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科学研究費助成事業　　研究成果報告書

平成　３０　年　　８　月　３１　日現在

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研究種目：　基盤研究(C)（一般）
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課題番号：　２６４６２３４１

研究課題名（和文）GABA抑制系による脳可塑性の制御と麻酔薬：鎮静と毒性の分子基盤に関する新研究

研究課題名（英文）GABAergic inhibitory control of synaptic plasticity and anesthetic actions in the brain: molecular basis of hypnotic action and neurotoxicity of general anesthetics

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,800,000円

研究成果の概要（和文）：我々は、小胞型GABA/Glycineトランスポター(VGAT)低下によるセボフルランの鎮痛・鎮静作用への影響を調べた。立ち直り反射消失（LORR）、尾ピンチ反応消失（LTWR）、後肢ピンチ反応消失（LHWR）の濃度を、野生型（WT）とVGATヘテロ（12-16週）で比較した。LORR、LTWR、LHWRいずれも両群差はなかった。VGATの発現低下（約50%）は、セボフルランの鎮静・鎮痛効果に影響がなかった。この結果は、VGAT低下で小胞内への抑制伝達物質取り込みが減少した状態でもセボフルランによる抑制系賦活は、シナプス後膜の受容体への作用によって可能であることを示唆する。

研究成果の概要（英文）：Aim of Investigation: GABA and glycine are principal inhibitory neurotransmitters in the central nervous system and are laded into synaptic visicles via the vesicular GABA transporter (VGAT). We have found that VGAT+/- mice showed enhanced sensitivity to thermal stimulation and chemical inflammation. We investigated the possible influence of down-regulation of VGAT on hypnotic and analgesic actions of sevoflurane and propofol in mice. Results and Discussion: Reduction of VGAT protein (about half of the normal) doesn't affect hypnotic/analgesic effect of sevoflurane on mice behavior. This result shows Sevoflurane can enhance the inhibitory system (sedation/analgesia) by the effect on postsynaptic receptors despite of the decreased loading inhibitory neurotransmitters into synaptic vesicles.Although this study demonstrated that growth/development and ansthetic sensitivity are the same between VGAT(+/-) and WT, VGAT protein has been the important potential target of anesthetic drugs.

研究分野：　麻酔科学・神経科学・神経薬理学

キーワード：　麻酔　GABA受容体　可塑性　鎮静　神経毒性

１．研究開始当初の背景

1. We have previously reported that, in VGAT (+/-) mice, the VGAT protein levels are reduced to almost the half of the normal mice. On the other hand, neurotransmitter levels such as GABA and glycine in the CNS are normal in VGAT (+/-) mice.

2. The phase 2 responses of the formalin test were significantly enhanced in VGAT (+/-) mice, which shows not a response to chemical nociception as a result of tissue damage but one to subsequent inflammation.

２．研究の目的

GABA and glycine are principal inhibitory neurotransmitters in the central nervous system and are laded into synaptic vesicles via the vesicular GABA transporter (VGAT).

We have found that VGAT+/- mice showed enhanced sensitivity to thermal stimulation and chemical inflammation. We investigated the possible influence of down-regulation of VGAT on hypnotic and analgesic actions of sevoflurane and propofol in mice.

３．研究の方法

1. Mice and anesthetics

All animal procedures and protocols used in this study were approved by the Animal Care Committee of the Gunma University Graduate School of Medicine (protocol no. 05-71) and were performed according to the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). The phenotypes of heterozygous VGAT knockout (VGAT+/-) mice were compared to wild-type (WT) mice. All mice used for experiments were from 12 to 16 weeks old weighing 23 to 28 g. We used two different anesthetics, which has different mechanism of action, sevoflurane and propofol. The former is considered to has multiple effects on various receptors and the latter is regarded as a positive allosteric modulator of GABA A receptors. None of the animals were used for more than two experiments and at least 1 week was allowed between the two treatments for the mice to recover.

2. Behavioral assays

We compared anesthetic sensitivity to these anesthetics, using three behavioral assays, loss of righting reflex (LORR), loss of tail-pinch withdrawal response (LTWR) and loss of hind-limb withdrawal reflex (LHWR). LORR was considered as a surrogate measure for hypnosis, and LTWR and LHWR were used as a surrogate measure for immobilization reflecting a hypnotic action. In LORR assays, mice were judged with the following rules. Score 0: awake, Score 1: difficult to right itself or walking, Score 2: fail to completely right itself (LORR) for 2-10 sec, Score 3: LORR for over 10 sec. In LORR assays, mice were judged with the next rules. Score 0: quick response to pain, Score 1: not quick response (<2sec), Score 2: no response (≧2sec).

3. Statistics

Statistical significance between curves fitted to LORR and LTWR data was performed by comparing EC50 values via t-test. The level of statistical significance was set at $P < 0.05$ in all tests.

４．研究成果

< Results >

LORR produced by sevoflurane was similar between both genotypes (ED50, 1.20% [1.16-1.23] for WT mice, n=8; 1.17% [1.12-1.21] for VGAT+/-, n=7).

No genotype difference was found for propofol-induced LORR (ED50, 5.11 g/mg [4.43-5.76] for WT mice; 5.05 g/mg [4.24-5.81] for VGAT+/-).

LTWR produced by sevoflurane was similar between both genotypes (ED50, 1.97% [1.94-2.00] for WT mice, n=10; 2.03% [2.00-2.06] for VGAT+/-, n=7). LHWR produced by sevoflurane was also similar between both genotypes (ED50, 2.69% [2.65-2.74] for WT mice, n=7; 2.68% [2.65-2.74] for VGAT+/-, n=8).

< Discussion and Conclusions >

1. Reduction of VGAT protein (about half of the normal) doesn't affect hypnotic/analgesic effect of sevoflurane on mice behavior. This result shows Sevoflurane can enhance the inhibitory system (sedation/analgesia) by the effect on postsynaptic receptors despite of the decreased loading inhibitory neurotransmitters into synaptic vesicles.

2. VGAT knockout leads to embryonic

lethality before birth and VGAT is essential to maintain neural activity. Although this study demonstrated that growth/development and anesthetic sensitivity are the same between VGAT (+/-) and WT, VGAT protein has been the important potential target of anesthetic drugs.

3. Long-time exposure to Sevoflurane, which means long-term potentiation of the inhibitory system, may lead to alter drug sensitivity because of recycling dysfunction of inhibitory neurotransmitters such as GABA and Glycine.

4. It has been unclear whether other transporters than VGAT load the inhibitory neurotransmitters or another protein compensates the function of VGAT when VGAT expression is reduced.

5. This study is characteristic of using intra-venous administration of propofol, which is more difficult than intra-abdominal administration. Previous research was often performed by intro-abdominal administration.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　3　件）

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〔学会発表〕（計　4　件）

Komatsu S, Nishikawa K: The role of vesicular GABA transporter (VGAT) in hypnotic and analgesic actions of general anesthetics. 16th World Congress on Pain at Yokohama, PW-334, Sep 28, 2016

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小林 磨巧、佐藤 友彦、若原 志保、藤田 喜久、赤津 賢彦、西川 光一: [P19-02] エリスロポエチン産生子宮筋腫による多血症患者に対する周術期管理の一例 日本麻酔科学会　関東甲信越支部・第57回合同学術集会 2017、新宿

〔図書〕（計　0　件）

〔産業財産権〕

○出願状況（計　0　件）

名称：
発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

○取得状況（計　0　件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

６．研究組織

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