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氏名	Tarek Abdulsamad Ali Balam
授与した学位	博　　士
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学位論文題名	Experimental tooth movement upregulates preproenkephalin mRNA in the rat trigeminal nucleus caudalis and oralis. （実験的歯の移動によりpreproenkephalin mRNAの発現が三叉神経脊髄路核尾側亜核および吻側亜核で亢進する。）
論文審査委員	教授　杉本　朋貞　教授　松尾　龍二　教授　山本　照子

学位論文内容の要旨

[Introduction]The trigeminal subnucleus caudalis (Vc) is the most caudal subdivision of the brain stem sensory trigeminal complex. The dorsomedial part of the trigeminal subnucleus oralis (Vodm) also receives intraoral primary afferent innervation. Orthodontic force causing continuous pressure to the teeth induces pain and discomfort to the patient. These sensations are classified into two responses: the first response appears at the moment when the orthodontic force is applied and then disappears immediately, whereas the second response appears much later with a peak intensity on day one or two, and lasts for a few day. Clinically the later response is more problematic. Experimental tooth movement induced nociceptive Fos response in the trigeminal subnucleus caudalis in a biphasic manner. Endogenous pain control system involves multiple pathways, such as various monoamine systems and endogenous opioid systems. It has been shown that the serotonergic system is activated by nociception caused by experimental tooth movement at the medulla and in the periaqueductal grey. However, there are few studies that have investigated the possible involvement of endogenous opioid systems in response to trigeminal nociception. In the present study, we evaluated possible deviation in the *preproenkephalin* mRNA (*PPE*) expression pattern in trigeminal subnucleus caudalis and oralis 24 hours after the experimental tooth movement, when c-Fos expression and serotonin turnover are increased in central nervous system.

[Material and Methods] Eight male Sprague-Dawley rats weighing 180-200g and 7-weeks-old were used. Under sodium pentobarbital anesthesia, orthodontic force was applied by inserting an orthodontic elastic module between the first and second molar. The sham rats were treated in a similar manner, but the elastic module was not retained (n=4). Twenty-four h following the induction of the experimental tooth movement, the rats were deeply anesthetized and transcardiacally perfused with 0.9% saline, followed by 4% paraformaldehyde. Brainstems (5mm up to obex and 4mm down to it) were carefully removed, stored in the same fixative overnight at 4℃, and immersed for 24 hours in phosphate-buffered 20% sucrose at 4℃ for cryoprotection. The tissues were embedded and stored at –80℃ until use. Specimens were serially cut at a thickness of 50 μm with a microtome. In situ hybridization histochemistry was carried out. As for the Vc, *PPE*-positive neurons were counted in laminae I and II of the dorsal third of subnucleus in the rostral most three sections. Vodm cell counts were made in three sections at the mid-rostrocaudal level. For statistical analysis, the average number of *PPE*-positive neurons defined as the average number of *PPE*-positive neurons per section (n/s), was recorded for each side in each animals. Statistical significance was determined by 2-way analysis of variance (ANOVA) and Schffe`s F test for post hoc comparison.

[Result] Numerous *PPE* mRNA-positive neurons were identified by in situ hybridization. In the Vc area of sham rats, transcripts were observed in the sensory nucleus, especially in superficial layer of Vc, in the nucleus of the solitary tract and in the nucleus of the spinal trigeminal tract. Twenty-four hours after initiating experimental tooth movement, upregulation of the mRNA expression was observed in the superficial dorsomedial edge of Vc and in the Vodm of experimental rats. *PPE* mRNA expression was significantly upregulated about two-fold ($P<0.01$) in the dorsomedial trigeminal subnucleus caudalis (Vc) on the ipsilateral side. Significant upregulation was also observed on both sides in the trigeminal subnucleus oralis (Vo) ($P<0.01$). In the present study, the experimental tooth movement upregulates *PPE* mRNA only in the dorsomedial edge of the superficial layer of Vc. Furthermore, this upregulation of the expression was confirmed only on the ipsilateral side in contrast to that in sham rats.

[Discussion] The dorsomedial part of the Vc receives primary afferent innervation from the tooth region, and the peripheral nociceptive information induced by tooth movement is mainly transmitted to the ipsilateral Vc as shown by studying the change of Fos immunoreactivity, a marker of nociception. Present findings together with previous findings suggested that endogenous enkephalinergic system could be activated presumably by the nociception caused by experimental tooth movement. In the present study we confirmed that experimental tooth movement induced significant upregulation of *PPE* mRNA expression only in the dorsomedial edge without affecting constitutive expression in other regions on either the ipsilateral or contralateral side. In molecular levels, immediate-early genes of the Fos and Jun families dimerize to form the transcription factor AP-1, which can bind to the target DNA sequence of the promoter regions of *PPE* genes. This transcription factor has been proposed to regulate the *PPE* mRNA expression in some brain regions. It is possible that Fos protein might be involved in the activation of brain *PPE* gene transcription induced by experimental tooth movement. In response to tooth movement, significant increase in 5-HT and 5-HIAA levels, and 5-HIAA/5-HT, an index of serotonin turnover, are detected in the brain stem in the Vc level, indicating that nociception induced by experimental tooth movement activates the endogenous descending serotonergic system. A recent study showed that 5-HT upregulates the *PPE* mRNA expression in cultured spinal cord cells. Furthermore, this upregulation of *PPE* mRNA is attenuated by application of c-fos antisense oligonucleotide. Taken together, in the antinociception mechanism during experimental tooth movement, serotonergic descending systems exert their inhibitory roles by activating the *PPE* gene and c-fos are associated with this serotonergic regulation of endogenous opioid ligand genes. Vodm is also the termination site for intraoral primary neurons. Noxious stimulation of the dental pulp and of the oral regions upregulated c-Fos expression in the Vodm, while innocuous dentin stimulation did not result in significant up-regulation. Hence, these data support that Vo is involved in orofacial nociception, as well as Vc. In response to orthodontically applied force to the teeth, Fos expression is upregulated both in Vc and in Vodm. Our present study showed that experimental tooth movement upregulated *PPE* expression there, indicating that enkephalinergic system is also activated in Vodm, as well as Vc. Hence, it is possible that enkephalinergic cells in Vo participate in inhibitory modulating systems in response to nociception evoked in the trigeminal subnucleus by experimental tooth movement.

論 文 審 査 結 果 の 要 旨

本研究は,歯の移動によって生じる痛みの伝達が内因性オピオイドによって下降性に制御される可能性を検討するために、ラットの実験的歯の移動モデルをもちいて、歯の移動開始 24 時間後に preproenkephalin の mRNA の発現を三叉神経脊髄路核において検討したものである。

その結果，歯の移動開始 24 時間後、三叉神経脊髄路核尾側亜核の背側近心部の移動部位と同側において、preproenkephalin の mRNA の発現が約 200％有意に上昇した。さらに三叉神経脊髄路核吻側亜核では、両側で約 150%の有意な増加が見られた。これらの部位では、歯の移動による Fos タンパクの発現の亢進も観察された。以上の結果，内因性オピオイドの一つである Enkephalin は歯の移動によって生じる痛みの制御に延髄レベルで関わっていることが示唆された。矯正治療中に生じる痛みは治療における大きな問題のひとつであり、患者によってその程度が異なること、また不快感などをともなう点で特徴がある。しかし、その伝達および制御のメカニズムについてほとんど知られていない。本研究はその機序を明らかにするための基盤として臨床的価値は高いと考えられる。

よって、本研究は臨床と密接に関係した基礎的研究であることが高く評価され，本申請論文は博士（歯学）の学位論文に値するものと認めた。