Subclassified Acutely Dissociated Cells of Rat DRG: Histochemistry and Patterns of Capsaicin-, Proton-, and ATP-Activated Currents

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INTRODUCTION

Acute and primary cultures of dorsal root ganglion (DRG) cells are commonly employed in investigations of sensory physiology. Interpretation of the data from such studies is confounded by the complexity of the DRG cell population. Cells harvested from the DRG could represent more than 25 functionally diverse afferent populations that are specialized to encode a variety of sensory events. Cultured cell bodies formerly gave rise to peripheral processes unique to muscle (anulospiral, flowerspray, golgi), joint (Ruffini, Pacinian), touch (Pacinian, Merkel, Meisner, Ruffini, guard hair, down hair, Aδ and C itch), warmth (C warm), cooling (Aδ and C cooling), and pain (noted below) sensations (Darian-Smith 1984a,b; Iggo 1985). While nociceptors are probably the afferents of greatest interest, they are composed of highly specialized subpopulations.

Classic sharp electrode and crushed end recordings have revealed a diverse and highly specialized nociceptive population, in vivo. A number of functional subtypes have been characterized in numerous preparations. Careful characterization of afferent properties have revealed a nociceptor population that includes representatives of Aβ, Aδ, and C fiber, cutaneous, deep, muscle, and visceral nociceptive subgroups that express single and multiple transduction capacities in various combinations. This diverse nociceptor pool would include Aβ high-threshold mechanoreceptors (HTM), Aδ HTM, Aδ polymodal nociceptors (PMN), Aδ mechanoreceptors (MM) nociceptors, Aδ cold nociceptors, C HTM, C PMN, C δ nociceptors, C heat nociceptors, C chemonociceptors, and Aδ and C “silent” nociceptors (Belmonte and Giraldez 1981; Campbell et al. 1990; Cervero 1994; Cooper and Sessle 1993; Mense 1993; Schable and Grubb 1993; Tanelian and Beuer- man 1984). The advantage of being able to study these subpopulations in vitro is manifest. However, identification of specific nociceptive subgroups in DRG culture with anything approaching the detail that is possible with classic in vivo methods has proven difficult.

To distinguish nociceptive from nonnociceptive groups in vitro, laboratories have relied on evidence that a number of...
anatomic and functional properties of nociceptors covary with cell size and/or action potential shape (e.g., presence of a “hump” or “shoulder”). These criteria have been shown to be correlates of thinly myelinated and unmyelinated fiber groups that include the majority of nociceptive subpopulations (Harper and Lawson 1985; Yoshida and Matsuda 1979). Cell size and action potential shape also correlate well with high-threshold mechanoreception (Djouhri et al. 1998; Koerber et al. 1988; Ritter and Mendel 1992; Rose et al. 1986), a property that is present in most nociceptive subgroups (see above). However, laboratories working in vitro have relied mainly on a binary, pharmacological classification scheme in which nociceptive cells are identified a posteriori, according to capsaincin sensitivity. These schemes, while useful, cannot hope to reveal the rich diversity of a nociceptive population that is comprised of 10 or more distinct phenotypes that include a number of important capsaincin insensitive subtypes.

It is likely that capsaincin sensitivity can identify perhaps four subsets of heat-reactive nociceptive cells, but it cannot distinguish between them. In vivo, capsaincin activates a large portion of the C heat nociceptive, C PMN, and at least half of the C MH nociceptive pool (Baumann et al. 1991; Belmonte et al. 1991; Chen et al. 1997; Szelcsányi et al. 1988). A portion of the Aδ MH and Aδ silent nociceptor group is also activated (Baumann et al. 1991; Meyer et al. 1991; Szelcsányi et al. 1988; Treede et al. 1998). Moreover, it is not generally acknowledged that warm fibers, as well as some Aβ low-threshold mechanoreceptors, are also capsaincin sensitive (Baumann et al. 1991; Szelcsányi et al. 1988). Therefore the identification of nociceptors based solely on capsaincin reactivity will result in inclusion of several functional cell types, many of which may be nociceptive, but belong to functionally distinct nociceptive subclasses, and still others cells that are not a part of the pain system. In addition, reliance on identification of nociceptive populations by capsaincin sensitivity alone will certainly leave an important portion of the nociceptive population (Aδ and C high-threshold mechanoreceptors, Aδ and C mechanocold, Aδ and C silent, C chemosensitive) inaccessible to investigation in vitro.

To identify functional nociceptive DRG subpopulations in vitro, it is first necessary to develop criteria that can readily classify cells into distinct, but internally homogeneous subpopulations whose properties can be determined and combined across a series of experiments. Additional criteria are then needed to determine which cells are nociceptive and the specific nociceptive subpopulations they represent.

The work of the Scroggs and Lawson laboratories represent two recent, and complementary approaches to cell classification and nociceptor specification (Cardenas et al. 1995; Djouhri et al. 1998; Lawson 1996; McCarthy and Lawson 1997). Although differing in detail, both of these methods are ultimately based on the notion that functionally uniform cell populations can be identified by their repertoire of voltage-activated currents. Due to the great diversity of voltage-activated currents in DRG (Dib-Hajj et al. 1998; Gold et al. 1996; Mayer and Westbrook 1983; Roy and Narahashi 1992; Tate et al. 1998), these techniques have the potential to provide criteria by which a large number of DRG subpopulations might be distinguished. Four subpopulations of cells (types 1, 2, 3, and 4) have been characterized by Scroggs and associates. These populations are internally uniform with respect to reactivity to capsaincin and serotonin (Cardenas et al. 1995, 1997a,b).

Methods developed by the Lawson laboratory complement this approach by providing criteria to more completely identify nociceptive subgroups. Using sharp electrode recordings and traditional characterization methods, Lawson and colleagues (Djouhri et al. 1998; Lawson 1996; McCarthy and Lawson 1997) have shown that the duration of afterhyperpolarization distinguishes 1) nociceptive C and Aδ subgroups from nonnociceptive Aβ fibers, 2) nociceptive from nonnociceptive groups from within the Aδ and C fiber categories, and 3) mechanically sensitive from mechanically insensitive (silent) nociceptor populations. Importantly, these criteria do not rely on capsaincin sensitivity, thus permitting capsaincin-insensitive nociceptive populations to be revealed. While this technique has been shown to work well in vivo, it is not clear that it can be successfully applied in vitro.

In the experiments described below, we used cluster analysis to form nine subclasses of cells that were distinguished by voltage-activated current signatures. The current signature method is an extension of procedures that were first applied to DRG cell classification by Scroggs and Cardenas (Cardenas et al. 1995). To determine whether these nine subgroups (clusters) had predictive validity, we have examined whether each subtype was homogenous with respect to histochemical phenotype (binding of the B4 isoelectin from *Griffonia simplicifolia* (IB4), immunoreactivity (IR) for substance P (SP) and calcitonin gene–related peptide (CGRP), algesic reactivity (capsaincin, protons, ATP), and action potential features (duration, afterhyperpolarization). In the present report, current signatures of the nine cell clusters and the properties of five of these subclassified cell groups are presented in detail. Some of these data have been published in abstract form (Cooper et al. 1999a,b).

Methods

Subjects

Adult male Sprague-Dawley rats (90–110 g) were anesthetized with a combination of xylazine (4 mg/kg) and ketamine (40 mg/kg). Following decapitation, the spinal cord was rapidly removed, and the dorsal root ganglia were dissected free. All animals were housed in American Association for Accreditation of Laboratory Animal Care–approved quarters, and all procedures were reviewed and approved by the local Institutional Animal Care and Use Committee.

Preparation of cells

Dissected ganglia were placed in a tube containing dispase (neutral protease, 5 mg/ml; Boehringer Mannheim) and collagenase (2 mg/ml; Sigma type 1). The tube was shaken for 60 min in a heated (35°) bath. Following wash and trituration, recovered cells were plated on 6–10 polylysine-coated Petri dishes. Dishes were mounted on a Nikon Diaphot inverted microscope or kept in an aerated holding bath for later use. All recordings were completed within 10 h of plating.

Whole cell patch recording

Glass pipettes (Scientific Products B4416-1) were prepared (2–4 MΩ) with a Brown and Flaming type horizontal puller (Sutter model P87). Whole cell recordings were made with an Axopatch 200B (Axon Instruments). Stimuli were controlled and digital records captured with pClamp 6.0 software and Digipack 1200b A/D converter.
among the smallest diameter cells (16–22 μm). Cell diameter was estimated from the average of the longest and shortest axis as measured through an eyepiece micrometer scale. From among the smallest diameter cells (16–22 μm), recordings were made only from cells with large nuclei (10–15 μm). Cells were classified according to patterns of voltage-activated currents (current signatures) that were revealed by three classification protocols. 1) Classification Protocol 1 (CP1) was used to examine the pattern of hyperpolarization-activated currents. With CP1, currents were evoked by a series of hyperpolarizing pulses presented from a V_{th} of −60 mV (10 mV per step to a final potential of −110 mV; 500-ms, 4-s interstimulus interval), 2) Classification Protocol 2 (CP2) was used to produce outward current patterns. From a V_{th} of −60 mV, a 500-ms conditioning pulse to −100 mV was followed by 200-ms depolarizing command steps (20 mV) to a final potential of +40 mV. 3) Classification Protocol 3 (CP3) was used to produce inward current patterns. With the cell held at −60 mV, a 500-ms conditioning pulse to −80 mV was followed by a series of depolarizing command steps (10-mV steps, 2.0 ms duration) to a final potential of +10 mV. The three protocols are illustrated in Fig. 1.

**Cluster analysis**

To determine whether cells could be subclassified according to their pattern of voltage-activated currents, cluster analysis was performed using up to five cluster variates that were derived from inward and outward current signatures. Two characteristics were quantified from the hyperpolarization test protocol. Hyperpolarization-activated currents (HAC pA) were assessed as the total inward current measured during the last hyperpolarization step (−110 mV). Transient outward current (TOC pA) was measured as the peak current, in pA (relative to baseline) during the final 500 ms that followed repolarization to −60 mV. The decay constant τ_{CP3} was derived from single or double exponential fits to the final outward current trace (+40 mV). The activation threshold of A-current peaks is indicated as well (AT). The decay constant τ_{CP2} was derived from single exponential fits to the inactivation phase of the first complete inward current trace. Fits were made between arrowheads. When double exponentials were required, the fastest component was used.

**Cell classification protocols**

Cells were preselected according to diameter. Recordings were made exclusively from cells with diameters between 16 and 48 μm. Cell diameter was estimated from the average of the longest and shortest axis as measured through an eyepiece micrometer scale. From among the smallest diameter cells (16–22 μm), recordings were made only from cells with large nuclei (10–15 μm). Cells were classified according to patterns of voltage-activated currents (current signatures) that were revealed by three classification protocols. 1) Classification Protocol 1 (CP1) was used to examine the pattern of hyperpolarization-activated currents. With CP1, currents were evoked by a series of hyperpolarizing pulses presented from a V_{th} of −60 mV (10 mV per step to a final potential of −110 mV; 500-ms, 4-s interstimulus interval), 2) Classification Protocol 2 (CP2) was used to produce outward current patterns. From a V_{th} of −60 mV, a 500-ms conditioning pulse to −100 mV was followed by 200-ms depolarizing command steps (20 mV) to a final potential of +40 mV. 3) Classification Protocol 3 (CP3) was used to produce inward current patterns. With the cell held at −60 mV, a 500-ms conditioning pulse to −80 mV was followed by a series of depolarizing command steps (10-mV steps, 2.0 ms duration) to a final potential of +10 mV. The three protocols are illustrated in Fig. 1.

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**Action potential**

A 1-ms, 1,500-pA current step was used to determine afterhyperpolarization (AHP) and action potential duration at the base (APDb). To quantify AHP, we used a criterion of 80% recovery to baseline (AHP80) (Lawson et al. 1997). Measurements are detailed in Fig. 2.

**Drugs and solutions**

Plated cells were superfused in rat Tyrode’s solution containing (in mM) 140 NaCl, 4 KCl, 2 MgCl₂, 2 CaCl₂, 10 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH. Recordings were made 3–10 h after plating at room temperature. Test solutions were applied via gravity-fed pipette positioned approximately 1 mm from the cell (sewer pipe). The recording electrodes were filled with (in mM) 120 KCl, 5 Na₂ATP, 0.4 Na₂GTP, 5 EGTA, 2.25 CaCl₂, 5 MgCl₂, and 20 HEPES, adjusted to pH 7.4 with KOH; osmolarity was approximately 315–325 mosm. Capsaicin was prepared from a 10-nM stock solution (in 100% ethanol) to a final concentration of 500 to 5,000 nM. The final concentration of ethanol was <0.1%. ATP and αβ-m-ATP were...
Cluster analysis

Cells were classified based on currents evoked by a series of three classification protocols (Fig. 1). Classification protocols 1, 2, and 3 produced distinct patterns of hyperpolarization and depolarization activated inward and outward currents (Fig. 3). Five measures of current amplitude and inactivation were used to form variables that were suitable for cluster analysis (HAC, TOC, \( \tau \text{CP2}, \tau \text{CP3}, \text{AT} \)).

Using a K means procedure, we identified nine clusters of cells from very small (<22 \( \mu \text{m} \); \( n = 38 \)), small (24–32 \( \mu \text{m} \); \( n = 56 \)), and medium (32–48 \( \mu \text{m} \); \( n = 59 \)) -sized neurons. Within the small cell population, two major subpopulations were formed by clustering on two current signatures variables (TOC, \( \tau \text{CP2} \)). These were labeled types 1 and 2. Among very small-sized cells, cluster analysis identified two major subpopulations based on two cluster variables (HAC, \( \tau \text{CP3} \)). These cells were labeled types 3 and 7. Large GCC scores were obtained with both solutions (see Table 1). The greatest variety of cells was observed in the medium cell range. Here, using five cluster variables (HAC, TOC, \( \tau \text{CP2}, \tau \text{CP3}, \text{AT} \)), we identified five cell subpopulations. These were given labels types 4, 5, 6, 8, and 9. Current signatures that were representative of the cell clusters are illustrated in Fig. 3. Predictive validity of the clusters was determined by examining the physiological, pharmacological, and histochemical properties of the cells within each cluster (\( n = 170 \)). If the cell properties of each cluster are uniform, a good cluster solution has been found.

Type 1 cell

CELL IDENTIFICATION AND ACTION POTENTIAL. Cells clustering in the type 1 group (\( n = 32 \)) exhibited little hyperpolarization or transient outward current (Fig. 3; Table 1). All type 1 cells had small cell body diameters (24–30 \( \mu \text{m} \); 31.4 ± 1.1 pF, mean ± SE), and broad action potentials with a pronounced deflection on the falling phase. A substantial AHP was observed (Fig. 4; Table 2).

ALGESIC PROFILE. Type 1 cells exhibited uniform algesic response profiles. Capsaicin sensitivity was present in most cases (22 of 28 cases). Capsaicin-induced currents (1 \( \mu \text{M} \)) were typically slow in onset with a relatively modest peak amplitude (Fig. 4). Some current was present within 15 s, but peak currents required 30 or more seconds to fully develop. Regardless of capsaicin sensitivity, all type 1 cells manifested weak inward current in the presence of acidic solutions. These currents developed simultaneously with proton application, but onset kinetics were slow and current was maintained for the duration of proton application (Fig. 4). Nondesensitizing currents were observed at both pH 6.1 (\( n = 17 \)) as well as pH 5.0 (\( n = 24 \)); however, those at pH 6.1 were barely suprathreshold (2.62 ± 1.3 pA/pF). In contrast, powerful, rapidly activating, slowly desensitizing currents were evoked by ATP in all cases tested (10 \( \mu \text{M} \); \( n = 6 \); Fig. 4, Table 2). The kinetics of these currents were internally consistent (Table 3). As shown below, this form of ATP-activated current was markedly different from those in other capsaicin-sensitive cell types (types 2 and 7).

Cells clustering in this category exhibited uniform histochemistry. Six type 1 cells were prepared for immunocytochemistry following recordings. Five of the six cases contained CGRP-IR,
FIG. 3. Current signatures from cells of acutely dissociated adult rat dorsal root ganglion (DRG). Nine distinct signatures were identified by cluster analysis from very small (<22 μm), small (24–32 μm), and medium cell (35–48 μm) populations. Current signature patterns evoked by classification protocols CP1, CP2, and CP3 are shown for each cluster. Visually, CP1 and/or CP2 are usually sufficient to distinguish types 1, 2, 4, 5, 6, 8, and 9. CP3 is particularly important to distinguish types 3 and 7. Note the fast inactivation of inward current traces in type 3 relative to type 7. Vertical scale bars: for CP1: 100 pA (A); 500 pA (B); 500 pA (C); 500 pA (D); for CP2: 2,000 pA (A); 15,000 pA (B); 8,000 pA (C); 15,000 pA (D); for CP3: 2,000 pA (A); 5,000 pA (B); 5,000 pA (C); 10,000 pA (D); for CP1: 200 pA (E); 500 pA (F); 100 pA (G); 300 pA (H); 1,000 pA (I); for CP2: 10,000 pA (E); 4,000 pA (F); 2,000 pA (G); 10,000 pA (H); 10,000 pA (J); for CP3: 15,000 pA (E); 20,000 pA (F); 1,000 pA (G); 15,000 pA (H); 20,000 pA (I). Waveforms for CP1, CP2, and CP3 are presented in Fig. 1.


and five of six bound IB4 (see Fig. 5). One IB4 binding, CGRP-positive case was weakly SP-IR positive, while all others lacked SP-IR. Cell properties are summarized in Table 2.

Type 2 cell

CELL IDENTIFICATION AND ACTION POTENTIAL. All cells that clustered in this subgroup exhibited large transient outward current (Fig. 3; n = 24); no hyperpolarization-activated currents were observed. Transient outward currents were totally blocked by local perfusion with external solution containing 4-aminopyridine (4-AP, 10 mM; CP2, 102.7 m; 33.0 pF) with long duration action potentials (APDb) of type 3 cells were significantly shorter than all other cells with the exception of type 4 (U = 0, P < 0.002, all comparisons); the afterhyperpolarization differed from all subclassified cells (U = 0, P < 0.002, all comparisons). Unlike the type 1 and type 2 cells, there was no hump on the falling phase (Fig. 4).

ALGESIC PROFILE. Responses to ATP, capsaicin, and protons were examined in 21 type 3 cells. All 21 cells were capsaicin and ATP insensitive (500–1,000 nM capsaicin; 10 μM ATP). In contrast to capsaicin-sensitive cells, type 3 cells exhibited powerful, rapidly activating, and desensitizing currents in acidic solutions (see Fig. 4; Table 3). A small but significant nondesensitizing component (3.6 ± 1.4 pA/pF) followed the rapidly desensitizing phase.

In addition to differences in form and amplitude, the type 3 cell was substantially more sensitive to protons than capsaicin-sensitive subtypes. Solutions at pH 6.1 produced large currents in all type 3 cells tested (95.6 ± 12.5 pA/pF; n = 7). While no current could be evoked at pH 7.0, rapidly desensitizing current was present at pH 6.8 (12.3 pA/pF; n = 1).

The rapidly desensitizing proton activated currents of type 3 cells were susceptible to block by amiloride (100 μM; n = 4). Block was rapid (within 2 min), relatively complete (12 ± 8.0% residual current), and was significantly greater than that expected from tachyphylaxis (58.5 ± 4.1% residual desensitizing current; n = 4).

Immunocytochemistry was performed on all 21 type 3 cells exposed to multiple algesics. Twenty-one cases were IB4 negative; 20 of 21 cases lacked CGRP-IR; none of the cases contained SP-IR (Fig. 5).

Type 4 cell

CELL IDENTIFICATION AND ACTION POTENTIAL. From among medium-sized neurons (35–48 μm; 56.8 ± 1.9 pF), a cluster of
Type 1 (IB4+ SP−CGRP+)

Type 2 (IB4+ SP−CGRP−)

Type 3 (IB4− SP−CGRP−)

Type 4 (IB4− SP−CGRP−)

Type 7 (IB4+ SP−CGRP+)

27 cells was subclassified as type 4. The principle distinguishing signature for these cells was the appearance of very large hyperpolarization-activated currents (Fig. 3; Table 1). The action potential duration of the type 4 cell was significantly less than types 1, 2, and 7 ($U = 0$, $P < 0.002$ in all comparisons; Table 2 and Fig. 4) but did not differ from type 3. Cells of the type 4 cluster exhibited a substantial AHP80 that was comparable to capsaicin-sensitive cells. While the AHP did not differ
In the presence of ATP, all type 4 cells exhibited powerful inward currents that resembled those of type 1 cells (type 1, 22; type 2, 6). These currents resembled those in capsaicin-sensitive activated currents were present in all type 4 cells (16 of 16 cases). These currents resembled those in capsaicin-sensitive cells (types 1 and 2) but were smaller in amplitude. The kinetics of activation and desensitization were relatively slow. The rise time of ATP-activated currents were significantly slower in type 1 cells (\( U = 14, P < 0.05 \)). There was no difference in decay time constants. In contrast to the type 2 cell, reactivity to \( \alpha \beta \)-m-ATP was weak (1.6 ± 0.5 pA/pF vs. 44.6 ± 10.3 pA/pF for type 2; \( n = 5 \) and 8).

Immunocytochemistry was performed on 16 cells that had been exposed to capsaicin, ATP, and protons. Cells classified as type 4 were very weakly IB4 positive using the biotinylated tyramide amplification procedure (Fig. 5; 6 of 10 cases). However, in six cells with tyramide-coumarin amplification, all cases were negative. None of the 16 cells could be shown to contain CGRP-IR or SP-IR.

### Type 7 cell

**CELL IDENTIFICATION AND ACTION POTENTIAL.** Recordings were obtained from 10 very small diameter cells (16–22 \( \mu \)m; 18.2 ± 1.3 pF) that clustered together based on small hyperpolarization-activated currents and a slowly inactivating inward current signature (HAC, \( \tau_{CP23} \)). Like other capsaicin-sensitive cells (types 1 and 2), the type 7 cell manifested a broad action potential and a substantial shoulder on the falling phase (Fig. 4). The duration of the AP differed significantly from types 1, 3, and 4 (see above). The AHP80 of the type 7 cluster was exceptionally long (110.2 ± 9.8 ms) and differed significantly from all other cell types.

### Table 2. Properties of subclassified cells

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Rise Time (s)</th>
<th>Decays</th>
<th>Rise Time (s)</th>
<th>Cases</th>
<th>Decay (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>239.2 ± 33.6*</td>
<td>6</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Type 2</td>
<td>173.8 ± 41.2</td>
<td>22</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Type 3</td>
<td>n/a</td>
<td>n/a</td>
<td>505.3 ± 83.8†</td>
<td>21</td>
<td>683.3 ± 53.4†</td>
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<tr>
<td>Type 4</td>
<td>354.9 ± 44.4</td>
<td>24</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Type 7</td>
<td>196.0 ± 36.0</td>
<td>8</td>
<td>189.3 ± 36.6</td>
<td>8</td>
<td>254.9 ± 45.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. Rise time and decay constants for desensitizing currents evoked by ATP (left) or protons (right). Rise time was determined as the time (ms) between the 1st inward deflection to 90% of the peak current. All decay constants were derived from single exponential fits. * \( P < 0.05 \) vs. type 4. † \( P < 0.02 \) and \( P < 0.002 \) vs. type 7 for rise time and decay, respectively. Doses: ATP, 10 \( \mu \)M; protons, pH 5.0.
ALGESIC PROFILE. The type 7 cell class was uniform with respect to capsaicin, ATP, and proton sensitivity (n = 8). Following application of ATP, a transient peak was followed by a weak, nondesensitizing inward current (Table 2). Capsaicin-activated currents were similar to those of the type 1 cell, but significantly less in amplitude than the type 2 cells (U = 7, P < 0.002; Table 2). However, unlike capsaicin-sensitive cells that expressed only nondesensitizing proton-activated currents, protons evoked large amplitude currents with fast kinetics in type 7 cells. The form and amplitude of these currents were similar to type 3 cells. Despite this general similarity, the kinetics of proton-activated currents of type 7 cells differed significantly from those of type 3. Both rise time and decay constants were significantly shorter than proton-activated currents in type 3 cells (U = 21, P < 0.02 and U = 3, P < 0.002 for rise time and decay, respectively; Table 3). The transient portion of pH 5.0 activated currents could be blocked by brief application (2 min, local superfusion) of 100 μM amiloride (28.2 ± 15.4% residual current; n = 4). A substantial amiloride-insensitive, nondesensitizing component was also present (6.0 ± 1.6 pA/pF; see Fig. 4).

Like the type 3 capsaicin-insensitive cell, there were very substantial proton-activated currents at pH 6.1 (45.8 ± 18.6 pA/pF; n = 6). It was noteworthy that the nondesensitizing component that was prominent at pH 5.0 was negligible at pH 6.1 (0.96 ± 0.42 pA/pF). Possibly, VR1 makes contributes to the nondesensitizing portion of the proton response at pH 5.0. No current could be evoked at pH 7.0, but significant transient and nondesensitizing current were present at pH 6.8 (28.3 pA/pF and 2.9 pA/pF, respectively; n = 1).

Eight type 7 cells were prepared for immunocytochemistry. All were IB4 positive and contained both SP-IR and CGRP-IR (Fig. 5).

DISCUSSION

We have used a current signature classification scheme to subclassify acutely dissociated DRG cells harvested from adult...
groups within the Aβ electrode recordings, in vivo, Lawson and colleagues (1992; Villiere and McLachlan 1996). Using sharp electrode recordings, in vivo, Lawson and colleagues were able to distinguish nociceptive from nonnociceptive subgroups within the Aδ and C fiber populations. Although specific subtypes within nociceptive populations could not be distinguished by AHP80 in vivo (e.g., Aδ MH vs. Aδ HTM; C MH vs. C PMN vs. C HTM vs. CH, etc.), all nociceptive classes (both capsaicin sensitive and insensitive) were clearly set apart from other groups (including silent type nociceptors). Because we were able to report that subclassified cells bodies also have uniform AHP80 in vitro, it is possible that this feature will be an aid to detailed nociceptor identification. Moreover, by combining algesic reactivity and AHP80, the ability to identify specific linkages between classic nociceptive subpopulations and subclassified cells is enhanced.

Although the afterhyperpolarization and action potential duration of cell clusters were internally consistent, they diverged from previous reports (Cardenas et al. 1995; see also Villiere and McLachlan 1996). In the studies by McLachlan, the full AHP was measured (complete return to baseline). This resulted in substantially longer and more variable records. In the studies of Cardenas, little or no AHPs were observed. Using a technique common in most patch-clamp studies, action potentials were evoked using a 30-ms, threshold current injection step (typically 200–400 pA). This technique precludes accurate measurement of AHP. The long current step (30 ms) is coincident with the AHP phase, thereby causing much of the AHP to be obscured. Therefore we adopted an alternative method (1-ms, 1,500- to 3,000-pA step) that permitted the AHP to be fully visualized. While we could not expect the AHP of patch-clamped cells to be identical with those recorded with sharp electrodes (Djourhi et al. 1998; Villiere and McLachlan 1996), it was likely that a similar ordering of AHPs would be observed. As we recorded only from small- and medium-sized cells, we were more likely to encounter nociceptive subtypes (Harper and Lawson 1985; Yoshida and Matsuda 1979). Accordingly, the AHP80 of type 1 (57 ms), type 2 (55 ms), type 4 (47 ms), and type 7 (110 ms) cells were clearly consistent with nociceptive function. The AHP80 of the type 3 cells (18 ms) was more likely to be associated with nonnociceptive function.

Current signatures, AHP, and nociceptive function

Action potential AHP appears to be a promising means of identifying cell bodies of nociceptors of all classes (Djourhi et al. 1998; Lawson et al. 1997; see also Koerber et al. 1988; Ritter and Mendell 1992; Villiere and McLachlan 1996). Using sharp electrode recordings, in vivo, Lawson and colleagues were able to distinguish nociceptive from nonnociceptive subgroups within the Aδ and C fiber populations. Although specific subtypes within nociceptive populations could not be distinguished by AHP80 in vivo (e.g., Aδ MH vs. Aδ HTM; C MH vs. C PMN vs. C HTM vs. CH, etc.), all nociceptive classes (both capsaicin sensitive and insensitive) were clearly set apart from other groups (including silent type nociceptors). Because we were able to report that subclassified cells bodies also have uniform AHP80 in vitro, it is possible that this feature will be an aid to detailed nociceptor identification. Moreover, by combining algesic reactivity and AHP80, the ability to identify specific linkages between classic nociceptive subpopulations and subclassified cells is enhanced.

Although the afterhyperpolarization and action potential duration of cell clusters were internally consistent, they diverged from previous reports (Cardenas et al. 1995; see also Villiere and McLachlan 1996). In the studies by McLachlan, the full AHP was measured (complete return to baseline). This resulted in substantially longer and more variable records. In the studies of Cardenas, little or no AHPs were observed. Using a technique common in most patch-clamp studies, action potentials were evoked using a 30-ms, threshold current injection step (typically 200–400 pA). This technique precludes accurate measurement of AHP. The long current step (30 ms) is coincident with the AHP phase, thereby causing much of the AHP to be obscured. Therefore we adopted an alternative method (1-ms, 1,500- to 3,000-pA step) that permitted the AHP to be fully visualized. While we could not expect the AHP of patch-clamped cells to be identical with those recorded with sharp electrodes (Djourhi et al. 1998; Villiere and McLachlan 1996), it was likely that a similar ordering of AHPs would be observed. As we recorded only from small- and medium-sized cells, we were more likely to encounter nociceptive subtypes (Harper and Lawson 1985; Yoshida and Matsuda 1979). Accordingly, the AHP80 of type 1 (57 ms), type 2 (55 ms), type 4 (47 ms), and type 7 (110 ms) cells were clearly consistent with nociceptive function. The AHP80 of the type 3 cells (18 ms) was more likely to be associated with nonnociceptive function.

Current signatures, algesic response, and cell classification

Current signature identified cells were histochemically uniform. Types 1 and 7 contained peptides SP and/or CGRP, while types 2, 3, and 4 did not. It is well recognized that SP and CGRP are present in some nociceptor subpopulations; however, it has not been clear whether these peptides are associated with specific subclasses or distributed throughout the nociceptor family (Lawson et al. 1997; Leah et al. 1985; McCarthy and Lawson 1990, 1997). In either case, the presence of these peptides suggests a functionally distinct role for the subpopulations in which they are contained. Peptides CGRP and SP have been implicated in important peripheral and central components of the inflammatory response including vasodilation (Bharali and Lisney 1992; Holzer 1988; Lembreck and Holzer 1979), central sensitization (Dougherty et al. 1994; Honore et al. 1999; Neugebauer et al. 1995; Neumann et al. 1996), and posttraumatic sprouting (Belyantseva and Lewin 1999). Therefore cells expressing these peptides are of unique importance. If peptide-containing cells can be reliably identified by current signature alone, the study of these important subpopulations would be greatly simplified.

Cells classified by current signature also fell into uniform classes of IB4-positive and -negative populations. The binding of IB4 has been associated with unmyelinated afferent populations (Kitchener et al. 1993; Streit et al. 1985, 1986; Wang et al. 1998) and with important functional specializations (Stucky and Lewin 1999). Accordingly, cells classified as types 1, 2, and 7 are predicted to be unmyelinated afferents, while cells classified as types 3 and 4 are presumably myelinated. Further, as it has been shown that the IB4-binding population innervates cutaneous structures almost exclusively (Kitchener et al. 1993; Petruska et al. 1997; Plenderleith and Snow 1993; Wang et al. 1998), most, if not all, type 1, 2, and 7 neurons were likely to be cell bodies of cutaneous sensory afferents. Of course, the absence of IB4 binding in types 3 and 4 would not exclude these from the cutaneous afferent pool.

Taken together, the pattern of peptide expression and IB4 binding has a number of implications for subclassified cells. Type 1 neurons contained CGRP-IR and had IB4 binding but lacked SP-IR. While many DRG neurons that express CGRP also express SP (Lee et al. 1985a,b), the singular overlap of CGRP-IR with IB4-binding neurons was not unexpected. Based on this profile, type 1 and type 2 cells are likely to correspond to an IB4 binding, glial cell line derived neurotrophic factor (GDNF)-dependent, DRG population (Bennett et al. 1998; Molliver et al. 1997). Because they express CGRP, but not SP, yet bind IB4, type 1 cells are likely to belong to a subgroup of the GDNF-responsive population that innervates cutaneous tissue and expresses somatostatin (SOM) (Gibbins et al. 1987; Hokfelt et al. 1976; Perry and Lawson 1998; Wang et al. 1994). In a recent report, we have confirmed that 78% of type 1 cells express SOM-IR (Petruska et al. 2000b). In contrast, neurons positive for all three markers (type 7) were likely to be part of the nerve growth factor–dependent, small-diameter DRG population expressing tyrosine kinase (e.g., Molliver et al. 1995; Verge et al. 1989).

Current signatures and histochemical phenotype

Current signatures and histochemical phenotype

Our current signature classification scheme is based on methods first used in DRG by Cardenas and Scroggs (Cardenas et al. 1995; see also Villiere and McLachlan 1996). In the studies by McLachlan, the full AHP was measured (complete return to baseline). This resulted in substantially longer and more variable records. In the studies of Cardenas, little or no AHPs were observed. Using a technique common in most patch-clamp studies, action potentials were evoked using a 30-ms, threshold current injection step (typically 200–400 pA). This technique precludes accurate measurement of AHP. The long current step (30 ms) is coincident with the AHP phase, thereby causing much of the AHP to be obscured. Therefore we adopted an alternative method (1-ms, 1,500- to 3,000-pA step) that permitted the AHP to be fully visualized. While we could not expect the AHP of patch-clamped cells to be identical with those recorded with sharp electrodes (Djourhi et al. 1998; Villiere and McLachlan 1996), it was likely that a similar ordering of AHPs would be observed. As we recorded only from small- and medium-sized cells, we were more likely to encounter nociceptive subtypes (Harper and Lawson 1985; Yoshida and Matsuda 1979). Accordingly, the AHP80 of type 1 (57 ms), type 2 (55 ms), type 4 (47 ms), and type 7 (110 ms) cells were clearly consistent with nociceptive function. The AHP80 of the type 3 cells (18 ms) was more likely to be associated with nonnociceptive function.
et al. 1995). Their procedure specifies both capsaicin testing and Ca²⁺ current identification in addition to hyperpolarization-activated currents to identify four cell subtypes. We have demonstrated that simplified procedures, which include hyperpolarization and depolarization tests, can subclassify a larger number of DRG cells without the presentation of capsaicin or dissection of voltage-activated Ca²⁺ currents (Table 4). We have retained the original nomenclature for the four subtypes identified by Scroggs. Because we did not rely on capsaicin sensitivity or Ca²⁺ currents to subclassify cells, some variation in cell assignment might occur.

The type 1 cluster was a small (31.4 pF), capsaicin-sensitive cell, with weak nondesensitizing proton-activated currents and slow desensitizing response to ATP. The size of the cells and the amplitude and form of capsaicin-activated currents were consistent with those reported by Scroggs for their type 1 cell (26 pF) (Cardenas et al. 1995). The type 1 cell of Scroggs was identified as a subtype that lacked both hyperpolarization-activated or transient outward currents, and also by capsaicin sensitivity and by the presence of L, N, and T type Ca²⁺ currents. Although we did not examine the Ca²⁺ currents in these cells, our type 1 cluster matches the type 1 cell identified by Scroggs. Because we did not rely on capsaicin sensitivity or Ca²⁺ currents to subclassify cells, some variation in cell assignment might occur.

We have recently reported that type 1 cells co-expressed P2X₁, P2X₂, and P2X₃ subunits (Petruska et al. 2000b). Assembly of P2X₂ and P2X₃ subunits in expression systems have been associated with large amplitude slowly desensitizing ATP-activated currents that are consistent with those of type 1 cells (Lewis et al. 1995). It is not entirely clear whether P2X₁, P2X₂, and P2X₃ subunits can combine to form functional receptors that are distinct from heteromers of P2X₂ and P2X₃; however, differences in amplitude and kinetics of the responses of type 1 cells from those of type 4 suggest that a distinct combination is present and functional. It remains to be determined whether other properties associated with the type 1 cell of Scroggs (projection into superficial lamina of the spinal cord, sensitivity to 5-HT, TTX-insensitive Na⁺ current) can be attributed to the type 1 cluster (Cardenas et al. 1995, 1997a,b; Del Mar and Scroggs 1996), but it is clear that this CGRP and somatostatin-expressing, capsaicin-sensitive cell population could contribute in an important fashion to posttraumatic pain. We suggest that it may be the cell body of a CMH or C polymodal nociceptor.

The premier capsaicin-sensitive DRG cell cluster was the type 2 cell. The Scroggs group identified a type 2 cell as a small (29 pF), capsaicin-sensitive cell that expressed L and N type Ca²⁺ currents, and A-type, transient outward current. We believe this cell can be identified reliably by the presence of transient outward current alone. Transient outward current is characteristic of cells expressing A-currents. We confirmed that the TOC of the type 2 cluster was sensitive to 4-AP and cadmium. Inhibition by 4-AP or divalent cations is a common property of A-currents (Conner and Stevens 1971; Gold et al. 1996; Talukder and Harrison 1995). Cells that clustered as type 2 were similar in size (31 pF) and had large amplitude (135 pA/pF) capsaicin-activated currents that were consistent with the type 2 cell of Scroggs (117 pA/pF) (Cardenas et al. 1995). The presence of a minor shoulder on the falling phase of the action potential was further evidence that cells clustering as type 2 are identical to those identified by Scroggs and colleagues using more invasive methods.

We found that the capsaicin sensitivity of type 2 cells was unsurpassed by other capsaicin-sensitive subtypes. Comparisons between capsaicin reactivity of the type 2 cell cluster and that of the other capsaicin-sensitive clusters (types 1 and 7) show virtually no overlap. It is somewhat surprising that the premier capsaicin-sensitive cell of the DRG contained no SP or CGRP. However, other cell clusters with powerful capsaicin-induced currents (types 5, 8, and 9) contain peptides SP and/or CGRP (Cooper et al. 2000). The algesic response profiles of these cells are distinct from those of type 2 cells. We will contrast the properties of these cells with type 2 cells in a subsequent report on the remaining cell clusters. With respect to pharmacology, form, sensitivity, and kinetics, the proton-activated currents of type 2 cells were typical of cells expressing VR1 homomers (Caterina et al. 1997; Tominaga et al. 1998). Therefore proton-activated currents of the type 2 cell

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**TABLE 4. Classification decision table**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>CP1</th>
<th>HAC</th>
<th>AT</th>
<th>CP3</th>
<th>Size Range, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>NR</td>
<td>24-30</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>No</td>
<td>NR</td>
<td>NR</td>
<td>25-32</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>&lt;100 pA</td>
<td>NR</td>
<td>Fast inactivation</td>
<td>18-22</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>&gt;300 pA</td>
<td>NR</td>
<td>33-40</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>&lt;100 pA</td>
<td>-40 mV</td>
<td>NR</td>
<td>35-45</td>
</tr>
<tr>
<td>6</td>
<td>No</td>
<td>&lt;100 pA</td>
<td>NR</td>
<td>35-45</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>No</td>
<td>&lt;100 pA</td>
<td>-20 mV</td>
<td>Slow inactivation</td>
<td>16-22</td>
</tr>
<tr>
<td>8</td>
<td>No</td>
<td>&lt;100 pA</td>
<td>NR</td>
<td>35-45</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>No</td>
<td>NR</td>
<td>35-45</td>
<td></td>
</tr>
</tbody>
</table>

A classification decision table for 9 DRG cell types. Application of classification protocols 1, 2, and 3 (CP1, CP2, CP3), provides enough information, by simple inspection, to classify all cell types without application of any statistical procedures. Using the presence and absence of transient outward current (TOC) and the amplitude of hyperpolarization-activated currents (HAC) in combination with the threshold of A-current peaks (AT), it is possible to classify all cell types. CP3 is only required to separate types 3 and 7. The actual $\tau_{\text{decay}}$ need not be computed, as the apparent inactivation rates are distinct by inspection (Fig. 2, C and G). The necessary and sufficient conditions for each cell type are shown in bold print. Note that size range simplifies the classification, but is not required. NR, test is not required.
cluster were likely to reflect proton sensitivity of the capsaicin receptor.

The pharmacology and kinetics of currents activated by ATP were consistent with the “mixed fast current” described recently by Burgard and others (Burgard et al. 1999; Petruska et al. 2000a). We found that, in contrast to other forms of ATP-activated currents, these exceptionally fast, large amplitude currents are exclusively expressed in the type 2 cell. The functional significance of this unique specialization is unclear, but certainly, the large amplitude and fast kinetics of these currents would provide a secure detection of any ATP that might be liberated in the periphery by tissue damage. The kinetics of these currents are consistent with those shown by co-expression of P2X3 and P2X7 receptor subunits in expression systems (Chen et al. 1995; Lewis et al. 1995; Ueno et al. 1999).

We have recently found that all cells (10 cases) classified as type 2 co-express P2X7 and P2X3-IR (Petruska et al. 2000b).

The type 3 cell cluster was composed of very small (21 pF) ATP- and capsaicin-insensitive cells with minor hyperpolarization-activated currents and rapidly inactivating inward current signatures (CP3). Scroggs identified the type 3 cell as a small cell (15 pF), with weak hyperpolarization-activated currents, that expressed L, N, and T type Ca2+ currents and was insensitive to capsaicin. Using only current signatures, we identified a homogenous capsaicin-insensitive population that was a good match for the type 3 cell of Scroggs. While we are confident that we have thoroughly sampled cells in the small and medium size range, we are also confident that we have not thoroughly sampled cells in the very small range (<22 µm).

Because sampling was confined to cells with large diameter nuclei, a number of interesting cell clusters may yet be identified among this population. Moreover, as we have not replicated other characteristics associated with the type 3 cell of Scroggs (Ca2+ currents and TTX-sensitive Na+ currents) (Cardenas et al. 1997a), we cannot be certain that these two groups are identical. Whether these cells were identical or not, their properties offered an interesting contrast to other cell populations.

Cells clustering as type 3 were uniquely capsaicin- and ATP-insensitive cells and exhibited powerful, rapidly desensitizing, amiloride-sensitive proton-activated currents (Cooper et al. 1999a). The form and pharmacology of the latter was consistent with the expression of acid-sensing ion channels (ASIC/DRASIC) (Chen et al. 1998; Lingueglia et al. 1997; Waldmann et al. 1997a,b, 1999). The exceptionally short AHP80 of the type 3 cluster further suggested that it was an important cell group whose properties should contrast markedly with capsaicin-sensitive and -insensitive cells with much longer AHP80s. A better understanding of the relationship between AHP and functional sub-specialization of DRG neurons requires such contrasts.

The type 4 cell cluster was a medium-sized (57 pF), capsaicin-insensitive cell with weak proton reactivity and narrow action potential. Slowly desensitizing currents were always evoked by ATP. The response profile for capsaicin, protons, and ATP were consistent in all cases. Scroggs and Cardenas identified the type 4 cell as a medium-sized (54 pF) capsaicin-insensitive cell with very large hyperpolarization-activated currents (>800 pA), narrow action potential, and large N and T type calcium currents (Cardenas et al. 1995). Our type 4 cluster was composed exclusively of cells with exceptionally large hyperpolarization-activated current (590 pA). While these currents were present in other medium-sized cells (types 5, 6, and 8), they were far smaller and could readily be distinguished by visual inspection alone. As cell clusters 5 and 8 are capsaicin-sensitive cells and type 6 has very weak hyperpolarization-activated current, we are confident that the type 4 cluster can be identified by this signature and is identical to that presented by the Tennessee group.

The type 4 cell cluster was particularly interesting due to properties implicating it as a capsaicin-insensitive, myelinated nociceptor. Regarding the latter, we have confirmed in a separate report that type 4 cells do not bind IB4 (10 of 11 cases) but do express NF-M-IR (9 of 9 cases). This pattern is strongly associated with myelinated afferents (see Petruska et al. 2000b). Despite the absence of a significant capsaicin response, this cell type consistently expressed properties associated with nociceptive cells. Similar to type 1, the type 4 cell manifested powerful, ATP-activated currents that represented co-expression of P2X3 and P2X7 subunits (Petruska et al. 2000b).

Moreover, while there was a relatively narrow APDb (3.1 ms, no shoulder), the AHP80 (47 ms) was similar to those of capsaicin-sensitive subtypes (55 and 57 ms) and significantly longer than capsaicin-insensitive, ATP-insensitive cells (type 3). Given its long AHP80, and previous demonstrations that the type 4 cell of Scroggs was sensitive to PGE2 and 5-HT (Cardenas et al. 1997a, 1999), it is likely that this cell belongs to a subgroup of capsaicin-insensitive nociceptors. The most likely candidates include the A6 HTM or A6 cold nociceptor.

We identified a fifth cell cluster that was not previously classified by the Scroggs group. The type 7 cell was a very small neuron (18 pF) with weak, but significant, capsaicin and ATP sensitivity. It was distinct from other capsaicin-sensitive cells due to the expression of fast desensitizing, amiloride-sensitive, proton-activated currents with a strong nondesensitizing component and the presence of peptides SP and CGRP. The long duration AHP80 (110 ms) and broad action potential (6.5 ± 0.2 ms) contrasted greatly with the type 3 cell (18 and 3 ms, respectively) and were consistent with a nociceptive function. Lawson has shown that the longest AHP80s were observed in the silent nociceptive classes (Djourhi et al. 1998; Lawson et al. 1997). The exceptional length of the AHP in the type 7 cell easily set it apart from other characterized cell groups and suggested that it might represent a portion of the silent (or mechanically insensitive) nociceptor class in vitro (Cooper et al. 1991a,b; Meyer et al. 1991; Perl 1968; Schäible and Schmidt 1983; Weidner et al. 1999).

Cluster analysis confirmed that nine distinct cell populations could be found in DRG cells with diameters ranging from 16 and 45 µm. However, we have not found it necessary to assign cells statistically. Rather, a simple decision table (Table 4) can be used to place cells in one of nine subgroups based on visual inspection of currents produced mainly by two classification protocols (CP1 and CP2). In most instances the assignment is simplified by cell diameter measurement. Because we have found that certain cell types are confined to distinct, nonoverlapping size ranges, the number of possible assignments will be reduced by taking cell size into account. Because this method was derived in acutely dissociated, adult DRG, some caution should be taken with other DRG preparations.
Profile construction in classified cell types

Classification of cells by current signature has important advantages over schemes that simply divide all cells into two mutually exclusive subtypes (e.g., IB4 positive/negative or capsaicin-sensitive/insensitive), and rely on post hoc cell identification. While binary schemes have utility, they cannot capture the rich functional diversity of the nociceptive afferent pool. Using the current signature method, we have been able to identify nine distinct clusters of cells. We have shown that at least five of these subclasses have uniform properties with respect to algesic and histochemistry. Four of the five exhibited action potential properties consistent with those of nociceptors, in vivo.

The classification of cells by this method has the substantial advantage of being able to predict algesic response profiles and histochemical phenotype by current signature alone. This has several applications in patch clamp experiments. If experiments require targeting of substance P expressing cells, current signature identification permits this determination without immunocytochemistry. Alternately, if ASIC expressing cells are targeted, current signatures offer the advantage of identifying such cells at the beginning rather than at the end of the experiment. More importantly, due to the uniform properties exhibited by subclassified cells, identification of a neuron by its unique signature allows its prior history to be transferred from experiment to experiment.

If current signatures reliably predict properties from experiment to experiment, an extensive profile of properties can be developed for each cell type. This building of profiles is best exemplified by the properties that have been attributed to the type 2 cell. A series of studies from this and the Scroggs laboratory have demonstrated that the type 2 cell (1) is the premier capsaicin-sensitive DRG cell (Cardenas et al. 1995 and above); (2) possesses weak, amiloride-insensitive, proton-activated currents that are characteristic of the vanilloid receptor; (3) is highly sensitive to ATP and expresses currents consistent in form, pharmacology, and histochemistry with the expression of P2x1 and P2x3 subunits (above and Petruska et al. 2000b); (4) projects into lamina I and II of the dorsal horn (Del Mar and Scroggs 1996); (5) uniquely expresses TTX-insensitive currents consistent with unmyelinated afferent pools (Cardenas et al. 1997a); (6) is sensitive to 5-HT via coupling of TTX-insensitive currents to a 5HT4 receptor (Cardenas et al. 1997a); (7) possesses TTX-insensitive currents that are amplified by PGE2 (Cardenas et al. 1997a); (8) has a long duration AHP80 consistent with nociceptors; and (9) belongs to a group of IB4-positive, neurofilament-negative cells that do not express either substance P or CRGP (above and Petruska et al. 2000b). It is possible to build a profile of cells if classification predicts properties with great accuracy and reliability. It is clear from previous reports that this was possible with respect to capsaicin, 5-HT, and PGE2 reactivity. In the present report, we have shown that this uniformity includes histochemical phenotype as well as sensitivity to protons, ATP, and afterhyperpolarization.

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REFERENCES


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