Cav2.3 calcium channels play an important role in pain transmission in peripheral sensory neurons. Six Cav2.3 isoforms resulting from different combinations of three inserts (inserts I and II in the II–III loop and insert III in the carboxyl-terminal region) have been identified in different mammalian tissues. To date, however, Cav2.3 isoforms unique to primary sensory neurons have not been identified. In this study, we determined Cav2.3 isoforms expressed in the rat trigeminal ganglion neurons. Whole tissue reverse transcription (RT)-PCR analyses revealed that only two isoforms, Cav2.3a and Cav2.3e, are present in TG neurons. Using single cell RT-PCR, we found that Cav2.3e is the major isoform, whereas Cav2.3e expression is highly restricted to small (<16 μm) isolectin B4-negative and tyrosine kinase A-positive neurons. Cav2.3e was also preferentially detected in neurons expressing the nociceptive marker, transient receptor potential vanilloid 1. Single cell RT-PCR following calcium imaging and whole-cell patch clamp recordings provided evidence of an association between an R-type calcium channel component and Cav2.3e expression. Our results suggest that Cav2.3e in sensory neurons may be a potential target for the treatment of pain.

The trigeminal ganglion (TG) neurons are involved in the transmission of orofacial sensory information, including pain (1). Physical, chemical, and inflammatory damage in peripheral tissues gives rise to increased excitability of nociceptive neurons (2). Calcium plays a key role in cellular processes under these conditions, and the major routes for calcium entry into the cell are the voltage-activated calcium channels (VACCs) (3, 4). Indeed, VACCs have a variety of physiological functions, including regulation of firing patterns, synaptic modulation, and neurotransmitter release in the nervous system (5).

VACCs are formed by one of several pore-forming α subunits (α1A, 1B, and 1D) and auxiliary subunits. Molecular characterizations have determined that α1C, α1D, α1F, and α1S subunits encode L-type (Ca2.1.1–1.4) Ca2+ channels (6, 7); α1A encodes P/Q-type (Ca2.2.1) channels (8); α1B encodes N-type (Ca2.2.2) channels (9); α1E encodes R-type (Ca2.3.3) channels (10); and α1G, α1H, and α1I encodes T-type (Ca3.1–3.3) channels (11, 12). R-type currents with diverse biophysical properties were described in different types of neurons in the central nervous system (13, 14). Although the molecular nature of R-type currents in neurons is not fully understood (15, 16), to date, six Cav2.3 spliced variants (Cav2.3a to Cav2.3f) have been described in various mammalian species (14, 17–20).

In primary sensory neurons, Cav2.3 calcium channels have been suggested to contribute to pain transmission (21). Immunohistochemical and in situ hybridization analysis showed heterogeneous expression of Cav2.3 calcium channels in sensory neurons (22, 23). Electrophysiological studies also demonstrated the presence of R-type currents in subsets of sensory neurons that result from Cav2.3 expression (15, 24). However, Cav2.3 calcium channel isoforms expressed in sensory neurons have not been characterized.

In this study, we therefore determined expression patterns of Cav2.3 isoforms in the rat TG nociceptive neurons by the combination of molecular and functional analyses. Nociceptive TG neurons were identified by size and the expression of isolectin B4 (IB4)-binding protein, tyrosine kinase A (trkA), and transient receptor potential vanilloid 1 (TRPV1). As a result, we found that Cav2.3e is the major Cav2.3 isoform in TG neurons, and Cav2.3e is preferentially expressed in small (<16 μm) IB4-negative/trkA-positive and TRPV1-positive neurons. In addition, we demonstrate that SNX-482-sensitive R-type calcium channel component is associated with Cav2.3e expression in these neurons.

**MATERIALS AND METHODS**

**Preparation of TG Neurons**—All surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the School of Dentistry,


**Ca\textsubscript{2.3e} Isoform in Nociceptive Neurons**

Seoul National University. TG neurons from 2- to 5-day-old neonatal rats were prepared as described previously (25). Briefly, TG neurons were washed several times in cold (4 °C) Hanks’ balanced salt solution (Invitrogen) and then incubated for 20 min at 37 °C in Hanks’ balanced salt solution containing trypsin. The cells were washed in Dulbecco’s modified Eagle’s medium and triturated with a flame-polished Pasteur pipette to separate cells and remove processes. Subsequently, cells were centrifuged, resuspended, and placed on poly-L-ornithine-coated glass coverslips (25 mm in diameter). Cells were maintained in an incubator at 37 °C equilibrated with 5% CO\textsubscript{2}.

**Immunocytochemistry**—For immunocytochemistry, trigeminal ganglion neurons were seeded on poly-L-ornithine-coated coverglass and maintained in a 5% CO\textsubscript{2} incubator at 37 °C. The TG neurons were used for the experiments at 1 day after culture. After rinsing in 0.1 M phosphate-buffered saline (PBS), the TG neurons were fixed in −20 °C methanol (Merck) for 10 min. Afterward, the TG neurons were preincubated in PBS containing 5% normal goat serum and 0.1% Triton X-100 for 1 h at room temperature and then incubated in rabbit anti-Ca\textsubscript{2.3} (α1E voltage-gated calcium channel, R-type) (1:200; Chemicon) in the same solution at 4 °C overnight. The cells were washed three times with PBS and then incubated with FITC-conjugated rabbit IgG antibody (1:200; Jackson Immuno-Research) for 1 h at room temperature. After washing with PBS, the samples were covered with VectaShield mounting (Vector Laboratories), and fluorescent images were obtained under a confocal microscope (FV-300, Olympus, Japan). The specificity of primary antibody was confirmed by control antigen for anti-Ca\textsubscript{2.3}. For negative control, 1 μg of blocking peptide was preincubated with the same volume of primary antibody for 1 h at room temperature.

**Whole Tissue RT-PCR Analysis**—Total RNA was isolated from 2- to 5-day-old rats TG neurons using the TRIzol® reagent (Invitrogen). Following digestion with DNase I, 3 μg of total RNA was used for cDNA synthesis with the Superscript® first-strand synthesis system (Invitrogen) according to the manufacturer’s instructions. After the reverse transcription reaction, 1 ng of cDNA was then used as a template for amplification. Primers for PCR were specifically designed to differentiate the presence or the absence of three inserts (inserts I, II, and III) in the Ca\textsubscript{2.3} transcripts based on GenBank”™ rat cDNA sequences (Table 1). After a denaturation step of 5 min at 94 °C, the amplification was carried out at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s for 35 cycles. The PCR was completed by maintaining temperatures at 72 °C for 10 min. As a positive control, cDNA from the same preparations was subjected to 35 cycles of PCR with primers for β-actin. All PCR products were resolved on 2% agarose gels.

**Single Cell RT-PCR**—We adopted methods described by Silber et al. (26) for single cell RT-PCR. An entire single cell or the intracellular content of a single cell (when performed just after electrophysiological recording) was aspirated into a patch pipette using negative pressure under visual control. Patch pipettes used for an entire neuron harvest had a tip diameter range of 12–30 μm and were filled with RNase-free water. The tip of the pipette and its contents were broken into a reaction tube containing RT reagents. To avoid genomic DNA contamination, digestion with DNase I was performed before RT. RT was carried out for 1 h at 50 °C (Invitrogen), and the cDNA product was used in a separate PCR. The forward and reverse primers were designed from unique sequence to avoid amplifying homologous genes. The first round of PCR was performed in 50 μl of PCR buffer containing 0.2 mM dNTPs, 0.2 μM “outer” primers, 5 μl of RT product, and 0.2 μl of platinum Taq DNA polymerase (Invitrogen). The protocol included a 5-min initial denaturation step at 95 °C followed by 60 cycles of 40 s of denaturation at 95 °C, 40 s of annealing at 60 °C, and 40 s of elongation at 72 °C. The reaction was completed with 7 min of final elongation. For the second round of amplification, the reaction buffer (20 μl) contained 0.2 mM dNTPs, 0.2 μM “inner” primers, 5 μl of the first round PCR products, and 0.1 μl of platinum Taq DNA polymerase. “Insert” primers, designed to detect the presence of insert fragments, were also used in second round amplifications. The reaction procedure for these primers was the same as the first round. The sequences of all the primers used for single cell PCR are presented in Table 1. For positive controls, β-actin primers were used in parallel PCRs. A negative control was obtained from pipettes that did not harvest any cell contents but were submerged in the bath solution. The PCR products were displayed on ethidium bromide-stained 2% agarose gels. Gels were photographed using a digital camera (Bio-print 2000 x-press zoom, Vilber Lourmat, France).

**Classification of Sensory Neurons**—As described previously (27), TG neurons were classified into three groups as follows: small (10–16 μm), medium (16–20 μm), and large (20–30 μm) neurons. *Griffonia simplicifolia* IB4 was also utilized to classify TG neurons as either IB4-positive or IB4-negative neurons (28). Before single cell collections, TG neurons were incubated with 10 μg/ml IB4-FITC (Sigma) in a balanced salt solution (BSS (in mM) as follows: 145 NaCl, 5 KCl, 2 CaCl\textsubscript{2}, 1 MgCl\textsubscript{2}, 10 HEPES, and 10 glucose) for 10 min and then rinsed in BSS. For electrophysiological experiments, neurons were stained with 10 μg/ml IB4-FITC for 10 min and then rinsed for 10 min in extracellular solution before recording. IB4-FITC staining was visualized with standard FITC filters.

**Intracellular Calcium Imaging**—Intracellular calcium imaging was performed as described previously (25). Briefly, neurons were loaded with fura-2 AM (2 μM; Molecular Probes, Eugene, OR) for 40 min at 37 °C in BSS. The cells were then rinsed and incubated for 30 min to de-esterify the dye. The cells were plated onto poly-L-ornithine-coated coverslips, mounted onto the chamber, placed on an inverted microscope (Olympus IX70, Japan) and perfused continuously with BSS at 2 ml/min. All measurements were made at 36 °C (temperature controller PTC-20; ALA Scientific Instrument Inc.). Cells were illuminated with a 175-watt xenon arc lamp, and excitation wavelengths (340/380 nm) were selected by a Lambda DG-4 monochromator wavelength changer (Shutter Instrument, Novato, CA). Intracellular free calcium concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) was measured by digital video microfluorometry with an intensified CCD camera (CasCade, Roper Scientific, Trenton, NJ) coupled to a microscope and software (Metafluor, Universal Imaging Corp., Downingtown, PA) on a Pentium 4 computer.

**Electrophysiological Recordings**—We performed whole-cell patch clamp recordings to measure barium currents (I\textsubscript{Ba}) with
an Axopatch-1C amplifier (Axon Instruments, Union City, CA). The pipette resistance was 2–5 megohms. Series resistance was compensated for (80%), and leak subtraction was performed. Data were low pass-filtered at 2 kHz and sampled at 10 kHz. The pClamp8 (Axon Instruments) software was used during experiments and analysis. The pipette solution for IBa contained the following (mM): 100 CsCl, 1 MgCl₂, 10 HEPES, 10 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid, 3.6 Mg-ATP, 14 phosphocreatine, 0.1 GTP, and 50 units/ml creatine phosphokinase, adjusted to pH 7.4 with CsOH. The extracellular solution for IBa contained the following (mM): 151 tetraethylammonium chloride, 10 HEPES, 5 BaCl₂, 1 MgCl₂, and 10 glucose, adjusted to pH 7.4 with tetraethylammonium OH. The IBa was evoked by a test pulse to 0 mV from the holding potential, –80 mV every 10 s.

Drugs—Nimodipine (Sigma) was dissolved in methanol. ω-Conotoxin-GVIA (ω-CgTx), ω-agatoxin-IVA (ω-Aga; Alomone Labs, Jerusalem, Israel), SNX-482, and cadmium chloride (Sigma) were dissolved in distilled water to make a stock solution and kept at –20 °C. The drugs were diluted to the final concentration in the extracellular solution and applied by gravity through the bath perfusion system.

RESULTS

Ca₂⁺,3 Is Heterologously Expressed in Sensory Neurons—The distribution of Ca₂⁺,3 was determined by immunocytochemical approach in rat TG neurons that were prepared from primary culture. We found that Ca₂⁺,3 was not evenly distributed in the cell bodies of TG neurons. As shown in Fig. 1, the staining of Ca₂⁺,3 in TG neurons was heterogeneous with different levels of immunoreactivity, being predominantly expressed in small sensory neurons than large neurons. These results suggest the potential role of Ca₂⁺,3 in nociceptive neurotransmission. Thus, we further examined which isoforms of Ca₂⁺,3 are specifically expressed in nociceptive sensory neurons.

Two Ca₂⁺,3 Isoforms, Ca₂⁺,3a and Ca₂⁺,3e, Are Expressed in Sensory Neurons—The presence of three major inserts was investigated to determine splice variants of Ca₂⁺,3. The inserts tested were I and II in the loop between domains II and III and a 43-amino acid segment (insert III) in the proximal carboxyl terminus. Eight possible isoforms deduced from the Ca₂⁺,3 sequence. Ca₂⁺,3a-Ca₂⁺,3f is a newly proposed set of names by Perervez et al. (20). The isoform names in parentheses are initial names. The illustration shows the locations in Ca₂⁺,3 subunit of the primers designed for RT-PCR analysis.
outside the region (Fig. 2C). In this scenario we only obtained amplification when the insert was present. As a result, we only found amplification of insert II in the II–III loop. In the carboxyl terminus, however, we obtained both short and long cDNA fragments (absence and presence of the 129-bp insert III fragment) (Fig. 3A). These two isoforms correspond to Cav2.3a and Cav2.3e, respectively (Fig. 3B).

**Cav2.3e Is the Major Cav2.3 Isoform in Sensory Neurons**—From whole tissue RT-PCR analysis, we found that the Cav2.3a and Cav2.3e isoforms are expressed in TG neurons. We further determined the expression pattern of these two isoforms at the single cell level. When we analyzed inserts I, II, and III, using single cell RT-PCR, the splicing patterns were consistent with those obtained in whole tissue. We detected the Cav2.3a and Cav2.3e isoforms in a subpopulation of TG neurons (Fig. 3C). In further experiments, we only analyzed insert III to discriminate between Cav2.3a and Cav2.3e isoforms in individual neurons (n = 78, chosen irrespective of size). Of the neurons analyzed, Cav2.3e and Cav2.3a mRNAs were found in 19.2% (n = 15/78) and 2.5% (n = 2/78), respectively (Fig. 3D). We failed to observe the expression of Cav2.3e and Cav2.3a expressed together in the same TG neuron. Our data demonstrate that Cav2.3e is the major Cav2.3 isoform in rat TG neurons.

**Expression of Cav2.3e Isoform Is Predominant in Small Sensory Neurons**—The observation that Cav2.3e is the major isoform in TG neurons led us to analyze only Cav2.3e in subsequent single cell RT-PCR analyses. TG neurons were categorized into small (10–16 μm), medium (16–20 μm), and large (20–30 μm) neurons, as described previously (27). Cav2.3e mRNA was detected in 30.9% (n = 13/42) of small neurons and 11.1% (n = 2/18) of medium neurons but was absent from the large neurons (n = 0/18) (Fig. 4A).

Preferential expression of Cav2.3e in small neurons suggests that Cav2.3e might be involved in nociception (29). Small diameter sensory neurons can be divided into two groups based on their neurochemical properties (30). One group contains neuropeptides such as calcitonin gene-related neuropeptide and substance P and expresses the high affinity nerve growth factor receptor trkA. The other group lacks neuropeptides but binds IB4 and expresses P2X3 (31–33). Therefore, we further characterized the expression of Cav2.3e in both groups. In agreement with the previous report (30), the majority of IB4-positive and IB4-negative neurons expressed P2X3 mRNA (n = 10/12) and trkA mRNA (n = 24/30), respectively. Cav2.3e mRNA was not detected in IB4-positive/P2X3-positive neurons (n = 0/10), but it was detected in IB4-negative/trkA-positive neurons (n = 13/24) (Fig. 4B).

The TRPV1 receptor, a member of the vanilloid receptor subfamily of the transient receptor potential channel superfamily, is a well known nociceptive marker in sensory neurons (34, 35). We examined the correlation between Cav2.3e mRNA expression and TRPV1 mRNA in small TG neurons. 76.9% (n =

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**TABLE 1**

| Primers Size (bp) | Primer sequence Length |
|-------------------|------------------------|
| Cav2.3 Insert I (57 bp) | Outer Forward (1751–1770) ATG GGA CTC CTT CGG CTA AT 20 |
| Inner (521/445) | Reverse (2232–2251) CAAGAGCTTTGAGCTTTGGGAG 20 |
| Cav2.3 Insert II (21 bp) | Outer Forward (2305–2324) AACCACCACTACTCTCTCCAT 20 |
| Inner (516/495) | Reverse (2800–2820) CATCAGCTTGTGCTCTCTCT 21 |
| Cav2.3 Insert III (129 bp) | Outer Forward (5738–5757) GTCTGGAAGATGACCTCA 20 |
| Inner (520/391) | Reverse (5986–6004) AACGTTACCTCGACGAT 20 |
| Inner (513/278) | Reverse (6159–6178) AGGTCTGAGCTAGACTTG 20 |
| Inner (513/278) | Inner Forward (5738–5757) GTCTGGAAGATGACCTCA 20 |
| Inner (520/391) | Inner Forward (5986–6004) AACGTTACCTCGACGAT 20 |
| Inner (513/278) | Inner Forward (6159–6178) AGGTCTGAGCTAGACTTG 20 |
| B-Actin NM_031144 | Outer Forward (5946–5965) AGGATCCACCACGGAAG 20 |
| Inner (513/278) | Inner Forward (5738–5757) GTCTGGAAGATGACCTCA 20 |
| trkA NM_021589 | Outer Forward (5738–5757) GTCTGGAAGATGACCTCA 20 |
| Inner (513/278) | Inner Forward (5986–6004) AACGTTACCTCGACGAT 20 |
| P2X3 NM_031075 | Outer Forward (5738–5757) GTCTGGAAGATGACCTCA 20 |
| Inner (513/278) | Inner Forward (5986–6004) AACGTTACCTCGACGAT 20 |
| TRPV1 AF029310 | Outer Forward (5738–5757) GTCTGGAAGATGACCTCA 20 |
| Inner (513/278) | Inner Forward (5986–6004) AACGTTACCTCGACGAT 20 |
| Inner (513/278) | Inner Forward (6159–6178) AGGTCTGAGCTAGACTTG 20 |
10/13) of neurons expressing Ca\textsubscript{2.3}e were TRPV1-positive (Fig. 4C). Taken together, these results indicate that Ca\textsubscript{2.3}e is restricted to trkA-positive, IB4-negative neurons and is preferentially expressed in TRPV1-positive small TG neurons.

**Ca\textsubscript{2.3}e Expression Is Associated with a Functionally Identified R-type Calcium Channel in Subsets of Sensory Neurons**—Using calcium imaging and whole-cell patch clamp recording, we then examined whether Ca\textsubscript{2.3}e expression is functionally linked to the R-type calcium channel in TG neurons. High K\textsuperscript{+} (30 mM) application readily elicits large calcium transient that was not blocked with the pretreatment of 1 \mu M thapsigargin in the bath solution (Fig. 5B), indicating that this calcium transient results from calcium influx in TG neurons (Fig. 5A). In some neurons (n = 9/15), the residual calcium transients remained, even after applying a mixture of calcium channel blockers (Fig. 5C). Because of complete blockade by CdCl\textsubscript{2} (200 \mu M), this remaining calcium transient was attributed to R-type calcium channel components (Fig. 5C). The mixture contains L-, N-, and P/Q-type Ca\textsuperscript{2+} channel blockers (500 nM nimodipine, 500 nM ω-CgTx, and 200 nM ω-AgaIVA). In the other neurons (n = 6/15), the calcium transients were completely abolished by applying the mixture (Fig. 5D). When the expression of Ca\textsubscript{2.3}e was determined by single cell RT-PCR after imaging, Ca\textsubscript{2.3}e mRNA was found in a subset of TG neurons that have an R-type calcium channel component (n = 5/9) (Fig. 5C). In contrast, Ca\textsubscript{2.3}e mRNA was not detected in neurons, with no residual calcium transient (n = 0/6) (Fig. 5D). Ca\textsubscript{2.3}a was not detected in any TG neurons tested (n = 0/15).

We next performed whole-cell patch clamp recordings from the identified nociceptive TG neurons using IB4-FITC (Fig. 6A). In all IB4-negative neurons tested (n = 10), we observed that residual calcium currents remained even after applying a mixture solution of calcium channel blockers, and this residual
current was sensitive to SNX-482 as the majority (80%) of the residual currents was abolished by SNX-482 (Fig. 6B). Ca\textsubscript{2.3e} mRNA was found in 70% of IB4-negative neurons tested (n = 7/10) (Fig. 6B). However, in IB4-positive neurons tested (n = 13), Ca\textsubscript{2.3e} mRNA was not found regardless of the presence of residual calcium currents after applying calcium channel blockers (Fig. 6C). Furthermore, the residual currents in IB4-positive neurons were not abolished by SNX-482 (Fig. 6C, panel b). Consistent with calcium imaging results, Ca\textsubscript{2.3a} was not found in IB4-negative or IB4-positive neurons (Fig. 6, B and C). Taken together, these results suggest that Ca\textsubscript{2.3e} mRNA is associated with the generation of SNX-482-sensitive R-type calcium channel components in IB4-negative nociceptive neurons.

**DISCUSSION**

R-type calcium channels have been demonstrated to contribute to pain transmission in sensory neurons. In this study, we have identified specific isoforms of the Ca\textsubscript{2.3} subunit expressed in TG nociceptive neurons. Two Ca\textsubscript{2.3} isoforms, Ca\textsubscript{2.3a} and Ca\textsubscript{2.3e}, were found, with Ca\textsubscript{2.3e} being the predominant form. The Ca\textsubscript{2.3e} isoform is preferentially expressed in small TRPV1-positive nociceptive neurons and only limited expression in IB4-negative and trkA-positive neurons. Ca\textsubscript{2.3e} mRNA expression is associated with a functionally identified SNX-482-sensitive R-type calcium channel component in these neurons.

**Ca\textsubscript{2.3e} Is the Major Ca\textsubscript{2.3} Isoform in TG Neurons**—R-type currents, which may result from Ca\textsubscript{2.3}, are found in most neurons, such as neocortical and striatal neurons (36), CA1 neurons (37), dentate granule cells, cerebellar granule neurons (18), and sensory neurons (24). The Ca\textsubscript{2.3} transcripts are widely expressed throughout the brain, as shown by in situ hybridization techniques (38). The full-length cDNA from human Ca\textsubscript{2.3d} with a genomic fragment from the human genome (GenBank\textsuperscript{TM} accession number NT_004552.7) revealed the intron-exon structure of the Ca\textsubscript{2.3} gene, which is composed of 49 exons including a 58-bp 3′-noncoding segment. Exon 19 encodes insert I in the II–III loop, and exon 45 encodes insert III in the carboxyl-terminal region, and the 21-bp insert II is located within exon 20. Six isoforms (Ca\textsubscript{2.3a} to Ca\textsubscript{2.3f}) have been reported in mammalian tissues, with the neuronal Ca\textsubscript{2.3c} and the endocrine Ca\textsubscript{2.3e} being the predominant isoforms detected in vivo. Although Ca\textsubscript{2.3a} has been detected in rat cerebellar granule cells (18), Ca\textsubscript{2.3e} was initially identified in the rat and human kidney, insulinoma cell line INS-1 cells, and the islets of Langerhans (19). In this study, we found that TG neurons express only two Ca\textsubscript{2.3} isoforms, Ca\textsubscript{2.3a} and Ca\textsubscript{2.3e}. Of these two isoforms, Ca\textsubscript{2.3e}, which is known to be a major endocrine Ca\textsubscript{2.3} isoform (19), was the predominant isoform in TG neurons. It is not clear at
this moment how Cav2.3e regulates neurotransmitter release from nociceptive nerve terminals as well as insulin secretion from islets of Langerhans. However, it is possible that Cav2.3e might be an important molecular mediator, which is involved in both neurotransmitter release and hormone secretion.

Several previous studies support that R-type calcium channels are crucial for nociception. R-type calcium channels are located at primary synapses (39) and contribute to neurotransmitter release and presynaptic plasticity (40). αIR−/− mice showed reduced response to somatic inflammatory pain (21). Based on our results, Cav2.3e might be the Cav2.3 isoform responsible for the nociception mediated by Cav2.3. Different Cav2.3 isoforms are known to display distinct biophysical properties (41, 42). They might have distinct interactions with specific proteins/modulators.

**Physiological Implications**—Sensory neurons expressing Cav2.3e exhibited several principal characteristics of nociceptors. First, Cav2.3e was preferentially present in small diameter neurons in the rat TG. Second, Cav2.3e expression was restricted to trkA-positive/IB4-negative nociceptors. Third, Cav2.3e was the predominant isoform in TRPV1-positive neurons. Thus, Cav2.3e is likely to play an important role in nociceptive neurotransmission. Likewise, a Cav2.2 isoform, preferentially present in neurons containing nociceptive markers, TRPV1 and Nav1.8, has been demonstrated to contribute to pain transduction in nociceptive neurons (43).

It is interesting to note that Cav2.3e expression was predominant in trkA-positive/IB4-negative and TRPV1-positive nociceptors. It has been suggested that IB4-positive and IB4-negative populations have different physiological roles in acute and chronic pain conditions (44). Given that IB4-negative neurons contain neuropeptides (30), Cav2.3e might be involved in secretion of calcitonin gene-related neuropeptide and substance P.

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**Correlation between a Functionally Identified R-type Channel Component and Cav2.3e Expression**—We verified the molecular nature of R-type calcium channel components by a combination of single cell RT-PCR and functional analyses, including calcium imaging and whole-cell patch clamp recording. A subpopulation of sensory neurons tested definitely showed R-type calcium channel components as demonstrated by residual calcium transients or currents in the presence of L-, N-, and P/Q-type blockers. Single cell RT-PCR following calcium imaging and whole-cell patch clamp recording revealed that Cav2.3e was present in the IB4-negative nociceptive neurons with SNX-482-sensitive R-type calcium channel components, suggesting a correlation between R-type channel and Cav2.3e expression in these neurons. However, there should be another calcium channel in the other subset of IB4-positive neurons, which generates R-type calcium channel components. Previous studies have demonstrated that there may be non-Cav2.3 R-type current in dorsal root ganglion neurons (15, 24). In line with these findings, using the combined approach of antiseense strategy and electrophysiology with pharmacological tool, the existence of a non-Cav2.3 R-type current that is resistant to SNX-482 has been reported (41, 42). The molecular identity of this channel remains to be determined.

In summary, this study provides the first evidence of expression of a nociceptor-specific Cav2.3 calcium channel isoform in sensory neurons. Cav2.3e in nociceptive neurons may be a potential target for the treatment of pain.
Ca\(_{2.3e}\) Isoform in Nociceptive Neurons

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