氏　　　名	山口　純弥
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論文審査委員	主　査　　教授　久保　義弘 教授　鍋倉　淳一 教授　井本　敬二 教授　小田　洋一　名古屋大学

論文内容の要旨

Fast inhibitory neurotransmissions in the central nervous system are mediated by γ-aminoburyric acid (GABA) and glycine. These transmitters can be co-released from the same synaptic vesicles at synapses in the cerebellum, the brain stem and in the spinal cord. The shared transport of GABA and glycine by vesicular inhibitory amino acid transporter (VIAAT), which is also named vesicular GABA transporter (VGAT), make it possible to co-release GABA and glycine from the same synaptic vesicles. However, the mechanisms that specify packaging of GABA+glycine into synaptic vesicles are not fully understood.

In the development of inhibitory synapses, the transmission was shown changes between the GABAergic and the glycinergic and this changes were also reflected in an increase of glycine content and a decrease in the extent of GABA in presynaptic terminals where both transmitters are present (Nabekura et al., 2004; Muller et al., 2006). Therefore, the author examined switching mechanism of the GABA/glycine transmission by the intracellular concentrations of inhibitory neurotransmitters, using paired whole-cell patch recording from monosynaptically coupled cultured spinal cord neurons derived from VGAT-Venus transgenic rat. Short depolarizing steps applied to presynaptic neurons evoked unitary (cell-to-cell) inhibitory postsynaptic currents (IPSCs). Under normal conditions, fractional contribution of postsynaptic GABA or glycine receptors to the unitary IPSC was not changed during 1 hr recording. When high concentrations of glycine were loaded into presynaptic neurons, glycinergic IPSC was enhanced but GABAergic IPSC was markedly reduced. On the other hand, when high concentrations of GABA were loaded into presynaptic neurons, GABAergic IPSC was enhanced but glycinergic IPSC was markedly reduced. Similar modulation was observed when presynaptic neurons were intracellularly perfused with high concentrations of glutamate via patch-pipette.

A fundamental property of neuronal circuits is the ability to adapt to altered inputs. Prolonged alteration in the activity of excitatory synapses causes adaptations to inhibitory synapse in order to maintain a level of network activity. Glutamate is a predominant excitatory neurotransmitter in the mammalian central nervous system. It has been reported that the neuronal subtype of glutamate transporter EAAT3 is located at inhibitory terminals (Rothstein et al 1994; He et al 2000), and that glutamate uptake modulates GABAergic synaptic strength in hippocampal neurons (Mathews and Diamond, 2003; Hartmann et al., 2008). In contrast, little is presently known about glutamate-induced changes of synaptic efficacy at GABA/glycine mixed synapses. Therefore, he next studied dynamic control of the GABA/glycine co-transmission by neuronal glutamate transporter, using paired recording from coupled cultured spinal cord neurons. Raising extracellular glutamate levels increased the amplitude of GABAergic IPSC by enhancing glutamate uptake, while it reduced the

glycine release.

Interestingly, high-frequency trains of stimuli easily caused depletion of glycinergic, but not GABAergic, unitary IPSCs. In addition, $GABA_A$ receptor mediated inhibition may have greater efficacy than glycine receptor mediated inhibition because of slower decay kinetics. Thus, the enhancement of GABA release by glutamate uptake may provide an advantage for rapid vesicular refilling of the inhibitory transmitter and thus may constitute a protective action against hyperexcitability.

The present results obtained from cultured spinal cord neurons raises the possibilities that the type of transmitter released from presynaptic nerve terminals defines the phenotype of fast inhibitory transmission, and that proportions of GABAergic or glycinergic transmission at inhibitory synapse depends on presynaptic cytoplasmic transmitter content. Thus, he next performed model experiment to study whether presynaptic cytoplasmic inhibitory transmitters defines transmission phenotype at inhibitory synapse using cultured rat hippocampal neurons. Although cultured hippocampal neurons usually show no glycinergic IPSC, immunocytochemical studies suggested the clustering of glycine receptors at GABA synapses (Lévi et al., 2004; Meier and Grantyn, 2004). Thus, he hypothesized that intracellular loading of glycine switches hippocampal GABAergic synapse to mixed GABAergic/glycinergic transmission.

Before intracellular application of glycine, he examined whether functional postsynaptic glycine receptors are present at hippocampal GABAergic synapse. Loading of glycine into presynaptic vesicles by endocytosis produced distinct glycinergic IPSC. Thus, hippocampal cultured GABAergic synapse expresses functional postsynaptic glycine receptors, yet lack presynaptic glycine release. In the paired recording from hippocampal neurons with presynaptic intracellular solution containing 100 mM glycine, glycinergic IPSC was appeared after waiting for several minutes. This glycinergic IPSC was fully blocked by tetrodotoxin or Ca^{2+} channel antagonists, suggesting the involvement of the exocytosis of synaptic vesicles. In addition, single bouton stimulation reveals that co-release of GABA and glycine occurs at single nerve terminal. The glycinergic component at 60 min after starting the paired recording was concentration-dependent. Furthermore, glycinergic transmission could be evoked from GlyT2-transfected hippocampal interneurons. These results suggest that hippocampal neurons possess presynaptically silent glycinergic synapse and that presynaptic cytoplasmic transmitter content defines the phenotype of inhibitory transmission.

In conclusion, his findings suggest that the phenotype of an inhibitory synapse is regulated by the nature of the presynaptically released transmitter. GABA/glycinergic inhibitory transmission is dynamic, and may be able to easily change the component in response to changes in neuronal activity.

博士論文の審査結果の要旨

中枢神経系における抑制性シナプス伝達を担う神経伝達物質であるGABAとグリシンは、両者とも小胞型抑制性アミノ酸輸送体 VIAAT によりシナプス小胞へと取り込まれ、脊髄や脳幹の一部の抑制性シナプスでは GABA とグリシンが同一の神経終末部から共放出される。しかし、GABA もしくはグリシンを特異的にシナプス小胞に取り込む機構はいまだ十分に明らかにされていない。

出願者、山口純弥氏は、この機構を明らかにすることを目的とし、神経細胞内の GABA およびグリシンの濃度および濃度比が決定因子であるという仮説の下に研究を開始した。抑制性神経細胞が蛍光により同定できるVGAT-Venusラットの脊髄神経細胞を分離培養し、ホールセルパッチクランプ下で、細胞質の GABA 及びグリシンの濃度をコントロールしながら、単シナプス結合している神経細胞間の抑制性シナプス伝達の性質を解析した。

シナプス前細胞内にパッチ電極から GABA を充填すると、その濃度依存的に抑制性シナプス電流の GABA 成分が増加し、グリシンを充填するとグリシン成分が増加した。このことから、GABA とグリシンによるシナプス伝達の割合が、両者の細胞質内の濃度に依存することが示された。また、山口氏は、通常グリシンによるシナプス伝達を通常示さない初代培養海馬神経細胞において、シナプス前細胞にグリシンを充填すると、抑制性シナプス電流にグリシン成分が出現することを見出した。また、海馬培養細胞にグリシン輸送体 GlyT2 を過剰発現させると、グリシン性成分が観察された。さらに、単一シナプスブートン刺激の実験により、単一ベシクル内で、神経伝達物質の変化が起きていることを示した。

興奮性神経伝達物質のグルタミン酸は、抑制性神経細胞のシナプス前終末にある興奮性アミノ酸輸送体 EAAT3 により細胞質内に取り込まれ、グルタミン酸脱炭酸酵素 GAD により、グルタミン酸から GABA が合成される。そこで、次のステップとして、山口氏は、初代培養脊髄神経細胞を用いてグルタミン酸の影響を解析した。細胞内へのグルタミン酸を充填、また、細胞外のグルタミン酸を EAAT3 により細胞内に取り込ませることにより、抑制性シナプス電流における GABA 成分の割合が増加することが明らかになった。高頻度刺激時に、GABA 成分はグリシン成分よりも長く持続するため、神経回路活動が高まりグルタミン酸が放出されるときに、GABA 成分が増えることは、抑制性シナプス伝達を確実に行うための機能的メリットがあると考えられる。

このように、山口氏は、抑制性シナプスにおいて用いられる 2 種の神経伝達物質が、両者の細胞質内の濃度に依存して変化することを明確に示し、さらに、その変化をひきおこす因子の一つとして神経回路の活動依存性があることを示した。

これらの知見は、抑制性シナプス伝達のダイナミックな変化の機構を明らかにしたもので、その科学的価値は極めて高く評価できる。以上の理由から、審査委員会は、全員一致で、本論文が学位論文として相応しいものであると判断した。