Nicotinic AChR in Subclassified Capsaicin-Sensitive and -Insensitive Nociceptors of the Rat DRG

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Rau, K. K., R. D. Johnson, and B. Y. Cooper. Nicotinic AChR in subclassified capsaicin-sensitive and -insensitive nociceptors of the rat DRG. J Neurophysiol 93: 1358–1371, 2005; doi:10.1152/jn.00591.2004. Nicotinic currents were determined. nAChRs were present on both capsaicin-sensitive and insensitive nociceptors. The distribution and form of nicotinic ACh receptors were classified, in vitro, according to patterns of voltage-activated currents. The presence of two distinct channels based on annicotinic agonists [ACh, nicotine, dimethyl phenyl piperazinium (DMPP), cytisine] evidence emerged of two distinct nAChR differentially expressed in type 4 (α2β2) and types 5 and 8 (α6β2α5). Although identification could not be made with absolute certainty, patterns of potency (type 4: DMPP > cytisine > nicotine = ACh; type 5 and type 8: DMPP = cytisine > nicotine = ACh) and efficacy provided strong support for the presence of two distinct channels based on an α6β2 platform. Studies conducted on one nonnociceptive class (type 3) failed to reveal any nAChR. After multiple injections of Di-I (1,1-dilinoleyl-3,3,3,3-tetramethylindocarbocyanine perchlorate) into the hairy skin of the hindlimb, we identified cell types 2, 4, 6, 8, and 9 as skin nociceptors that expressed nicotinic receptors. We conclude that at least three nicotinic AChR are diversely distributed into discrete subclasses of nociceptors that innervate hairy skin.

INTRODUCTION

Trauma liberates a variety of pro-inflammatory agents that contribute to the acute and hyperesthetic pain of trauma as well as to the healing of injured tissue thereafter (Cooper and Sessle 1993). The influence of these agents has been extensively investigated, yet our understanding of the interplay among the pain, vascular, immune, and endocrine systems over the course of trauma and healing remains fairly limited. Acetylcholine (ACh) is a prototypical pro-inflammatory agent that contributes to both pain and healing. ACh is liberated from fibroblasts, keratinocytes, epithelial, endothelial, and immune cells after trauma (Buchli et al. 1999; Grando et al. 1993; Parnavelas et al. 1985; Wessler et al. 1998, 1999). Once liberated, ACh can activate Aδ and C fiber nociceptors in superficial and deep tissues (Bernardini et al. 2001; Bessou and Perl 1969; Fjallbrant and Iggo 1961; Haegerstam et al. 1975; Lang et al. 2003; Schmelz et al. 2003; Steen and Reeh 1993; Tanelian 1991) and activate CNS relays (Carstens et al. 2000). Either ACh or nicotinic agonists can be painful to humans when applied at a variety of superficial and deep tissues sites including blister base, artery, cornea, and tongue (Armstrong et al. 1993; Dessirier et al. 1998, 1999; Keele and Armstrong 1964; Wilson and Stoner 1947).

Heteromeric neuronal nicotinic receptors (nAChR) can be formed from the pentameric assembly of 7 alpha (α2, α3, α4, α5, α6, α9, α10) and 3 beta (β2, β3, β4) subunits. Functional nAChR heteromers manifest distinct pharmacology, permeability, and kinetics (Albuquerque et al. 1997; Dani 2001; McGeehe and Role 1995; Papke 1993). Additional alpha subunits (α7, α8, α9) combine to form homomeric channels with exceptional Ca2+ permeability (Couturier et al. 1990; Elgoyhen et al. 1994; Gerzanich et al. 1994; Seguela et al. 1993). The composition and distribution of heteromeric nAChR, in vivo, is not known with any certainty, but the predominant forms in the mammalian CNS and PNS are believed to be the α6β2 and α6β3, respectively (Flores et al. 1992; Genzen et al. 2001; Liu et al. 1998; Wada et al. 1989). All of these alpha and beta subunits are expressed in sensory ganglia (α2, α3, α4, α5, α6, α7, α9, α10, β2, β3, β4) (Boyd et al. 1991; Flores et al. 1996; Genzen et al. 2001; Lips et al. 2002; Liu et al. 1998). These subunits could assemble into a variety of functional nAChR. The distribution of nAChR in nociceptive and nonnociceptive populations is not known, but it is reported that ≤50% of DRG cells respond to nicotinic agonists (Genzen et al. 2001; Liu et al. 1993; Sucher et al. 1990). A portion of these neurons are likely to be nociceptive.

There may be functional diversity among the nAChR expressing peripheral afferent pool. In addition to its clear nociceptive role, nicotinics may be able to confer a degree of analgesia (Carstens et al. 2001; Kesingland et al. 2000; Reuter et al. 2003). These influences might arise from modulation of peptide release (Bannon et al. 1998a,b; Donnelly-Roberts et al. 1998), cross desensitization (Sudo et al. 2002), tachyphylaxis (Dessirier et al. 2000; Jinks and Carstens 1999), or direct interaction with voltage-dependent channels (Liu et al. 2004). Contrasting nicotinic influences could also arise from separate
pools of afferents that contribute distinct analgesic or algesic influences or could reflect a segregated distribution of algesic and analgesic conferring afferent pools to distinct tissue sites. Little is known about the distribution of nicotinic channels in nociceptors or the sites that they innervate.

Our laboratory has been concerned with primary afferent sub-specializations within the pain system. Scroggs and colleagues originally devised a method of classifying dorsal root ganglion cells, in vitro, that was based on the distribution of hyperpolarization-activated currents and voltage-activated Ca\(^{2+}\) channels (Cardenas et al. 1995). Using methods derived from Scroggs and colleagues, we have recently shown that patterns of hyperpolarization and depolarization activated currents form signatures that could identify nine distinct afferent subpopulations with internally uniform properties and histology (Petruska et al. 2000a,b, 2002). Based on a variety of evidence, eight of these cell populations were likely to be nociceptive and represent members of the distinct subtypes of nociceptive afferents that have been characterized in vivo (C polymodal, C mechanoheat, C high-threshold mechanoreceptor, C cold, C-silent, A\(\delta\) high-threshold mechanoreceptor, A\(\delta\) mechanoreceptor, A\(\delta\) polymodal, A\(\delta\) cold, A\(\delta\)-silent (mechanically insensitive afferent) (Ringkamp et al. 2001; Treede et al. 1992).

Consistent with the substantial diversity of the nociceptive population in vivo, investigations in vitro have revealed that the sensory cells of the DRG were composed of discrete, internally homogenous, classes of capsaicin-sensitive (types 1, 2, 5, 7, 8 and 9) and -insensitive (types 3, 4, 6) populations with internally uniform properties and histochemistry (Petruska et al. 2000a,b, 2002). Based on a variety of evidence, eight of these cell populations were likely to be nociceptive and represent members of the distinct subtypes of nociceptive afferents that have been characterized in vivo (C polymodal, C mechanoheat, C high-threshold mechanoreceptor, C cold, C-silent, A\(\delta\) high-threshold mechanoreceptor, A\(\delta\) mechanoreceptor, A\(\delta\) polymodal, A\(\delta\) cold, A\(\delta\)-silent (mechanically insensitive afferent) (Ringkamp et al. 2001; Treede et al. 1992).

Preparation of cells

Adult male rats (Harlan Sprague-Dawley, 90–150 g) were anesthetized with halothane and rapidly decapitated (Braintree Scientific, RG-100). The spinal cord was quickly removed, and 10–15 dorsal root ganglia were dissected free. Dissected cervical, thoracic, and lumbra ganglia were placed in a heated bath (35°C for 70 min) containing dispase (neutral protease, 5 mg/ml; Boehringer Mannheim) and collagenase (2 mg/ml; Sigma type I). After wash and trituration, recovered cells were plated on 12 polylysine-coated petri dishes. Recordings were made at room temperature, 2–10 h after plating. Plated cells were maintained in a rat Tyrobe’s solution containing (in mM) 140 NaCl, 4 KCl, 2 MgCl\(_2\), 2 CaCl\(_2\), 10 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH. The pipette solution contained (in mM) 120 KCl, 5 Na\(_2\)-ATP, 0.4 Na\(_2\)-GTP, 5 EGTA, 2.25 CaCl\(_2\), 5 MgCl\(_2\), and 20 HEPES, adjusted to pH 7.4 with KOH. These methods were consistent with the Panel on Euthanasia of the American Veterinary Medical Association. All animal purchases, housing, and veterinary care was provided by Animal Care Services. A local IACUC committee reviewed and approved all procedures involving animals prior to any experimentation.

Cell classification and testing

Recordings were made exclusively from cells with diameters between 17 and 45 \(\mu\)m. Cell diameter was estimated from the average of the longest and shortest axis as measured through an eyepiece micrometer scale. All recordings were made at room temperature. After the whole cell mode was achieved, series resistance was compensated 30–60%. A junction potential error of 4 mV was not corrected. Other cell and membrane properties are presented in Table 1.

All neurons examined were classified according to patterns of voltage-activated currents (current signatures) that were revealed by three classification protocols. Neurons that did not fit these classification criteria were excluded from the study. 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 \(V_0\) of -60 mV (10 mV/step to a final potential of -110 mV; 500-ms, 4-s interstimulus interval). Classification protocol 2 (CP2) was used to produce outward current patterns. From a \(V_1\) of -60 mV, a 500-ms conditioning pulse to -100 mV was followed by 200-ms depolarizing command steps (20 mV steps) to a final potential of +40 mV. 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 patterns of voltage-activated currents were used to classify cells into nine subpopulations (Petruska et al. 2000a, 2002).

When recording from small cells, application of CP1 revealed cells that expressed small amplitude H currents (<100 pA). These could be divided into types 3 and 7 using CP3. Type 3 cells had fast decaying, low-threshold inward currents. In contrast, type 7 cells exhibited high-threshold, slow decaying inward currents. (Fig. 1G). Both type 3 and 7 manifested similar CP2 patterns. Other small cells did not express H current and had distinct outward current patterns that were devoid of sharp A-current peaks (Fig. 1A). These cells were classified as type 1. A fourth group of small-diameter cells exhibited no \(I_H\) but a strong transient outward current appeared on repolarization (Fig. 1B). Such neurons were classified as type 2. From the medium-diameter pool, we encountered many neurons that exhibited slow activating, large-amplitude hyperpolarization-activated currents (>500 pA). These were recognized as type 4 cells (Fig. 1D). Less frequently, we encountered medium-sized cells with fast-activating, weak hyperpolarization-activated currents (<200 pA at -110 mV). CP2 distinguished between patterns of outward currents that differentiated types 5 and 8. Both of these classes exhibited classic A-cur-
rent peaks but differed in the number (threshold) of peaks revealed by CP2. If three peaks were observed, the cell was type 5 (Fig. 1E). If four peaks were observed, the cell was type 8 (Fig. 1H). If five peaks were observed, the cell was type 6 (Fig. 1F). The number of A-current peaks can also be defined as an A-current threshold (AT/H11005/H11002 20 and –40 mV). This is equivalent to counting peaks when using CP2 as defined in the preceding text. Another group of medium-sized cells were devoid of hyperpolarization-activated currents and exhibited intermediate threshold A-current peaks (4 peaks or AT = –20 mV). These cells were classified as type 9 (Fig. 1I). CP3 was not required to classify types 5, 6, 8, and 9. These cells classes have proven to have highly uniform properties (Petruska et al. 2000a, 2002).

After initial characterization, cells were exposed to a variety of nicotinic agonists [ACh, nicotine, cytisine, dimethyl phenyl piperazinium (DMPP), 4-OH-GTS21, TC 2403, choline] and antagonists [aBgTX (alpha-bungarotoxin), MLA (methyllycaconitine), mecamylamine, atropine, muscarine, and biccuculine], aBgTX, 4-OH-GTS21, and TC 2403 were kindly provided by Dr. R. Papke. All other agents were purchased from Sigma-Aldrich. Inhibitors were applied for 2 min prior to test application and were contained within test solutions. Inhibitors were washed out over a 2- to 3-min interval. Concentration response curves were formed from an ascending series of agonist superfusions that were separated by 2-min intervals. A maximal dose of ACh (640–1280 μM) was applied 4 min after the agonist peak response was determined. EC50s and efficacies were determined from these concentration response curves. All substances were presented by gravity fed sewer pipe positioned 1 mm distant from the recorded cell.

**Afferent tracing**

Under aseptic conditions, five young adult male rats (80–100 g) were anesthetized with a mixture of ketamine and xylazine (ip injection; 80 mg/kg ketamine; 10 mg/kg xylazine). The following signs were monitored during surgery: heart rate, respiratory rate, ventilatory status (end-expired pCO2), and body temperature. Anesthetic depth was assessed by corneal, palpebral, and pinna reflexes. The animals were placed on a heating pad to maintain ideal body temperature (36–37°C). Anesthetic supplements were administered by ip injection when necessary. A small transverse incision was made through the hairy skin, lateral and caudal to the gastrocnemius muscle. Intradermal injections of the fluorescent tracer FastDiI oil (1,1'-
dilinoleyl-3,3',3'-tetramethylindocarbocyanine perchlorate; Molecular Probes) were then made several centimeters rostral to the incision site in the underside of the skin with a 33-gauge needle coupled to a Hamilton microsyringe (20 ml volume per animal divided into 10 injections per limb of 1 ml each). After each injection, the needle was slowly removed, any leakage was controlled by cotton-tipped applicators, and the site was rapidly sealed with n-butyl cyanoacrylate monomer glue (either Nexaband Liquid or SteriTac-B). After injections were completed in a limb, the incision was closed with cyanoacrylate. Rats were monitored daily and allowed to recover for 7 days. They were then killed for in vitro electrophysiological studies. Cells were plated in the usual manner but protected from ambient light. Dishes were mounted on a Nikon Diaphot inverted microscope with an epifluorescence attachment. Tracer-labeled cells were viewed with the appropriate Vivid filter set (XFI02, Omega Optical), and ultraviolet light exposure to all fields was <1 min in duration. Only intensely fluorescent cells were considered positive. Cells were classified as type 1–9 or unknown (Fig. 1). Only one cell was recorded per dish.

![Diagram](image)

**FIG. 2.** Form and pattern of nicotinic currents in subclassified DRG neurons. A: ACh did not evoke any currents in cell classes 3 or 7. B: type 2 cells expressed a small-amplitude fast current with rapid, monophasic, decay kinetics. In contrast, type 4 cells exhibited large-amplitude slow currents with biphasic decay. C: type 5 and type 8 cells expressed large-amplitude current with monophasic decay. Much-smaller-amplitude fast currents could also be observed with specific agonists (4-OH-GTS21; inset). D: cell types 1, 6, and 9 exhibited slowly decaying currents that were similar to types 5 and 8 in appearance. E: for each subclassified cell type, either fast or slow current forms were consistently present (types 1, 2, 4, 5, 6, 8, 9) or were consistently absent (types 3, 7). Type 2 exhibited only fast currents. For type 8, n = 61 represents dominant slow currents. All cells shown were treated were with ACh (320–640 μM).
After a recording was completed, digital images of the brightfield and fluorescent fields of view were captured using a Dage MTI RC300 camera coupled to a PC running Scion Image 4.0.2.

To assess the possible spread of DiI from injection sites, injected tissue and underlying muscle tissues were harvested prior to plating the DRG cells. The tissues were placed in vials containing 4% paraformaldehyde in phosphate-buffered saline (PBS) for a 24-h period. Subsequently, this fixative solution was replaced by 30% sucrose in PBS for cryoprotection. Once the tissue equilibrated it was embedded in TBS Tissue Freezing Medium (Triangle Biomedical Sciences) and 10-μm sections were cut on a cryostat (HM 550; Microm). Sections were thaw-mounted onto slides and placed in a -20°C freezer until viewed under fluorescent microscopy. Cases in which DiI had leaked into underlying muscle tissue were not included.

Statistics

EC_{50}s were determined by fit of the normalized data to a function of the form: \( I = I_{\text{max}}/[1 + (EC_{50}/[Ag])^n] \), where \( I_{\text{max}} \) is the peak current and \([Ag]\) is the agonist concentration. Student’s t-test was used to compare EC_{50}’s and efficacy (agonist_{max}/ACh_{max}) derived from concentration response curves of distinct nociceptive cell types. The alpha level was set at 0.05. In some instances, a \( \tau_{\text{decay}} \) was also determined. The exponential decay constants (\( \tau \)) were derived from the expression \( A_1 \exp[-(t - k)/\tau_1] + C \) (Clampfit 6.0). Fits were made at points between 10% of the peak current and 90% of the return to baseline using Clampfit software (Axon Instruments).

RESULTS

Whole cell recordings were made from nine subclasses of capsaicin-sensitive and -insensitive, medium (35–45 μm; \( n = 285 \)) and small-diameter cells (17–30 μm; \( n = 105 \)). Most of the nine cells types expressed one or more forms of nicotinic AChR. Although reactivity to ACh was common in most cell types, ACh did not induce ionic currents in type 3 (\( n = 29; 640 \mu M \)) or type 7 cells (\( n = 13; 640 \mu M; \) Fig. 2A). Both of these cell classes were from the small diameter populations (17–22 μm) that we have extensively characterized with respect to capsaicin, protons, and ATP (Petruska et al. 2000a,b). All other classes exhibited some form of ACh evoked current, and in some cases, multiple currents could be demonstrated (Fig. 2C). In reactive classes, application of ACh produced large-amplitude currents that were highly consistent, in form, within each cell class. Across all classes, the proportion of cells responding to ACh approached 100 or 0% in each cell class (Fig. 2, E–M). The main exception was the type 5 cell class in which a portion of cells (13/58) failed to respond to ACh. We believe this represents a genuine subphenotype of the type 5 class.
Detailed studies were carried out in types 2, 4, 5, and 8. Some of these cell classes expressed multiple AChR (fast and slowly decaying). When multiple currents were present, it was necessary to use highly specific agonists and antagonists to separate them for further study. Cell types 1, 6, and 9 expressed large, slowly decaying currents. The characterization of these nAChR was limited. In the following text, specific agonists and antagonists were applied to characterize the nicotinic channels in selected heat and/or capsaicin sensitive cell classes. These nicotinic currents proved to have diverse properties, leading us to believe that different DRG nociceptors expressed distinct nicotinic channels. Because some cells may have co-expressed α7 or muscarinic receptors, detailed characterization of the large, slowly adapting currents were carried out in the presence of methyllycaconitine (MLA, 50 nM) and atropine (500 nM). These antagonists were preapplied (2 min) and were also contained in agonist solutions. In selected cases, we replicated capsaicin sensitivity after application of nicotinic agonists. As previously reported, all type 1, 2, 5, and 8 cells were capsaicin sensitive (n = 7/7, 25/25, 20/20, and 12/12, respectively).

**FIG. 4.** Representative currents evoked by nicotinic agonists. The form of currents evoked by various nicotinic agonists in cell types 4, 5, and 8. The response to ACh was distinct in type 4, where biphasic kinetics were observed (see Fig. 2). A similar and powerful distinction was apparent for nicotine as well. Little difference can be seen when the agonist was cytisine or dimethyl phenyl piperazinium (DMPP). All 12 traces from different cells.
contrast, type 4 cells with nAChR were never capsaicin sensitive (0/21).

**Fast currents with α7-like properties**

Two afferent neuron subclasses expressed currents consistent with those of α7 homomeric channels. In the type 8 cell, α7-like currents were present with other nAChR, but they were expressed in isolation in the type 2 cell. Presentation of ACh (640 μM; n = 33) to the capsaicin sensitive type 2 cells consistently evoked a small-amplitude current that rapidly decayed with monophasic kinetics (τ = 165 ± 0.009 ms; Fig. 2B). Fast currents in type 2 cells could be activated by choline (500 μM) or the α7-specific agonist 4-OH-GTS21 (Fig. 3A–C), blocked by MLA (n = 3; 50–200) or αBGTX (3 μM, n = 3; not shown). When activated by ACh or choline, α7-like cur-

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**Fig. 5.** Concentration curves for 4 nicotinic agonists. Concentration response curves were formed in 3 cell classes exhibiting slowly decaying currents. A: with ACh as the agonist, type 4 cells exhibited a sharply reduced sensitivity relative to types 5 and 8. B: blockade of αi and muscarinic receptors with atropine (ATR)-MLA corrected most of the sensitivity shifts, but type 4 was still significantly shifted relative to type 5 and efficacy differences remained in both cases (see Table 2). C: MLA did not influence concentration response curves to ACh in type 4 cells. D and E: there were no differences in sensitivity to nicotine or DMPP. F: type 4 cells exhibited decreased sensitivity to cytisine. ATR-MLA present in B and D–F.
rents were classic, fast decaying currents. When activated with 4-OH-GTS21, the α7-like currents were slowly desensitizing. The α7 agonist oxotremorine (100 μM) failed to evoke any currents in type 2 cells (n = 2; not shown). Therefore choline-activated currents were unlikely to be mediated by α7 containing channels (Verbitsky et al. 2000).

Other subclassified cells (type 8) exhibited α7-like currents, but these could only be demonstrated with specific agonists as large, slowly decaying, nicotinic currents were also present (see following text). Application of 4-OH-GTS21 confirmed that α7 was present in the type 8 cell (Fig. 3, F and G). These were fast decaying currents. In contrast, there was little indication of 4-OH-GTS21-sensitive currents in type 4 or 5 cells (0/8 and 1/6 cases, respectively). We did not apply 4-OH-GTS21 to cell types 1, 6, or 9.

**Slow currents with β4-like properties**

Combinations of alpha and beta subunits can yield nicotinic AChR with varying potency, efficacy, kinetics, and permeability (Albuquerque et al. 1997; Dani 2001; McGhee and Role 1995). Relative to channels formed from α7 proteins, those formed as αβ or αβα heteromers exhibit currents that decay relatively slowly. Many DRG cell classes, particularly medium-sized capsaicin-sensitive (type 5, 8, and 9) and -insensitive (type 4 and 6), expressed slowly decaying currents that could be evoked by a variety of nicotinic agonists (Fig. 4). These large-amplitude currents were completely blocked by mecamylamine (40 μM; not shown; n = 6, 5, and 3, respectively, types 4, 5, and 8).

A variety of evidence suggested that the slowly decaying currents in cell types 4, 5, and 8 were distinct nAChR, and that these medium-diameter cells may have also expressed muscarinic receptors. Concentration response curves formed from the application of ACh (20–1,380 μM) revealed EC50s that were significantly right-shifted in type 4 cells relative to types 5 and 8 (Fig. 5A). The relative positioning of these curves might reflect the presence of multiple nicotinic or muscarinic receptors. As noted in the preceding text, α7 was present in type 8, and this channel (as well as muscarinic receptors) could distort the apparent ACh potency in this cell classes by binding ACh or through initiating secondary messenger or Ca2+-dependent regulatory pathways. Although α7 was absent in types 4 and 5 (see preceding text), the presence of atropine and MLA significantly shifted the EC50 for ACh in both of these cell classes (Table 2; Fig. 5B). When MLA was excluded (ACh atropine-MLA vs. ACh-atropine), there was little indication of any influence of MLA (Fig. 5C). It was possible that muscarinic receptors were present and responsible for the shifted potency. Accordingly, all concentration response curves formed on members of these cell classes were carried out in the presence of atropine and MLA (ATR-MLA).

In concentration response curves formed in the presence of ATR-MLA, the differences in ACh potency and efficacy were partially maintained (Table 2). Concentration response curves in type 4 cells remained significantly right shifted relative to type 5, but were normalized with respect to type 8 (Fig. 5B). Significant differences in efficacy were still present in both comparisons with type 4 (Table 2). When nicotine or DMPP was the agonist, no detectable differences in potency were observed between cell classes, but significant shifts in DMPP efficacy were found in comparisons between type 4 and both other classes. These distinct patterns of potency and efficacy were strong evidence of a distinct distribution of nAChR in these nociceptive cells. Cytisine is a useful agonist due to its high efficacy in β4 containing heteromers (Chavez-Noriega et al. 1997; Luetje and Patrick 1991; Papke and Heineman 1994). All subclassified DRG nociceptors exhibited a high efficacy and potency to cytisine over an application range of (5–300 μM; Fig. 4; Table 2). Once again, cytisine evoked currents were significantly right-shifted in type 4 cells relative to those from types 5 and 8. Efficacy shifts were also apparent with the

### TABLE 2. Nicotinic agonist potency and efficacy in subclassified nociceptive cells

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Type 4</th>
<th>Type 5</th>
<th>Type 6</th>
<th>Type 8</th>
<th>Type 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>239 ± 17, P &lt; 0.03 vs. A-MLA</td>
<td>95.3 ± 15, P &lt; 0.03 vs. A-MLA</td>
<td>125 ± 39</td>
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<tr>
<td>EC50 n</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td></td>
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<tr>
<td>Acetylcholine*</td>
<td>191 ± 9.8 (1.9)</td>
<td>174 ± 28 (2.7)</td>
<td>148 ± 18 (1.7)</td>
<td>P &lt; 0.05 vs. type 4</td>
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<tr>
<td>EC50 n</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td></td>
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<tr>
<td>Efficacy</td>
<td>1</td>
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<td>1</td>
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<tr>
<td>Nicotine*</td>
<td>205 ± 14 (2.8)</td>
<td>216 ± 20 (2.7)</td>
<td>213 ± 9 (1.9)</td>
<td>125</td>
<td></td>
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<tr>
<td>EC50 n</td>
<td>5</td>
<td>4</td>
<td>6</td>
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<tr>
<td>Efficacy</td>
<td>0.81 ± 0.06</td>
<td>0.68 ± 0.07</td>
<td>0.99 ± 0.14</td>
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<td>5</td>
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<tr>
<td>DMPP*</td>
<td>33.4 ± 5 (2.7)</td>
<td>23.6 ± 5 (3.1)</td>
<td>20</td>
<td>23.5 ± 5 (3.1)</td>
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<tr>
<td>EC50 n</td>
<td>7</td>
<td>4</td>
<td>5</td>
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<tr>
<td>Efficacy</td>
<td>0.55 ± 0.04</td>
<td>0.55 ± 0.05</td>
<td>P &lt; 0.03 vs. type 4</td>
<td>0.55 ± 0.06, P &lt; 0.04 vs. type 4</td>
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<td>5</td>
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<tr>
<td>Cytisine*</td>
<td>72.8 ± 4 (1.8)</td>
<td>28.3 ± 4.9 (1.69), P &lt; 0.0001 vs type 4</td>
<td>20</td>
<td>38.4 ± 5.8 (2.8), P &lt; 0.05 vs type 4</td>
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<tr>
<td>EC50 n</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
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<tr>
<td>Efficacy</td>
<td>0.58 ± 0.05</td>
<td>0.95 ± 0.05</td>
<td>P &lt; 0.005 vs type 4</td>
<td>0.81 ± 0.07, P &lt; 0.03 vs type 4</td>
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Potency and efficacy patterns in five nociceptive cell classes treated with a variety of nicotinic agonists. Hill coefficients are provided in parentheses. Entries are means ± SE. Statistical significance is noted if appropriate. *, in the presence of atropine (500 nM) and MLA (50 nM).
same pattern obtained (Fig. 5F; Table 2). It is likely that these shifts in potency and efficacy for cytisine reflected distinct composition of channels expressed in nociceptive cells and, in particular, the number of \( \beta_4 \) subunits. Difference in the relative potency of these nicotinic agonists in capsaicin-insensitive (type 4) and sensitive types 5 and 8 cells further emphasized the distinct distribution of nAChR (type 4: DMPP > cytisine > nicotine = ACh; types 5 and 8: DMPP = cytisine > nicotine = ACh).

A series of experiments were performed to further specify the nAChR of the type 4 nociceptor. Despite the relatively smooth curves that were obtained, it was possible that these cells contained multiple functional nAChR, the presence of which were responsible for shifts in potency and efficacy relative to other subclassified cells. We used specific agonists and select antagonists to examine this possibility. To study the influence of antagonists, we used a procedure of repeated application of ACh in the presence and absence of selected agents (ACh 640 \( \mu \text{M}; \) 3-min intervals). After the first application, there was a significant tachyphylaxis. However, reactivity stabilized from application two through four so that highly consistent responses could be obtained (Fig. 6B; \( n = 5 \)). The selected antagonist was applied during the interval between the second and third ACh presentation. The third application of ACh (also containing the selected antagonist) served as a test, and the fourth application was used as a wash. Neither atropine nor MLA was present in these experiments.

Initial studies suggested that the ACh evoked currents of type 4 cells might contain \( \alpha_9 \) or \( \alpha_9\alpha_{10} \) like protein. These proteins have been reported in trigeminal and dorsal root ganglion neurons (Lips et al. 2002; Liu et al. 1998). Pretreatment (2 min) with bicuculline (20 \( \mu \text{M} \)) significantly and reversibly reduced ACh-evoked current in type 4 cells (Fig. 6C; \( n = 8 \)). These reductions were consistent with \( \alpha_9 \) or \( \alpha_9\alpha_{10} \) (but not inconsistent with \( \alpha_3\beta_4 \) or \( \alpha_2\beta_4 \)) (Demuro et al. 2001). Although a relatively small portion of the total current was reduced (<33%), it was possible that a weakly co-expressed channel imparted the distinct properties found in this cell class. Further and more specific examinations failed to confirm the presence of \( \alpha_9 \) containing nAChR. Muscarine is an antagonist at \( \alpha_9 \) containing AChR (Elgoyhen et al. 2001; Verbitsky et al. 2000). We were unable to demonstrate that any portion of the stable ACh-evoked current could be inhibited by muscarine (Fig. 6E). Moreover, \( \alpha_9 \) and \( \alpha_9\alpha_{10} \) agonists choline (500 \( \mu \text{M}, \)

![Figure 6](http://jn.physiology.org/)

**FIG. 6.** Lack of co-expression of multiple nAChR in type 4 cells. We investigated whether the unique pattern of potency and efficacy in type 4 cells was due to the expression of multiple nAChR. **A:** representative traces illustrating the response to ACh, nicotine, and cytisine. **B:** at maximal doses, ACh produces a rapid tachyphylaxis that stabilizes within 2 presentations. Two minutes between each application. **C:** bicuculline (20 \( \mu \text{M} \)) significantly reduces ACh-evoked current (640 \( \mu \text{M} \); \( P < 0.009 \)). **D:** neither TC 2403, choline, or oxotremorine evokes any current in type 4 cells. **E:** muscarine (300 \( \mu \text{M} \)), an \( \alpha_9 \) or \( \alpha_9\alpha_{10} \) antagonist, failed to block ACh-evoked current in type 4 cells. ATR-MLA not present in these experiments.
n = 3) or oxotremorine (100 μM; n = 8) also failed to evoke any current in type 4 cells (Fig. 6D) (Elgoyen et al. 1994, 2001; Rothlin et al. 1999; Verbitsky et al. 2000). The specific α4β2 agonist TC 2403 was also ineffective in type 4 or 5 cells (120 μM, n = 2 and 3, respectively) (Papke et al. 2000). Therefore we could not demonstrate a co-expressed nAChR in type 4 cells (or type 5) that could impart distinct characteristics observed in concentration response curves.

Projection fields of nAChR-expressing nociceptors

It has been reported that pain and/or nociceptor activity occurs after application of nicotinic agonists to skin (see INTRODUCTION). One or more of those nociceptive populations we have characterized could mediate these influences. Accordingly, we made a series of small (1 μl) injections of the tracer, Di-I, into the hairy skin overlying the gastrocnemius. After a 2-wk period to allow for transport to the ganglion, the rat was killed, and ganglia were harvested in the usual manner. Recordings made exclusively from intensely fluorescent cells revealed that populations of skin afferents corresponded to our nociceptive cell class types 2, 4, 6, 8, and 9 (n = 13, 7, 3, 2, and 3, respectively; Fig. 7). Fluorescent cells that were exposed to ACh (640 μM) responded with characteristic fast or slow decaying currents (Fig. 7). Other fluorescent cells were observed, and a portion of these were also ACh responsive (2/8 small and 5/7 medium sized neurons; not shown). Most of these noncategorized cells were capsaicin sensitive (6/8 small and 4/7 medium-diameter cells; 1 μM) and likely to be nociceptive.

DISCUSSION

Recordings from isolated cells of mammalian sensory ganglia have long demonstrated that some small- and medium-sized sensory neurons expressed nicotinic AChRs (Genzen et al. 2001; Liu and Simon 1996; Liu et al. 1993; Papadopolou et al. 2004; Sucher et al. 1990). We now know that DRG neurons can be classified by physiological current signatures into distinct groups that exhibit highly uniform reactivity to algesic substances. Subpopulations of myelinated and unmyelinated afferents that uniformly express receptors for capsaicin, ATP, and protons have been extensively characterized using these methods (Cardenas et al. 1995; Petruska et al. 2000a,b, 2002). We now demonstrate that these same populations also differentially expressed nAChR (Table 3) and include subfamilies that innervate hairy skin. These nicotinic receptors were observed both in small- and medium-sized cell classes and in both capsaicin-sensitive and -insensitive cells. It is possible that portions of such afferent groups contribute to nociceptor discharge, nocifensive behavior and pain report after application of ACh or nicotinic agonists to skin (Bernardini et al. 2001; Lang et al. 2003; Schmelz et al. 2003; Steen and Reeh 1993).

Using in vivo preparations, a number of laboratories have reported activation of C fibers, reduction of activation threshold (Bernardini et al. 2001; Lang et al. 2003; Schmelz et al. 2003; Steen and Reeh 1993), and release of peptides by nicotinic agonists (Benarroch and Low 1991; Parkhous and Lequesne 1988; Walmsley and Wiles 1990). These findings, for both rat and human, are consistent with our observations of nicotinic AChR in C-fiber peptidergic nociceptors in skin. As previously reported, capsaicin-sensitive cell types 5 and 8, bind the C fiber marker isolecitin B4, and co-express SP and CGRP (Petruska et al. 2002). While not directly demonstrated here, the Ca2+-permeable nicotinic currents would likely support release of peptides as would any action potentials that would occur after evocation of these powerful currents.

A restricted portion of cells expressed fast decaying currents that were physiologically and pharmacologically consistent with homomeric α7 channels. Time constants derived on the falling phase of the currents activated by ACh or choline were consistent with α7 (Cuevas et al. 2000; McGehee and Role 1995). More significantly, MLA and αBtx-sensitive currents could be reliably activated by the highly specific α7 agonist 4-OH-GTS21 (Meyer et al. 1998; Papke et al. 2004). The fast decaying currents were consistent with classic α7 nAChRs and differed from some α7 variants recently reported in superior cervical ganglion (Cuevas et al. 2000). In DRG, we observed previously reported, capsaicin-sensitive cell types 5 and 8, bind the C fiber marker isolecitin B4, and co-express SP and CGRP (Petruska et al. 2002). While not directly demonstrated here, the Ca2+-permeable nicotinic currents would likely support release of peptides as would any action potentials that would occur after evocation of these powerful currents.

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TABLE 3. Properties of subclassified cells

<table>
<thead>
<tr>
<th>Type</th>
<th>C/A</th>
<th>Peptides</th>
<th>CAPS/heat</th>
<th>Protons</th>
<th>ATP</th>
<th>ACh</th>
<th>Projection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A*</td>
<td>CGRP, Som</td>
<td>Pos</td>
<td>TASK-1, TASK-3</td>
<td>P2X1, P2X2, P2X3</td>
<td>Pos</td>
<td>?</td>
</tr>
<tr>
<td>2</td>
<td>Cb</td>
<td>Neg</td>
<td>Pos</td>
<td>TASK-2, TASK-3</td>
<td>P2X1, P2X2</td>
<td>α7</td>
<td>Hairy skin</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>Neg</td>
<td>Neg</td>
<td>ASIC-like</td>
<td>Neg</td>
<td>Neg</td>
<td>?</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>Neg</td>
<td>Pos</td>
<td>TASK-1, TASK-2, and TASK-3</td>
<td>P2X2</td>
<td>α7β2</td>
<td>Hairy skin</td>
</tr>
<tr>
<td>5</td>
<td>C</td>
<td>SP and CGRP</td>
<td>Pos</td>
<td>ASIC-like, TASK-2, TASK-3</td>
<td>P2X1, P2X2</td>
<td>α7β2, α7</td>
<td>Hairy skin</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
<td>AGRP</td>
<td>Neg</td>
<td>ASIC-like, TASK-3</td>
<td>Pos</td>
<td>α7β2</td>
<td>Hairy skin</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
<td>SP and CGRP</td>
<td>Pos</td>
<td>ASIC-like, TASK-3</td>
<td>P2X1, P2X2</td>
<td>α7, α7β2</td>
<td>Hairy skin</td>
</tr>
<tr>
<td>8</td>
<td>C</td>
<td>SP and CGRP</td>
<td>Pos</td>
<td>ASIC-like, TASK-3</td>
<td>P2X1, P2X2</td>
<td>α7, α7β2</td>
<td>Hairy skin</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>CGRP</td>
<td>Pos</td>
<td>ASIC-like, TASK-2, TASK-3</td>
<td>P2X1, P2X2</td>
<td>α7</td>
<td>Hairy skin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Entries represent immunological [peptides, TWIK-related acid-sensitive K+ channel (TASK) proteins, purinergic proteins], pharmacological [capsaicin, acid-sensing ion channels (ASICs), cholinergic] and physiological evidence (heat, protons, ATP, ACh) of features uniformly present in each cell class. Each entry represents testing for all possible combinations in a column with the following exception: the presence of P2X2 was not determined in types 5, 6, 8, or 9. The entry “pos” (positive) refers to the presence of reactivity to the given algesic (or label), but the composition and/or identity of the channels that support the reactivity has not been identified. “Neg” (negative) indicates absence of a response or label. SP, substance P; CGRP, calcitonin gene related peptide; Som, somatostatin. Fiber: C type or Aδ type axon. Projection fields were traced using Dil. †, based upon expression of Nm; ‡, based upon binding of IB4; *, based upon one μM capsaicin or 52° C; †, immunocytochemistry; ‡, physiology and pharmacology; †, physiology and immunocytochemistry; ‡, immunocytochemistry. Data from Petruska et al. 2000a,b; Petruska et al. 2002; Rau et al. 2004.

α7 in isolation from other nicotinics in the capsaicin-sensitive small-diameter, nonpeptidergic type 2 class as well as co-expressed with other nAChR in the capsaicin sensitive, peptidergic medium-diameter type 8 cells. These classes are likely to be C fiber nociceptors based on binding of isolectin B4 and capsaicin sensitivity (Petruska et al. 2000b, 2002). Tracing experiments demonstrated that both of these cell classes were found in hairy skin. We did not observe α7 in myelinated, capsaicin insensitive nociceptors (type 4) or the unmyelinated capsaicin sensitive peptidergic type 5 cells. Nor did we observe α7 in other capsaicin-sensitive nociceptors of hairy skin. Therefore α7 was not uniformly expressed in C fiber nociceptors but was present in select, functionally distinct subgroups that differ not only with respect to peptide content, but also proton sensitivity (Petruska et al. 2000b, 2002). The α7 channel is frequently found at presynaptic sites where it is believed to be involved in neurotransmitter release in CNS neurons (MacDermott et al. 1999; Wonnacott 1997). It may play the same role in central processes of some C fiber nociceptors. Type 2 cells are known to terminate in the lamina I and IIo of the dorsal horn (Del Mar and Scroggs 1996). The central terminals of type 8 cells are not known, although peptidergic afferents are also known to terminate in lamina I, IIo as well as V (Snider and McMahon 1998).

Most DRG nociceptive cells expressed a slowly decaying nicotinic current (type 1, 4, 5, 6, 8, 9). Although slowly decaying nicotinic currents have been previously reported in DRG, the molecular identity of this current(s), and its distribution among functional groups (i.e., nociceptors) is not known (Genzen et al. 2001; Liu and Simon 1996; Liu et al. 1993; Sucher et al. 1990). A substantial variety of nicotinic subunits can combine to mediate slowly decaying currents. Many of these proteins have been identified in rat DRG or in central processes of primary afferents (α3, α4, α5, α7, α8, α10, β2, β3, β4) (Flores et al. 1996; Genzen et al. 2001; Lips et al. 2002; Vincier and Eisenach 2004). These subunits assemble into a diverse family of functional channels (Albuquerque et al. 1997; Dani 2001; McGehee and Role 1995) and have been shown to co-elute in chick ganglia in heteromers consisting of up to four different protein subunits (Conroy and Berg 1995). Because of the potential complexity, and the lack of specific agonists/antagonists, precise identification of a channel is not always possible. Nevertheless, many candidates can be ruled out. We were unable to evoke currents with the α4β2 specific agonist TC 2403 in types 4 or 5 (Papke et al. 2000). Moreover, the patterns of potency and efficacy were inconsistent with the presence of α4β2 in subclassified DRG nociceptors (Table 4). In limited testing, we could not find any evidence of functional α9 or α9α10 in DRG nociceptors. The relative sensitivity of these channels to cytisine indicated that β3 expressing nAChR were present on all myelinated nociceptors we examined (types 4, 6, and 9). These include both capsaicin-sensitive (type 9) and insensitive populations (type 4 and 6).

Previous studies on sensory afferents are generally consistent with the notion that cells of the DRG express α3β4; however, there is only indirect evidence for this contention, and it is mainly based on single dose, relative cytisine potency (Genzen et al. 2001). When nicotinic channels are reconstituted in expression systems, distinct patterns of potency and efficacy can be observed when diverse nicotinic agonists are presented.

TABLE 4. Nicotinic agonist potency of reconstituted channels

<table>
<thead>
<tr>
<th>Agonist</th>
<th>αβ4</th>
<th>αβ2</th>
<th>αβ4</th>
<th>αβ4</th>
<th>αβ2</th>
<th>αβ2</th>
<th>αβ2</th>
<th>αβ2</th>
<th>αβ2α5</th>
<th>αβ2α5</th>
<th>α9</th>
<th>α9α10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>163, 110*, 145*, 202*</td>
<td>26, 28</td>
<td>0.8†</td>
<td>2, 68</td>
<td>20, 14*</td>
<td>68*, 83</td>
<td>69</td>
<td>122</td>
<td>0.5</td>
<td>100†</td>
<td>11*</td>
<td>14*</td>
</tr>
<tr>
<td>Nicotine</td>
<td>40, 80, 31*, 106</td>
<td>132, 68</td>
<td>0.3, 8, 0.8†</td>
<td>5</td>
<td>21</td>
<td>19</td>
<td>105</td>
<td>1.9</td>
<td>3, 12†</td>
<td>Ant</td>
<td>Ant</td>
<td></td>
</tr>
<tr>
<td>DMPP</td>
<td>10, 28*, 12, 19</td>
<td>56, 2</td>
<td>18</td>
<td>19</td>
<td>23</td>
<td>11</td>
<td>20</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cytisine</td>
<td>72, 26, 24*, 76</td>
<td>67, 71</td>
<td>3</td>
<td>0.9</td>
<td>39</td>
<td>25</td>
<td>20</td>
<td>70</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Data from human, rat, and chick are shown with rat and chick signified (*, †). All values expressed as micromolar EC50 reported from publications identified. Recording conditions and methods vary with each laboratory. Values are means ± SE. Data from Leutje and Patrick (1991); Elgoyen et al. (1994); Wang et al. (1995); Wang et al. (1996); Ramírez-Latorre et al. (1996); Stetzer et al. (1996); Olale et al. (1997); Chavez-Noriega. (1997); Zwart and Vijverberg. (1997); Gerzanich et al. (1998); Stauderman et al. (1998); Demuro et al. (2001); Meyer et al. (2001); Verbitsky et al. (2001); Ibañez-Tallon et al. (2002). Ant, antagonist.
This approach can be applied to neurons when homogenous populations of cells can be identified. The classification procedure we employed made it possible to examine the nicotinic pharmacology of cells in a systematic fashion and in substantial detail in DRG neurons. We found that these slow currents exhibited properties that were highly consistent within cells classes but clearly distinct between some classes. Despite the substantial potential for diversity that was possible (based on a large number of subunits combinations predicted), the cell classes we examined expressed a limited subset of the slow decaying nAChR family. It was likely that mainly two isoforms were present. Based on the patterns of relative potency for nicotinic agonists, it was likely that the channel expressed by the type 4 cells (DMPP > Cyt > Nic = ACh) was significantly different from that of types 5 and 8 (DMPP = Cyt > Nic = ACh). Neither of these relative potency patterns for nicotinic agonists were identical to that reported for reconstituted rat αβ4 (DMPP = Cyt = Nic > ACh; see Table 4 references). Nevertheless, an αβ4 platform (αβ4 and possibly additional subunits) was likely given the pattern of potency and efficacy observed (Tables 2 and 4). Cell types 4, 5, 6, 8, and 9, all displayed a high potency and efficacy to cytisine and were likely to contain one or more β3 subunits (20–70 μM) (Chavez-Noriega et al. 1997; Luetje and Patrick 1991; Papke and Heineman 1994). Additionally, both the absolute and relative potency of four nicotinic agonists were very consistent with α3β2 or a channel containing α4β2 and other protein. If two distinct nAChR were expressed by cell types 4, 5, and 8, then the simplest prediction is that one of these expressed α4β2 (e.g., type 4) and the other expressed α3β3 (e.g., types 5 and 8). This scenario is consistent with the observed a shift in cytisine potency in types 5 and 8 cells relative to type 4 and consistent with heteromerization of α3 with α3β3 as reported by Gerzanich and colleagues for human nAChR (70–20 μM) (Gerzanich et al. 1998; see also Wang et al. 1996; Yu and Role 1998). We cannot rule out the possibility that addition subunits could be present or unrecognized postranslational modifications might complicate interpretations.

It is clear from our studies that nAChR are widely distributed in capsaicin-sensitive and -insensitive nociceptive cells, are present in both myelinated and unmyelinated cell classes in peptidergic and nonpeptidergic groups, and are present in several populations of skin nociceptors (types 2, 4, 6, 8, and 9). It is likely that the powerful currents of these nicotinic channels play a major role in algesic reactivity. As previously observed with respect to ATP and protons, the nociceptor population has evolved multiple strategies to detect each particular algesic. Quite distinct nicotinic, purinergic and proton-sensitive channels can be found in each nociceptor population (Table 3). The utility of this complexity is not certain but likely reflects particular nervous system adaptations required to detect the spectrum of algesics that are likely to be encountered in specialized tissues (skin, muscle, joint, etc.).

ACh is contained within a substantial variety of cells that are distributed into cutaneous and deep tissues. The release of ACh from keratinocytes, fibroblasts, endothelial cells, immune cells, and linings of viscera would result from local trauma or other release mechanisms (Buchli et al. 1999; Grando et al. 1993; Parnavas et al. 1985; Wessler et al. 1998, 1999). As noted, ACh-dependent nociceptor activation and pain have been demonstrated from cutaneous and deep tissues (Bernadini et al. 2001; Bessou and Perl 1969; Fjallbrant and Iggo 1961; Hagerstrom et al. 1975; Keesee and Armstrong 1964; Lang et al. 2003; Schmelz et al. 2003; Steen and Reeh 1993; Tanelian 1991; Wilson and Stoner 1947). It is likely that select populations of nociceptors, expressing large powerful nAChR, would be closely associated with tissues populated by ACh-expressing cells. It is further possible that the mechanical allosthyria that is common with wounds has a peripheral nicotinic component. Fragile tissue, in and around wound margins, are subject to mechanical disruption for several days following a trauma. Consequent to excessive movement or manipulation of healing tissues, the release of ACh from keratinocytes or endothelial cells would serve as a mechanical-chemo algesic signal. A mecanno-chemo mechanism of this sort would include not only nociceptor activation and acute pain but also Ca2+-dependent upregulatory events through Ca2+-permeable nAChR (Albuquerque et al. 1997; Dani 2001; McGhee and Role 1995). In this manner, nicotinic receptors could play a key role in the regulation of mechanical allodynia by contributing important sensory feedback relevant to the mechanical state of wound margins. This sensory component would complement known nicotinic contributions to wound healing (Zia et al. 2000).

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References


