doi:10.1111/j.1365-2591.2007.01297.x

Hyperpolarization-activated channels in trigeminal ganglia innervating healthy and pulp-exposed teeth

J. E. Wells[1], K. C. Rowland[1] & E. K. Proctor[2]

[1]School of Dental Medicine, Southern Illinois University, Alton, IL; and [2]School of Medicine, Washington University, St Louis, MO, USA

Abstract

Wells JE, Rowland KC, Proctor EK. Hyperpolarization-activated channels in trigeminal ganglia innervating healthy and pulp-exposed teeth. *International Endodontic Journal*, **40**, 715–721, 2007.

Aim To use immunocytochemistry for determining the expression of HCN1, HCN2 and HCN3 (three subunits of the hyperpolarization-activated cyclic nucleotide-gated current channel) in rodent trigeminal ganglia (TG) that innervate healthy teeth and determine if expression of HCN subunits is increased in TG following pulp exposure.

Methodology Pulps were exposed in right maxillary incisors of male Sprague-Dawley rats. After fixation, TG were removed, cryostat sectioned, and immunocytochemistry was utilized to study the expression of HCN1-3 subunits. Immunoreactivity of individual neurons from the maxillary region of the TG was determined with ImageJ software. Differences in the number immunopositive neurons amongst groups were tested for statistical significance with either a Yates or Pearson's chi-square or Fisher's exact probability tests depending on neuron sample size. Differences in the intensity of immunoreactivity between groups were tested for statistical significance with a Student's *t*-test.

Results The majority of TG neurons were immunopositive for HCN1-3. Moreover, statistically significant increases in the number of TG neurons immunopositive for HCN1 and the intensity of HCN1-3 immunoreactivity were observed within hours of exposing the tooth pulp.

Conclusions HCN1-3 expression, as determined by immunocytochemistry, is increased within hours after injury. Given that I_h can facilitate neuronal excitability, results of the current study suggest that antagonists to HCN1-3 subunits could work as analgesics in the alleviation of orofacial pain.

Keywords: hyperpolarization-activated cyclic nucleotide-gated gene, I_h, immunocytochemistry, pain, pulp.

Received 16 January 2007; accepted 16 April 2007

Introduction

Tooth pulp, the most common origin of odontalgia (Bender 2000), is one of the more heavily innervated tissues in the body (Byers 1985, Byers & Narhi 1999) and has a large population of nociceptive axons (Virtanen 1985, Byers & Narhi 1999). Pulpal nociceptive neurons have somata in the trigeminal ganglion (TG), whose axons extend peripherally into the tooth pulp (Gregg & Dixon 1973, Shellhammer *et al.* 1984) and function to convey nociceptive stimuli into the brainstem.

Following injury, neuronal excitability is increased and contributes to the pathological states of hyperalgesia and allodynia (Iwata *et al.* 1999, Costigan & Woolf 2000, Hu *et al.* 2004, Tsuboi *et al.* 2004). For example, decreased K^+ current (Everill & Kocsis 1999, Tsuboi *et al.* 2004) or increased Na^+ current (Abdulla & Smith 2002) increases the excitability of neurons after injury. The hyperpolarization-activated cationic current (Pape 1996), I_h, is a particularly attractive candidate for increasing the excitability of neurons post-injury because I_h does not inactivate, has large

Correspondence: Jason E. Wells, PhD, Southern Illinois University, School of Dental Medicine, 2800 College Avenue, Alton, IL 62002, USA (Tel.: 001 618 474 7202; fax: 001 618 474 7071; e-mail: jwells@siue.edu).

amplitude and a reversal potential well above the action potential firing threshold (Tabata & Ishida 1996, Clapham 1998, Accili *et al.* 2002).

I_h channels belong to the hyperpolarization-activated cyclic nucleotide-gated (HCN) gene family. The four known subunits (HCN1-4) differ in their voltage-dependence of activation and sensitivity to intracellular cAMP (Viscomi *et al.* 2001, Baruscotti *et al.* 2005, DiFrancesco 2006), and therefore, differential expression of HCN subunits has functional implications. Indeed, whilst I_h is increased in TG neurons after ligation and chronic compression of peripheral nerves rendering neurons more excitable (Yao *et al.* 2003, Tsuboi *et al.* 2004), the resultant increase in I_h could be due to increased number of channels, but HCN subunit expression has not yet been studied in TG neurons. It can be hypothesized that orofacial injury causes increased expression of HCN1, HCN2 and HCN3 channel subunits in TG neurons. Therefore, this study focused on the expression of HCN1-3 subunits in rodent TG that innervate both healthy intact teeth and teeth with injured pulp by utilizing immunocytochemistry.

Materials and methods

Surgery

Male Sprague-Dawley rats (250–300 g; Hilltop Lab Animals, Scottdale, PA, USA) received i.p. injections of xylazine : ketamine mixture (6 mg : 70 mg per kilogram bodyweight). For three rats, a separating disk on a high-speed drill was used to coronally section the right maxillary incisor and a ¼-round carbide bur was used to expose the pulp. Rats were perfused, as described below, following a 6-h post-surgery period, which was previously empirically determined to be an early time-point that still affected HCN expression (see Discussion section). Three additional control rats were anaesthetized and handled, but the tooth pulp was not exposed. The protocol was approved by and followed the guidelines of the Institutional Animal Care and Use Committee of Southern Illinois University Edwardsville.

Animal perfusion

Rats received i.p. injections of xylazine : ketamine mixture (18 mg : 210 mg per kilogram bodyweight). Upon deep anaesthesia, rats were perfused through the ascending aorta with 50 mL of 0.15 mol L^{-1} phosphate buffered saline (PBS) followed by 300 mL of 4% paraformaldehyde in PBS. TG were dissected, placed into 30% sucrose in PBS overnight, frozen to −20 °C, 12-μm horizontal sections were cut with a freezing-stage cryostat, and sections were collected onto Super-Frost Plus slides (Fisher Scientific, Pittsburgh, PA, USA).

Immunocytochemistry

Sections were rinsed (three 10-min washes in PBS), followed by 10-min incubation in 3% H_2O_2, and rinsed again. Sections were incubated in 4% normal goat serum (NGS) in PBS with 0.4% Triton X-100 (Sigma, St Louis, MO, USA) for 1 h, then transferred into primary antibody (rabbit anti-HCN1 IgG, rabbit anti-HCN2 IgG) (Alomone labs, Jerusalem, Israel) or rabbit anti-HCN3 IgG (Alpha Diagnostics, San Antonio, TX, USA) used at concentrations of 1 : 1000, 1 : 1000 and 1 : 250, respectively) at 4 °C in a humidified environment for 48 h and then rinsed. Primary antibodies were diluted in PBS with 4% NGS and 0.1% Triton X-100. Secondary antibody, biotinylated goat anti-rabbit (Vector Laboratories, Burlingame, CA, USA), was applied according to manufacturer directions at a concentration of 1 : 200. After rinsing, sections were incubated in avidin-biotin-peroxidase complex (Vector Laboratories) for 30 min, rinsed, and then reacted with Sigma-Fast DAB tablets (Sigma). After rinsing, sections were dehydrated and cleared in xylene and alcohol, then coverslipped with Permount (Fisher Scientific). Primary antibody specificity was verified by omitting each of the primary antibodies on at least one slide in every immunoreaction and by preadsorbing the primary antibody with the proper antigen at a ratio of 2 : 1 (antigen : antibody), completely eliminating positive immunoreactivity.

Image analysis

Digital images were acquired with an upright Nikon Eclipse microscope (Nikon, Melville, NY, USA) using a 40 × 0.75NA objective and a Nikon CoolPix 5400. Two 40× images from three sections of each ganglia were collected from the maxillary region of the TG as somatotopically defined (Gregg & Dixon 1973, Shellhammer *et al.* 1984) and as indicated in the inset of Fig. 1a for each of the primary antibodies. Each section was separated by at least 48 μm (along the dorsal/ventral axis in the ganglion) to ensure that the same neuron was not counted twice. Neurons were divided into three categories according to soma cross-sectional area: small (<399.9 μm^2), medium (400–799.9 μm^2)

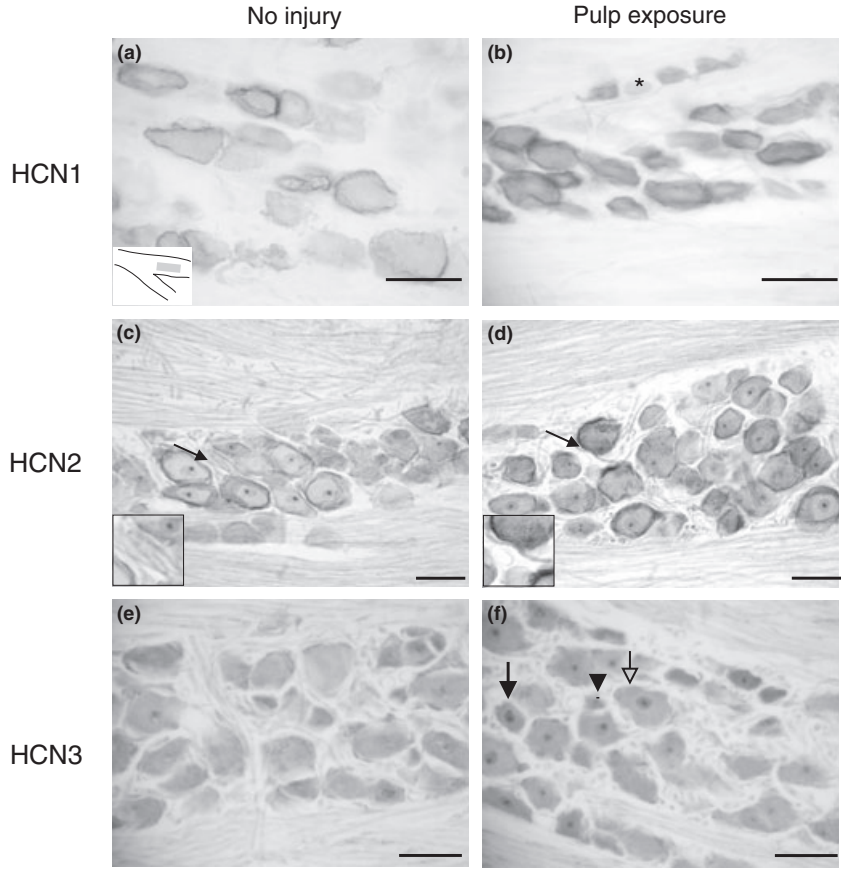

Figure 1 HCN immunoreactivity is found in the maxillary region of the rat trigeminal ganglion and is increased 6 h after pulp exposure. The immunoreactivity in the maxillary region of the rat trigeminal ganglion of HCN1-3 without injury (a, c, e) and with injury (b, d, f). Inset in (a) shows schematic diagram of rat trigeminal ganglion (*shaded* region indicates sampled area); *asterisk* in (b) indicates an immunonegative cell; (c, d) *arrows* indicate the region shown in the inset to illustrate examples of axonal immunoreactivity; (f) *arrowhead*, *solid arrow* and *hollow arrow* indicate small, medium and large neurons, respectively. Positive immunoreactivity in all panels is indicated by *dark precipitate*. Note increase in HCN1-3 channel immunoreactivity with pulp exposure compared to without peripheral injury. Scale bar equals 50 μm (all panels).

and large (>800 μm^2). Images were converted to greyscale and all images for each antibody were brightness/contrast enhanced identically, all neuronal somata in the field were outlined, then individual somata size and histograms of the number of pixels staining per grey value were collected using ImageJ (http://rsb.info.nih.gov/ij). Each neuron's histogram was normalized to the highest pixel value for its soma (converting pixel counts to proportion of total pixels) to normalize across experiments. From normalized histograms, cumulative probability curves were generated, which demonstrates the proportion of pixels within a soma above or below a particular grey value. To determine background nonspecific immunostaining, somata from control sections (processed without primary antibodies) were analysed identically. Neurons were considered immunopositive if 75% of their pixels were darker than 95% of the pixels for control (no primary antibody) somata within its size group (75 and 95% were chosen to increase the stringency and veracity of qualifying a neuron as immunopositive). Grey values of 255 and 0 indicate black and white saturation, respectively; positive immunoreactivity is indicated by higher grey values. Reported grey values were normalized so that the highest grey value in each group was 100. Immunoreactivity for all HCN members in the TG contralateral (CTG) to the injured pulp did not differ significantly from rats that received no

injury (n = 3; data not shown), therefore, all comparative analyses were performed between the TG ipsilateral (ITG) to the pulp exposure and the CTG to the pulp exposure.

Differences in the number of immunopositive neurons amongst groups were tested for statistical significance with either a Yates or Pearson's chi-square or Fisher's Exact Probability tests depending on neuron sample size. Differences in mean grey values between ipsilateral and contralateral neurons were tested for statistical significance with a Student's t-test. P-values <0.05 were considered statistically significant. Means are reported as mean ± SE.

Results

Trigeminal ganglion neurons are immunopositive for hyperpolarization-activated cyclic nucleotide-gated 1-3 subunits (HCN1-3)

Positive immunoreactivity for HCN1-3 was observed (Fig. 1) in TG in all three neuronal size groups from the maxillary region as previously defined (Gregg & Dixon 1973, Shellhammer *et al.* 1984) and as indicated in the inset of Fig. 1a. In basal conditions, typical HCN1 immunoreactivity was concentrated around neuronal membranes and within the somatic cytoplasm. After pulp exposure, both membranous and cytoplasmic HCN1 immunoreactivity were increased (Fig. 1). About 85.1% of all neurons (205 of 241 neurons) in the CTG were HCN1 immunopositive (Fig. 2). A significantly larger proportion of small neurons than large neurons (91.2% of 68 small neurons vs. 79.5% of 88 large neurons) in the CTG were immunopositive for HCN1 ($P < 0.05$; Fig. 2). Additionally, the TG innervating exposed pulp had more HCN1 immunopositive neurons than the TG innervating intact pulp. The number of HCN1 immunopositive neurons was significantly higher ($P < 0.01$) in the ITG (234 of 251 sampled neurons; 93.2%) compared with the CTG (205 of 241 sampled neurons; 85.1%; Fig. 2). Additionally, all of the sampled neurons in the CTG and ITG were immunopositive for HCN2 (562 neurons) and HCN3 (533 neurons). Under basal conditions, typical HCN2 immunoreactivity (Fig. 1c) was concentrated around the neuronal membrane and was also found within the somatic cytoplasm and axons (see arrows in Fig. 1c). After pulp exposure (Fig. 1d), the largest increase in HCN2 immunoreactivity was within the somatic membrane and cytoplasm. HCN3 immunoreactivity was not found in the cell membrane (Fig. 1e,f). Under basal conditions, typical HCN3 immunoreactivity was found within the somata and axons. Following pulp injury, somatic HCN3 immunoreactivity increased within the somata, particularly in the region of the cell nucleus (Fig. 1f).

Pulp exposure increases intensity of hyperpolarization-activated cyclic nucleotide-gated 1-3 immunoreactivity

Intensity of HCN1-3 expression in neurons was assessed by measuring grey value intensity of HCN1-3 immunoreactivity for each neuron (Fig. 3; see Methods

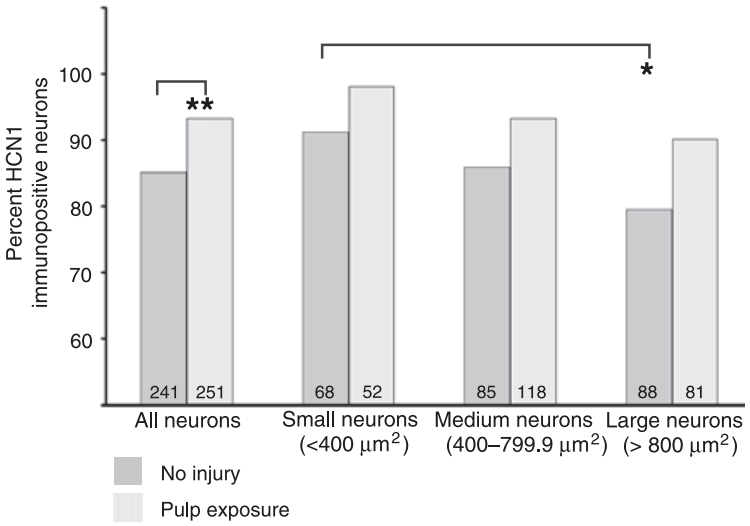

Figure 2 The percentage of HCN1 immunopositive neurons increases in response to pulp exposure. Note the increase in the percentage of immunopositive neurons with an injured pulp. In addition, note that without injury there is a significantly larger portion of small neurons than large neurons that are HCN1 immunopositive. *$P < 0.05$; **$P < 0.01$. Number of neurons in each group is indicated in the bottom of each bar.

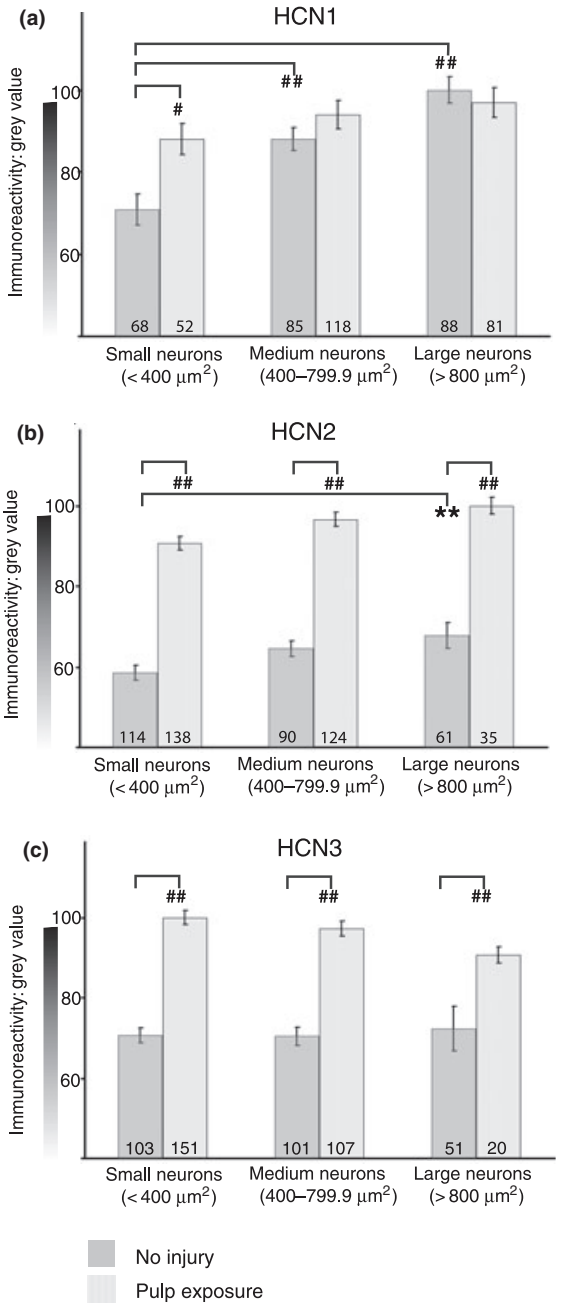

Figure 3 Pulp exposure increases HCN1-3 expression. Immunoreactivity measured as mean grey units for each group of neurons (small, medium and large) for HCN1 (a), HCN2 (b), HCN3 (c). Note increased HCN1-3 immunoreactivity with pulp exposure. *Larger grey units* indicate a stronger degree of positive immunoreactivity as denoted by the *vertical colour bar* to the left of each graph. $^{**}P < 0.01$; $^{\#}P < 0.002$; $^{\#\#}P < 0.001$. Number of neurons in each group is indicated in the bottom of each bar.

section). In the CTG, large (grey value: 100 ± 3.2) and medium (grey value: 88.2 ± 2.8) sized neurons were significantly more HCN1 immunoreactive than small neurons (grey value: 71.2 ± 3.8; $P < 0.001$; Fig. 3a). Additionally, small neurons were more intensely HCN1 immunoreactive in the ITG compared with CTG (grey values: 88.1 ± 3.8 vs. 71.2 ± 3.8; $P < 0.002$; Fig. 3a). Furthermore, large neurons were significantly more HCN2 immunoreactive than small neurons of the CTG (grey value: 67.9 ± 3.2 vs. 58.6 ± 1.9; $P < 0.01$). However, neurons of all size groups exhibited significant increases in HCN2 immunoreactivity in the ITG (small neuron grey value: 58.6 ± 1.9 vs. 90.7 ± 1.9; medium neuron grey value: 63.9 ± 1.9 vs. 96.4 ± 2.0; large neuron grey value: 67.9 ± 3.2 vs. 100.0 ± 2.1; $P < 0.001$ for all three groups; Fig. 3b). HCN3 immunoreactivity within all neuronal size groups in the CTG was similar, and all neuron size groups showed increased levels of immunoreactivity in the ITG (small neuron grey value: 70.7 ± 1.8 vs. 100.0 ± 1.7; medium neuron grey value: 70.5 ± 2.3 vs. 97.2 ± 1.8; large neuron grey value: 72.4 ± 5.5 vs. 90.6 ± 2.0; $P < 0.001$ for all three groups; Fig. 3c).

Discussion

Hyperpolarization-activated cyclic nucleotide-gated subunit immunoreactivity in trigeminal ganglion

The present study is the first to indicate that TG neurons are immunoreactive for HCN1, HCN2 and HCN3 channel subunits. Furthermore, the results demonstrate that hours after the pulp in the maxillary incisor is exposed there was an increase in the number of HCN1 immunopositive neurons, and the intensity of HCN1, HCN2 and HCN3 immunoreactivity is increased. Whilst not previously studied in the TG, HCN immunoreactivity has been studied in the dorsal root ganglion (DRG). In animal models of pain, despite increases in I_h, DRG neurons have decreased HCN1 and HCN2 immunoreactivity, and no change was observed HCN3 immunoreactivity (Chaplan *et al.* 2003), whilst TG neurons have increased I_h (Tsuboi *et al.* 2004) coupled with an increase in HCN1-3 immunoreactivity as determined in the present study. However, previous DRG pain models and the TG pain model used in the present study are not entirely similar, given that the DRG models damage an entire nerve whilst the TG model used here involves only a small peripheral injury

and could account for the different HCN1-3 channel expression following injury in these ganglia. Whilst the present model relates well to orofacial pain, it would be interesting to determine if similar results are replicated if a maxillary nerve ligation model is used.

Significance of hyperpolarization-activated cyclic nucleotide-gated subunits in nociception

The results suggest that the increased I_h and associated increases in excitability of TG neurons following injury (Tsuboi *et al.* 2004) results from increased HCN1-3 channel expression. Experiments examining HCN levels could be performed in conjunction with experiments to examine the levels of molecules associated with nociception, such as CGRP, substance P, TRPV1 and $P2X_3$ (Aoki *et al.* 2004) to determine if particular classes of nociceptive neurons have altered HCN expression levels. It is interesting, given that HCN channels are sensitive to cAMP (Viscomi *et al.* 2001, Baruscotti *et al.* 2005, DiFrancesco 2006), that opioids modulate pain by decreasing cAMP concentrations by mediating adenylyl cyclase (Ingram & Williams 1994). An increase in I_h via HCN1-3 could also explain the analgesic efficacy of ZD7288, an HCN channel antagonist.

As not only a systemic application of HCN channel antagonists will reduce mechanical allodynia (Lee *et al.* 2005), but also a perineural injection is effective (Dalle & Eisenach 2005), HCN subunits may also increase in pulpal nerve endings. Future experiments targeting HCN expression in pulpal axons may determine if axonal expression parallels somatic expression. Additionally, the present study demonstrated changes in HCN1-3 expression 6 h after the tooth pulp had been exposed, which is a relatively short amount of time following the injury. The present results suggest that HCN1-3 subunits, possibly along with other cation conducting channels, such as transient receptor potential (TRP) channels (Bezzerides *et al.* 2004, Zhang *et al.* 2005), can change the physiology of neurons within a short period of time after a stimulus.

Cross-excitation of trigeminal ganglion somata

Interestingly, although the number of neurons innervating the maxillary incisor is relatively small (Gregg & Dixon 1973, Shellhammer *et al.* 1984), increases in HCN1-3 immunoreactivity throughout the maxillary region of the TG were observed. It can be suggested that this response involved neurons throughout the maxillary region and was not limited to a small subpopulation of neurons because primary sensory neurons cross-excite one another through several mechanisms, including the release of diffusible chemical mediators, which occurs in several sensory ganglia, including DRG (Amir & Devor 1996, 2000), nodose ganglia (Oh & Weinreich 2002) and TG (Matsuka *et al.* 2001, Takeda *et al.* 2005). Increasing I_h in additional neurons by increasing HCN1-3 expression via cross-excitation offers a potential mechanism to facilitate hyperalgesia by recruiting additional neurons to respond to sensory stimuli.

Conclusions

HCN1, HCN2 and HCN3 subunit expression increases in TG neurons within hours of a tooth pulp injury. These results suggest that I_h is increased following injury via increased HCN1-3 expression in neurons throughout the TG, and that, future analgesics aimed at blocking HCN1-3 subunits could be used to combat orofacial pain.

References

Abdulla FA, Smith PA (2002) Changes in Na(+) channel currents of rat dorsal root ganglion neurons following axotomy and axotomy-induced autotomy. *Journal of Neurophysiology* **88**, 2518–29.

Accili EA, Proenza C, Baruscotti M, DiFrancesco D (2002) From funny current to HCN channels: 20 years of excitation. *News in Physiological Sciences* **17**, 32–7.

Amir R, Devor M (1996) Chemically mediated cross-excitation in rat dorsal root ganglia. *The Journal of Neuroscience* **16**, 4733–41.

Amir R, Devor M (2000) Functional cross-excitation between afferent A- and C-neurons in dorsal root ganglia. *Neuroscience* **95**, 189–95.

Aoki Y, Takahashi Y, Ohtori S, Moriya H, Takahashi K (2004) Distribution and immunocytochemical characterization of dorsal root ganglion neurons innervating the lumbar intervertebral disc in rats: a review. *Life Sciences* **74**, 2627–42.

Baruscotti M, Bucchi A, Difrancesco D (2005) Physiology and pharmacology of the cardiac pacemaker (''funny'') current. *Pharmacology and Therapeutics* **107**, 59–79.

Bender IB (2000) Reversible and irreversible painful pulpitides: diagnosis and treatment. *Australian Endodontic Journal* **26**, 10–4.

Bezzerides VJ, Ramsey IS, Kotecha S, Greka A, Clapham DE (2004) Rapid vesicular translocation and insertion of TRP channels. *Nature Cell Biology* **6**, 709–20.

Byers MR (1985) Terminal arborization of individual sensory axons in dentin and pulp of rat molars. *Brain Research* **345**, 181–5.

Byers MR, Narhi MV (1999) Dental injury models: experimental tools for understanding neuroinflammatory interactions and polymodal nociceptor functions. *Critical Reviews in Oral Biology and Medicine* **10**, 4–39.

Chaplan SR, Guo HQ, Lee DH, et al. (2003) Neuronal hyperpolarization-activated pacemaker channels drive neuropathic pain. *The Journal of Neuroscience* **23**, 1169–78.

Clapham DE (1998) Not so funny anymore: pacing channels are cloned. *Neuron* **21**, 5–7.

Costigan M, Woolf CJ (2000) Pain: molecular mechanisms. *The Journal of Pain* **1**, 35–44.

Dalle C, Eisenach JC (2005) Peripheral block of the hyperpolarization-activated cation current (Ih) reduces mechanical allodynia in animal models of postoperative and neuropathic pain. *Regional Anesthesia and Pain Medicine* **30**, 243–8.

DiFrancesco D (2006) Serious workings of the funny current. *Progress in Biophysics and Molecular Biology* **90**, 13–25.

Everill B, Kocsis JD (1999) Reduction in potassium currents in identified cutaneous afferent dorsal root ganglion neurons after axotomy. *Journal of Neurophysiology* **82**, 700–8.

Gregg JM, Dixon AD (1973) Somatotopic organization of the trigeminal ganglion in the rat. *Archives of Oral Biology* **18**, 487–98.

Hu WP, You XH, Guan BC, Ru LQ, Chen JG, Li ZW (2004) Substance P potentiates 5-HT3 receptor-mediated current in rat trigeminal ganglion neurons. *Neuroscience Letters* **365**, 147–52.

Ingram SL, Williams JT (1994) Opioid inhibition of Ih via adenylyl cyclase. *Neuron* **13**, 179–86.

Iwata K, Tashiro A, Tsuboi Y, et al. (1999) Medullary dorsal horn neuronal activity in rats with persistent temporomandibular joint and perioral inflammation. *Journal of Neurophysiology* **82**, 1244–53.

Lee DH, Chang L, Sorkin LS, Chaplan SR (2005) Hyperpolarization-activated, cation-nonselective, cyclic nucleotide-modulated channel blockade alleviates mechanical allodynia and suppresses ectopic discharge in spinal nerve ligated rats. *The Journal of Pain* **6**, 417–24.

Matsuka Y, Neubert JK, Maidment NT, Spigelman I (2001) Concurrent release of ATP and substance P within guinea pig trigeminal ganglia in vivo. *Brain Research* **915**, 248–55.

Oh EJ, Weinreich D (2002) Chemical communication between vagal afferent somata in nodose Ganglia of the rat and the Guinea pig in vitro. *Journal of Neurophysiology* **87**, 2801–7.

Pape HC (1996) Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annual Review of Physiology* **58**, 299–327.

Shellhammer SB, Gowgiel JM, Gaik GC, Weine FS (1984) Somatotopic organization and transmedian pathways of the rat trigeminal ganglion. *Journal of Dental Research* **63**, 1289–92.

Tabata T, Ishida AT (1996) Transient and sustained depolarization of retinal ganglion cells by Ih. *Journal of Neurophysiology* **75**, 1932–43.

Takeda M, Tanimoto T, Nasu M, Ikeda M, Kadoi J, Matsumoto S (2005) Activation of NK1 receptor of trigeminal root ganglion via substance P paracrine mechanism contributes to the mechanical allodynia in the temporomandibular joint inflammation in rats. *Pain* **116**, 375–85.

Tsuboi Y, Takeda M, Tanimoto T, et al. (2004) Alteration of the second branch of the trigeminal nerve activity following inferior alveolar nerve transection in rats. *Pain* **111**, 323–34.

Virtanen AS (1985) Electrical stimulation of pulp nerves – comparison of monopolar and bipolar electrode coupling. *Pain* **23**, 279–88.

Viscomi C, Altomare C, Bucchi A, et al. (2001) C terminus-mediated control of voltage and cAMP gating of hyperpolarization-activated cyclic nucleotide-gated channels. *The Journal of Biological Chemistry* **276**, 29930–4.

Yao H, Donnelly DF, Ma C, LaMotte RH (2003) Upregulation of the hyperpolarization-activated cation current after chronic compression of the dorsal root ganglion. *The Journal of Neuroscience* **23**, 2069–74.

Zhang X, Huang J, McNaughton PA (2005) NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. *The EMBO Journal* **24**, 4211–23.