様式第１９号

学位論文審査の結果の要旨

審査区分 ㊟・論	第657号	氏名	王　普
審査委員会委員		主査氏名	高橋尚彦
		副査氏名	松本重清
		副査氏名	石崎敏理

論文題目

Disruption of asparagine-linked glycosylation to rescue and alter gating of the $Na_v1.5$-Na^+ channel
(N-型糖鎖変調は $Na_v1.5$ 心筋型 Na^+チャネルの発現を制御しゲーティング機構を調節する)

論文掲載雑誌名

Heart and Vessels

論文要旨

・The aim of this study was to investigate impact of N-linked glycosylation disruption on the Na^+ channel, and the mechanism by which glycosylation regulates the current density and gating properties of the Na^+ channel.

・The NaV1.5-Na^+ channel isoform (α subunit) derived from human was stably expressed in human embryonic kidney (HEK)-293 cells (NaV1.5-HEK cell). We applied the whole-cell patch-clamp technique to study the impact of N-linked glycosylation disruption on the Na^+ channel gating properties and metabolic pathways by use of NaV1.5-HEK cell.

・Tunicamycin, an inhibitor of N-acetylglucosamine phosphotransferase, was without effect on I_{Na} when applied for 5-10 min; the maximum inward current, the steady-state inactivation and the activation curves of I_{Na} were unchanged. However, when applied for 24 hours, tunicamycin caused a significant increase of I_{Na}.

・Tunicamycin shifted the steady-state inactivation curve to the hyperpolarization direction, whereas the activation curve was unaffected.

・The use-dependent block of I_{Na} was significantly larger in tunicamycin-treated NaV1.5-HEK cells, which was even larger when the holding potentials were more depolarized.

・Recovery from inactivation was prolonged, while the fast phase and the slow phase of the current decay was unaffected by tunicamycin.

・I_{Na} was unaffected by tunicamycin when NaV1.5-HEK cells were treated by a proteasome inhibitor MG132 [N-[(phenyl- methoxy)carbonyl]-L-leucyl-N-[(1S)-1-formyl-3-methylbutyl]-L-leucinamide], while I_{Na} was significantly increased by tunicamycin in the presence of a lysosome inhibitor butyl methacrylate (BMA).

・Hyperpolarization-directed shift of the steady-state inactivation curve by tunicamycin was also preserved in the presence of BMA.

・These findings suggest that N-glycosylation disruption rescues the NaV1.5 channel possibly through the alteration of ubiquitin-proteasome activity, and changes gating properties of the NaV1.5 channel by modulating glycan milieu of the channel protein.

本研究は，N-glycosylation 阻害が ubiquitin- proteasome activity 変化を介して Na^+電流を増大することを示した。学術的にも意義あるものと考えられ、審査委員の合議により本論文は学位論文に値するものと判定した。

様式第２０号

最終試験
の結果の要旨
~~学力の確認~~

審査区分 (課)・論	第657号	氏名	王　普
審査委員会委員		主査氏名	高橋尚彦
		副査氏名	松本重清
		副査氏名	加藤毅

学位申請者は本論文の公開発表を行い，各審査委員から研究の目的，方法，結果，考察について以下の質問を受けた。

１．あなたの講座はBr J Pharmacol 2009で，ベプリジルが治療域の濃度でカルモジュリン活性を阻害し，Naチャネルαサブユニットの分解を減少させ，その結果，Na電流を増加させたことを報告している。今回，ツニカマイシンのNa電流増加作用を報告したが，ツニカマイシンとベプリジルのNaチャネルの効果に対する類似点と相違点につき説明せよ。

２．あなたの講座はJournal of Physiological Science 2019で，ツニカマイシンがCaV3.1-T-type Ca^{2+} channel currentを減少させることを報告している。なぜツニカマイシンが，Na電流を増加させ，CaV3.1-T-typeCa電流を抑制するという逆の効果になるのか。

３．Fig. 1Fでinactivationカーブが過分極方向にシフトしているが，このシフトの生理的意義について説明せよ。

４．細胞の薬物処理（tunicamycin, MG132, BMA）を24時間で実施した根拠は？また、その処理により細胞毒性は発現していないのか。

５．TunicamycinはERストレスにも関与していることが知られているが、ERストレスはNav1.5チャネルの機能には影響を与えないのか。

６．薬物処理により、Nav1.5チャネルの細胞内、細胞表面上での発現量に変化はあるのか。それを実際確認したのか。

７．実験にはNav1.5安定発現株を用いてINaを測定しているが、これは発現させたNav1.5特異的なものか。その親株（HEK293細胞）ではこのようなことは観察されなかったのか。

８．糖鎖付加を阻害することは、様々な細胞機能を変化させることが予想されるが、そのような機序を有する糖鎖付加阻害薬であっても臨床上のベネフィットを与えるものになりうるのか。

９．N型糖鎖変調は多くの心疾患で治療ターゲットとなる可能性を指摘され、それを今後も目指していくということであるが、どうして心筋細胞を使用せずにHEK細胞を使用したのか。HEK細胞と心筋細胞のNaチャネルのゲーティング特性は同じであると考えていいのか。

１０．Resultsで糖鎖変調は蛋白合成を促進しないと述べているが、その根拠はあるのか。あるいは他の実験にてそれを確認されているか。

これらの質疑に対して，申請者は概ね適切に回答した。よって審査委員の合議の結果，申請者は学位取得有資格者と認定した。

（注）不要の文字は２本線で抹消すること。

様式第８号 No. 1

学　位　論　文　要　旨

氏名　王 普

論　文　題　目

Disruption of asparagine-linked glycosylation to rescue and alter gating of the $Na_v1.5$-Na^+ channel

（N-型糖鎖変調は $Na_v1.5$ 心筋型 Na^+チャネルの発現を制御しゲーティング機構を調節する）

要　　旨

Background and aim of the study: SCN5A gene encodes the voltage-gated sodium channel $Na_V1.5$ which is composed of a pore-forming α subunit of the channel. Asparagine (N)-linked glycosylation is one of the common post-translational modifications in proteins. The aim of this study was to investigate impact of N-linked glycosylation disruption on the Na^+ channel, and the mechanism by which glycosylation regulates the current density and gating properties of the Na^+ channel.

Materials and methods: The $Na_V1.5$-Na^+ channel isoform (α submit) derived from human was stably expressed in human embryonic kidney (HEK)-293 cells ($Na_V1.5$-HEK cell). We applied the whole-cell patch-clamp technique to study the impact of N-linked glycosylation disruption on the Na^+ channel gating properties and metabolic pathways by use of $Na_V1.5$-HEK cell.

Results: Tunicamycin, an inhibitor of N-acetylglucosamine phosphotransferase, was without effect on I_{Na} when applied for 5-10 min; the maximum inward current, the steady-state inactivation and the activation curves of I_{Na} were unchanged. However, when applied for 24 hours, tunicamycin caused a significant increase of I_{Na}. Tunicamycin shifted the steady-state inactivation curve to the hyperpolarization direction, whereas the activation curve was unaffected. The use-dependent block of I_{Na} was significantly larger in tunicamycin-treated $Na_V1.5$-HEK cells, which was even larger when the holding potentials were more depolarized. Recovery from inactivation was prolonged, while the fast phase and the slow phase of the current decay was unaffected by tunicamycin. I_{Na} was unaffected by tunicamycin when $Na_V1.5$-HEK cells were treated by a proteasome inhibitor MG132 [N-[(phenyl-methoxy)carbonyl]-L-leucy-N-[(1S)-1-formyl-3-methylbutyl]-L-leucinamide], while I_{Na} was significantly increased by tunicamycin in the presence of a lysosome inhibitor bafilomycinA1 (BMA). Hyperpolarization-directed shift of the steady-state inactivation curve by tunicamycin was also preserved in the presence of BMA.

Conclusions: N-linked glycosylation is a common post-translational modification of many ion channels and play an important role in the function and maturation. Thus, in general, disruption of glycosylation leads to a reduction of ionic flow through the channels or malfunction of the channel gating. However, these findings suggest that N-glycosylation disruption rescues the $Na_V1.5$ channel possibly through the alteration of ubiquitin-proteasome activity, and changes gating properties of the $Na_V1.5$ channel by modulating glycan milieu of the channel protein. Based on the fact that down-regulation of the Na^+ channel in cardiomyocytes aggravates many heart diseases, distinct alteration of cellular glycosylation process may act as a potential therapeutic benefit for the rescue of the voltage-gated Na^+ channel-related disorders of the heart.