－原著－

歯根膜ルフィニ神経終末の再生過程における ASIC3 発現様式の変化

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Alterations in the Expression Pattern of the Acid-Sensing Ion Channel 3（ASIC3）during the Regeneration of Periodontal Ruffini Endings

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Abstract

The acid-sensing ion channel 3 (ASIC3), belonging to the epithelial sodium-channel/degenerin (ENaC/DEG) superfamily, is regarded as an essential ion channel for mechanosensation. Our recent study has confirmed the expression of ASIC3 in the mechanoreceptive Ruffini endings in the periodontal ligament of mice incisors, indicating that this molecule is a new marker for mechanoreceptors. Although the periodontal Ruffini endings have been reported to have a potential for high neuroplasticity, no information is available regarding the role of ASIC3 during the regeneration process after nerve injury. This study was therefore undertaken to examine changes in the expression pattern of ASIC3 in the periodontal ligament and trigeminal ganglion of the rats. After transection of the inferior alveolar nerve (IAN), cryostat sections – including the lingual ligament of the lower incisors and trigeminal ganglia – were processed for immunohistochemical procedures. As previously reported, the periodontal Ruffini endings disappeared on postoperative (PO) Day 3, and began regeneration from PO Day 7 to return to their normal morphology by PO Day 28, as shown by immunohistochemistry for protein gene product 9.5, a neuronal marker. However, no apparent immunoreaction for ASIC3 was ever recognizable in the periodontal ligament throughout this observation period. On the other hand, a strong immunoreaction for ASIC3 occurred in the satellite cells encircling the trigeminal ganglion neurons on the ipsilateral side. These findings indicate that ASIC3 is not directly involved in the axonal regeneration of the periodontal Ruffini endings but rather in neuron-glial interactions such as control of the neuronal activity in the trigeminal ganglion.

抄録

Acid-sensing ion channel 3 (ASIC3) は，上皮性ナトリウムチャネル/ degenerin (ENaC/DEG) 遺伝子スーパーファミリーに属する非電位依存性陽イオンチャネルで，機械刺激受容に重要な役割を担う。近年，我々は ASIC3 が歯根膜の機械受容器である歯根膜ルフィニ神経終末に発現することを報告し，機械受容器の新たなマーカーとなり得ることを示した。歯根膜ルフィニ神経終末は，高い神経可塑性を有することが報告されているが，神経傷害後の再生過程における ASIC3 の役割については全く不明である。従って本研究では，ラットの歯根膜ルフィニ神経終末および三叉神経節における下歯槽神経傷害後の ASIC3 の発現様式の変化を検討した。下歯槽神経切断後，切歯舌側歯根膜を

含む下顎骨と三叉神経節の凍結切片を作製し，神経線維のマーカーである protein gene product 9.5 (PGP 9.5) と ASIC3 の局在を免疫組織化学的手法を用いて検索した。過去の報告と同様に，切歯歯根膜ルフィニ神経終末は，下歯槽神経切断 3 日後に完全に消失したが，7 日後には再生を開始し，切断後 28 日目には正常なルフィニ神経終末の形態に回復した。しかしながら，観察期間を通して ASIC3 の免疫陽性反応は歯根膜ルフィニ神経終末には認められなかった。一方，切断側の三叉神経節では神経細胞周囲の衛星細胞が強い ASIC3 免疫陽性反応を示した。これらのことから，ASIC3 は歯根膜ルフィニ神経終末の再生過程において，軸索終末の形態回復に直接的には関与しないが，三叉神経節における神経活性の調節といった neuron-glia interaction に関与する可能性が示唆された。

Introduction

Acid-sensing ion channels (ASICs) belong to the epithelial sodium-channel/degenerin (ENaC/DEG) superfamily[1-3]. ASICs consisting of 7 isoforms[2,4] have been reported to be distributed throughout the central and peripheral nervous systems in mammals[5-7]. Previous studies have suggested the involvement of the neuronal ASICs in acid sensing as well as nociception and mechanoception[5,8-11]. In fact, ASIC1, 2, and 3 have been confirmed in the mechanoreceptive neurons of the dorsal root ganglia[12] as have ASIC2a and ASIC3 in cutaneous mechanoreceptors including Meissner corpuscles, Merkel nerve endings, and palisades of lanceolate nerve endings[5,8,13]. Predominant expressions of sensory neurons have suggested that ASIC3 is an essential channel for mechanosensation[5,8,9,13,14], as confirmed by ASIC3 expression in the trigeminal neurons, which participate in the sensation and modulation of nociceptive and mechanoreceptive signals[15-17].

The periodontal ligament is a dense connective tissue which receives a rich supply of sensory nerves. The periodontal sensory endings are divided into nociceptive free endings and mechanoreceptive specialized nerve terminals[18]. In spite of variance among species or kinds of teeth, a series of morphological studies have suggested that the Ruffini ending is a primary mechanoreceptor in the periodontal ligament[19,20]. Although the periodontal Ruffini ending lacks a distinctive fibrous capsule different from the Ruffini endings originally reported by Ruffini, this mechanoreceptive nerve terminal is morphologically characterized by dendritic ramifications of expanded axon terminals filled with abundant mitochondria and by an association with the terminal Schwann cells, analogues to lamellar cells of the Pacinian corpuscle or laminar cells of the Meissner corpuscle[18,19,21-23].

Active tissue remodeling takes place in the periodontal ligament with the constant exposure of occlusal forces[24]. Indeed, it has been shown that the turnover rate of the collagen fibers in the periodontal ligaments is about five times faster than that in other tissues[25-27]. This indicates that the periodontal nerve fibers have to adapt to the active tissue remodeling of the periodontal ligament. Previous experimental studies on tooth movement[28,29] and traumatic occlusion[30,31] have shown the rearrangement of the periodontal nerves and changes in the shape of the nerve endings. Furthermore, when the inferior alveolar nerve (IAN) is cut at the level of the mandibular foramen, the morphology and density of the periodontal Ruffini ending almost recover by postoperative 1 month[32,33]. Therefore, many researchers agree with the notion that the periodontal Ruffini ending has a high potential for neuroplasticity. Our regeneration studies using neurotrophin gene knockout mice have indicated the possibility that various kinds of molecules control the regeneration of the periodontal Ruffini endings in a stage-specific manner[34-36]. We have recently demonstrated ASIC3 immunoreaction in the axoplasm of the periodontal Ruffini endings as well as in the medium-sized trigeminal neurons which mediate mechanotransduction[17] in the mouse, suggesting a possible role for ASIC3 during the regeneration process of the periodontal Ruffini endings. To date, however, no information is available regarding changes in the ASIC3 expression in the periodontal ligament and trigeminal ganglion during nerve regeneration. Therefore, this study investigated changes in the localization of ASIC3 in the periodontal ligament and trigeminal ganglion using a rat IAN injury model to determine the role of ASIC3 in the regenerating Ruffini endings.

Materials and Methods

Animal preparation

All animal experiments were approved and performed according to the guidelines of the Niigata University Intramural Animal Use and Care Committee