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学位論文名	Long-Term Potentiation of Intrinsic Excitability in Hypoglossal and Trigeminal Motoneurons （舌下神経と三叉神経運動ニューロンにおける神経可塑性についての研究）
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論文内容の要旨

Introduction:

Rhythmical oral-motor activities are produced in response to action potentials mostly generated by hypoglossal and trigeminal motoneurons (HMNs and TMNs). The processes that determine the firing behavior of motoneurons are therefore important in understanding the transformation of neural activity to motor behavior. Changes in MNs intrinsic properties are critical for maintaining the excitability which is important to ensure appropriate output to muscles for execution of motor behaviors. Activity-dependent plasticity, referred to as long-term potentiation (LTP), in the central nervous system and recently in motor systems has been the subject of many studies. However, little is known about the presence of plasticity in the hypoglossal system and the main responsible pathway controlling the LTP in trigeminal system. Therefore, the present study was aimed at addressing these issues by using brainstem-spinal cord preparation and whole-cell patch clamp techniques in neonatal rats.

Method and Material:

1-1; In vitro brainstem-spinal cord preparation

Experiments were conducted on Sprague-Dawley rats, (1-4 days). The brainstem and spinal cord were isolated and transected at the pontomedullary and medulla-spinal cord junction. Motoneuron population discharges were recorded from the hypoglossal rootlet with the use of glass electrodes filled with artificial cerebrospinal fluid. Experiments were performed in the

presence of excitatory and inhibitory ionotropic transmission blockers. A fixed 500-ms test pulse (400–500 μV) every 30 seconds was injected into HMNs via a concentric bipolar electrode. We measured the extracellular responses of the hypoglossal motor branch. An electrical stimulus of 1.5 to 2 x the size of the test pulse (500 ms) (every 5 s for 5 min) was injected to stimulate induction. After 5 min of stimulation with the induction pulse, we recorded the extracellular response of the hypoglossal motor branch by a test pulse again.

1-2; Whole cell-patch clamp recording from HMNs

Whole-cell current recordings were performed on HMNs. Experiments were performed in the presence of excitatory and inhibitory ionotropic transmission blockers. A fixed 500-ms test pulse (every 30 seconds) was injected through the patch pipette to evoke four to five action potentials, and then a brief period of high-frequency repetitive firing (20 - 25 Hz for 500 ms every 5 s for 5 min) was induced by current application as an induction pulse through the patch pipette. After 5 min of stimulation with the induction pulse, we recorded the firing elicited by the same test pulse as used before the induction.

2; *Whole-cell patch clamp recording from TMNs*

The same protocol as previous step was performed. Role of calcium (Ca^{2+}) and different kinds of Ca^{2+}-dependent protein kinases [e.g., PKA, PKC and, Ca^{+2} dependent protein kinase II (CaMKII)] in long-term potentiation of intrinsic excitability (LTP-IE) were studied.

Results:

1-1 and 1-2; There was no increase in amplitude of the extracellular response from hypoglossal motor branch when the hypoglossal motor nucleus was stimulated electrically for a brief period by induction. Additionally, the single HMN showed any increase in response to the brief period of positive current injection. These results indicate that HMNs show no LTP-IE.

2; LTP-IE is calcium dependent on TMNs. Bath application of nominally 0 Ca^{2+} during the induction period prevented LTP-IE. BAPTA blocked the LTP-IE of TMNs. H7, a broad-spectrum protein kinase inhibitor, and H89, a specific PKA inhibitor, blocked the increase in excitability after induction on TMNs. Application of forskolin (forsk), an adenylyl cyclase activator to directly elevated cAMP and activate PKA, produced a long-lasting increase in excitability. The induction stimulus run following application of forsk did not cause any further change in excitability. Increase of neural activity by the application of PKC activator was smaller than that by forsk.

Discussion:

These studies indicated that hypoglossal motor system does not show LTP-IE, while trigeminal motor system does. This different behavior could be explained by following criteria; Based on McKay LC (2004) in vivo studies, episodic, but not continuous, hypoxia evokes LTP in genioglossus muscle. So, type of induction protocols could be effective in inducing plasticity in hypoglossal motor system. On the other hand, HMNs are involved in motor behaviors that must be functional at birth, such as swallowing and respiration. Therefore, these motor activities are already developed at the age of neonatal 1-4 days, but in trigeminal system there is a transition period in function of jaw movement from suckling to mastication. In this transition period applying the induction protocol could increase TMNs excitability and produce LTP-IE but applying induction to HMNs during neonatal 1-4 days old could not increase the motoneuronal excitability, and so HMNs do not show LTP-IE.

An intracellular Ca^{2+} increase during the induction protocol is necessary for the induction of LTP-IE of TMNs. The increase in excitability after induction depends on protein kinase activation. CaMKII activation is not essential for the induction of LTP-IE. LTP-IE is calcium dependent and PKA is a main regulator among other protein kinases.

論文審査の結果の要旨

本研究は、脳幹·脊髄標本およびホールセルパッチクランプ法を用いて、舌下神経及び三叉神経運動ニューロンの内因性の興奮を検討したものである。

舌下神経運動ニューロンはインダクション刺激によって内因性の興奮の長期増強（LTP-IE）を示さなかったのに対し、三叉神経運動ニューロンはLTP-IEを示す結果となった。また、インダクション刺激中の細胞内Ca^{2+}の増加は三叉神経運動ニューロンにおけるLTP-IEの誘導に必要であることが明らかとなった。さらに、インダクション後の興奮性の増加は、プロテインキナーゼの活性化に依存し、LTP-IEはカルシウム依存性であり、プロテインキナーゼAは他のプロテインキナーゼのうちの主な調節因子であることが明らかとなった。

以上の結果は、顎運動の生理的な機能の発達や回復などに関連する機能的順応に重要な知見を与えるものである。

よって、本研究論文は博士（歯学）の学位論文として価値のあるものと認める。