitude at a holding potential of −90 mV and a 200 msec test potential to −40 mV.

For measuring the above-mentioned K^+-dependent phenomena, cells were superfused externally with Tyrode's solution, and the patch electrodes were filled with (in mM): 120 KCl, 5 2Na-ATP, 0.4 2Na-GTP, 5 EGTA, 2.25 $CaCl_2$, 20 HEPES, adjusted to pH 7.4 with KOH. Total KCl after pH adjustment was 154 mM. Free $[Ca^{2+}]_i$ was calculated at 140 nM. Calcium channel currents (carried by Ba^{2+}) were isolated by changing the Tyrode's solution superfusing the outside of the DRG cell under study to one containing (in mM): 160 tetraethylammonium chloride (TEA-Cl), 2 $BaCl_2$, 10 HEPES, pH 7.4 with TEA-OH (Del Mar et al., 1994; Cardenas et al., 1995). To study Na^+ currents, the solution was changed to one that suppressed K^+ and Ca^{2+} currents; it contained (in mM): 60 NaCl, 90 TEA, 10 4-AP, 2 $BaCl_2$, 0.1 $CaCl_2$, 0.4 $CdCl_2$, 10 HEPES, pH 7.4 with TEA-OH.

In experiments regarding Na^+ current amplitude, measurements were made from plots of current versus time. It was often necessary to take into consideration the rate of Na^+ current run-up, which varied from cell to cell. To this end, a line was drawn through the control data points and extrapolated out to a position adjacent to the peak effect of the drug. This position was considered to be the control Na^+ current amplitude and was used to calculate the percent change in Na^+ current amplitude produced by the drug.

RESULTS

The effects of TTX on Na^+ currents were tested on small- and medium-diameter DRG cells (≤40 μm in diameter), which were classified as types 1, 2, 3, and 4 (Cardenas et al., 1995). Briefly, according to the classification system, cells are categorized as type 2 DRG cells if they are capsaicin sensitive and express I_A, and as type 1 if they are capsaicin sensitive but express neither I_H nor I_A. Cells are categorized as type 3 if they are capsaicin insensitive, express I_H, and have T-type Ca^{2+} currents ≤1 nA, or as type 4 if they share these properties but have T-type Ca^{2+} currents ≥2.4 nA (Cardenas et al., 1995).

The expression of TTX-sensitive and -insensitive Na^+ currents varied among types 1, 2, 3, and 4 DRG cells. Figure 1*A–D* illustrates the characteristic action potentials exhibited by types 1, 2, 3, and 4 DRG cells (*top*) and representative Na^+ current traces recorded from each type in the presence and absence of 1 μM TTX. The Na^+ current recorded from types 1 and 2 DRG cells was nearly completely resistant to blockade by 1 μM TTX, being reduced by 7 ± 3.6% (n = 11) in type 1 DRG cells and by 1.0 ± 0.6% (n = 16) in type 2 DRG cells. On the other hand, the Na^+ current in all type 3 and type 4 DRG cells tested (n = 10 and 15, respectively) was completely blocked by 1 μM TTX. In agreement with previous studies, the TTX-insensitive Na^+ current observed in type 1 and type 2 cells was more slowly activating and inactivating than the TTX-sensitive Na^+ current observed in type 3 and type 4 DRG cells (Ogata and Tatebayashi, 1993)

5HT (10 μM) produced a marked increase in the Na^+ current in type 2 cells and had little or no effect on types 1, 3, and 4 DRG cells (Fig. 2*A–C*). The increase in Na^+ current by 5HT in a type 2 cell is illustrated in Figure 2*A,B*, whereas the distribution of this effect among the four DRG cell types is illustrated by the bar graph in Figure 2*C*. In this series of experiments, superfusion with 10 μM 5HT increased Na^+ current by 69.7 ± 17.5% in eight of the type 2 cells studied. This was significantly greater than increases of 3.8 ± 3.8% (n = 10), 0 ± 0% (n = 7), and 0.1 ± 1.0% (n = 10) produced by 5HT in types 1, 3, and 4 DRG cells, respectively (Newman–Keuls multiple-range test; $p < 0.05$). Over the entire study, 47 of 48 type 2 cells tested with 10 or 1 μM 5HT responded with a >10% increase in Na^+ current.

As illustrated in Figure 2*A*, the onset of the 5HT-induced increases in Na^+ current was rather slow (20–30 sec). Because the solution surrounding the cell under study is completely changed in <4 sec, it is unlikely that this delay was an artifact of the drug delivery system. As illustrated in Figure 2*A*, the 5HT-induced increase in Na^+ current in type 2 cells was not readily reversible on washout of agonist: it lasted until termination of the experiment (as much as 10 min). The increase in Na^+ current amplitude by 5HT was attributable to an increase in TTX-resistant Na^+ current, as evidenced by the inability of TTX (1 μM) to reverse the 5HT-induced increase in Na^+ current amplitude (n = 9) (Fig. 2*D,E*).

Several 5HT receptor agonists were tested regarding their interaction with the 5HT receptor mediating the 5HT-induced increase in Na^+ current in type 2 cells (Fig. 3*A–C*). As summarized in Figure 3*C*, 5-methoxy-*N,N*-dimethyl tryptamine (10 μM) produced a 75.9 ± 4.7% increase in Na^+ current (n = 3); however, 5-carboxyamidotryptamine (1 μM), (+)8-OH-DPAT (10 μM), and 2-methyl-5HT (10 μM) had no effect on Na^+ current in type 2 cells tested with each compound (Fig. 3*A–C*). In the same

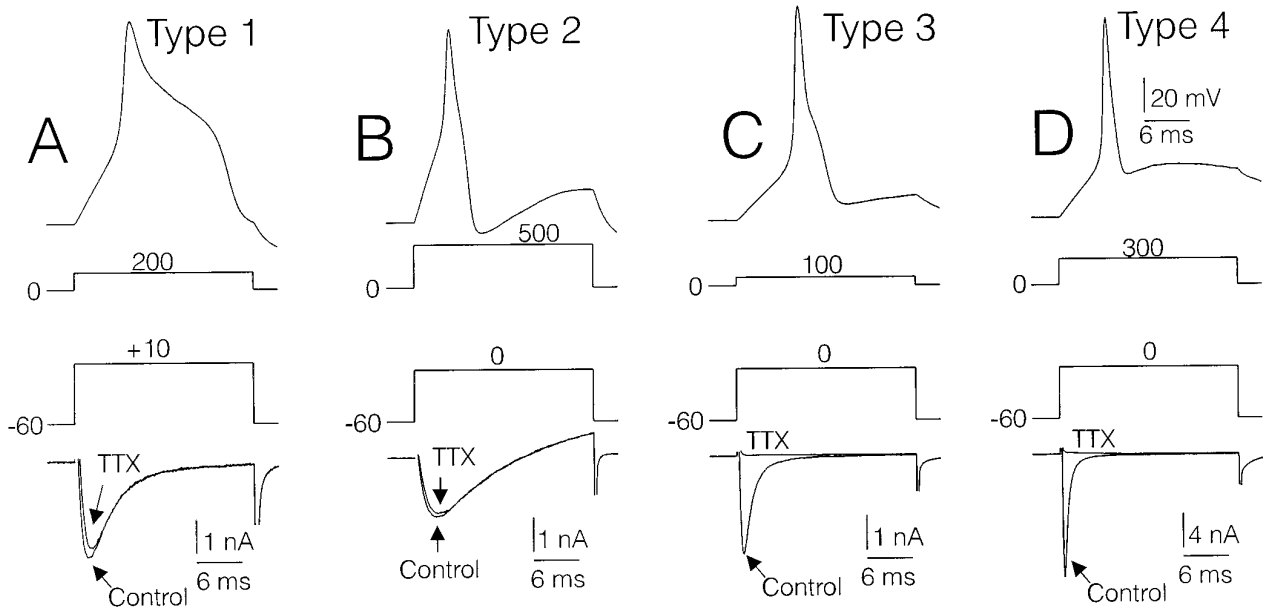

Figure 1. Distribution of TTX-sensitive and -insensitive Na^+ channels in types 1, 2, 3, and 4 DRG cells. In *A–D*, the *top panels* are characteristic action potentials recorded from each of the four types of DRG cells as indicated. The *bottom panels* are recordings of Na^+ currents, recorded from each cell type, before and during superfusion of the cells with 1 μM TTX. For all recordings the pipette solution contained (in mM): 120 KCl, 5 2Na-ATP, 0.4 2Na-GTP, 5 $MgCl_2$, 5 EGTA, 2.25 $CaCl_2$, 20 HEPES, adjusted to 7.4 with KOH (total KCl = 154 mM; free $[Ca^{2+}]_i$ was calculated to be 140 nM). For recording action potentials, the external solution (Tyrode's) contained (in mM): 140 NaCl, 4 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES, adjusted to pH 7.4 with NaOH. For recording Na^+ currents the external solution contained (in mM): 60 NaCl, 90 TEA, 10 4-AP, 2 $BaCl_2$, 0.1 $CaCl_2$, 0.4 $CdCl_2$, 10 HEPES, pH 7.4 with TEA-OH.

type 2 DRG cells in which the inactive agonists were tested, 5HT (10 μM) produced an increase in Na^+ current amplitude that averaged 63.5 ± 10.6% (n = 14) (Fig. 3*A–C*).

The 5HT receptor antagonists ketanserin and ICS-205-930 were tested for their ability to antagonize the increase in Na^+ currents by 5HT. The effect of 10 μM 5HT on Na^+ currents was not affected by ketanserin (1 μM) in three cells tested (Fig. 3*C*); however, as illustrated in Figure 4*A–D*, the $5HT_3/5HT_4$ receptor antagonist ICS-205-930 was an effective blocker of the 5HT-induced increase in Na^+ current. Figure 4*A–C* illustrates an example in which 1 μM 5HT produced only a 27% increase in Na^+ current when tested in the presence of 10 μM ICS-205-930, whereas a subsequent challenge with 1 μM 5HT after washout of ICS-205-930 resulted in a 69% increase in Na^+ current amplitude.

The results of several experiments with ICS-205-930 are summarized in Figure 4*D*. In this series of studies, 1 μM 5HT increased Na^+ current by an average of 94 ± 13% in nine control type 2 DRG cells, in which 5HT was the only substance tested. In additional type 2 cells, isolated from the same group of rats as the control cells, 1 μM 5HT increased Na^+ current by 52 ± 7.0% (n = 6), 28 ± 1.7% (n = 4), and 0 ± 0% (n = 4) in the presence of 1, 10, and 100 μM ICS-205-930, respectively (Fig. 4*D*). The increase in Na^+ currents produced by 5HT in the presence of each of the three concentrations of ICS-205-930 was significantly less than in control cells ($p < 0.05$; Neuman–Keuls multiple-range test). By extrapolation from the graph of ICS-205-930 antagonism of 5HT (Fig. 4*D*), it is estimated that the EC_{50} for ICS-205-930 regarding 5HT-induced increase in Na^+ current was ~2 μM.

As illustrated in Fig. 4*D*, there was an inverse relationship between the degree of antagonism produced by the different concentrations of ICS-205-930, and the increase in Na^+ current produced by a second challenge with 5HT after the ICS-205-930 was washed out. When the effect on Na^+ current amplitude of an initial challenge with 5HT was completely antagonized by 100 μM ICS-205-930, a subsequent challenge with 5HT, after washout of antagonist, produced an increase in Na^+ current that was similar in magnitude to that observed in control type 2 cells (Fig. 4*D*). When the effect of 5HT was only partially antagonized by lower concentrations of ICS-205-930, however, a subsequent challenge with 5HT after washout of antagonist produced a submaximal response, which was roughly inversely proportional to the degree of the preceding antagonism (Fig. 5*D*). This data could be explained by the idea that ICS-205-930 prevented activation of a population of 5HT receptors, thus preventing them from participating in activation of the persistent and saturable increase in Na^+ current. Thus, higher concentrations of ICS-205–930 left more of the response available for subsequent activation by 5HT.

Because the pharmacological profile of the 5HT receptor mediating the increase in Na^+ currents was consistent with a $5HT_4$ receptor (see Discussion) that has been shown to couple positively to adenyl cyclase in other systems, we tested the possibility that cAMP was involved in the increase in Na^+ current by 5HT. Superfusion of type 2 cells for up to 5 min with a 200 μM concentration of a membrane-permeant cAMP mimetic (8-chlorophenylthio-cAMP) did not produce any increase in the amplitude of Na^+ currents (Fig. 4*B,C*). In these same seven cells, 5HT (10 μM) produced a 54.6 ± 9.5% increase in Na^+ current.

It has been reported that PGE_2 and adenosine also increase TTX-resistant Na^+ currents in cultured DRG neurons (Gold et al., 1996b). We tested the effects of PGE_2 and 2-chloroadenosine (a general adenosine agonist) on Na^+ currents in types 1, 2, 3, and 4 DRG cells to observe how the effects of these agents on Na^+ currents overlapped with those of 5HT on these same cell types. We observed that PGE_2 produced an increase in TTX-resistant and TTX-sensitive Na^+ current in capsaicin-sensitive

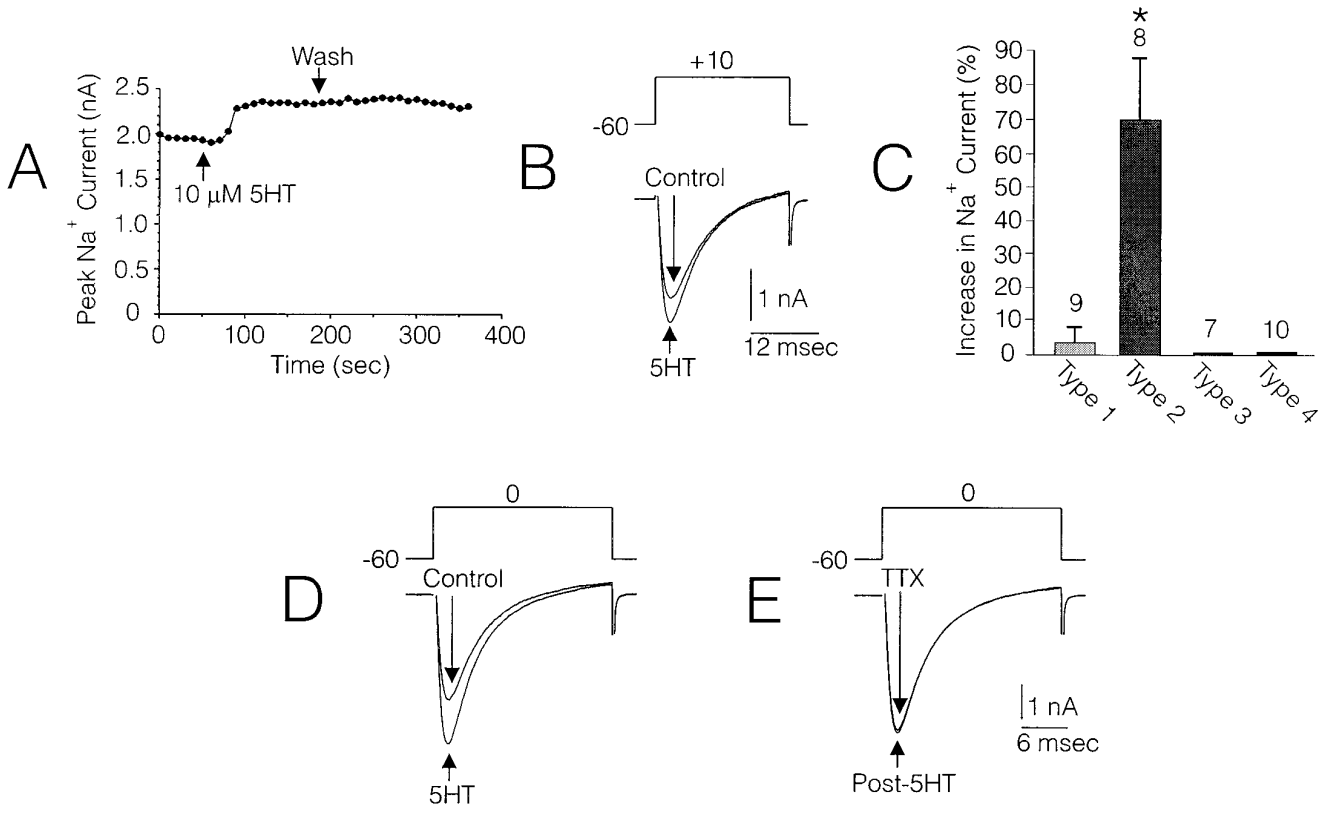

Figure 2. Effects of 5HT on Na^+ currents in types 1, 2, 3, and 4 DRG cells. *A*, Plot of peak Na^+ current versus time in a type 2 DRG cell, illustrating an increase in Na^+ current amplitude produced by superfusing the cell with 10 μM 5HT. Na^+ currents were evoked every 10 sec using a test command to +10 mV from a holding potential of −60 mV. *B*, Individual Na^+ current sweeps taken from the same experiment depicted in *A*, before (*Control*) and during (*5HT*) superfusion of the cell with 10 μM 5HT. *C*, *Bar graph* illustrating the average effect of 5HT on Na^+ current amplitude in types 1, 2, 3, and 4 DRG cells. Solutions were the same as those used to record Na^+ currents in Figure 1. *D, E*, Illustration that 5HT increased TTX-insensitive Na^+ current. *D*, Current sweep under control conditions (*Control*) and after exposure of the type 2 cell to 10 μM 5HT (*5HT*). *E*, Na^+ current sweeps are shown before (*Post-5HT*) and after superfusion with 1 μM TTX (*TTX*), after the Na^+ current had been increased by 5HT in the same cell as that depicted in *D*.

and capsaicin-insensitive acutely isolated DRG cells, respectively. PGE_2 (4 or 10 μM) increased Na^+ current by 5% or greater in 3 of 12 type 1 cells, in 13 of 17 type 2 cells, in 2 of 8 type 3 cells, and in 8 of 11 type 4 cells (Fig. 4). In the cells that responded to PGE_2, the average increase in Na^+ current was 88.9 ± 56.3% (n = 3), 29.8 ± 3.9% (n = 13), 18.9 ± 7.6% (n = 2), and 13 ± 2.5% (n = 8) in types 1, 2, 3, and 4 DRG cells, respectively.

Similar to the responses to 5HT, the increase in Na^+ current by PGE_2 was also slow in onset and persisted after washout of PGE_2. Also, as with 5HT, the increase in Na^+ current by PGE_2 was caused by an increase in TTX-insensitive Na^+ current in types 1 and 2 DRG cells. In contrast, the increase in Na^+ current by PGE_2 in types 3 and 4 cells was caused by an increase in TTX-sensitive Na^+ current. This was evidenced by the complete blockade of Na^+ current by 1 μM TTX after exposure to PGE_2 (n = 11).

Although adenosine (1 μM) has been reported to increase Na^+ currents in capsaicin-sensitive cultured DRG cells, the nonselective adenosine receptor agonist 2-chloroadenosine (10 μM) was observed to have no effect on Na^+ currents in nine of the type 2 cells tested. 2-Chloroadenosine was not tested on types 1, 3, or 4 DRG cells.

DISCUSSION

Several studies suggest the possibility that the release of 5HT and PGE_2 near sites of injury may contribute to hyperesthetic pain. In various *in vivo* preparations, exposure to 5HT and PGE_2 has been demonstrated to produce an increase in the excitability of several types of nociceptors (Handwerker, 1976; Kumazama and Mizumura, 1980; Mense, 1981; Martin et al., 1987; Schaible and Schmidt, 1988; Lang et al., 1990; Grubb et al., 1991; Herbert and Schmidt, 1992; Taiwo and Levine, 1992); however, the mechanisms underlying this action of 5HT and PGE_2 are not completely understood. One idea is that these agents promote edema in injured tissues, which facilitates the transmission of mechanical stimulus to the nerve endings (Cooper, 1993); however, the observation that 5HT and PGE_2 produced an increase in Na^+ currents in subgroups of DRG cell bodies with characteristics similar to nociceptors suggests the idea that these substances may produce a similar change in Na^+ current in nociceptor endings, which could also contribute to hyperesthetic pain. An increase in the availability of voltage-activated Na^+ current at sites of action potential initiation could increase nociceptor excitability as well as suprathreshold reactivity.

Our observations that 5HT and PGE_2 increased Na^+ current amplitude in various subpopulations of acutely isolated DRG cells is in general agreement with a previous study in cultured DRG cells (Gold et al., 1996b). In contrast to the previous study, however, we found that PGE_2 increased *TTX-sensitive* Na^+ currents in several type 3 and type 4 DRG cells. In the report by Gold et al. (1996b), PGE_2 increased only TTX-resistant Na^+

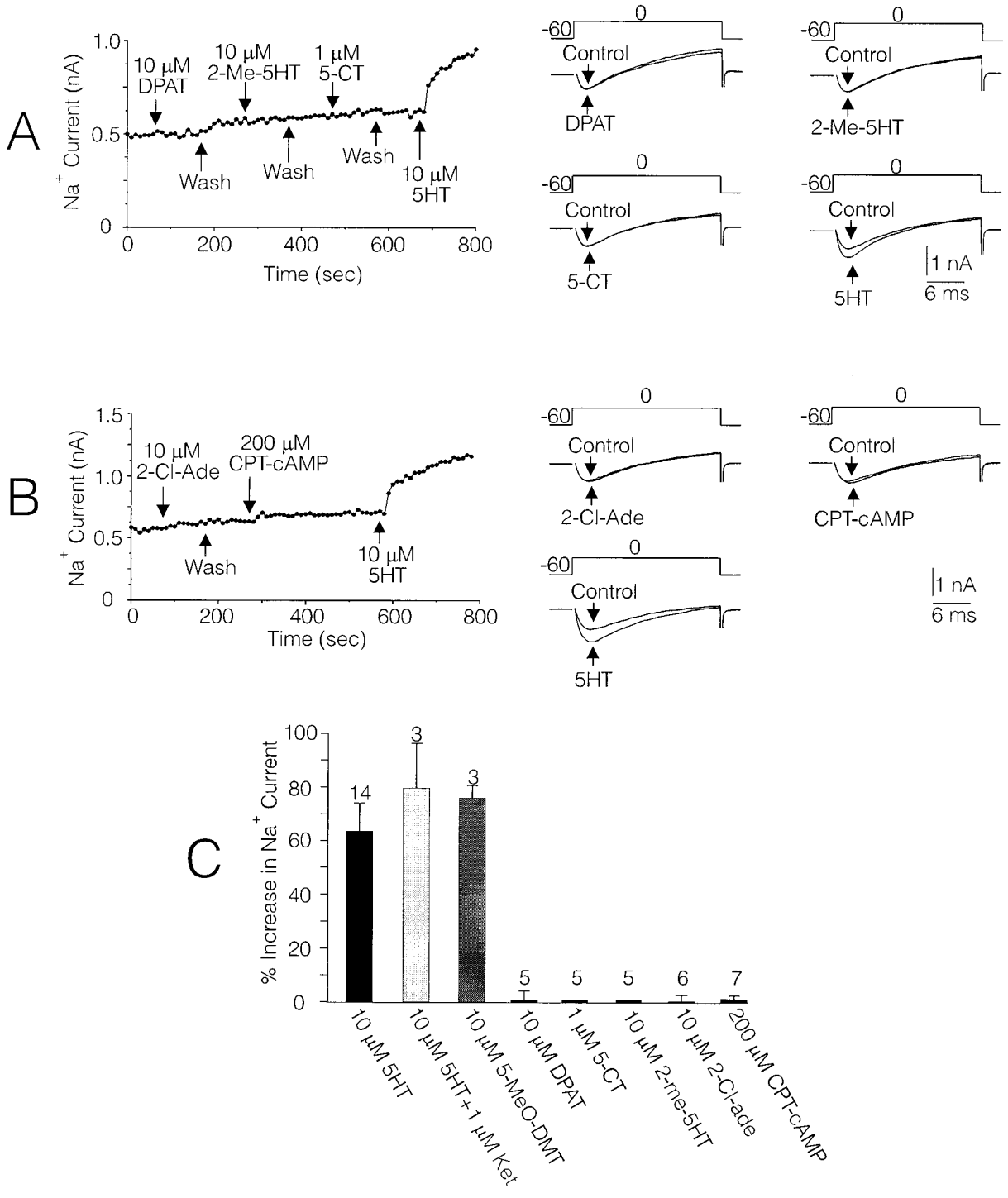

Figure 3. Lack of effect of several 5HT-receptor ligands, 2-chloroadenosine(2-Cl-ade), and 8-chlorophenylthio-cAMP (CTP-cAMP) on Na^+ currents in type 2 DRG cells. *A, B*, Plots of peak Na^+ current versus time in type 2 DRG cells, illustrating the effects of various agents on Na^+ current amplitude. To the *right* of each plot are individual Na^+ current sweeps taken from the corresponding experiments, before and during superfusion of the cell with the various agents as labeled. *C*, *Bar graph* illustrating the average effects of the different agents on Na^+ current amplitude as listed under each bar. The *small bars* corresponding to 5-CT and 2-me-5HT are markers only. The average change was zero in both cases. Solutions were the same as in Figure 2.

current. Also, in the Gold et al. (1996b) study, adenosine was observed to increase Na^+ currents in 64% of cultured DRG cells tested; however, we did not observe any affect of the adenosine receptor agonist 2-chloroadenosine on Na^+ currents in nine type 2 DRG cells tested. These disparities are possibly attributable to differences in sampling and survival of different types of DRG cells when subjected to the acute isolation procedure versus culture conditions. Also, some disparities may have arisen from changes in gene expression in cultured DRG cells caused by exposure to neurotrophins (e.g., Zur et al., 1996).

Data from the present study suggest that 5HT primarily affects Na^+ currents in nociceptors, whereas PGE_2 may affect Na^+

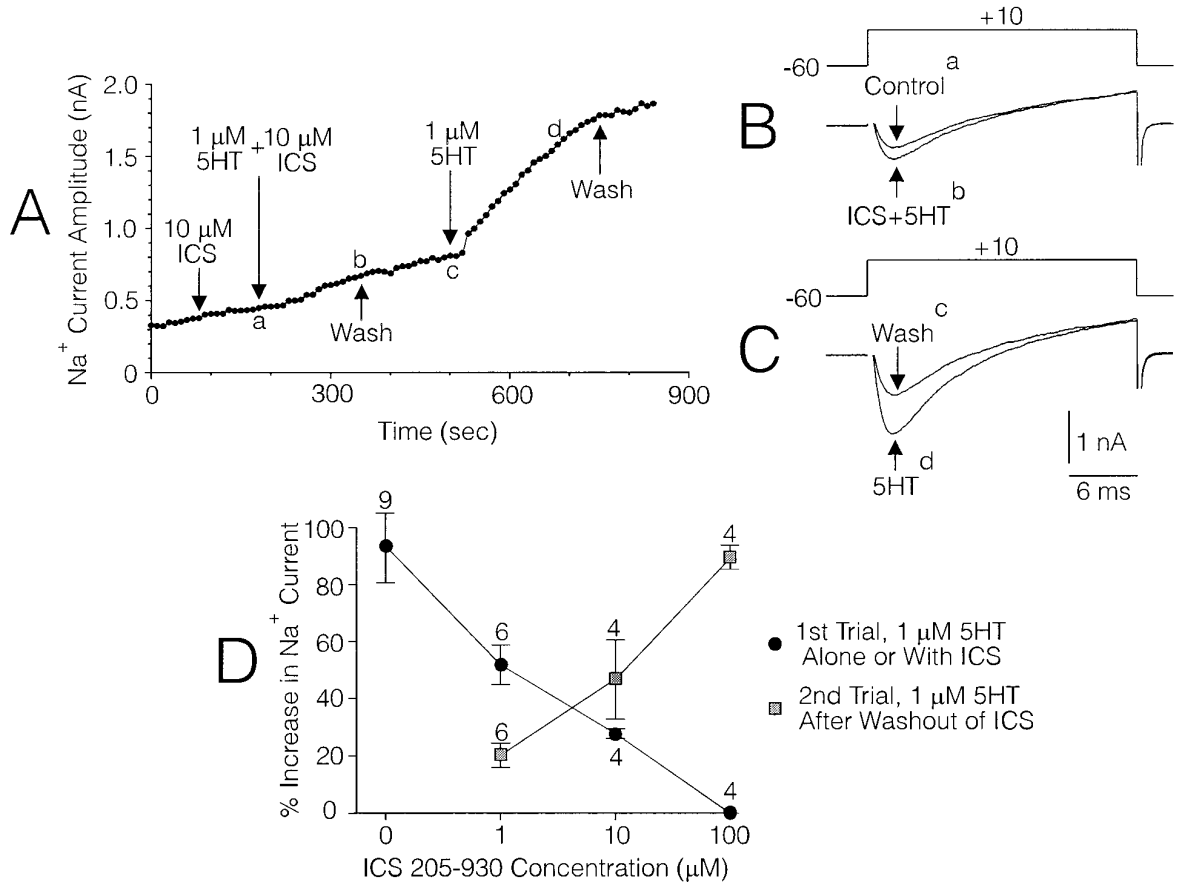

Figure 4. Antagonism of the 5HT-induced increase in Na^+ current amplitude by ICS-205-930. *A*, Plot of peak Na^+ current versus time illustrating the effects of 5HT on Na^+ current when administered in the presence of 10 μM ICS-205-930, and the effects of a subsequent challenge with 5HT after washout of ICS-205-930. *B, C*, Individual Na^+ current sweeps taken from the same experiment depicted in *A*, under control conditions (*Control*) and 170 sec after superfusion of the cell with 1 μM 5HT + ICS-205-930 (*ICS+5HT*) (*B*), and under control conditions after washout of ICS and 5HT, and 170 sec after superfusion of the cell with 1 μM 5HT. The *superscript letters* (*a–d*), adjacent to the sweep labels, correspond to the letters in the plot of current versus time, illustrating where the sweeps were from in the plot. *D*, Plot of the average increase in Na^+ current observed after superfusion of several type 2 cells with 1 μM 5HT only, or in the presence of increasing concentrations of ICS-205-930 (*black circles*), or the increase in Na^+ current produced by a second challenge of 5HT after washout of the initial treatment of ICS-205-930 and 5HT (*gray squares*). Error bars equal SEM. Solutions were the same as in Figure 2.

currents in subpopulations of both nociceptive and non-nociceptive sensory neurons. The observation of TTX-insensitive Na^+ currents in type 1 and type 2 DRG cells in this study adds to the list of characteristics previously observed by us in these cells (small-diameter cell bodies, capsaicin sensitivity, paucity of I_H) that are consistent with those of C-type nociceptors (Yoshida and Matsuda, 1979; Harper and Lawson, 1985a,b; Holzer, 1991; Scroggs et al., 1994; Cardenas et al., 1995; Villiere and McLachlan, 1996). Thus, the observation that 5HT selectively targeted Na^+ currents in type 2 cells suggests the possibility that 5HT primarily has this effect on nociceptors.

On the other hand, PGE_2 was observed to affect Na^+ currents in some type 3 and type 4 DRG cells as well as a portion of type 1 and type 2 DRG cells. The TTX-sensitive Na^+ currents, prominent I_H, and small- or medium-diameter cell bodies characteristic of type 3 and type 4 DRG cells overlap well with the characteristics of Aδ-type sensory neurons (Yoshida and Matsuda, 1979; Harper and Lawson, 1985a,b; Holzer, 1991; Scroggs et al., 1994; Villiere and McLachlan, 1996). In addition, the prominent I_{IR} and large T-type Ca^{2+} currents expressed by the medium-diameter type 4 DRG cells (Scroggs et al., 1994; Cardenas et al., 1995) are consistent with the electrical properties exhibited by a subpopulation of medium-diameter Aδ-type sensory neurons recorded from in whole ganglia (Villiere and McLachlan, 1996). The insensitivity of type 3 and type 4 DRG cells to capsaicin suggests that they are not nociceptors. Thus, the observation that PGE_2 increased Na^+ currents in type 3 and type 4 DRG cells suggests the possibility that PGE_2 can have this effect in both nociceptors and non-nociceptors. Insensitivity to capsaicin, however, is not conclusive evidence of non-nociceptive afferents, because some Aδ high-threshold mechanoreceptors (which may be nociceptors) are not capsaicin sensitive (Szolcsanyi et al., 1988).

Previous studies using classification schemes based on cell body diameter, action potential duration, capsaicin sensitivity, conduction velocity, or mechanothermal reactivity have been unable to demonstrate subpopulations of nociceptors that react uniformly to any endogenous proinflammatory agent (Mense, 1981; Fowler et al., 1985; Heppelmann et al., 1987; Lang et al., 1990; Herbert and Schmidt, 1992; Gold et al., 1996a,b). On the other hand, our DRG cell classification system (Cardenas et al., 1995) has produced subgroups with remarkable homogeneity regarding the effects of 5HT on ion channels. In the present investigation, 47 of 48 type 2 cells were shown to manifest 5HT-sensitive Na^+ cur-

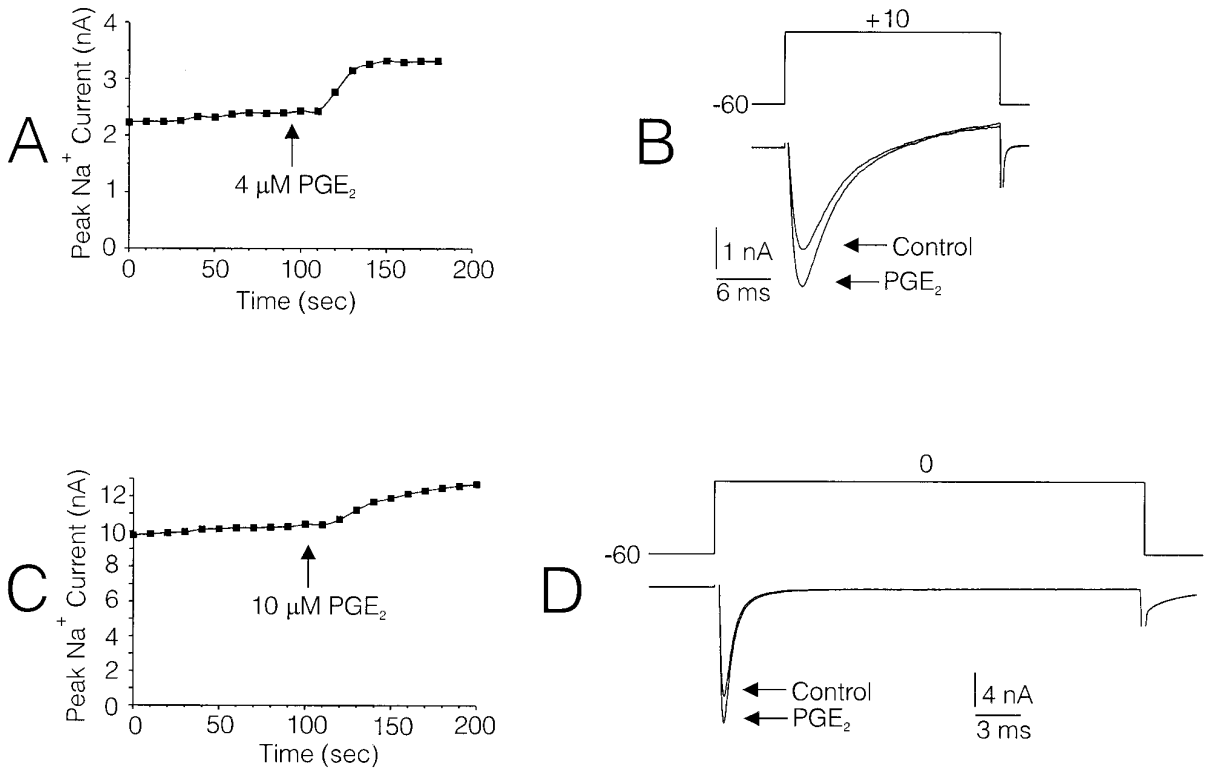

Figure 5. Effects of PGE_2 on Na^+ currents in a type 2 DRG cell and a type 4 DRG cell. *A, C*, Plots of peak Na^+ current versus time in a type 2 DRG cell (*A*) and a type 4 DRG cell (*C*), illustrating the increase in Na^+ current amplitude produced by superfusion of the cells with PGE_2. *B*, Individual Na^+ current sweeps taken from the same experiment depicted in *A*, before (*Control*) and during (*PGE_2*) superfusion of the cell with 4 μM PGE_2. *D*, Individual Na^+ current sweeps taken from the same experiment depicted in *C*, before (*Control*) and during (*PGE_2*) superfusion of the cell with 10 μM PGE_2. The solutions were the same as in Figure 2.

rents, whereas there was little evidence of 5HT-sensitive Na^+ currents in type 1 DRG cells (1 of 10 cases) or in type 3 or type 4 DRG cells (0 of 7 and 10 cases, respectively). Also, a previous study demonstrated that 5HT (acting at $5HT_{1A}$ receptors) produces substantial inhibition of voltage-activated Ca^{2+} currents in type 1 cells and has little effect on Ca^{2+} currents in types 2, 3, and 4 DRG cells (Cardenas et al., 1995). In addition, another previous study indicated that all type 2 DRG cells and ~50% of type 1 DRG cells, but not type 3 or type 4 DRG cells, expressed a lactoseries carbohydrate antigen (Galβ1–4GlcNAc-R) found on sensory neurons that terminate in lamina I and II of the spinal cord, similar to most nociceptors (Dodd and Jessell, 1985; Del Mar and Scroggs, 1996). These data support the idea that if appropriate criteria are used, classification of DRG cells by their repertoire of ion currents can generate categories that are functionally significant.

Several lines of evidence suggest that the 5HT receptors which mediate an increase in Na^+ currents in type 2 cells belong to the $5HT_4$ category. The sensitivity of these receptors to activation by 5-methoxy-*N,N*-dimethyl tryptamine (10 μM) but not by (+)-8-OH-DPAT (10 μM), 5-carboxyamidotryptamine (1 μM), or 2-methyl-5HT (10 μM) is consistent with the agonist profile of $5HT_4$ receptors and inconsistent with most other 5HT receptors, with the exception of the $5HT_{1E}$ and the $5HT_6$ receptors. Similarly, high concentrations of ICS-205-930 needed to produce significant antagonism and the lack of antagonism by ketanserin are consistent with the antagonist profile of $5HT_4$ receptors and inconsistent with most other 5HT receptors, including the $5HT_{1E}$ and $5HT_6$ receptors (Andrade and Chaput, 1991; Reeves et al., 1991; Bockaert et al., 1992; Fagni et al., 1992; Boess and Martin, 1994; Martin and Humphrey, 1994).

Gold et al. (1996b) presented evidence that 5HT receptors are coupled to Na^+ channels via a pathway involving a cytosolic diffusible component. The time course of the increase in Na^+ currents by 5HT in type 2 cells is consistent with this idea. The drug delivery system we use takes <4 sec to completely change the solution surrounding a cell under study. Thus the observation that onset of the 5HT-induced increase in Na^+ currents began 20–30 sec after superfusion with 5HT was initiated is more consistent with a slower cytosolic diffusible pathway versus the rapid transduction expected via membrane-delimited pathways (Brown, 1993).

There are numerous reports demonstrating that $5HT_4$ receptor activation can lead to an increase in cAMP levels (Boess and Martin, 1994); however, the lack of effect of the membrane-permeant cAMP analog 8-chlorophenylthio-cAMP (200 μM) in 5HT-sensitive type 2 cells suggests the possibility that cAMP is not involved in the $5HT_4$ receptor coupling to Na^+ channels in type 2 DRG cells. Previous experiments have shown that 50–200 μM concentrations of 8-chlorophenylthio-cAMP enter cells well within the time frame used in these experiments and strongly activate cAMP-dependent pathways (Artalejo et al., 1990; Surmeier et al., 1995). The above data do not rule out a role for cAMP in hyperesthesia, which is suggested by experiments both in cultured cells (Weinreich, 1986; Grega and MacDonald, 1987) and in whole animals (Taiwo et al., 1992; Khasar et al., 1995; Wang et al., 1996). It is possible that cAMP modulates ion

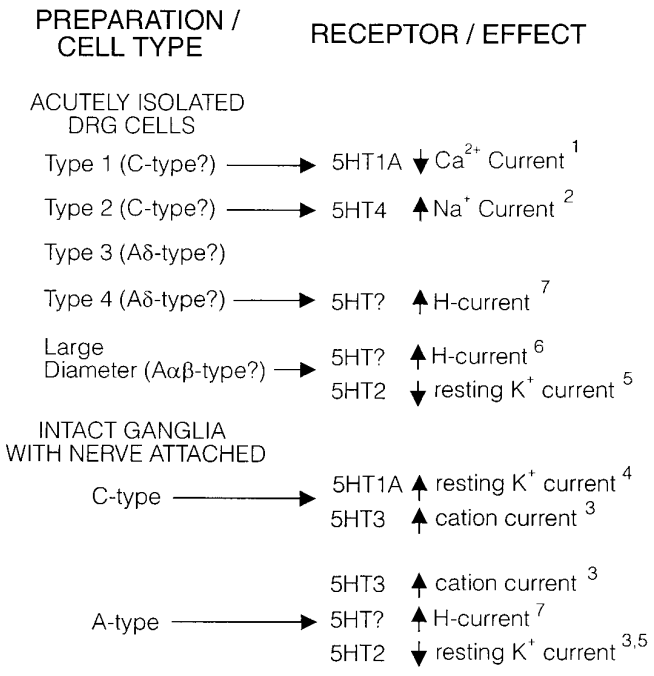

Figure 6. Diagram illustrating different 5HT receptor subtypes coupling to various ion currents in different subpopulations of DRG cells. For acutely isolated DRG cells the corresponding DRG neuron type (*C* or *A*, based on conduction velocity) is only speculation, based on various cell characteristics such as cell diameter. For DRG cells recorded from intact ganglia with nerve attached, the conduction velocity was measured. In some instances more than one response to 5HT is listed for a particular group of cells. Sometimes these multiple responses to 5HT were demonstrated in the same cell, whereas in other cells the responses occurred separately. The incompletely characterized 5HT receptor (*5HT?*) does not correspond to $5HT_{1A}$, $5HT_2$, $5HT_3$, or $5HT_4$ receptors (R. Scroggs, unpublished observations). [1] Cardenas et al. (1995); [2] this study; [3] Todorovic and Anderson (1990ab); [4] Todorovic and Anderson (1992); [5] Todorovic et al. (1997); [6] Anderson and Scroggs (1993); [7] R. Scroggs (unpublished observations).

currents other than Na^+ or that cAMP modulates Na^+ channels in cell types not sampled in the present study.

An interesting pattern of effects of 5HT on different subpopulations of DRG neurons is emerging (Fig. 6). Several different 5HT receptors, including the $5HT_{1A}$, $5HT_2$, $5HT_3$, $5HT_4$, and an incompletely characterized 5HT receptor that does not correspond to any of the above, are coupled to various ion currents (high-threshold Ca^{2+} current, resting K^+ current, ligand-gated cation current, Na^+ current, and I_H) in different subpopulations of DRG cells (Todorovic and Anderson, 1990a,b, 1992; Cardenas et al., 1995; Scroggs and Anderson, 1995; Todorovic et al., 1997) (R. Scroggs, unpublished observations). Thus, 5HT released into the spinal cord or in the periphery may target a wide variety of sensory information and affect the transmission of different types of information in different ways.

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