Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 1043 (2005) 225 – 230
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Short communication

Delta-opioid receptor-immunoreactive neurons in the rat cranial sensory ganglia

H. Ichikawa[a,b,*], S. Schulz[c], V. Höllt[c], T. Sugimoto[a,b]

[a]Department of Oral Function and Anatomy, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8525, Japan
[b]Biodental Research Center, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8525, Japan
[c]Department of Pharmacology and Toxicology, Otto-von-Guericke University, Magdeburg, Germany

Accepted 15 February 2005
Available online 7 April 2005

Abstract

Immunohistochemistry for delta-opioid receptor (DOR) was performed on the rat cranial sensory ganglia. The immunoreactivity was detected in 16%, 19% and 11% of neurons in the trigeminal, jugular and petrosal ganglia, respectively. The nodose ganglion was devoid of such neurons. DOR-immunoreactive (IR) neurons were mostly small to medium-sized (trigeminal, range = 62–851 μm^2, mean ± SD = 359 ± 175 μm^2; jugular, range = 120–854 μm^2, mean ± SD = 409 ± 196 μm^2; petrosal, range = 167–1146 μm^2, mean ± SD = 423 ± 233 μm^2). Double immunofluorescence method revealed that all DOR-IR neurons were also immunoreactive for calcitonin gene-related peptide. The cutaneous and mucosal epithelia in the oro-facial region, tooth pulp, taste bud and carotid body were innervated by DOR-IR nerve fibers. In the brainstem, IR nerve terminals were located in the superficial medullary dorsal horn and dorsomedial part of the subnucleus oralis as well as the solitary tract nucleus. The present study suggests that DOR-IR neurons may be associated with nociceptive and/or chemoreceptive function in the cranial sensory ganglia.

Theme: Sensory systems
Topic: Somatic and visceral afferents

Keywords: Calcitonin gene-related peptide; Delta-opioid receptor; Immunohistochemistry; Jugular ganglion; Petrosal ganglion; Trigeminal ganglion

Delta-opioid receptor (DOR) and its endogenous ligands, Met-enkephalin and Leu-enkephalin, are distributed in the spinal cord where they modulate the transmission of nociceptive neural activity [1,8,9,13]. Previous immunohistochemical studies have demonstrated that DOR is localized to small neurons in dorsal root ganglia (DRG) [1,8]. These neurons supply their receptive fields with free nerve endings and project to the superficial laminae in the spinal dorsal horn [1,9,15]. On the other hand, calcitonin gene-related peptide (CGRP) is thought to be a marker for small to medium-sized neurons in the sensory ganglia [2,7,11]. CGRP-containing DRG neurons have free nerve endings in peripheral tissues and project to the spinal dorsal horn [2,7]. Double immunofluorescence study revealed that a subpopulation of these neurons were also immunoreactive for DOR [1].

The cranial ganglia include trigeminal, glossopharyngeal and vagal sensory neurons. Somatic sensory neurons of the trigeminal and vagus nerves are located in the trigeminal and jugular ganglia, respectively. Visceral sensory neurons of the vagus and glossopharyngeal nerves are located in the nodose and petrosal ganglia, respectively. CGRP-containing trigeminal neurons supply the facial skin, oral mucosa and tooth pulp with free nerve endings and project to caudal and rostral parts of the trigeminal sensory nuclei [2,5,10,12,16]. CGRP-containing neurons in the jugular, petrosal and

* Corresponding author. Department of Oral Function and Anatomy, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8525, Japan. Fax: +81 86 235 6612.
E-mail address: hiroichi@md.okayama-u.ac.jp (H. Ichikawa).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.02.041

nodose ganglia innervate the carotid body and taste bud [3,4,10]. Their projection site is the solitary tract nucleus [2]. These neurons are considered to be associated with nociceptive and/or chemoreceptive transmission. However, little is known about DOR in the trigeminal, glossopharyngeal or vagal sensory ganglia.

In this study, we examine the distribution of DOR and its co-expression with CGRP in the cranial sensory ganglia.

Four trigeminal, jugular, petrosal and nodose ganglia and 3 brainstems as well as facial skins, tongues, maxillae, mandibles and carotid bodies were obtained from six male Sprague–Dawley rats (200–300 g). The 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 and post-fixed with the same fixative for 30 min. Maxillae and mandibles were decalcified with 4.13% ethylene diaminetetraacetic acid disodium salt in 0.1 M phosphate buffer (pH 7.4) for 2 weeks at room temperature. Materials were soaked in a phosphate-buffered 20% sucrose solution overnight, frozen sectioned at 12 μm and thaw-mounted on gelatin-coated glass slides. For demonstration of DOR, sections were incubated with rabbit anti-DOR serum (1:100,000) [9] for 24 h at room temperature, followed by biotinylated goat anti-rabbit IgG and avidin–biotin–horseradish peroxidase complex (Vector Laboratories Inc., Burlingame, CA). Following nickel ammonium sulfate-intensified diaminobenzidine reaction in tris-buffered saline (pH 7.6), the sections were dry-mounted on gelatin-coated glass slides, dehydrated in a graded series of alcohols, cleared in xylene and cover-slipped with Entellan (Merck, Germany). In this study, rostral and caudal halves of the jugular–petrosal complex were regarded as the jugular and petrosal ganglia, respectively. The number and cell size of DOR-positive and negative neurons were analyzed in every tenth of the serial sections of 2 vagal and glossopharyngeal ganglia from different animals. For the trigeminal ganglion,

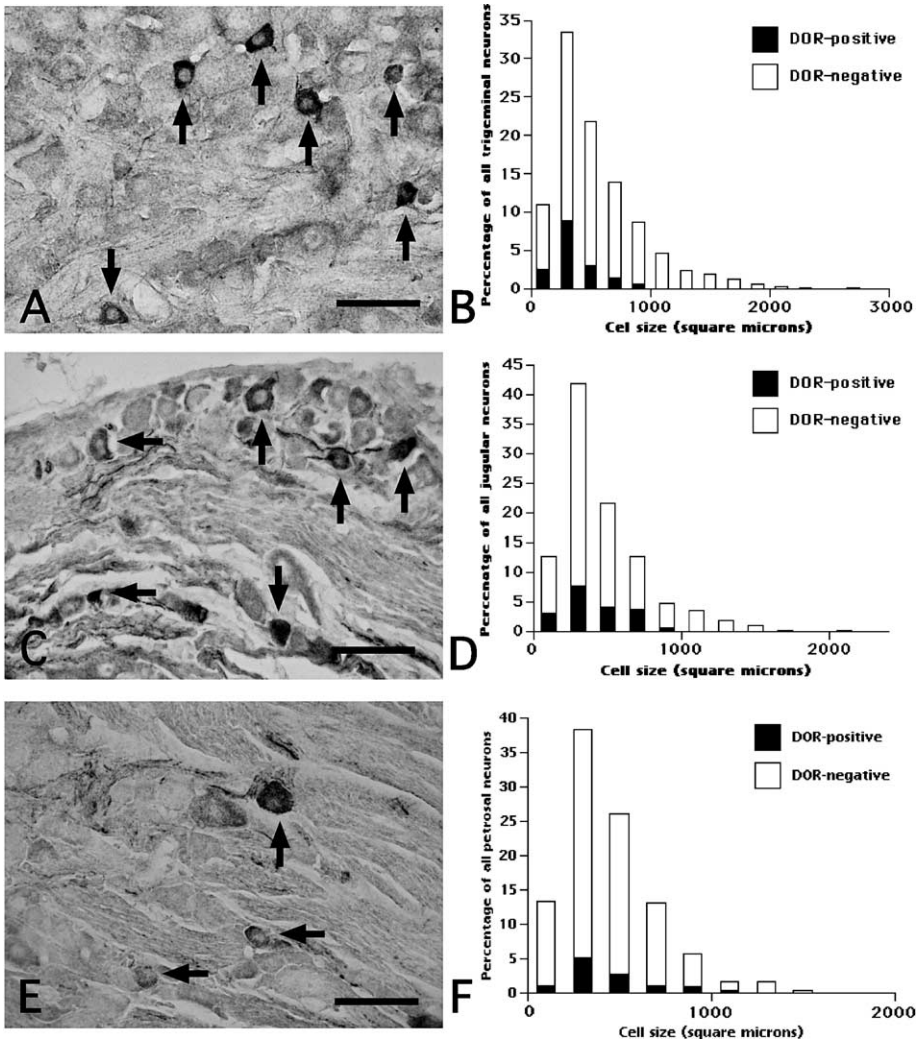

Fig. 1. Microphotographs for DOR-positive neurons and histograms showing the cell size spectra of DOR-positive and negative neurons in the trigeminal (A B), jugular (C, D) and petrosal ganglia (E, F). DOR-IR neurons are mostly small to medium size (arrows in panels A, C, E). The data were obtained from 768 trigeminal (B), 434 jugular (D) and 368 petrosal (F) neurons. Scale bars = 100 μm.