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Research Report

Peripheral and central distribution of TRPV1, substance P and CGRP of rat corneal neurons

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ABSTRACT

The rat corneal neurons expressing vanilloid receptor TRPV1, substance P (SP) and calcitonin-gene-related peptide (CGRP) were examined. In the cornea, some TRPV1-immunoreactive nerve fibers displayed either SP- or CGRP immunoreactivity also. For observing corneal neuronal elements in the trigeminal ganglion (TG) and in the medulla oblongata, retrograde and anterograde cholera toxin subunit B (CTB) tracing methods combining with triple immunofluorescence technique were performed. The corneal neuronal somata were located in the ophthalmic division of the TG; 37% of them were immunoreactive for TRPV1. One third and three quarters of the corneal TRPV1-immunoreactive neurons co-expressed SP and CGRP, respectively. All of SP-immunoreactive corneal neurons exhibited TRPV1 immunoreactivity. They were predominantly medium-sized (mean ± SE = 638.2 ± 49.5 μm^2) and significantly larger than SP-immunoreactive and TRPV1-immunonegative neurons in the ophthalmic division of the TG. The central projection fibers of corneal neurons co-expressing TRPV1 with SP and CGRP were observed at the subnucleus interpolaris/caudalis transition within trigeminal nucleus. The present study suggests that TRPV1 of the corneal neurons works in close relation to SP and CGRP both in the cornea and CNS for healing and nociceptive transduction.

1. Introduction

The cornea exhibits a high sensitivity to physical and chemical stimuli and is densely innervated by trigeminal sensory nerve fibers that terminate in the corneal epithelium as free nerve endings. Those corneal sensory neurons have been well documented that their somata are mainly located in the dorsal part of the ophthalmic region of the ipsilateral trigeminal ganglion (TG) and the central fibers of them project to the ventral part of the spinal tract of trigeminal nerve by using neuronal tracers (Marfurt and Del Toro, 1987; Takemura et al., 1991) and by c-fos detecting method following corneal stimulation (Meng and Bereiter, 1996).

The sensory neurotransmitter substance P (SP) (Miller et al., 1981; Shimizu, 1982; Tervo et al., 1981) and calcitonin-gene-related peptide (CGRP) (Colin and Kruger, 1986; Silverman and Kruger, 1988; Stone et al., 1986) have been detected in the sensory nerve fibers of the cornea. They are, when released at the peripheral end of the axon, principal mediators of neurogenic inflammation. SP functions as a modulator of corneal epithelial wound healing (Murphy et al., 2001; Yamada et al., 2005). SP and CGRP coexist in single cells of TG (Lee et al.,

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1985; Ma, 2002; Skofitsch and Jacobowitz, 1985) and possibly in the corneal fibers (Beckers et al., 1993).

The capsaicin (vanilloid) receptor TRPV1 is a Ca^{2+} permeant channel that is potentiated by heat (>43 °C) and decreased pH. In TG, TRPV1 is localized to small- and medium-sized neurons (Guo et al., 1999; Ichikawa and Sugimoto, 2001; Stenholm et al., 2002). TRPV1 immunoreactivity has been observed in the cornea and the spinal trigeminal nucleus where peripheral and central processes of TG neurons terminate (Bae et al., 2004; Guo et al., 1999).

TRPV1-containing neurons co-express CGRP in TG (Guo et al., 1999; Ichikawa and Sugimoto, 2001), and capsaicin evokes CGRP release via a vanilloid-receptor-mediated exocytotic mechanism from rat buccal mucosa of trigeminal field of innervation (Flores et al., 2001). TG neurons innervating the cornea in the mouse are studied about SP and CGRP (Felipe et al., 1999). However, TRPV1 in corneal neurons has not yet been studied enough. Thus, in this study, TRPV1 expression in the somata of the corneal TG neurons and terminals in the medulla oblongata of corneal neurons was examined by immunofluorescence and tracing methods. Furthermore, in order to understand the function of TRPV1 in the corneal peripheral and central nervous system, we examined the co-expression of TRPV1 and either SP or CGRP there.

2. Results

2.1. *Localization of TRPV1 with SP and CGRP in nerve fibers and terminals of the cornea*

TRPV1 immunoreactivity was observed in fibers of the cornea. These fibers ran beneath the stratified squamous epithelium and some branches of them went among the epithelial cells. There were some TRPV1-immunoreactive nerve fibers that contained either SP (Fig. 1) or CGRP (Fig. 2) immunoreactivity and some that contained no SP and no CGRP immunoreactivity.

2.2. *Corneal neurons in the TG*

CTB-labeled corneal neuronal somata were observed at the ophthalmic division in the ipsilateral TG (see Marfurt and Del Toro, 1987 for reference). Less than 10 CTB-labeled cells were seen in a section of the TG, and their total number in a ganglion was expected around 50. Half of CTB-labeled neurons are TRPV1-immunoreactive, and one third of them were immunoreactive with SP. And a half of SP-immunoreactive neurons were TRPV1-immunoreactive. Thirty-seven percent of corneal neurons were TRPV1-immunoreactive, and one third of them were SP-immunoreactive also. All SP-immunoreactive corneal neurons were TRPV1-immunoreactive (Table 1, Fig. 3). Three quarters of corneal TPRV1-immunoreactive neurons were CGRP-immunoreactive also. And 7% of corneal neurons were TRPV1-immunonegative and CGRP-immunoreactive (Table 1, Fig. 4).

Table 2 and Fig. 5 give the size and its frequency distribution of neuronal somata in each group. CTB-positive corneal neurons were larger than 450 μm^2. In particular, neurons positive for CTB only were significantly larger than

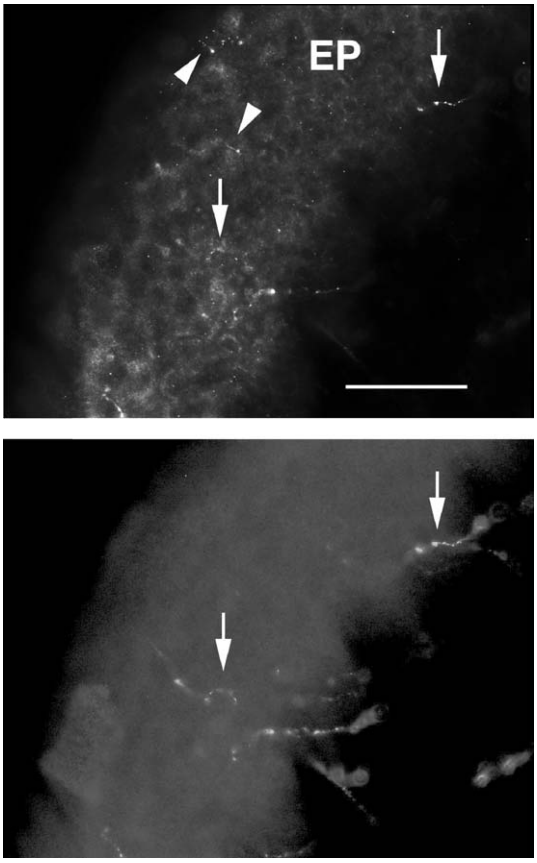

Fig. 1 – A pair of photomicrographs illustrating double immunofluorescent labeling using anti-TRPV1 (upper) and anti-SP (lower) antisera in the cornea. TRPV1-immunoreactive fibers with (arrows) and without (arrowheads) SP immunoreactivity run into the stratified squamous epithelium (EP). Scale bar = 100 μm.

those in the other groups. Neurons positive for CTB, TRPV1 and SP were significantly larger than those in four groups CTB-immunonegative except TRPV1- and SP-immunoreactive group. There is no significance between every pair groups SP- and CGRP-immunoreactive. This is reasonable because SP and CGRP are expressed in single neurons of the TG so often (Lee et al., 1985; Ma, 2002; Skofitsch and Jacobowitz, 1985).

2.3. *Central projection of corneal neurons into the medulla oblongata*

TRPV1-immunoreactive fibers were distributed at the lateral part of the ipsilateral spinal trigeminal nucleus, the ventrolateral medulla immediately dorsal to the lateral reticular nucleus and some dorsal parts in the spinal trigeminal. Much of them seemed to colocalize with SP or CGRP immunoreactivity. But the area including SP- or CGRP-immunoreactive fibers were wider than that including TRPV1-immunoreactive fibers and extended to the reticular formation as a previous study have indicated (Bae et al., 2004).

CTB tracer labels were observed in nerve fibers and terminals of the ventrolateral portion of the spinal trigeminal nucleus at the transition between trigeminal subnucleus