Voltage-gated Sodium Channels Confer Excitability to Human Odontoblasts

POSSIBLE ROLE IN TOOTH PAIN TRANSMISSION*

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Odontoblasts are responsible for the dentin formation. They are suspected to play a role in tooth pain transmission as sensor cells because of their close relationship with nerve, but this role has never been evidenced. We demonstrate here that human odontoblasts in vitro produce voltage-gated tetrodotoxin-sensitive Na+ currents in response to depolarization under voltage clamp conditions and are able to generate action potentials. Odontoblasts express neuronal isoforms of α2 and β2 subunits of sodium channels. Co-cultures of odontoblasts with trigeminal neurons indicate a clustering of α2β2 sodium channel subunits, and, at the sites of cell-cell contact, a co-localization of odontoblasts β2 subunits with peripherin. In vivo, sodium channels are expressed in odontoblasts. AnkyrinG and β2 co-localize, suggesting a link for signal transduction between axons and odontoblasts. Evidence for excitable properties of odontoblasts and clustering of key molecules at the site of odontoblast-nerve contact strongly suggest that odontoblasts may operate as sensor cells that initiate tooth pain transmission.

The mechanisms underlying dentin sensitivity still remain unclear because of the structural complexity of this tissue including odontoblasts, nerve endings, and the liquid content of surrounding dentinal tubules. Odontoblasts constitute a layer of cells responsible for dentin formation. Each cell has an extension running into the dentinal tubule and bathed in the dentinal fluid. Sensory unmyelinated nerve fibers belonging to the trigeminal ganglion enter the inner dentin and coil around the monopolar processes of odontoblasts (1, 2). A hydrodynamic concept, based on the spatial situation of odontoblasts, nerve endings, and fluid movements in dentinal tubules, postulated that nociceptive responses may result from an increase in intradental pressure, which in turn might activate nerve endings. However, nerve terminals do not reach the most sensitive zone of the dentin (dentin-enamel junction), and intradental axons could not be directly excited by stimuli producing pain when applied to the teeth (1, 3). Thus emerged the hypothesis that odontoblasts may initiate tooth pain sensation. Several lines of evidence support this assumption. Recently, we have shown that reelin, a large extracellular matrix glycoprotein elaborated by odontoblasts, could promote adhesion between nerves and cells (4). This close association suggested that odontoblasts and nerve endings may directly interact, although no synaptic structures or any junction could be detected between them (1–3). Along this line, two kinds of mechanosensitive K+ channels (KCa and TREK-1) have been identified in human odontoblasts (5, 6). This finding indicated that odontoblasts might be able to convert pain-evoking fluid displacement within dentinal tubules into electrical signals, strengthening their possible role as tooth sensor cells. The view that odontoblasts could detect and transduce painful stimuli into electric signals questioned the possibility that these cells display excitable properties and possess voltage-gated sodium channels. These later have indeed been detected in non-excitable mineralizing cells (7) like osteoblasts where sodium channel Na1.2 mRNA and protein were identified (8). In teeth, voltage-gated Na+ channels have been previously evidenced in vitro on dental pulp cell by electrophysiological investigation (9). However, the identity of the cultured cells under study and the expression of odontoblast key genes were not established, thus casting doubt about the cell type displaying voltage-gated sodium channel activity. To overcome these difficulties, we recently set up a unique cell culture system allowing the differentiation of human dental pulp cells into odontoblasts at the morphological, molecular, and functional levels (5, 10, 11). In the present study, we took advantage of this cell model to apply the patch clamp technique and determine whether a voltage-gated Na+ channel is functional in the odontoblast plasma membrane. In parallel, we investigated the molecular isoforms of sodium channel subunits and their spatial distribution in relation with key molecular components at sites of close contact between odontoblasts and nerves in odontoblasts co-cultivated with trigeminal ganglions and in human dental pulp in vivo.

EXPERIMENTAL PROCEDURES

Cell Culture—Dental pulps cells were obtained from sound human third molar germs that were extracted for orthodontics reasons. Informed consent was obtained from the patients in accordance with French legal requirements (article 672-1, public health code). Explants were grown in Eagle’s basal medium

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were size-fractionated by electrophoresis in a non-denaturating 6% polyacrylamide gel (mini-gel apparatus, Bio-Rad) using a constant 40 V field. Gel was stained for 30 min in Vistra Green and imaged on a FluorImager (Amersham Biosciences, Orsay, France).

In Situ Hybridization—The material consisted of culture samples and sound non-erupted human third molars prepared as described previously in Refs. 10 and 12. For detection of the SCN2A and SCN2B transcripts, in situ hybridization was performed using an antisense single-stranded DNA probe (12) with a specific activity of about 2.5 \times 10^6 cpm/pmol. Sense primers were used for the synthesis of the control probes. The images were processed using Adobe PhotoShop 6.0 (Adobe Systems). Finally, pulp tissue from tooth germs was routinely processed (Masson’s trichrome staining) for light microscopic observation.

Immunocytochemistry—Cryostat sections of pulps were reacted for double staining with anti-\(\alpha_2/\beta_2\) subunits (mouse monoclonal antibodies at 10 \(\mu\)g/ml, Upstate Biotechnology, Lake Placid, NY); rabbit polyclonal antibodies at 10 \(\mu\)g/ml; Alomone Labs, Jerusalem, Israel); anti-\(\beta_2\) subunits/anti-peripherin (mouse monoclonal Mab 1527, chemicon, Temecula, CA); anti-\(\beta_2\) subunits/anti-ankyrin\(_G\) (mouse monoclonal antibodies at 10 \(\mu\)g/ml, Zymed Laboratories Inc., South San Francisco, CA), and anti-ankyrin\(_G/\beta\)-tubulin (H-235, Santa Cruz Biotechnology, Santa Cruz, CA). Subsequently, the slices were rinsed and then incubated (45 min at room temperature) with Alexa Fluor 594 goat anti-mouse IgG (Molecular Probes, Eugene, OR) for \(\alpha_2\) subunits, ankyrin\(_G\), and peripherin and with Alexa Fluor 488 goat anti-rabbit IgG (Molecular Probes) for \(\beta_2\) subunits and \(\beta\)-tubulin. They were then washed, mounted in phosphate-buffered saline-glycerol, and observed under scanning laser confocal microscopy using \(\times40/1.3\) or \(\times63/1.4\) oil immersion objective. Peripherin, \(\alpha_2\) subunits, and ankyrin\(_G\) were assigned a red color; \(\beta_2\) subunits and \(\beta\)-tubulin a green color with the laser scanning software. Co-localization consequently resulted in a yellow color. Negative controls were carried out by omitting the primary antibodies or by incubating with normal mouse or rabbit IgG. Figures were processed using Adobe PhotoShop 6.0 (Adobe Systems).

Electrophysiology—Membrane currents and potentials were recorded in the whole cell configuration on cultured odontoblasts using a patch clamp amplifier (model RK 400; Bio-Logic, Claix, France). Data acquisition and generation of command voltage pulses were done using the pClamp9 software (Axon Instruments Inc.) driving an A/D, D/A converter (Digidata 1322A, Axon Instruments Inc.). Cell capacitance, used to calculate the density of currents (A/F), was determined by integration of a control current trace obtained with a 10-mV depolarizing pulse from −90 mV. Leak currents were subtracted from all recordings using a 10-mV depolarizing pulse from the holding potential supposedly a linear evolution of leak current with depolarization. Individual curves of the voltage dependence of the \(Na^+\) current density were fitted with Equation 1.

\[
I(V) = \frac{G_{max}(V - V_{rev})}{1 + \exp[(V_{0.5} - V)/k]} \tag{Eq. 1}
\]

where \(I(V)\) is the density of the current measured, \(V\) is the test...
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pulse, \( G_{\text{max}} \) is the maximum conductance, \( V_{\text{rev}} \) is the apparent reversal potential, \( V_{0.5} \) is the half-activation voltage, and \( k \) is a steepness factor. Individual curves of the voltage dependence of the steady-state inactivation of the \( Na^+ \) current were fitted with the Equation 2.

\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + \exp\left(\frac{V - V_{0.5}}{k}\right)}
\]

(Eq. 2)

where \( I_{\text{max}} \) is the maximal current, \( V \) is the conditioning pulse, \( V_{0.5} \) is the half-maximal inactivation voltage, and \( k \) is a steepness factor. All experiments were carried out at room temperature (20–24 °C).

Solutions and Chemicals—Pipettes were filled with (in mM): 120 potassium aspartate, 5 KCl, 5 MgCl\(_2\), 10 glucose, 3 K\(_2\)ATP, 5 Na\(_2\)-CP, 0.4 Na\(_3\)-GTP, 5 EGTA, 10 Hepes, pH 7.2. The bath solution corresponded to a Tyrode solution containing (in mM): 140 NaCl or 140 choline chloride, 5 KCl, 2.5 CaCl\(_2\), 2 MgCl\(_2\), 10 Hepes, pH 7.2. Tetrodotoxin (TTX) (Sigma) was diluted to the required concentration in the bath solution. Voltages were corrected for liquid junction potentials calculated to be 10 mV with the solutions used.

Dye Microinjection in Odontoblasts in Co-culture—For intracellular staining of odontoblasts co-cultivated with trigeminal ganglia, micropipettes filled with an internal solution containing Lucifer yellow CH (100 \( \mu \)M, Sigma) were sealed on odontoblasts, and the patch membrane was ruptured to allow the dye to diffuse into the cell. Cells were imaged using a \( \times20 \) objective on an inverted microscope (Olympus IMT2) equipped for epifluorescence. Images were captured every 2 min after dye injection with a Coolsnapfx charge-coupled device camera (Roper Scientific, Evry, France). Injected cells were chosen as a function of their apparent close vicinity with the thin nerve fibers from trigeminal ganglia.

Statistics—Non-linear least-squares fits were performed using a Marquadt-Levenberg algorithm routine included in MicroCal Origin. Data values are presented as means ± S.E.

RESULTS

Characterization of Voltage-gated Sodium Channels and Voltage Responses in Cultured Odontoblasts—Cultured odontoblasts were depolarized by steps of 50-ms duration from a holding potential of −90 mV. All the odontoblasts tested (\( n = 13 \)) displayed a voltage-gated inward current that rapidly inactivated (Fig. 1A). The average time to peak of this inward current was 2.9 ± 0.4 ms at 0 mV. Fig. 1B presents the mean current-voltage relationships established for the peak current. The threshold of activation was around −40 mV, and currents peaked at 0 mV and reversed at +55 ± 3.6 mV. For each cell, the current-voltage relationship was fitted using Equation 1 (see “Experimental Procedures”). Mean values for \( G_{\text{max}}, V_{\text{rev}}, V_{0.5}, \) and \( k \) were 336 ± 52 S/F, +55 ± 3.6 mV, −13 ± 1.9 mV, and 4.1 ± 0.4 mV, respectively. The voltage dependence of the inactivation process was then investigated using a steady-state inactivation protocol. A 50-ms test pulse was delivered to 0 mV, a membrane potential at which the current was maximal, and was preceded by a 50-ms depolarizing prepulse of increasing amplitude. Fig. 1C shows that for depolarizations up to −40 mV, the current that was activated during the test pulse remained unaltered. For higher depolarizations, the inward current during the test depolarization progressively decreased and then completely vanished for a prepolarization to 0 mV. For each cell, the inactivation curve was fitted using Equation 2 (see “Experimental Procedures”). The corresponding mean inactivation curve indicated mean values for \( V_{0.5} \) and \( k \) of −26 ± 1.5 and 8.6 ± 0.6 mV, respectively. Voltage dependence, kinetic properties, and reversal potential of the inward current strongly suggested that this current corresponds to a voltage-gated \( Na^+ \) current. Indeed, in all odontoblasts tested, we found that TTX completely and reversibly abolished the inward current elicited by a depolarizing pulse to 0 mV (Fig. 2A). Adding increasing concentrations of TTX from 10 to 1000 nM in a cumulative manner indicated a half-maximal inhibition of the current with 45 nM. Additionally, in two cells, the substitution of choline for \( Na^+ \) led to an almost complete and a reversible abolition of the inward current (Fig. 2C). Taken together, these data demonstrate that odontoblasts express a functional voltage-gated TTX-sensitive \( Na^+ \) channel.

Given the high density of the voltage-gated \( Na^+ \) inward current together with the low density of outward current that developed in response to depolarization, we speculated that odontoblasts might be able to produce regenerative voltage responses. We then investigated the electrical excitability of

![Figure 1. Voltage-gated sodium currents in voltage clamped odontoblasts.](image-url)

In A, membrane currents were elicited by applying voltage pulses of 50-ms duration in 10-mV increments from a holding potential of −90 mV. In B, the mean current-voltage relationship obtained in five cells is presented. The curve was fitted by using Equation 1 with values for \( G_{\text{max}}, V_{\text{rev}}, V_{0.5}, \) and \( k \) of 342 S/F, +58 mV, −13 mV, and 4 mV, respectively. In C, currents were elicited by the voltage protocol indicated below the current traces; a first step of 50-ms duration and various amplitude was followed by a 50-ms test pulse to 0 mV with a short interpulse of 0.5-msec duration to −90 mV. In three cells (D), the means of normalized currents were plotted against the voltage values of conditioning pulses, and the curve was fitted by using Equation 2 with values for \( V_{0.5} \) and \( k \) of −26 and 9 mV, respectively. EM, membrane potential.
Brain Sodium Channel Subunits in Cultured Odontoblasts—The presence of functional voltage-gated Na⁺ channel in cultured odontoblasts prompted us to look for what kind of sodium channel subunits transcripts are expressed by odontoblasts. Degenerate homology-PCR was used to co-amplify SCN1A, SCN2A, and SCN3A genes. For this purpose, these sequences (SCN1A: S71446, SCN2A: M94055, SCN3A: AF035685, in GenBank™) were aligned using the MultAlin program (13). Two degenerate primers were designed to amplify a 277-bp fragment for SCN1A and SCN2A and a 268-bp fragment for SCN3A. RT-PCR realized on cultured odontoblast RNA amplified a single PCR product of about 277 bp. The gel did not allow us to discriminate between the 277 and 268 bp fragments. This product was submitted to four sets of digestion by restriction enzymes having specificity for each subtype. Fig. 3 shows that SCN1A, SCN2A, and SCN3A were expressed. For SCN2A and SCN3A, restriction produced two fragments of the expected size (Table 1). Digestion of the PCR product by TaqI gave rise to a fragment of 155 bp. The two other expected fragments (64 and 58 bp) were not detectable. A band of 399 bp was amplified from these RNA using the SCN2B primers. Restriction of the product gave rise to two fragments of 255 and 144 bp. In contrast, SCN5A coding for Na₁,5 (found in cardiac tissue) was not expressed in odontoblasts (data not shown). All these data confirm that the PCR products accurately represent the co-expression of the β2 subunit and the three isoforms SCN1A, SCN2A, and SCN3A (corresponding to Na₁,1.1, Na₁,1.2, and Na₁,1.3 α subunits, respectively, in another nomenclature) found in the central nervous system.

In Situ Hybridization of SCN2A and SCN2B Transcripts—SCN2A and SCN2B transcripts were detected in cultured odontoblasts (Fig. 4, a and b). Experiments conducted in vivo on human dental pulp tissue clearly demonstrated that the highest density of transcripts was detected in odontoblasts as opposed to pulp cells (Fig. 4, c and d). Hybridization with sense SCN2A (data not shown) and SCN2B probes showed negligible signal in odontoblasts (Fig. 4f). On Fig. 4e, the location and spatial organization of odontoblast layer is clearly evidenced at the periphery of the pulp tissue.

Co-culture Assays—Odontoblasts are known to be closely associated with nerves. To mimic the in vivo situation and to explore how Na⁺ channel subunits localize when nerves make contacts with odontoblasts, odontoblasts were co-cultivated with rat trigeminal ganglia. Analysis by confocal microscopy clearly showed a co-localization (yellow patches) of a2 and β2 around −80 mV by applying constant negative current. In all three cells tested with this protocol, infralaminar stimulations induced electrotonic response, whereas a spike bringing the membrane potential around +45 mV developed in response to current injection higher than 18 pA (Fig. 2D, left panel).

Fig. 2C, right panel, shows that train of action potentials could even be elicited in response to supralaminar repetitive stimulation of the cells at a frequency up to 18 Hz without any decrease in spike amplitude. Finally, as expected, spikes were totally inhibited by the addition of TTX in the bath or substitution of choline for Na⁺ in the external solution, confirming that the spike resulted from the activation of the voltage-gated TTX-sensitive Na⁺ current (Fig. 2E).
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1A 2A 3A α subunits

β2 subunit

FIGURE 3. Detection of transcripts of SCN1A, SCN2A, SCN3A, and SCN2B genes coding for sodium channel α and β2 subunits by RT-PCR. The expected restriction enzymes fragment sizes are listed in Table 1. The PCR products analyzed represent the co-expression of the β2 subunit and the three isoforms, the Na\(_1\).1, Na\(_1\).2, and Na\(_1\).3 α subunits. *, DNA ladder V (Roche Applied Science, Meylan, France).

TABLE 1

| Sodium channel subunits | Size     |
|-------------------------|----------|
|                         | TaqI     | AsnI | BspLU11 | MaeIII |
| SCN1A                   | 155 bp   |      |         |        |
| SCN2A                   | 143–125 bp | 152–125 |       |        |
| SCN3A                   | 255–144 bp | 144–125  |       |        |
| SCN2B                   | 125 bp   |      |         |        |

subunits in the odontoblast cell membrane, whereas α2 subunits were often densely expressed at the apical pole of the cells (Fig. 5, a–c). When a single neurite ran close to the odontoblast cell membrane, α2 and β2 subunits clustered in the contact area (Fig. 5, d–f). Staining with antibodies raised against peripherin, a component of trigeminal axons (14), clearly identified the nerves from the trigeminal ganglion, and the double labeling with β2 subunits showed a dot-like co-localization where a close contact was evidenced between odontoblasts and neurites (Fig. 5, g–i).

To explore the possibility that electric signals may propagate from odontoblasts to nerve cells via gap junctions, we injected Lucifer yellow into odontoblasts, a reported freely diffusible dye tracer through gap junctions (15). Microinjection was preferentially performed in odontoblasts making apparent close contact with nerves. Fig. 5j shows an intense fluorescence in a microinjected cell that spread after 30 min to adjacent odontoblast (Fig. 5k). However, in the 10 cells tested, the dye failed to migrate to axons located in the close vicinity of the injected cell.

Immunohistochemistry in Odontoblasts in Vivo—Double staining of α2 and β2 subunits in odontoblasts demonstrated a strong fluorescence for the two subunits at the apical pole of the cells (Fig. 6, a and b). In addition, α2 subunits labeling clearly underlined the membranes of the basal pole of the odontoblasts (Fig. 6b). Confocal analysis showed co-localizations of α2 and β2 subunits on the cell membrane and at the apical pole corresponding to the terminal web connecting odontoblasts in this region (Fig. 6c). In mature odontoblasts, β2 subunits decorated as dots profiled the cell membrane and labeled thin axons (Fig. 6d). Peripherin immunoreactivity revealed nerves running to the odontoblast layer (Fig. 6e). Confocal microscopy clearly showed co-localizations of peripherin positive nerves with β2 at the apical pole of the cells without co-localization of β2 and peripherin in the nerve fibers (Fig. 6f).

β2 subunits were also shown to co-localize with ankyrin\(_{\text{C}}\) (an intracellular anchoring protein directly linked to β2 subunit intracellular domains) (Fig. 6g) at the apical pole of the odontoblast layer and along the cell bodies only as cell processes did not show any ankyrin immunoreactivity (Fig. 6, h and i). Finally, β-tubulin, a major brain component of microtubules suspected to bind ankyrin\(_{\text{C}}\) (16), was mainly associated with the odontoblast cell membrane (Fig. 6j) and co-localized with ankyrin\(_{\text{C}}\) particularly at the base of the cell processes (Fig. 6, k and l). On in vitro and in vivo control experiments, negligible staining could be detected (data not shown). Intracellular staining was observed in some experiments (Fig. 6g); it might correspond to β2 subunits in the progress of synthesis related to the secretory stage of odontoblasts.

DISCUSSION

In this study, we present evidence that voltage-gated TTX-sensitive sodium channels are functional in cultured odontoblasts originating from human dental pulp. In response to depolarization, odontoblasts exhibited a fast sodium current that inactivated rapidly. This sodium current displayed biophysical characteristics comparable with those classically reported for the voltage-gated sodium current in axons (17, 18). Although less sensitive to TTX than voltage-gated Na\(^{+}\) channels present in nervous cells, Na\(^{+}\) currents in odontoblasts were found to be as sensitive as cloned brain channels expressed in host systems (19). In agreement with our electrophysiologically data, PCR experiments and in situ hybridization performed in cultured odontoblasts demonstrated expression of the transcripts of four genes (SCN1A, SCN2A, SCN3A, and SCN2B) encoding, respectively, the pore-forming α subunit isoforms.
Nav1.1, Nav1.2, and Nav1.3 and β2 subunits of voltage-gated Na⁺ channels broadly expressed in neurons of the central nervous system (20–22). Taken together, these data strongly emphasize the neural phenotype of odontoblasts not only in vitro but also in vivo as evidenced by our in situ hybridization and immunolabeling data.

More importantly, we demonstrated that odontoblasts were excitable and produced all or none spikes in response to injection of depolarizing currents. The fact that the spikes were totally inhibited in the presence of TTX or after removal of external Na⁺ demonstrated that action potentials were supported by the voltage-gated Na⁺ channels and excludes the possibility that other ion channels, such as Ca²⁺ channels, detected previously in dental pulp cells (23), might contribute to the generation of spikes. This finding has relevant physiological consequences. Indeed in vivo, intracellular recordings have shown that odontoblasts have a resting membrane potential around ~80 mV, values comparable with excitable cells (24). Previous electrophysiological studies have described the presence of ion channels in odontoblasts whose activity was modulated by mechanical stimuli (5). It is thus tempting to speculate that odontoblasts might be able to transduce and integrate diverse somatosensory signals known to elicit nociceptive responses in the pulp (drilling, dentin fluid flow, and heat and cold, for example) and initiate bursts of regenerative voltage responses. If true, this hypothesis raises the question of how the firing of odontoblasts is transmitted to the neighboring nerve cells.

We have utilized a co-culture system comprised of odontoblasts and trigeminal neurons to investigate axon-odontoblast interactions and mimic the in vivo situation. Our results in line with recent studies (4) indicate that we can successfully recapitulate in vitro the specific in vivo relationship between nerves and odontoblasts (1) by evidencing for the first time the close adhesion of β2 proteins of odontoblast with peripherin filaments expressed by trigeminal axons. β subunits modulate channel activity but also function as cell adhesion molecules and participate in cell adhesion (25). β2 subunits were suggested to preferentially associate with α2 (26), leading us to believe that these Na⁺ channel proteins present in vivo and in vitro odontoblast cell membrane may participate in the intimate nerve-odontoblast relationship described previously (1–3). In co-culture, α2 and β2 subunits co-localized at the sites of axon-odontoblast contact, indicating that a clustering of Na⁺ channels occurred. Sodium channel aggregation is a highly dynamic process contributing to the efficiency of conduction and excitability (27). Such a clustering may lower the threshold for generation of action potentials (28). Additionally, channel clustering in specific membrane microdomains could be modulated by the cytoskeletal linker protein ankyrinG since, as we showed, ankyrinG co-localized with β2 subunits and β-tubulin in odontoblasts in vivo. Ankyrins are expressed in most tissues and are able to interact with multiple proteins (29). Several lines of evidence indicate that ankyrinG is involved in Na⁺ channel clustering, promoting rapid and efficient conduction of action potentials.

**FIGURE 4. In situ hybridization with SCN2A (α2 subunit) and SCN2B (β2 subunit) cDNA.** In a (β2) and b (α2), a signal is clearly detected in cultured odontoblasts. On a human dental pulp section, the concentration of transcripts is well evidenced in odontoblasts (od) c, d (β2) d, e (α2) when compared with the underlying pulp cells (arrows). e, light microscopic micrograph (Masson’s trichrome staining) of a longitudinal section of a pulp sample carefully removed from the dentine walls. Odontoblast cell bodies are organized as a layer of palisade cells at the periphery of the pulp, whereas pulp cells (arrows) are scattered in the underlying tissue. f, in situ hybridization with a sense SCN2B probe as control. Bars are 50 μm.
potentials at the node of Ranvier as well as firing properties in Purkinje cells (20, 26, 30, 31). A similar process could occur in odontoblasts by inducing and stabilizing a high density of Na$^{+}$ channels at the sites of contact (mainly the terminal web) between these cells and unmyelinated axons. The co-localization of ankyrinG with $\alpha$-tubulin in odontoblasts, particularly at the apical pole of the cells, suggests that these molecules may act in restricting the mobility of the channels and maintaining the architecture of the anchoring complex (32).

The way odontoblasts and nerve cells may communicate remains unclear. To determine whether there are active intercellular communications allowing the action potentials from stimulated odontoblasts to produce depolarization in the sites of contact with axons, we injected Lucifer yellow in odontoblasts co-cultivated with trigeminal ganglia. The dye appeared to stain intensely the injected cells and spread to adjacent odontoblasts but failed to migrate to nerve fibrils in the close vicinity of cultured odontoblasts. This confirms a previous experiment with the same tracer on odontoblasts, demonstrating the presence of active gap junctions between odontoblasts themselves only, without any dye detection in related nerves (15). Thus, the connexin 43, later on identified in odon-
toblast cell membrane and trigeminal axons (33), probably does not participate in intercellular communications between these respective cells. A possibility is that the propagation of the action potentials from odontoblasts to axons in teeth might occur via an ephaptic communication process. This coupling refers to interactions between neurons mediated by current flow through the extracellular space (34). Considering the close relationship between nerves and odontoblasts and the cluster-
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...ing of Na⁺ channels in the sites of cell-cell contact, it is possible that a firing odontoblast may induce a supraliminal depolarization in the unmyelinated axon to impulse a spike. This type of interaction was recently suggested to occur under physiological conditions in the olfactory nerve in mammals (35). However, the release in the gap space of mediators from stimulated odontoblasts transducing the signal to the nerve could not be excluded.

In conclusion, patch clamp recordings and gene transcript analyses show for the first time that cultured human odontoblasts express functional Na⁺ channels composed of neural forms of α and associated β2 subunits. Considering that odontoblasts are able to generate action potentials and that α2 and β2 subunits cluster at the sites of contacts between odontoblast membranes and neurites, it is tempting to propose that odontoblasts could participate in the sensory transduction process in teeth through interactions with nerve fibrils whose nature remains to be elucidated. Moreover, in vivo, nerves appear to preferentially co-localize with sodium channels at the apical pole of odontoblasts and correlates with the spatial distribution of mechanosensitive KCa channels and L-type calcium channels (5, 36). Ion channels, concentrated at this borderline between cell processes and bodies, could thus operate as molecular transducers between dentine fluid flow and the underlying layer of odontoblasts, which in turn may initiate tooth pain sensation.

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