Immunohistochemical Analysis of the Purinoceptor $P2X_7$ in Human Lingual Nerve Neuromas

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Aims: Recent evidence suggests that the purinoceptor $P2X_7$ may be involved in the development of dysesthesia following nerve injury, therefore, the aim of the present study was to investigate whether a correlation exists between the level of $P2X_7$ receptor expression in damaged human lingual nerves and the severity of the patients' symptoms. ***Methods:*** *Neuroma-in-continuity specimens were obtained from patients undergoing surgical repair of the damaged lingual nerve. Specimens were categorized preoperatively according to the presence or absence of dysesthesia, and visual analog scales scores were used to record the degree of pain, tingling, and discomfort. Indirect immunofluorescence using antibodies raised against S-100 (a Schwann cell marker) and $P2X_7$ was employed to quantify the percentage area of S-100 positive cells that also expressed $P2X_7$.* ***Results:*** *$P2X_7$ was found to be expressed in Schwann cells of lingual nerve neuromas. No significant difference was found between the level of $P2X_7$ expression in patients with or without symptoms of dysesthesia, and no relationship was observed between $P2X_7$ expression and VAS scores for pain, tingling, or discomfort. No correlation was found between $P2X_7$ expression and the time between initial injury and nerve repair.* ***Conclusion:*** *These data show that $P2X_7$ is expressed in human lingual nerve neuromas from patients with and without dysesthesia. It therefore appears that the level of $P2X_7$ expression at the injury site may not be linked to the maintenance of neuropathic pain after lingual nerve injury.* J OROFAC PAIN 2009;23:65–72

Key words: dysesthesia, immunohistochemistry, lingual nerve, nerve injury, $P2X_7$

Injury to the lingual nerve is common and usually results from iatrogenic damage during routine surgery such as the removal of mandibular wisdom teeth. The injury results in sensory loss to the ipsilateral side of the tongue and some patients develop abnormal and unpleasant sensations of dysesthesia, which can be either spontaneous or evoked by moving or touching the tongue. The management of dysesthesia is difficult, and none of the treatment methods are entirely satisfactory.[1] Surgical repair of the damaged nerve can ameliorate the symptoms in some patients, but does not reduce the number of patients suffering from the condi-

tion.[2] The pharmacological management of dysesthesia is also difficult, with antidepressants or membrane-stabilizing drugs offering limited efficacy and, often, significant side effects.[3] A better understanding of the etiology of nerve injury-induced sensory disorders could help in the development of new treatment options and novel analgesics. In this study, the role of the purinoceptor $P2X_7$ in the development of dysesthesia following lingual nerve injury in man was investigated.

P2X receptors are ligand-gated ion channels activated by extracellular adenosine triphosphate (ATP). Seven subtypes of this family have been identified, $P2X_{1–7}$, and are expressed on a variety of different cell types.[4] Within this family, $P2X_7$ is of interest as it is predominantly expressed by cells involved in immune responses, including astrocytes and microglia.[5–7] $P2X_7$ appears to be involved in the regulation of cytokine and inflammatory mediator expression and secretion (see Sperlagh et al[8] for review).

Recent studies have also implicated $P2X_7$ in inflammatory and neuropathic pain conditions. Following cerebral ischemia, a notable expression of $P2X_7$ was observed in astrocytes which show limited expression of this receptor prior to injury,[9] and increased $P2X_7$ protein expression was reported in Schwann cells of injured nerves from chronic neuropathic pain patients.[10] Chessell and colleagues[10] also demonstrated that in $P2X_7$-null mice, inflammatory and neuropathic hypersensitivity was completely abolished to noxious mechanical and thermal stimuli, while normal nociceptive behavior was maintained. Antagonist studies have also been conducted; after acute spinal cord injury, the $P2X_7$ antagonist oxidized ATP protected against neuronal cell death and improved functional recovery after injury.[11] Antinociceptive effects of $P2X_7$ antagonists have also been noted in animal models of both inflammatory[12–14] and neuropathic pain.[15]

In the authors' laboratory, a unique archive of human neuromas has been obtained from patients referred for microsurgical repair of an injured lingual nerve. The repair procedure involves the excision of the neuroma that has developed at the injury site, thereby providing the specimens used in the present study. Detailed clinical histories were obtained for each patient. The aim of the present study was to investigate whether a correlation exists between $P2X_7$ receptor expression in damaged human lingual nerves and the patients' symptoms of dysesthesia.

Materials and Methods

Specimens

Ethical approval for the study was obtained from the South Sheffield Research Ethics Committee, and all specimens were collected with the patients' informed consent. All patient details were kept confidential, and each specimen was given a unique code that was used throughout the study. Neuroma specimens were obtained from an archive of 84 neuromas taken from patients referred to the authors' department for treatment during the period 1999 to 2004. The light and electron microscopic characteristics of these neuromas have been described previously.[16,17] We selected 13 neuroma-in-continuity specimens in which there were still some elements of the nerve bridging the gap between the central and distal stumps of the damaged nerve. Ten specimens were from female patients and three from male patients, and the mean age of the patients at the time of neuroma removal was 31.25 ± 1.36 years (range, 23 to 40 years). The mean time between nerve injury and repair was 17.75 ± 2.84 months (range, 7 to 41 months).

The clinical histories and symptoms experienced by the patient were obtained prospectively, prior to the surgery to remove the neuroma, using a form completed by one of two clinicians. Patients were asked whether the affected part of the tongue was painful and whether they had tingling either spontaneously or initiated by moving or touching the tongue. Patients also scored their level of pain, discomfort, or tingling on visual analogue scales (VASs). In addition, a series of standardized sensory tests were performed to assess the ability to detect light touch, pin-prick, and gustatory stimuli and to record 2-point discrimination thresholds, as previously described in detail by Robinson et al.[2] In all patients, these tests revealed either anesthesia or a significant degree of hypoesthesia (in addition to varying degrees of dysesthesia) on the affected side of the tongue, thus confirming the need for exploration and repair. Patients were categorized as having no symptoms if they showed reduced sensation in the tongue but no pain or unpleasant tingling. Patients were categorized as having symptoms if they experienced pain or unpleasant tingling either spontaneously or initiated by touching or moving the tongue; patients with a relatively high level of symptoms were selected, on the assumption that this would increase the likelihood of revealing differences between the two groups.

Immunohistochemistry

Immediately following surgical removal of the neuroma specimen, the central end was marked with a 9/0 Ethicon (Ethicon, Edinburgh, UK) suture and the neuroma was placed in 2% Zamboni's fixative (0.1 mol/L phosphate buffer, pH 7.4, containing 4% paraformaldehyde and 0.2% picric acid) for 24 hours at 4°C. The specimen was cryoprotected in a 30% sucrose solution for 6 hours at 4°C and embedded longitudinally in Tissue-Tek OCT compound (Sakura). Serial sections (14 μm) were cut on a cryostat and thaw-mounted onto poly-D-lysine (Sigma-Aldrich) coated glass slides in 20 sets so that each section was 280 μm from the adjacent section on the same slide. Sections were left to air dry for 1 hour at room temperature prior to storage at –80°C until ready for use.

One set of slides from each specimen was removed from storage and left to air dry for 1 hour at room temperature. Sections were washed in phosphate buffered saline (PBS) containing 0.2% Triton-X-100 (PBST) for 2 × 10 minutes. To reduce non-specific staining, sections were first incubated in PBST containing 20% normal donkey serum (NDS, Jackson ImmunoResearch) for 1 hour in a moisture chamber at room temperature. Sections were then double-labeled with a mixture of a polyclonal antibody to the $P2X_7$ receptor raised in rabbit (1:1000, Chemicon) and a monoclonal Schwann cell marker, S-100 raised in mouse (1:100, Chemicon). The antisera were diluted in PBST containing 5% NDS, and the slides were left to incubate in the primary antisera solution for 24 hours at 4°C in a moisture chamber.

Following overnight incubation, slides were washed in PBST, 2 × 10 minutes, prior to a 90-minute incubation period with the secondary antibodies donkey anti-rabbit IgG conjugated to indocarbocyanine (Cy3, 1:200, Jackson ImmunoResearch) and donkey anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC, 1:20, Jackson ImmunoResearch) diluted in 1.5% NDS in PBST solution.

Immunohistochemical controls were performed by incubating tissue sections with the secondary antibody alone (S-100) or by preabsorption of the $P2X_7$ primary antibody with an excess of its specific antigen (10 μg/mL) for 24 hours prior to applying to the tissue.

Analysis of Labeling

All analysis was performed blind to the symptom group. Sections were viewed using a Zeiss Axioplan fluorescent microscope. Descriptive analysis of the immunolabeling of each sample was undertaken followed by quantitative analysis using the image processing program Image-pro Plus (v3.0, Media Cybernetics) with which the percentage of $P2X_7$ labeling in S-100 positive cells could be calculated.

A section from approximately the middle of each neuroma was selected for analysis to ensure that tissue across the entire width of the nerve was sampled. The very central and distal portions of the neuroma were eliminated from analysis, as these regions have often been manipulated during the surgical procedure. Each neuroma was divided into quarters longitudinally, with analysis starting in the second quarter from the distal end and continuing proximally along a length of at least 1,800 μm of the neuroma with a minimum of 30,000 μm^2 of positively labeled Schwann cells included. Each field of interest was initially viewed through the FITC filter and the area of positively labeled Schwann cells was measured. The same field was then viewed using the Cy3 filter to determine the percentage of Schwann cells that also expressed $P2X_7$.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (version 12.0). Independent sample *t* tests were used to test for any significant differences between levels of $P2X_7$ expression in neuromas from patients with or without dysesthesia, for differences between neuromas from male and female patients, and between neuromas from females with and without dysesthesia. There were no significant differences between the two groups in terms of patient age and time between injury and repair (independent sample *t* test), indicating these groups to be matched. All significance levels were set at $P < .05$. Pearson's correlation coefficients were used to determine whether there was any correlation between the expression of $P2X_7$ and the VAS scores for pain, tingling, or discomfort, the age of the patient, or the time between injury and repair.

Results

Qualitative Analysis

Immunoreactivity to S-100 and $P2X_7$ was present in all neuromas analyzed. This immunoreactivity varied widely between different specimens, with some neuromas containing large areas of S-100

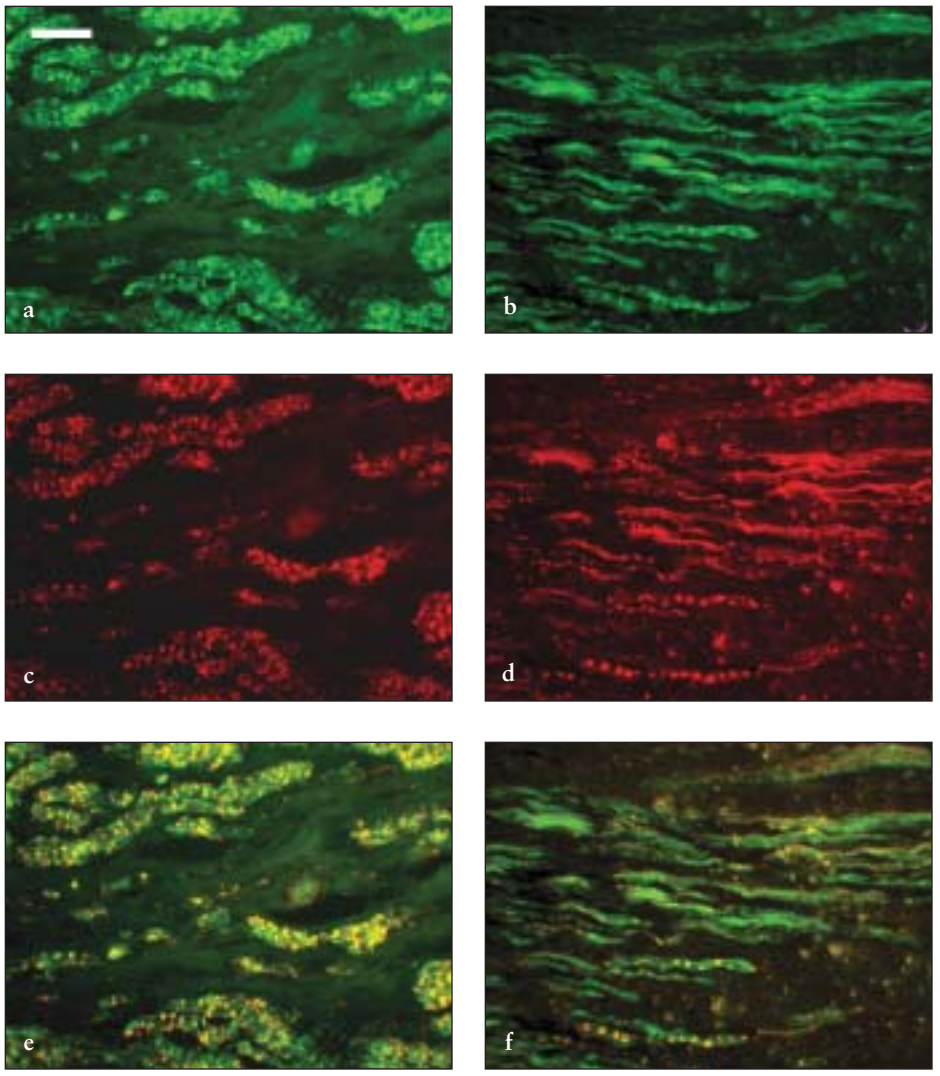

Fig 1 Photomicrographs of lingual nerve neuromas from patients with *(a, c, e)* and without *(b, d, f)* symptoms of dysesthesia, showing double labeling of S-100, a Schwann cell marker *(green, a and b)*, and $P2X_7$ receptor *(red, c and d)*. Photomicrographs *(e and f)* show colocalization of $P2X_7$ in Schwann cells of the lingual nerve. Labeling of $P2X_7$ in Schwann cells was observed in all specimens but varied greatly from high *(a, c, e)* to low *(b, d, f)* levels of expression. Scale bar = 30 μm.

and $P2X_7$ positively labeled tissue, whilst in others, only a small proportion of S-100 labeled Schwann cells contained $P2X_7$ (Fig 1). No $P2X_7$ labeling was seen in S-100-negative structures. In all samples, $P2X_7$ labeling was observed throughout the length of the neuroma. All positive immunoreactivity was abolished by preincubation of $P2X_7$ with its respective peptide and by replacing the S-100 antibody with normal serum, indicating both labeling processes to be specific (Fig 2).

Quantitative Analysis

$P2X_7$ Expression and Symptoms of Dysesthesia. In the patients with symptoms, the VAS scores were: pain 58.86 ± 7.71 (SD); tingling 75.57 ± 13.08; discomfort 67.86 ± 8.48. In the patients without symptoms, the VAS scores were: pain 0.83 ± 0.17 (SD); tingling 1.00 ± 0.37; discomfort 46.17 ± 9.41. $P2X_7$ expression ranged from 2.37% to 23.72% (mean = 9.14%) in neuromas from patients without dysesthesia, and values ranged from 4.10% to 20.37% (mean = 10.47%) in neuromas from patients with symptoms of dysesthesia (Fig 3). There was no significant difference between these two groups (Independent samples *t* test, $P > .05$). Correlation analysis revealed no relationship between the level of $P2X_7$ expression and levels of pain ($P = .29$, $r = 0.32$), tingling ($P = .98$, $r = -0.09$), or discomfort ($P = 0.85$, $r = 0.06$).

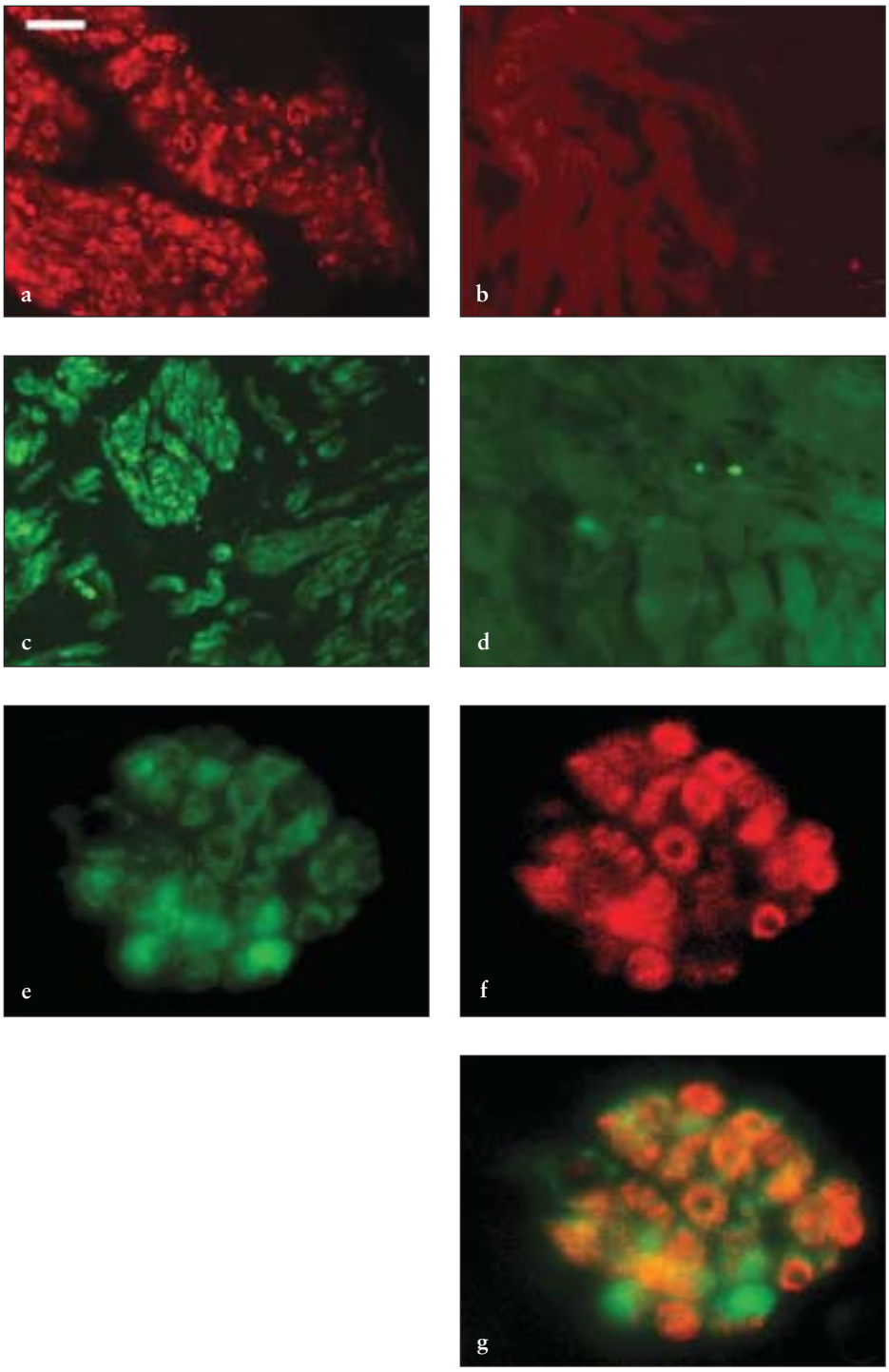

Fig 2 Photomicrographs showing *(a)* $P2X_7$ expression in a human lingual nerve neuroma and *(b)* following preabsorption with the $P2X_7$ antibody blocking peptide. *(c)* S-100 positive Schwann cells in human lingual nerve neuroma and *(d)* following omission of primary S-100 antibody. All positive staining is abolished. Photomicrographs *e* and *f* show S-100 and $P2X_7$ expression in Schwann cells of human lingual nerve neuromas respectively. A transverse section showing the colocalization of S-100 and $P2X_7$ observed in the Schwann cells *(g)*. Scale bar = 15 μm.

$P2X_7$ Expression and Gender. No significant difference in levels of $P2X_7$ expression was seen between neuromas from male (n = 3, mean = 15.03%) and female patients (n = 10, mean = 8.30%; independent samples *t* test, *P* = .09). All three of the male patients had symptoms of dysesthesia, whereas six of the female patients did not suffer painful symptoms. Differences in expression levels of $P2X_7$ were also analyzed in just the female patients; there was no significant difference between the percentage of S-100 positive Schwann cells in females with symptoms (mean = 7.06%) or females without symptoms (mean = 9.14%, *P* = .97).

$P2X_7$ Expression and Time Following Initial Injury. Analyses using Pearson's correlation coefficients revealed no correlation between $P2X_7$

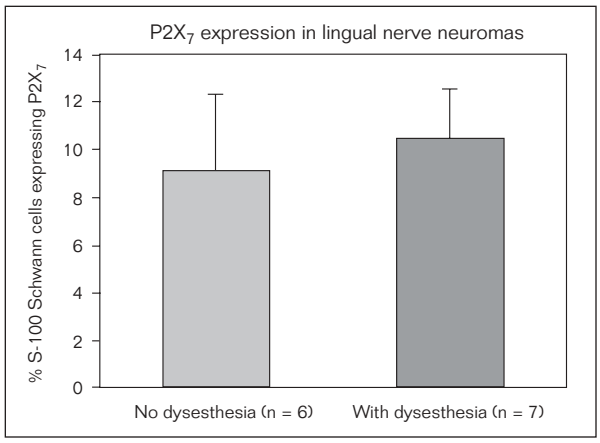

Fig 3 Bar chart showing mean percentage (± SEM) of S-100 labeled Schwann cells expressing $P2X_7$ receptors in neuromas from patients with (n = 7) and without (n = 6) symptoms of dysesthesia. No differences were found between these two groups ($P > .05$, independent t test).

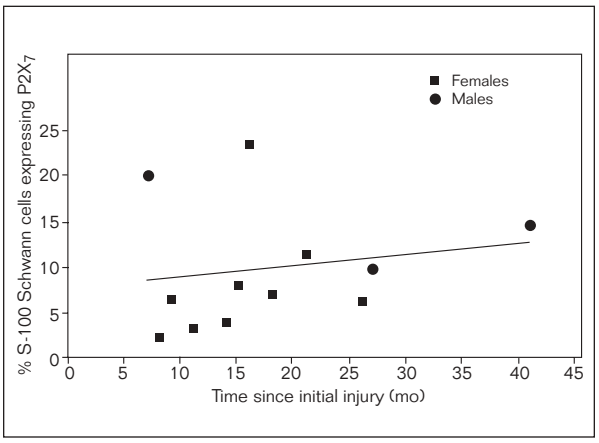

Fig 4 Scatter plot showing correlation between expression of $P2X_7$ and the time between injury and repair (pooled data from symptomatic and nonsymptomatic groups, $P > .05$, $r = 0.34$).

expression and the time between the initial injury to the nerve and repair ($P = .28$, $r = 0.34$) (Fig 4).

$P2X_7$ Expression and Age of Patient. The mean age of the patients with dysesthesia was 29.00 (± 1.82) years and without symptoms, 30.83 (± 2.39) years ($P = .56$). Correlation analysis revealed there was no significant relationship between the level of $P2X_7$ expression and age ($P = .11$, $r = –0.46$).

Discussion

Chessell et al[10] reported that $P2X_7$ was present in injured sensory nerves and was co-localized with the glial marker GFAP, indicating the presence of $P2X_7$ in Schwann cells. This is in direct agreement with the present study, which showed co-localization of $P2X_7$ with the Schwann cell marker, S-100, in human lingual nerve neuromas. Chessell and colleagues[10] showed $P2X_7$ to be markedly upregulated in painful injured nerves when compared to uninjured control nerves but were unable to investigate expression changes in injured nerves from patients who did not suffer from neuropathic pain; the present study has filled that gap. In contrast, one of the limitations of the present study was the lack of uninjured control tissue, and so $P2X_7$ expression could not be examined in normal human lingual nerve. In previous investigations the authors have used control lingual nerves obtained at the time of organ donor retrieval,[17] but these specimens proved to be unsatisfactory for immunohistochemistry. Therefore, the present investigation was confined to studying differences in receptor expression in injured nerves from patients with or without symptoms. Using this approach we found no differences between the two groups.

Functional $P2X_7$ has previously been demonstrated on peripheral glial cells in rat dorsal root ganglia,[18] and $P2X_7$ transcripts have been identified in the rat thoracolumbar sympathetic ganglia.[19] The authors are not aware of any investigations demonstrating $P2X_7$ expression in the trigeminal ganglion, but in view of these previous studies it seems likely to be present.

Importance of $P2X_7$ in Inflammation and Pain Processes

Many recent studies have implicated $P2X_7$ in inflammatory and neuropathic pain processes. Activation of $P2X_7$ results in the release of the proinflammatory cytokine interleukin 1β (IL-1β).[10,20,21] This action is depleted in $P2X_7$-knockout mice.[22] Increased concentrations of IL-1β can lead to induction of inducible nitric oxide synthase (iNOS) and increased production of tumor necrosis factor (TNFα) and cyclooxygenase 2 (COX-2), which in turn can lead to increased pain sensitivity.[23,24] In $P2X_7$ knockout studies, null mice show markedly reduced inflammation in a mouse model of arthritis[25] and a reduction in the behavioral changes thought to be indicative of nerve-injury induced neuropathic pain.[10]

Other studies have used $P2X_7$ antagonists to try to elucidate its role. A-740003, a highly specific antagonist for mammalian $P2X_7$ receptors, was found to dose-dependently reduce nociception in a rat model of neuropathic and inflammatory pain.[15] Following both chronic constriction injury of the sciatic nerve and vincristine-induced neuropathic pain, A-740003 significantly reduced mechanical allodynia. Furthermore, thermal hyperalgesia was significantly attenuated in two models of inflammatory pain.[15] Another $P2X_7$ antagonist, A-438079, both attenuates formalin-induced nociceptive behaviors and reduces noxious and innocuous-evoked activity of spinal neurones in a neuropathic rat model using in vivo electrophysiology.[26]

These data all seem to indicate a specific role for $P2X_7$ activation in chronic pain states. It appears that in the periphery, following inflammation or a neuropathic insult, the release of ATP results in $P2X_7$ activation, inducing the release of IL-1β from macrophages, mast cells, and Schwann cells leading to a cascade of events including release of COX-2, iNOS, and nerve growth factor.[23] However, the present study has shown that $P2X_7$ expression, and presumably the subsequent cascade of events, occurs in all damaged lingual nerves but is not always associated with the development of neuropathic pain symptoms. Although this does not exclude a possible role for $P2X_7$ in the etiology of neuropathic pain, it suggests that the level of expression at the injury site is not critical in this process.

Other Factors Affecting $P2X_7$ Expression

Perception and responses to pain differ between genders and with age. Females tend to have lower pain thresholds and higher pain ratings (for review see Berkley[27]), indeed, a high proportion of patients presenting with trigeminal dysesthesia are female.[28] Older patients are also more vulnerable to pain and pain-associated events (see Gibson and Farrell[29]). In this study, no differences were found between the level of $P2X_7$ expression in females and males. In addition, no relationship was evident between the age of the patient and $P2X_7$ expression. Thus, differences in $P2X_7$ expression after lingual nerve injury cannot be attributed to gender or age. However, the number of samples obtained from male patients was small and may account for the lack of age and gender differences.

No correlation was evident between the time from initial injury to resection of the specimen and the level of $P2X_7$ expression. There is limited evidence on the time-course of changes in $P2X_7$ expression in animal studies, but following cerebral ischemia in rats, $P2X_7$ expression in astrocytes and microglial cells showed a time-dependent upregulation at 1, 4, and 7 days after injury.[9] It is possible that $P2X_7$ expression in our lingual neuromas would have been significantly higher in the early stages after injury, but as most of the neuromas were obtained at a relatively late stage, this relationship would not have been identified.

In summary, the present study has shown that $P2X_7$ is expressed in Schwann cells of human lingual nerve neuromas. This level of expression was found to vary greatly between specimens, and no relationship was apparent between the level of $P2X_7$ expression and the patients' symptoms of dysesthesia. This suggests that the level of expression at the injury site is not critical in the development of nerve injury-induced pain. It remains possible that changes in $P2X_7$ expression at other sites after nerve injury could play a more important role.

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References

1. Gregg JM. Studies of traumatic neuralgia in the maxillofacial region: Symptom complexes and response to microsurgery. J Oral Maxillofac Surg 1990;48:135–140.
2. Robinson PP, Loescher AR, Smith KG. A prospective, quantitative study on the clinical outcome of lingual nerve repair. Br J Oral Maxillofac Surg 2000;38:255–263.
3. Gregg JM. Post-traumatic trigeminal neuralgia: Response to physiological, surgical and pharmacological therapies. Int Dent J 1978;28:43–51.
4. North RA. Molecular physiology of P2X receptors. Physiol Rev 2002;82:1013–1067.
5. Collo G, Neidhart S, Kawashima E, Kosco-Vilbois M, North RA, Buell G. Tissue distribution of the P2X7 receptor. Neuropharmacology 1997;36:1277–1283.
6. Di Virgilio F, Sanz JM, Chiozzi P, Falzoni S. The P2Z/P2X7 receptor of microglial cells: A novel immunomodulatory receptor. Prog Brain Res 1999;120:355–368.
7. Duan S, Anderson CM, Keung EC, Chen Y, Chen Y, Swanson RA. P2X7 receptor-mediated release of excitatory amino acids from astrocytes. J Neurosci 2003;23:1320–1328.
8. Sperlagh B, Vizi ES, Wirkner K, Illes P. P2X7 receptors in the nervous system. Prog Neurobiol 2006;78:327–346.
9. Franke H, Gunther A, Grosche J, et al. P2X7 receptor expression after ischemia in the cerebral cortex of rats. J Neuropathol Exp Neurol 2004;63:686-699.
10. Chessell IP, Hatcher JP, Bountra C, et al. Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. Pain 2005;114:386–396.

11. Wang X, Arcuino G, Takano T, et al. P2X7 receptor inhibition improves recovery after spinal cord injury. Nat Med 2004;10:821–827.
12. Dell'Antonio G, Quattrini A, Cin ED, Fulgenzi A, Ferrero ME. Relief of inflammatory pain in rats by local use of the selective P2X7 ATP receptor inhibitor, oxidized ATP. Arthritis Rheum 2002;46:3378–3385.
13. Dell'Antonio G, Quattrini A, Dal Cin E, Fulgenzi A, Ferrero ME. Antinociceptive effect of a new P(2Z)/P2X7 antagonist, oxidized ATP, in arthritic rats. Neurosci Lett 2002;327:87–90.
14. Fulgenzi A, Dell'Antonio G, Foglieni C, et al. Inhibition of chemokine expression in rat inflamed paws by systemic use of the antihyperalgesic oxidized ATP. BMC Immunol 2005;6:18.
15. Honore P, Donnelly-Roberts D, Namovic MT, et al. A-740003 [N-(1-{[(cyanoimino)(5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective P2X7 receptor antagonist, dose-dependently reduces neuropathic pain in the rat. J Pharmacol Exp Ther 2006;319:1376–1385.
16. Vora AR, Loescher AR, Boissonade FM, Robinson PP. Ultrastructural characteristics of axons in traumatic neuromas of the human lingual nerve. J Orofac Pain 2005; 19:22–33.
17. Vora AR, Loescher AR, Craig GT, Boissonade FM, Robinson PP. A light microscopical study on the structure of traumatic neuromas of the human lingual nerve. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 99:395–403.
18. Zhang XF, Han P, Faltynek CR, Jarvis MF, Shieh CC. Functional expression of P2X7 receptors in non-neuronal cells of rat dorsal root ganglia. Brain Res 2005;1052: 63–70.
19. Allgaier C, Reinhardt R, Schadlich H, et al. Somatic and axonal effects of ATP via P2X2 but not P2X7 receptors in rat thoracolumbar sympathetic neurones. J Neurochem 2004;90:359–367.
20. Perregaux D, Gabel CA. Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. J Biol Chem 1994;269:15195–15203.
21. Ferrari D, Pizzirani C, Adinolfi E, et al. P2X7 receptor: A key player in IL-1 processing and release. J Immunol 2006;176:3877–3883.
22. Solle M, Labasi J, Perregaux DG, et al. Altered cytokine production in mice lacking P2X(7) receptors. J Biol Chem 2001;276:125–132.
23. Woolf CJ, Allchorne A, Safieh-Garabedian B, Poole S. Cytokines, nerve growth factor and inflammatory hyperalgesia: The contribution of tumour necrosis factor alpha. Br J Pharmacol 1997;121:417–424.
24. Samad TA, Moore KA, Sapirstein A, et al. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 2001;410:471–475.
25. Labasi JM, Petrushova N, Donovan C, et al. Absence of the P2X7 receptor alters leukocyte function and attenuates an inflammatory response. J Immunol 2002;168: 6436–6445.
26. McGaraughty S, Chu KL, Namovic MT, Donnelly-Roberts DL, Harris RR, Zhang XF. P2X(7)-related modulation of pathological nociception in rats. Neuroscience 2007;146:1817–1828.
27. Berkley KJ. Sex differences in pain. Behav Brain Sci 1997;20:371–380.
28. Robinson PP, Loescher AR, Yates JM, Smith KG. Current management of damage to the inferior alveolar and lingual nerves as a result of removal of third molars. Br J Oral Maxillofac Surg 2004;42:285–292.
29. Gibson SJ, Farrell M. A review of age differences in the neurophysiology of nociception and the perceptual experience of pain. Clin J Pain 2004;20:227–239.