Biochemical and Biophysical Research Communications 458 (2015) 596–600

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Phenotypes of ATP-activated current associated with their genotypes of P2X1-6 subunits in neurons innervating tooth-pulp

Yuwei Liu [a, b], Xiang Tian [a], Yuxiang Wu [a], Lin Chen [a], Chu-Li Yi [b], Zhi-Wang Li [a], Ying Zhang [a], Chao-Ying Li [a, *]

[a] Wuhan Institutes of Biomedical Sciences, Jianghan University, Wuhan, 430056, Hubei Province, China
[b] School of Medicine, Jianghan University, Wuhan, 430056, China

ARTICLE INFO

Article history:
Received 23 January 2015
Available online 11 February 2015

Keywords:
P2X
Tooth-pulp
Trigeminal ganglion
Retrograde fluorescence-tracing
Immunohistochemistry

ABSTRACT

To explore the association of the phenotype of ATP-activated current with the genotype of P2X1-6 subunits in nociceptors, we developed a method that allows us to label nociceptive neurons innervating tooth-pulp in rat trigeminal ganglion (TG) neurons using a retrograde fluorescence-tracing method, to record ATP-activated current in freshly isolated fluorescence-labeled neurons, and then to conduct single cell immunohistochemical staining for P2X1-6 subunits in the same neuron. We found that fast application of 100 μM ATP to fluorescence-traced TG neurons produced robust inward current in 87% (96/110) of cells tested. The diameter of cells varied from 16 to 56 μm. Three types of ATP-activated current (F, I and S) were recorded with distinct rise times of the current (R_{10-90}, $P < 0.05$). There was a positive correlation between the cell diameter and the value of R_{10-90} ($P < 0.05$): the value of R_{10-90} increased with increases in the cell diameter. Cells responsive to ATP with the type F current mainly showed positive staining for P2X3 and P2X5, but negative staining for P2X2; cells responsive to ATP with the type I current showed positive staining for P2X1-3 and P2X5, but negative staining for P2X4; and cells responsive to ATP with the type S current showed positive staining for P2X1-5, but negative staining for P2X6. The present findings suggest that in addition to P2X3 subunits, P2X5 subunits are also involved in the generation of the F type of ATP-activated current in small-sized nociceptive neurons. In addition to the P2X2/3 subunit-containing channels, more complex uncharacterized combinations of P2X1-5 subunits exist in native medium-sized nociceptive neurons exhibiting the I and S types of ATP-activated current. In addition, the P2X6 subunit is not a main subunit involved in the nociceptive signal in rat TG neurons innervating tooth-pulp.

1. Introduction

P2X receptors are ligand-gated ion channels that are activated by extracellular ATP, and a growing body of evidence suggests that P2X receptors have important functions in the nervous system [1,2]. To date, seven mammalian P2X receptor subunits, designated P2X1-P2X7, have been identified that assemble as either homo- or heterotrimeric receptors [1,2]. All of these subunits are thought to consist of two transmembrane domains, a large extracellular loop, and intracellular amino- and carboxy-terminals [1,2]. With the exception of P2X7, all P2X receptor subunits are expressed in various primary sensory neurons including tooth-pulp neurons [1,2,3,4]. In particular, the P2X3 homomeric and P2X2/3 heteromeric receptors have been associated with peripheral nociceptive mechanisms, since these subtypes occur in a subset of putative nociceptive sensory neurons [5,6], and recent studies with antagonists selective for P2X3 and P2X2/3 subunit-containing channels and antisense oligonucleotides that reduce the level of expression of P2X3 subunits in primary afferent cells as well as observations on P2X3 and P2X2/3 gene knock-out mice indicate the involvement of P2X3 and P2X2/3 receptors in the generation and transmission of nociceptive signals [5,6]. However, compared to the P2X3 and P2X2/3 receptors, the association of other P2X receptor subunits with the peripheral nociceptive mechanisms remains unknown.

Neurons innervating tooth-pulp can be regarded as pain-sensing neurons or nociceptors in trigeminal ganglion (TG) because pain is the only sensation reported when such fibers are stimulated in man [3,4,7]. Although transcripts and

* Corresponding author. Fax: +86 27 84225827.
E-mail address: licy.whibs@aliyun.com (C.-Y. Li).

http://dx.doi.org/10.1016/j.bbrc.2015.01.156
0006-291X/

immunoreactivities for six subunits (P2X1-6) have been found in TG neurons [8,9], so far the P2X3 receptor is the only one that has been reported to associate with neurons innervating tooth-pulp [3,4]. In those studies, Cook and colleagues successfully labeled and characterized nociceptors innervating tooth-pulp in rat TG neurons using a retrograde fluorescence-tracing method, and found that P2X3, not P2X1, subunits were present in cultured fluorescence-labeled TG neurons with small-diameter cell bodies [3]. In order to evaluate whether other P2X subunits are also involved in nociception, in the present study we developed a method that allows us to label nociceptive neurons innervating tooth-pulp in rat TG neurons using a retrograde fluorescence-tracing method, to record ATP-activated current in freshly isolated fluorescence-labeled neurons and then to conduct single cell immunohistochemical staining for P2X1-6 subunits in the same neuron. Our results provide the first evidence at the level of single native nociceptive neurons for correlating the characteristics of ATP-activated current with their composition of P2X1-6 subunits and cell size.

2. Materials and methods

2.1. Retrograde fluorescence-tracing of nociceptors

Spraque-Dawley rats, two-three weeks old, were anesthetized by injection of chloral hydrate (400 mg/kg, i.p.) and then placed on a platform in supine position. The first and/or second maxillary molars were drilled and fluorescence dye, DiIC18 [3,4], was placed into drilled cavities, thereafter holes were filled with ZnO dental cement. After 7 days trigeminal ganglia were taken out, and neurons were freshly isolated (see below) and nociceptors were identified (see Fig. 1A,B). All animal experimental procedures were reviewed and approved by the Animal Care and Use Committee at Jianghan University, and were performed in accordance with the National Institutes of Health guidelines on animal care.

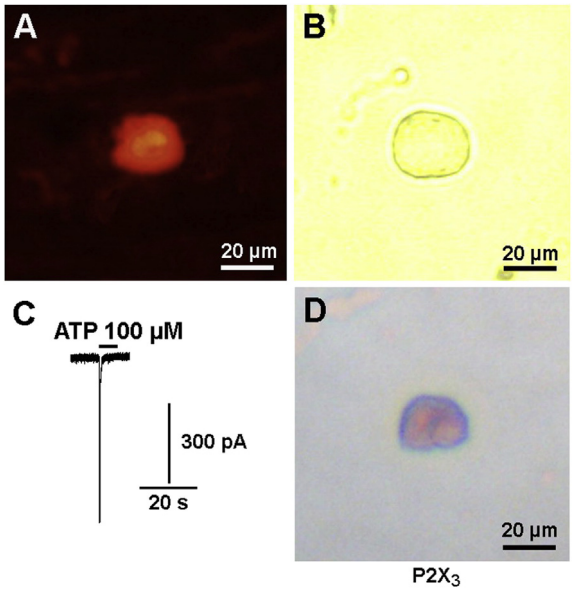

Fig 1. Detection of the phenotype of ATP-activated current associated with its genotype of P2X1-6 subunits in a single fluorescence-labeled nociceptor. (A,B) Fluorescence and phase micrographs of a freshly isolated nociceptor. (C) Record showing current activated by 100 μM ATP. The horizontal bar above the trace indicates the ATP application. (D) Single cell immunohistochemical result showing positive staining for P2X3. (A–D) were obtained from the same neuron.

2.2. Isolation of TG neurons

Trigeminal ganglia were taken out from decapitated animals. The skull was opened to expose the trigeminal ganglia and transferred immediately into Dulbecco's modified Eagle's medium (DMEM) at pH 7.4. After removal of the surrounding connective tissues, the trigeminal ganglia were minced with fine spring scissors and the ganglion fragments were placed in a flask containing 5 ml DMEM in which trypsin (type II-S) 0.5 mg/ml, collagenase (type I-A) 1.0 mg/ml and DNase (type IV) 0.1 mg/ml had been dissolved, and incubated at 35 °C in a shaking water bath for 30–35 min. Soybean trypsin inhibitor (type II-S) 1.25 mg/ml was then added to stop trypsin digestion. Dissociated neurons were placed into a 35 mm Petri dish and kept for at least another 30 min before electrophysiological recording.

2.3. Electrophysiological recordings

Whole-cell patch-clamp recordings were carried out at room temperature (22–24 °C) as described previously [10,11]. In brief, the micropipettes were filled with internal solution containing (mM): KCl 140, $MgCl_2$ 2.5, HEPES 10, EGTA 11 and ATP 5; its pH was adjusted to 7.4 with KOH and osmolarity was adjusted to 310 mOsm/L with sucrose. The resistance of the recording pipette was in the range of 2–5 MΩ. Cells were bathed in an external solution containing (mM): NaCl 150, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 2, HEPES 10, D-glucose 10; its osmolarity was adjusted to 330 mOsm/L with sucrose and pH was adjusted to 7.4 with NaOH. The membrane potential was held at −60 mV.

Drug solutions were prepared in extracellular medium and applied to neurons by a rapid solution exchange system (SF–77B Perfusion Fast-Step, Warner Instruments, LLC, Hamden, CT). With this system, the 10–90% rise time of the junction potential at an open pipette tip was <2 ms.

2.4. Single cell immunohistochemistry

Single cell immunohistochemical staining for P2X1-6 subunits was conducted as described previously [10]. In brief, after electrophysiological recording (see Fig. 1C), the cell was carefully moved to a gelatin-coated slide and dried at room temperature for 2 h. Then the cell was fixed for 10 min with a few drops of freshly prepared 4% paraformaldehyde in 0.1 M PBS (PBS: 0.1 M phosphate; 0.9% w/v saline; pH 7.3) and rinsed in 0.1 M PBS three times for 10 min. The endogenous peroxidase was blocked by incubation with 0.3% hydrogen peroxide in methanol. The neuron was then incubated in 10% normal bovine serum in 0.1 M PBS for 30 min at room temperature. After further washing in PBS, the cell was incubated overnight with affinity-purified antibodies for P2X1, P2X2, P2X3, P2X4, P2X5 or P2X6. All antibodies were diluted at 1:100 with 0.1 M PBS. Between the subsequent incubations the cell was washed in PBS three times for 5 min. Goat anti-rabbit secondary antisera diluted in the same diluents were applied for 1 h at room temperature, followed by incubation for 20 min in tertiary antibody prepared in 0.1 M PBS. After several rinses with PBS, 0.05% 3,3′-diaminobenzidine and 0.003% H_2O_2 were applied for color reaction. Then, the neuron was dehydrated and coverslipped. The staining for the specific P2X subunit was reviewed under a microscope (see Figs. 1D and 3).

2.5. Drugs and chemicals

The drugs and chemicals used in these experiments were purchased from Sigma–Aldrich Chemical Co. (St Louis, MO), except for P2X1-6 antibodies which were purchased from

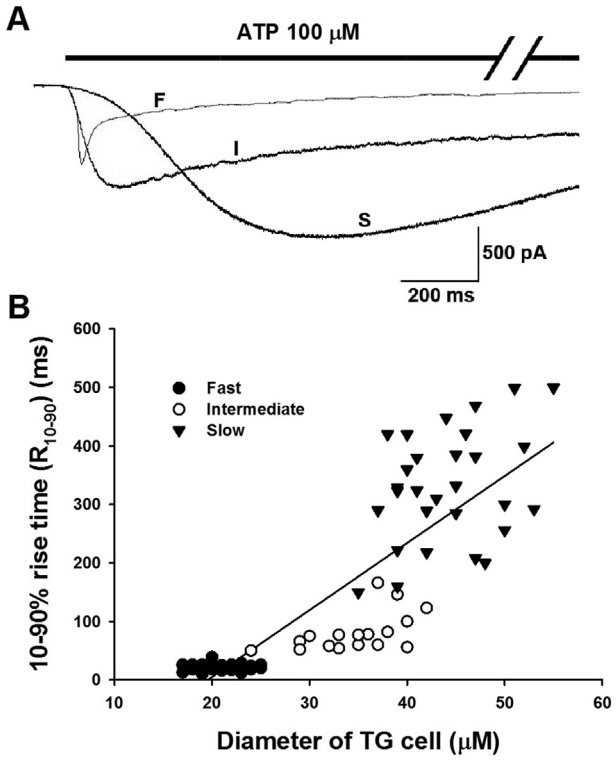

Fig 2. Correlation of R_{10-90} with cell size for the three types of ATP-activated current. (A) Traces of types F, I and S of ATP (100 μM)-activated current with time axis expanded. (B) Graph plotting R_{10-90} of different types of ATP (100 μM)-activated current vs. cell size. Each point represents a single cell. The correlation coefficient was calculated to be 0.6 ($P < 0.05$).

Alomone Labs, Ltd (Jerusalem, Israel), goat anti-rabbit secondary antisera which were obtained from Vector Labs (Burlingame, CA) and fluorescence dye DiIC18 which was obtained from Molecular Probes (Eugene, OR).

2.6. Data analysis

Statistical significance of results was assessed using analysis of variance (ANOVA), as noted. Average values are expressed as mean ± s.e.m. with n equal to the number of cells studied. The rise time of ATP-activated current was fitted with one exponential.

3. Results

3.1. Fluorescence-tracing, patch-clamp recording and immunohistochemical staining in the same nociceptor

7 days after injection of DiIC18 into the drilled cavity in rat maxillary molars, isolation of TG neurons innervating tooth-pulp was carried out by mechanical and enzymatic digestion (see Methods). The fluorescence-labeled neurons could be detected as bright red (in web version) against the dark field background under fluorescence microscopy (Fig. 1A). The objective of the microscope was then switched from fluorescence to normal light, to allow the morphological characteristics of labeled neurons to be seen clearly (Fig. 1B), and the cell was available for membrane current recording using patch-clamp technique. Usually, the fluorescence-labeled neurons accounted for 15–20 % of the total cells in one Petri dish isolated from two trigeminal ganglia. In the neuron shown in Fig. 1A and B, 100 μM ATP activated a fast inward current (Fig. 1C). After patch-clamp recording, the same neuron was harvested by lifting it from the surface of the dish, then single cell immunohistochemical staining was conducted and the result showed positive staining for P2X3 (Fig. 1D).

3.2. ATP-activated current in fluorescence-traced nociceptors

Fast application of 100 μM ATP to fluorescence-traced TG neurons produced robust inward current in 87% (96/110) of cells tested; the diameter of cells varied from 16 to 56 μm. It has previously been reported that in freshly isolated rat TG capsaicin-sensitive neurons extracellular ATP activates three types of current with distinct kinetic properties [10]. Similar results were observed in the present

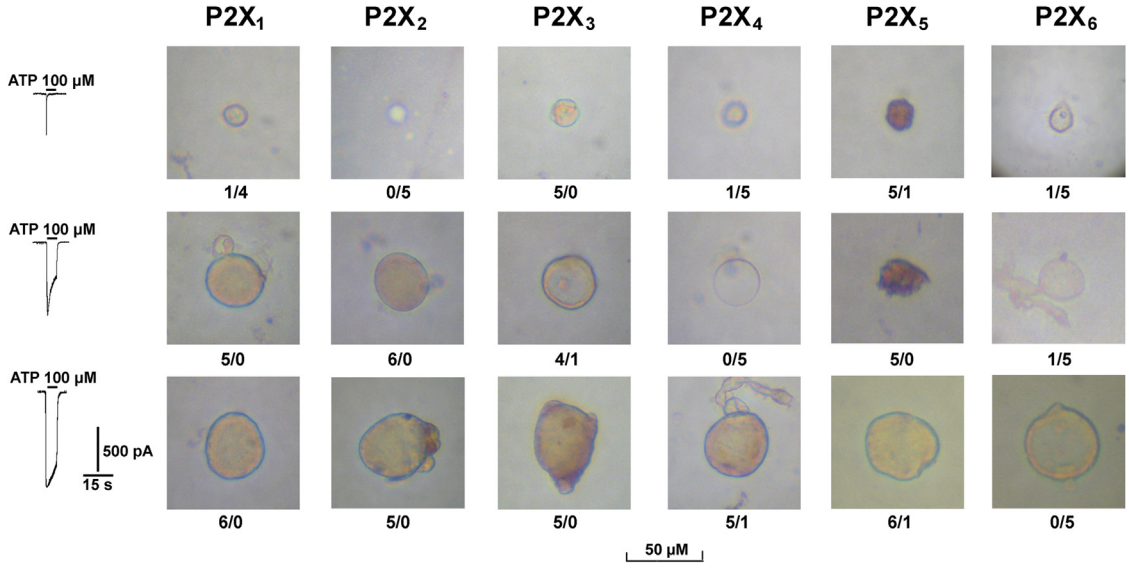

Fig 3. Relevance of expression of P2X1-6 subunits with three types of ATP-activated current and cell size. The immunohistochemical results revealed positive or negative staining for P2X1-6 subunits, which correlated with three types of ATP-activated current and cell size. Samples in each row were from 5 to 6 different neurons that responded to 100 μM ATP with different types of ATP-activated current (type F at the top, type I in the middle, and type S at the bottom). The numerator represents the number of positive cells reacting with the indicated antibody, and the denominator is the number of negative cells that responded to the indicated antibody.

study. As shown in Fig. 2A, 100 μM ATP activated three types of inward current with different rise and decay rates, namely, fast (F), intermediate (I) and slow (S) currents. Of a total of 96 cells tested, 51% of cells exhibited type F, 19% of cells showed type I and 30% of cells showed type S ATP-activated currents (Table 1). To further explore their kinetic features, we measured the rise time of ATP-activated current in relation to the cell size using a rapid solution exchange system. The 10–90% ($R_{10\text{-}90}$) rise time of ATP-activated current has been reported to be a useful measure to separate ATP-activated currents in TG neurons [10]. In the present study, the values of $R_{10\text{-}90}$ for three types of ATP-activated current were significantly different from each other (Table 1, ANOVA, $P < 0.05$). In addition, there was a positive correlation between the cell diameter and the value of R_{10-90}: the value of R_{10-90} increased with increases in the cell diameter (Fig. 2B). The correlation coefficient was calculated to be 0.6 ($P < 0.05$). Note that the points representing the three types of ATP current were rather scattered around the slope line, and overlap can be seen between both type F and type I, and between type I and type S currents.

3.3. Association of three types of ATP-activated current with expression of P2X1-6 subunits

The different phenotypes of ATP-activated current in the different types of neurons may be attributed to differences in subunit composition. In order to test this directly, we determined the subunit assembly using single cell immunohistochemistry in the same individual cells used for patch-clamp recording [10]. As shown in Fig. 3 and Table 1, cells responsive to ATP with the type F current mainly showed positive staining for P2X3 and P2X5, but negative staining for P2X2; cells responsive to ATP with the type I current showed positive staining for P2X1-3 and P2X5, but negative staining for P2X4; cells responsive to ATP with the type S current showed positive staining for P2X1-5, but negative staining for P2X6.

4. Discussion

As is well known from practical observations in the dental clinic as well as the common experience of human beings, sensations originating from tooth-pulp are pain-sensing or nociceptive, since any stimulation of tooth-pulp, including thermal, osmotic, chemical, or mechanical, produces pain [3,4,7]. Therefore, the sensory afferents originating from tooth-pulp can be regarded as pain-sensing and thus the TG neurons innervating tooth-pulp can be considered to be nociceptive neurons.

A growing body of evidence suggests that P2X3 receptors play an important role in the generation and transduction of sensory nociceptive signals [5,6]. Cook et al. labeled nociceptors innervating tooth-pulp in rat TG neurons using a retrograde fluorescence-tracing method, and found that P2X3, but not P2X1, subunits were present in cultured fluorescence-labeled TG neurons [3], which supports a role of P2X3 receptors in pain sensation. *In situ* hybridization and immunohistochemical studies, however, revealed that of the cloned P2X receptor subunits, six (P2X1-P2X6) were expressed in sensory (including TG) neurons [8,9,10,12,13], thus raising the possibility that the native channels, including the channels expressed in nociceptive neurons, may be heteromultimers with diverse composition. Indeed, it has been reported that extracellular ATP can evoke different types of ion current, differing in a number of characteristics, such as current–voltage relationships, agonist and antagonist selectivities, modulation by endogenous agents, and desensitization rate, in nodose ganglion neurons [14,15,16], dorsal root ganglion neurons [17,18,19,20,21], and in TG neurons [3,4,10]. The molecular basis for the different types of ATP-activated current in different types of neuronal preparations, including nociceptive neurons, has not been established.

Table 1
Properties of three types of ATP-activated current.

Parameter	F (fast)	I (intermediate)	S (slow)
Incidence	51% (49/96)	19% (18/96)	30% (29/96)
R_{10-90} (ms)	24.5 ± 3.8 (n = 49)	66.1 ± 9.8 (n = 18)	358.2 ± 82.2 (n = 29)
Cell size (μm)	16–26	24–43	35–56
Major subunit composition	P2X3, P2X5	P2X1, P2X2, P2X3, P2X5	P2X1-5

In order to evaluate the association of P2X receptor subunits from P2X1 to P2X6 with nociceptors innervating tooth-pulp in rat TG neurons, in the present study we developed a method which combined two methods previously reported by Cook et al. [3,4] and this group [10]. Cook et al. labeled nociceptors innervating tooth-pulp in rat TG neurons using a retrograde fluorescence-tracing method, and found that P2X3, not P2X1, subunits were present in cultured fluorescence-labeled TG neurons with small-diameter cell bodies [3]. In these cultured nociceptive neurons, they also recorded two kinetically distinct currents: transient and persistent currents, but the molecular basis for the two different types of current has not been established [3,4]. In our previous study, we characterized three types of ATP-activated current in freshly isolated rat TG neurons that were sensitive to capsaicin using a method combining the whole-cell patch-clamp recording technique with single-cell immunohistochemical detection to explore the association of the phenotype of ATP-activated current with the genotype of P2X1-4 subunits, and found that both P2X1 and P2X3 receptors were expressed in TG neurons and mediated similar rapidly desensitizing currents [10]. However, as this study was not directly associated with nociceptive neurons as reported by Cook et al. [3,4], whether the characterized phenotype of ATP-activated current with the genotype of P2X1-4 subunits is related to nociceptive signals is uncertain.

In the present study, we developed a method that allows us to label nociceptive neurons innervating tooth-pulp in rat TG neurons using a retrograde fluorescence-tracing method, to record ATP-activated current and to conduct single cell immunohistochemical staining for P2X1-6 subunits in these neurons after acute isolation. We found that fast application of 100 μM ATP to fluorescence-traced TG neurons innervating tooth-pulp produced robust inward current in 87% of cells tested. In the total of 96 cells tested, the diameter of the cells was ≤50 μm (with the exception of four cells that was between 52 and 56 μm) indicating that the majority of nociceptive TG neurons innervating tooth-pulp tested in the present study are small-to medium-sized sensory neurons [17,21,22]. In these fluorescence-traced TG nociceptive neurons, we recorded fast and slow as well as intermediate (or mixed) types of ATP-activated current, and the current type was correlated well with cell size; these results are consistent with previous observations [3,4,10].

Results shown in Fig. 3 were obtained by combining whole-cell patch-clamp technique with a single-cell immunohistochemical method, which offered direct and convincing evidence at the level of single native nociceptive neurons for correlating the characteristics of ATP-activated current with their composition of P2X1-6 subunits and cell size. Our findings did confirm the previous observation [3] that the P2X3 rather than the P2X1 subunit was the main subunit that mediated the fast ATP-activated current in small-sized nociceptive neurons. As mentioned above, our previous study showed that the P2X1 subunit was also often detected in small-sized neurons with fast kinetics [10]. The reason for this discrepancy could be that the cells expressing the P2X1 subunit in our

previous study may not be nociceptive neurons even if they were sensitive to capsaicin, as these cells were not directly associated with nociceptive signals as in the present study. This suggests that the small-sized cells expressing the P2X1 subunit in rat TG neurons are not nociceptive. It is noteworthy that cells with the F type of ATP-activated current were all small-sized neurons and mainly showed positive staining for P2X3 and P2X5, but negative staining for P2X2; cells with the I type of ATP-activated current were medium-sized neurons (with one exception) and mainly showed positive staining for P2X1-3 and P2X5, but negative staining for P2X4; and the majority of cells with the S type of ATP-activated current were also medium-sized neurons (with four exceptions in the total of twenty-nine cells tested) and mainly showed positive staining for P2X1-5, but negative staining for P2X6 (also see Table 1). Therefore, these results suggest that in addition to P2X3 subunits, P2X5 subunits are also involved in the generation of the F type of ATP-activated current in small-sized nociceptive TG neurons. Furthermore, in addition to the P2X2/3 subunit-containing channels, more complex uncharacterized compositions of P2X subunits exist in native medium-sized nociceptive TG neurons with the I and S types of ATP-activated current, as P2X1-5 subunits were all expressed in these neurons. The results presented in Fig. 3 and Table 1 also indicate that the P2X6 subunit was not often detected in all cells tested, suggesting that this subunit is not a main subunit involved in the nociceptive signal in rat TG neurons innervating tooth-pulp. By contrast, like the P2X3 subunit, the P2X5 subunit is a main subunit expressed in both small- and medium-sized nociceptive neurons which showed the F, I and S types of ATP-activated current. Interestingly, a recent study using an experimental approach combining bioluminescence resonance energy transfer, bifunctional fluorescence complementation and protein biochemistry revealed that P2X5 subunits could interact with P2X1, P2X2, or P2X4 subunits at the plasma membrane, which supports the idea that rodent P2X5 subunits are auxiliary subunits associating with P2X1, P2X2, and P2X4 subunits to generate molecular diversity among P2X receptors [8,23]. Indeed, heteromeric receptors of P2X1/5 and P2X2/5 have been characterized in a cell expression system and in native CNS tissues [23,24]. Determining the functional role of P2X5 subunits in nociceptive signals will be an interesting subject for future studies.

The present findings suggest that in addition to P2X3 subunits, P2X5 subunits are also involved in the generation of the F type of ATP-activated current in small-sized nociceptive TG neurons, and in addition to the P2X2/3 subunit-containing channels, more complex uncharacterized compositions of P2X subunits exist in native medium-sized nociceptive TG neurons exhibiting the I and S types of ATP-activated current, as P2X1-5 subunits were all expressed in these neurons. In addition, our finding that the P2X6 subunit was not often detected in all cells tested suggests that this subunit is not a main subunit involved in the nociceptive signal in rat TG neurons innervating tooth-pulp. Our results provide the first direct and convincing evidence at the level of single native nociceptive neurons that the characteristics of ATP-activated current are correlated with their P2X1-6 subunit composition and cell size.

Conflict of interest

None.

Acknowledgments

We thank Dr. Robert W. Peoples for his helpful comments on the manuscript. This work was supported by the National Natural Science Foundaton of China (81371235, 81273846 and 31371090) and Wuhan Science and Technology Foundation (2014060101010040 and 201250499145-27).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.01.156.

References

[1] A. Surprenant, R.A. North, Signaling at purinergic P2X receptors, Annu. Rev. Physiol. 71 (2009) 333–359.
[2] K. Kaczmarek-Hajek, E. Lorinczi, R. Hausmann, A. Nicke, Molecular and functional properties of P2X receptors–recent progress and persisting challenges, Purinergic. Signal. 8 (2012) 375–417.
[3] S.P. Cook, L. Vulchanova, K.M. Hargreaves, R. Elde, E.W. McCleskey, Distinct ATP receptors on pain-sensing and stretch-sensing neurons, Nature 387 (1997) 505–508.
[4] S.P. Cook, E.W. McCleskey, Desensitization, recovery and Ca(2+)-dependent modulation of ATP-gated P2X receptors in nociceptors, Neuropharmacology 36 (1997) 1303–1308.
[5] R.A. North, P2X3 receptors and peripheral pain mechanisms, J. Physiol. 554 (2004) 301–308.
[6] G. Burnstock, Purinergic receptors and pain, Curr. Pharm. Des. 15 (2009) 1717–1735.
[7] H.Y. Kim, G. Chung, H.J. Jo, Y.S. Kim, Y.C. Bae, S.J. Jung, J.S. Kim, S.B. Oh, Characterization of dental nociceptive neurons, J. Dent. Res. 90 (2011) 771–776.
[8] G. Collo, R.A. North, E. Kawashima, E. Merlo-Pich, S. Neidhart, A. Surprenant, G. Buell, Cloning OF P2X5 and P2X6 receptors and the distribution and properties of an extended family of ATP-gated ion channels, J. Neurosci. 16 (1996) 2495–2507.
[9] Z. Xiang, X. Bo, G. Burnstock, Localization of ATP-gated P2X receptor immunoreactivity in rat sensory and sympathetic ganglia, Neurosci. Lett. 256 (1998) 105–108.
[10] J. Luo, G.F. Yin, Y.Z. Gu, Y. Liu, J.P. Dai, C. Li, Z.W. Li, Characterization of three types of ATP-activated current in relation to P2X subunits in rat trigeminal ganglion neurons, Brain. Res. 1115 (2006) 9–15.
[11] Y.W. Liu, J.L. Luo, H. Ren, R.W. Peoples, Y.X. Ai, L.J. Liu, Y.P. Pang, Z.W. Li, Y.F. Han, C.Y. Li, Inhibition of NMDA-gated ion channels by bis(7)-tacrine: whole-cell and single-channel studies, Neuropharmacology 54 (2008) 1086–1094.
[12] L. Vulchanova, M.S. Riedl, S.J. Shuster, G. Buell, A. Surprenant, R.A. North, R. Elde, Immunohistochemical study of the P2X2 and P2X3 receptor subunits in rat and monkey sensory neurons and their central terminals, Neuropharmacology 36 (1997) 1229–1242.
[13] K. Kobayashi, T. Fukuoka, H. Yamanaka, Y. Dai, K. Obata, A. Tokunaga, K. Noguchi, Differential expression patterns of mRNAs for P2X receptor subunits in neurochemically characterized dorsal root ganglion neurons in the rat, J. Comp. Neurol. 481 (2005) 377–390.
[14] B.S. Khakh, P.P. Humphrey, A. Surprenant, Electrophysiological properties of P2X-purinoceptors in rat superior cervical, nodose and guinea-pig coeliac neurones, J. Physiol. 484 (Pt 2) (1995) 385–395.
[15] C. Li, R.W. Peoples, F.F. Weight, Cu2+ potently enhances ATP-activated current in rat nodose ganglion neurons, Neurosci. Lett. 219 (1996) 45–48.
[16] Y. Tan, B. Zhao, Q.C. Zeng, C.M. Shi, F.B. Zhao, Z.W. Li, Characteristics of ATP-activated current in nodose ganglion neurons of rats, Neurosci. Lett. 459 (2009) 25–29.
[17] C. Li, R.W. Peoples, T.H. Lanthorn, Z.W. Li, F.F. Weight, Distinct ATP-activated currents in different types of neurons dissociated from rat dorsal root ganglion, Neurosci. Lett. 263 (1999) 57–60.
[18] B.D. Grubb, R.J. Evans, Characterization of cultured dorsal root ganglion neuron P2X receptors, Eur. J. Neurosci. 11 (1999) 149–154.
[19] S. Ueno, M. Tsuda, T. Iwanaga, K. Inoue, Cell type-specific ATP-activated responses in rat dorsal root ganglion neurons, Br. J. Pharmacol. 126 (1999) 429–436.
[20] E.C. Burgard, W. Niforatos, T. van Biesen, K.J. Lynch, E. Touma, R.E. Metzger, E.A. Kowaluk, M.F. Jarvis, P2X receptor-mediated ionic currents in dorsal root ganglion neurons, J. Neurophysiol. 82 (1999) 1590–1598.
[21] J.C. Petruska, B.Y. Cooper, R.D. Johnson, J.G. Gu, Distribution patterns of different P2x receptor phenotypes in acutely dissociated dorsal root ganglion neurons of adult rats, Exp. Brain. Res. 134 (2000) 126–132.
[22] J.M. Caffrey, D.L. Eng, J.A. Black, S.G. Waxman, J.D. Kocsis, Three types of sodium channels in adult rat dorsal root ganglion neurons, Brain. Res. 592 (1992) 283–297.
[23] V. Compan, L. Ulmann, O. Stelmashenko, J. Chemin, S. Chaumont, F. Rassendren, P2X2 and P2X5 subunits define a new heteromeric receptor with P2X7-like properties, J. Neurosci. 32 (2012) 4284–4296.
[24] G.E. Torres, W.R. Haines, T.M. Egan, M.M. Voigt, Co-expression of P2X1 and P2X5 receptor subunits reveals a novel ATP-gated ion channel, Mol. Pharmacol. 54 (1998) 989–993.