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Short communication

The co-expression of ASIC3 with calcitonin gene-related peptide and parvalbumin in the rat trigeminal ganglion

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Abstract

The co-expression of ASIC3 with calcitonin gene-related peptide (CGRP) or parvalbumin (PV) was examined in the trigeminal ganglion (TG) by a double immunofluorescence method. ASIC3-immunoreactivity (IR) was detected in 23% of TG neurons. These neurons were of various sizes (range=43–1768 μm^2, mean±S.D.=651±356 μm^2); 26% and 14% of ASIC3-immunoreactive (IR) neurons co-expressed CGRP- and PV-immunoreactivity (IR), respectively; 33% and 13% of the TG neurons retrogradely labeled from the tooth pulp and facial skin, respectively, exhibited ASIC3-IR; 36% of CGRP-IR TG neurons which innervate these tissues co-expressed ASIC3-IR. Only 4% of ASIC3-IR cutaneous TG neurons showed PV-IR, while 25% of ASIC3-IR tooth pulp neurons were also immunoreactive for PV. The present study suggests that ASIC3-IR TG neurons supply the tooth pulp and facial skin with unmyelinated or finely myelinated axons. ASIC3-IR neurons which have large myelinated axons may be common in the tooth pulp but not the facial skin. The axonal morphology of ASIC3-IR TG neurons may depend on the variety of their receptive fields.

Theme: Sensory systems

Topic: Somatic and visceral afferents

Keywords: ASIC3; Facial skin; Immunohistochemistry; Tooth pulp; Trigeminal ganglion

1. Introduction

ASIC3 belongs to the family of acid-sensing ion channels [4]. The mRNA is expressed by sensory ganglia, brain and many internal tissues including lung and testis [1,2]. Immunohistochemical methods have revealed that ASIC3 was localized to small neurons in the dorsal root ganglion [18]. Therefore, this channel is thought to play a role in nociception by functioning as a sensor of tissue acidosis [14,17]. However, little is known about the distribution of ASIC3 in the trigeminal ganglion (TG).

Calcitonin gene-related peptide (CGRP) has been recognized to be a marker for small to medium-sized neurons in the TG [12,15,16]. These neurons supply the peripheral receptive fields with free nerve endings [11,15,16]. Immunoelectron microscopy revealed that CGRP-immunoreactive (IR) neurons have unmyelinated or finely myelinated axons [11]. Therefore, CGRP-IR neurons are considered to be associated with nociceptive transmission [13]. On the other hand, parvalbumin (PV) is mostly localized to large neurons in the TG [3,7–10]. Our previous studies have demonstrated that PV-IR TG neurons supply the tooth pulp with large myelinated axons [5,6]. Because the tooth pulp has been considered to be innervated exclusively by nociceptive afferents, PV-containing TG neurons may participate in nociception of oro-facial structures.

In this study, we examine the co-expression of ASIC3 with CGRP or PV in TG neurons which innervate the tooth pulp and facial skin.

2. Materials and methods

Five TGs were obtained from four male Sprague–Daw-

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ley rats (200–300 g). Rats were anesthetized with ether to the level at which respiration was markedly suppressed, and transvascularly perfused with 50 ml of saline followed by 500 ml of 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4). The materials were dissected, frozen-sectioned at 12 μm, and thaw-mounted on gelatin-coated glass slides.

For simultaneous visualization of ASIC3 with CGRP or PV, a double immunofluorescence method was used. The sections were incubated for 24 h at room temperature with a mixture of guinea-pig anti-ASIC3 serum (1:200, Neuromics, USA) and either rabbit anti-CGRP serum (1:1000, Peninsula Labs., USA) or monoclonal anti-PV antibody (1:1000, Sigma, USA). The sections were then treated with a mixture of fluorescein isothiocyanate-conjugated goat anti-guinea pig IgG (1:100, Jackson ImmunoResearch, for ASIC3) and either lissamine rhodamine B chloride-conjugated donkey anti-rabbit IgG (1:500, Jackson ImmunoResearch Labs, for CGRP) or lissamine rhodamine B chloride-conjugated donkey anti anti-mouse IgG (1:100, Jackson ImmunoResearch Labs., for PV).

For demonstration of ASIC3-IR neurons innervating the tooth pulp and facial skin, four male rats (300–350 g) were used. Under deep anesthesia by i.p. injection with ethyl carbamate (650 mg/kg) and pentobarbital sodium (20 mg/kg), 0.1–0.2 μl of 1% fluorogold (FG, Fluorochrom, USA) in distilled water was injected into the right first and second maxillary molar tooth pulps or infraorbital skin. After 3 days, the animals were reanesthetized with ether, and transvascularly perfused with 4% formaldehyde. The right TGs were frozen-sectioned at 12 μm, mounted on gelatin-coated glass slides, and processed for the co-expression of ASIC3 with CGRP or PV as described above.

The percentages of TG neurons which exhibited ASIC3-IR and co-expressed ASIC3-IR with CGRP- or PV-IR were counted per area of photograph. For cell size analysis of ASIC3-IR neurons, tooth pulp neurons and cutaneous neurons, the cross-sectional area of ASIC3-IR or FG-labeled cell bodies that contained nuclear profiles was measured on glossy prints (×165). Because of the halo surrounding the positive cells, however, accuracy of measurement was compromised.

For the control, guinea-pig anti-ASIC3 serum was preabsorbed with rat ASIC3 (100 μg/ml, Neuromics). The specificities of other antibodies have been described elsewhere [5,6].

3. Results

3.1. The co-expression of ASIC3 with CGRP or PV in the TG

The TG contained many ASIC3-, CGRP- and PV-IR neurons (Fig. 1B and D); 22.8% (225/987) of TG neurons were immunoreactive for ASIC3. These neurons were of various sizes (range=43–1768 μm^2, mean±S.D.= 651±356 μm^2). As described previously, CGRP-IR neurons were small to medium-sized whereas PV-IR neurons were mostly large [7,15,16]. These neurons were scattered throughout the TG. Our double immunofluorescence method revealed the co-expression of ASIC3 with CGRP or PV in the TG (Fig. 1A–D); 26.2% (81/309) of ASIC3-IR neurons exhibited CGRP-immunoreactivity (IR) and 27.5% (81/295) of CGRP-IR TG neurons co-expressed ASIC3-IR.

TG neurons which co-expressed ASIC- and CGRP-IR measured 103–1106 μm^2 (mean±S.D.=392±221 μm^2). More than half (57.6% or 53/92) of small (<400 μm^2) ASIC3-IR neurons were also immunoreactive for CGRP, whereas large (>800 μm^2) ASIC3-IR neurons were mostly devoid of CGRP-IR (4.1% or 4/98) (Fig. 2). On the other hand, 13.7% (40/291) of ASIC3-IR neurons exhibited PV-IR and 26.7% (40/291) of PV-IR TG neurons showed ASIC3-IR. TG neurons which co-expressed these substances measured 378–1140 μm^2 (mean±S.D.= 692±185 μm^2); 23.5% (27/115) and 12.6% (12/95) of medium-sized and large ASIC3-IR neurons, respectively, co-expressed PV-IR (Fig. 3). Small ASIC3-IR neurons were mostly devoid of PV-IR (1.2% or 1/81).

3.2. ASIC-IR neurons innervating the tooth pulp and facial skin

At 3 days after FG application to the upper molar tooth pulps or infraorbital skin, many cell bodies were labeled in the TG (Fig. 1E, H, K, N). They were mostly located in the maxillary division of the ganglion. Retrograde tracing and immunohistochemical methods revealed that tooth pulp and cutaneous TG neurons contained ASIC3-IR (Fig. 1E, F, H, I, K, L, N, O, 5, 6); 33.3% (61/183) and 13.2% (44/334) of TG neurons innervating the tooth pulp and facial skin, respectively, were immunoreactive for ASIC3 (Figs. 4 and 5). About 30% of small (30.8% or 8/26, <400 μm^2), medium-sized (35% or 21/60, 400–800 μm^2) and large (33.0% or 32/97, >800 μm^2) tooth pulp neurons showed ASIC3-IR (Fig. 4). On the other hand, 15.0% (19/127) and 20.9% (18/86) of medium-sized and large cutaneous neurons, respectively, exhibited the IR (Fig. 5). Small ASIC3-IR neurons which innervate the facial skin were rare in the TG (5.8% or 7/121).

A double immunofluorescence method revealed the co-expression of ASIC3 with CGRP or PV in tooth pulp and cutaneous TG neurons. All ASIC3-IR tooth pulp neurons contained CGRP-IR (25/25) and 36.2% (25/69) of CGRP-IR tooth pulp neurons showed ASIC3-IR (Fig. 1E–G); 25% (9/36) of ASIC3-IR pulpal neurons exhibited PV-IR and 42.9% (9/21) of PV-IR ones were also immunoreactive for ASIC3 (Fig. 1H–J). ASIC3-IR cutaneous neurons mostly showed CGRP-IR (81.0% or 17/21) and 37.0% (17/46) of CGRP-IR cutaneous neurons exhibited ASIC3-IR (Fig. 1K–M). ASIC3-IR cutaneous neurons

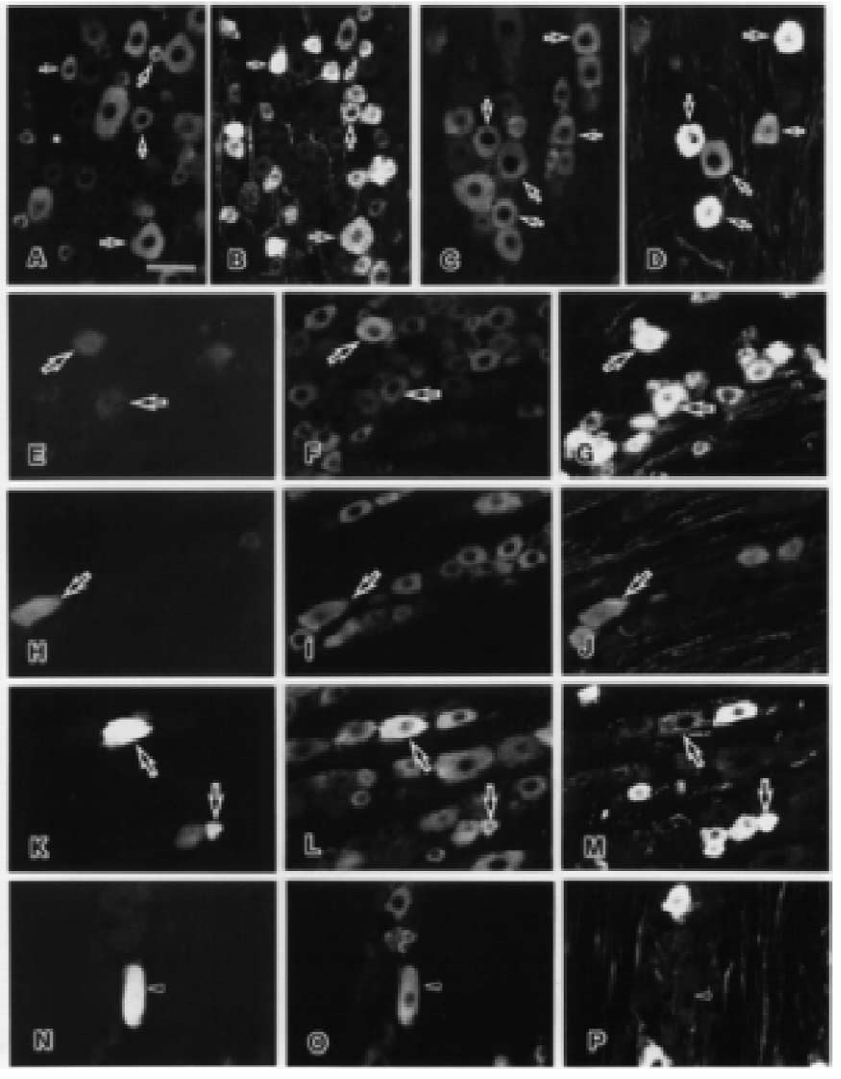

Fig. 1. Microphotographs of ASIC3-IR (A, C, F, I, L, O), CGRP-IR (B, G, M), PV-IR (D, J, P) and FG (E, H, K, N) in the TG. Panels A and B, C and D, E–G, H–J, K–M, and N–P show the same fields of view, respectively. The TG contains abundant ASIC3-IR neurons (A, C). These neurons are of various sizes and scattered throughout the ganglion. A double immunofluorescence method reveals the co-expression of ASIC3 with CGRP or PV. Many ASIC-IR neurons (arrows in A, C) are also immunoreactive for CGRP (arrows in B) or PV (arrows in D). TG neurons retrogradely labeled from the molar tooth pulp with FG (arrows in E, H) co-express ASIC3 (arrows in F, I) with CGRP (arrows in G) or PV (arrows in J). TG neurons which co-express ASIC3- and CGRP-IR innervate the facial skin (double arrows in G). Arrows in N–P point to a cutaneous TG neuron which shows ASIC3-IR but not PV-IR. Bars=50 μm (A, B). All panels are at the same magnification.

which co-expressed PV-IR were rare in the TG (4.3% or 1/23) (Fig. 1N–P).

4. Discussion

The present study demonstrated that the TG contained abundant ASIC3-IR neurons; 23% of TG neurons were immunoreactive for ASIC3. These neurons were of various sizes. Our double immunofluorescence method also revealed that ASIC3-IR neurons co-expressed CGRP- or PV-IR. The axons of CGRP-IR sensory neurons are unmyelinated or finely myelinated [11,13,16], whereas those of PV-IR ones are large and myelinated [6]. Therefore, both myelinated and unmyelinated neurons probably contain ASIC3 in the TG.

Our retrograde tracing method demonstrated that ASIC3-IR TG neurons innervate the tooth pulp and facial skin. The proportion of ASIC3-IR neurons among tooth pulp neurons (33%) appeared to be larger than among cutaneous TG neurons (13%). It is likely that the content of ASIC3 in TG neurons is different between their receptive fields. In addition, 36% of CGRP-IR tooth pulp and cutaneous TG neurons were also immunoreactive for ASIC3. This suggests that ASIC3-IR neurons supply the tooth pulp and facial skin with unmyelinated or finely myelinated axons [6,11]. On the other hand, the co-expression of ASIC3 and PV was frequent in tooth pulp neurons

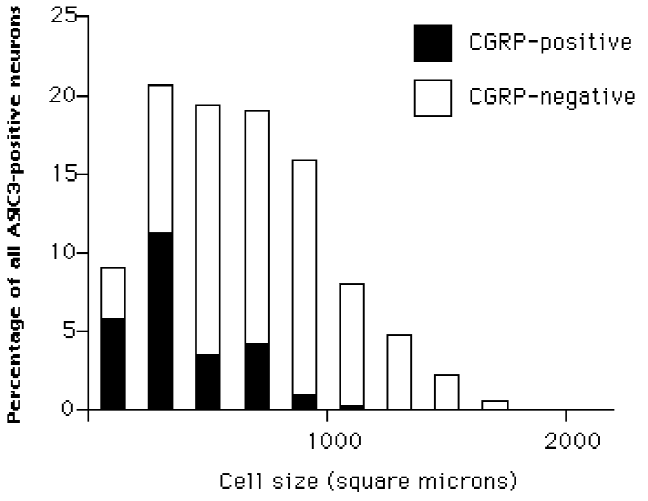

Fig. 2. A histogram showing the cell size spectrum of ASIC3-IR TG neurons which are immunoreactive or immunonegative for CGRP. The data were obtained from 309 ASIC3-IR neurons.

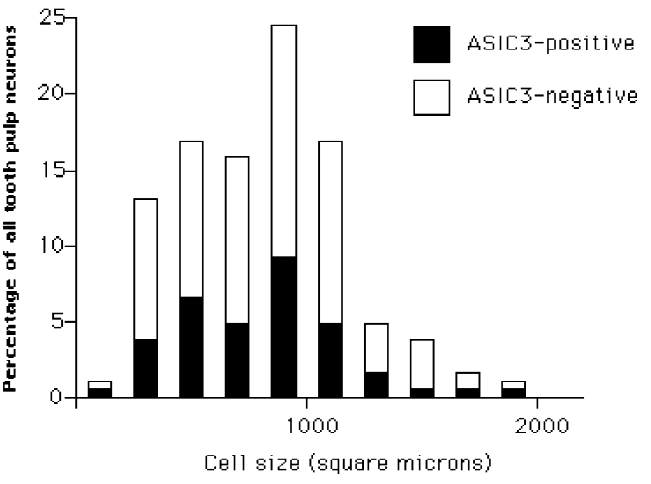

Fig. 4. A histogram showing the cell size spectrum of ASIC3-positive and negative tooth pulp neurons. The data were obtained from 183 neurons.

but not cutaneous TG neurons; 25% of ASIC3-IR pulpal neurons were also immunoreactive for PV. Only 4% of ASIC3-IR cutaneous neurons co-expressed PV-IR. ASIC3-IR neurons which have large myelinated axons may be common in the tooth pulp but not the facial skin [6]. The axonal morphology of ASIC3-IR TG neurons may depend on the variety of their receptive fields. ASIC3 has been considered to function as a sensor of tissue acidosis. Rat ASIC3 requires very acidic pH ($<$4.5) for activation of the sustained current [4]. Therefore, ASIC3-IR TG neurons with large myelinated axons may play a role in the perception of pulpal pain that accompanies tissue acidosis.

In conclusion, we have described ASIC3-IR neurons in the TG. ASIC3-IR neurons were of various sizes and co-expressed CGRP- or PV-IR. CGRP-IR neurons which co-expressed ASIC3-IR were frequent in the tooth pulp and facial skin. On the other hand, the co-expression of ASIC3 and PV was common in tooth pulp neurons but not cutaneous TG neurons. The axonal morphology of ASIC3-IR TG neurons may depend on the variety of their receptive fields.

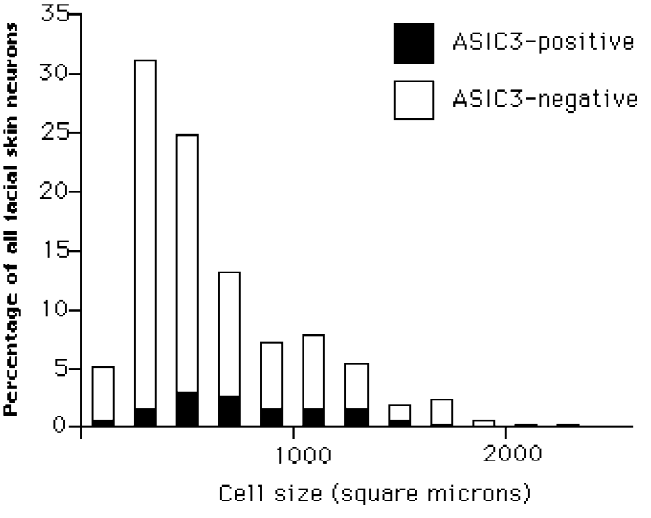

Fig. 5. A histogram showing the cell size spectrum of ASIC3-positive and negative cutaneous TG neurons. The data were obtained from 183 neurons.

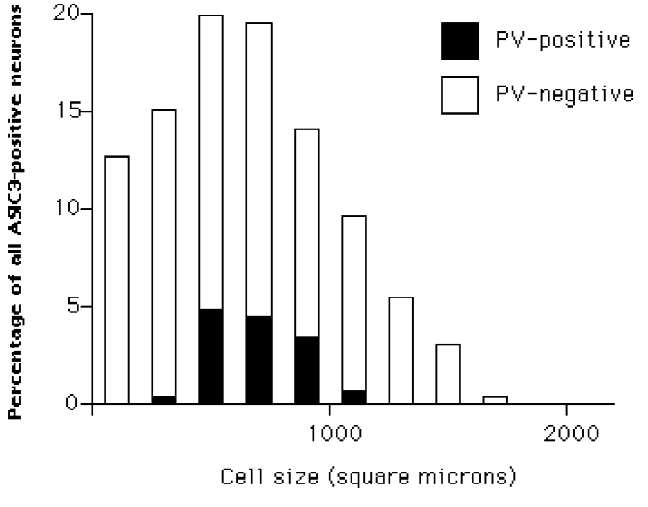

Fig. 3. A histogram showing the cell size spectrum of ASIC3-IR TG neurons which are immunoreactive or immunonegative for PV. The data were obtained from 291 ASIC3-IR neurons.

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