氏　　名	賀（ガ）　剣（ケン）　飛（ヒ）
博士の専攻分野の名称	博　士（歯　学）
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学位授与の要件	学位規則第４条第１項該当 歯学研究科統合機能口腔科学専攻
学 位 論 文 名	Differential responses of mesencephalic trigeminal neurons and trigeminal motoneurons after masseteric nerve axotomy in adult and neonatal mice （成獣及び幼若マウス咬筋神経切断による三叉神経中脳路核、三叉神経運動核ニューロンの変化）
論 文 審 査 委 員	（主査） 教　授　矢谷　博文 （副査） 教　授　脇坂　聡　講　師　豊田　博紀　講　師　小泉　英彦

論 文 内 容 の 要 旨

Purpose：

After peripheral nerve injury, degeneratition and subsequent regeneration of impaired neurons occur. It is known that nerve injury results in alteration of various bioactive substances such as trophic factors, growth factors and neuropeptides both in the injured sensory and motoneurons, and such alterations are different between sensory neurons and motoneurons. Moreover, the neuronal responses against peripheral nerve injury are different in neonatal animals and adult animals. Most studies have been carried out in spinal nervous system of adult animals. It is thought that the trigeminal sensory system is similar to the spinal sensory system, but there are two major differences between them, 1) trigeminal sensory system has two clusters of sensory neurons, trigeminal ganglion neurons which is analogue to the dorsal root ganglion neurons, and trigeminal mesencephalic neurons (MesV)－the only sensory neurons located within the central nervous system, and their sensory modality is proprioception, and 2) spinal cord has laminar cytoarchtecture while such lamilar cytoarchtecture is observed only in caudal portion of the trigeminal sensory nuclear complex. In the present study, the responses against peripheral nerve injury in the MesV and trigeminal motoneurons (Vmo) in both neonatal and adult mice were examined. Nerve injury was subjected to masseteric nerve which contains both sensory nerves from trigeminal ganglion and MesV, and motor nerve from Vmo. In addition, degeneration and regeneration of muscle spindle in masseter muscle which are innervated by MesV, and motor endplates which are effector from Vmo.

Materials and methods：

Animals and surgery：Six-week-old DDY male mice and neonatal mice at postnatal day 3 (PN3d) at the time of surgery were used. Under anesthesia, left masseteric nerve was exposed and cut at the level before branching.

Immunohistochemistry : Under deep anesthesia, animals were perfused transcardically with 4% paraformaldehyde at post-operation day 1 (PO1), PO3, PO7, PO14, PO28 and PO42 (adults) or at PO1, PO3, PO7 and PO14 (neonatal animals). Brainstem and left masseter muscle were removed, post-fixed, immersed in 20% sucrose, and section at thickness of 50 μ m with freezing microtome. Every second section from brainstem was immunostained by active transcription factor-3 (ATF-3), a maker of injured neurons. The numbers of neurons in MesV and Vmo were also counted from every second section. Sections from masseter muscle were labeled with protein gene product 9.5 (PGP 9.5), a marker of neurons and S-100, a marker of Schwann cells.

Results:

Adult brainstem : In normal adult, approximately (345.3±28.4) and (512.7±37.1) neurons were counted in MesV and Vmo, respectively. There were no apparent changes in number of Vmo neurons following injury, while number of MesV neurons decreased from PO14. There was no immunoreactivity of ATF-3 in normal animals. At PO1, approximately 27.3±6.2% of MesV neurons and 42.4±9.3% of Vmo neurons expressed ATF-3 immunoreactivity ; immunoreactivity was restricted in nuclei. Percentage of ATF-3 immunoreactive neurons increased gradually, reaching maximal levels at PO7 (46.1±78% in MesV and 50.7±12.3% in Vmo). The percentages of ATF-3 immunoreactive neurons decreased gradually from PO14, and became very few in Vmo at PO42. However, approximately 17.6±9.5% of MesV neurons still showed ATF-3 immunoreactivity at PO42.

Neonatal brainstem : At postnatal day 3, approximately 246.7±50.3 and 438.2±52.3 neurons were counted in MesV and Vmo, respectively. Number of neurons decreased gradually in both MesV and Vmo after injury. Following masseteric nerve axotomy, approximately 23.5±4.7% and 58.3±11.6% of neurons expressed ATF-3 in MesV and Vmo at PO1 respectively. The percentages of ATF-3 neurons decreased rapidly from PO1, and very few ATF-3 neurons were detected in Vmo on PO7. In the MesV, however, significant number of ATF-3 neurons was remained even at PO42.

Masseter muscles : In adult animals, spiral PGP 9.5 immunoreactive structures, presumably muscle spindles were occasionally detected. Motor endplates were clearly labeled with PGP 9.5 and S-100, showing array distribution. Following axotomy, regenerated muscle spindles were rarely detected. PGP9.5 immunoreactive motorendplates disappeared, and re-appeared around PO42, while S-100 reappeared around PO28.

Discussion and Conclusion

l) In adult mice Vmo neurons were more resistant against nerve injury compared to MesV neurons. Nerve injury caused neuronal cell death in MesV

2) In neonatal mice, both MesV and Vmo neurons were very sensitive against nerve injury. Although peripheral nerve injury evoked neuronal cell death in both MesV and Vmo, Mes V neurons were more severely damaged.

3) Regeneration of neurons and reconstruction of sensory receptors (muscle spindles) (motorendplates) were almost simultaneously following injury.

論 文 審 査 の 結 果 の 要 旨

本研究は成熟および幼若マウスでの咬筋神経切断による三叉神経中脳路核ニューロンおよび三叉神経運動核ニューロンの変化を検討したものである。

その結果、三叉神経運動核ニューロンよりも三叉神経中脳路核ニューロンのほうが咬筋神経切断による変化が顕著であり、また成熟動物よりも幼若動物のほうが影響が大きいことが分かった。

以上の研究結果は、神経損傷による三叉神経系におけるニューロンの反応機構の解明に重要な知見を与えるものであり、博士（歯学）の学位を授与するに値するものと認める。