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学位の種類	博士（医学）
学位記番号	博士第927号
学位授与の要件	学位規則第４条第１項
学位授与年月日	令和４年３月１０日
学位論文題目	Selective activation of adrenoceptors potentiates /Ks current in pulmonary vem cardiomyocytes through the protein kinase A and C signaling pathways （肺静脈心筋細胞の IKs チャネルに対するアドレナリン受容体の調節機構）
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別紙様式３（課程博士・論文博士共用）

論　文　内　容　要　旨

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学位論文題目	Selective activation of adrenoceptors potentiates I_{Ks} current in pulmonary-vein cardiomyocytes through the protein kinase A and C signaling pathways (肺静脈心筋細胞の I_{Ks} チャネルに対するアドレナリン受容体の調節機構)		

Background and purpose: Atrial fibrillation (AF) is the most common of all sustained cardiac arrhythmias in humans, manifesting more frequently with age and being typically caused by stroke, although the mechanisms underlying the initiation of AF are still not fully understood. A reduction in the pulmonary veins (PVs) focus through ablation is effective for treating acute and sustained AF, suggesting dynamic interaction between the left atrial (LA) and PVs ectopic activity.

The slow component of the delayed rectifier K^+ channel (I_{Ks}) has been shown to exist in the cardiac myocytes of various mammalian species. Our group previously reported that suppression of the I_{Ks} current by its selective blocker delays the repolarization process and markedly reduces the pacemaker activity in SA node cells of guinea pigs suggesting that I_{Ks} can contribute to the repolarizing process in SA node cells, which is important for determining the pacemaker activity. We hypothesized that I_{Ks} might be expressed in the pulmonary vein cardiomyocyte (PVC), and I_{Ks} might modify the property of PVC automaticity, especially under adrenoceptor-stimulated conditions. The objective of this study was to investigate the electrophysiological properties of I_{Ks} and compared the effects of β_1-, β_2- and α_1-adrenergic receptor stimulation on I_{Ks} in PVC.

Methods: Female Hartley guinea pigs (4-6 weeks old, 250-400 g) were used in the present experiments. PVC and left atrial myocytes were isolated using Langendorff perfusion method. The expression and localization of I_{Ks} channel in PVC was analyzed by western blotting and immunocytochemistry. Perforated and ruptured whole-cell patch-clamp techniques were used to record the action potentials and I_{Ks} currents from PVC in the current- and voltage-clamp modes, respectively, with an EPC-8 patch-clamp amplifier. The actions of β_1-, β_2- and α_1-adrenoceptor stimulation on I_{Ks} in PVC, involved in the cAMP-protein kinase A (PKA), the protein kinase C (PKC) signaling pathway were examined by SQ 22536 (AC inhibitor, 300 μM), H89 (PKA selective inhibitor, 25 μM) or BIS-I (selective PKC inhibitor, 0.5 μM). The functional role of I_{Ks} in PVC automaticity (action potentials) under basal and β-adrenergic stimulation conditions was testified by the I_{Ks} selective blocker HMR-1556.

Result: The present study examined the electrophysiological properties of I_{Ks} and its modulation by stimulating the β_1-, β_2- and α_1-adrenergic receptors in PVC. Our findings show that (1) the functional I_{Ks} channel (*KCNQ1*) was expressed and located on the cell membrane; (2) I_{Ks} amplitude was markedly enhanced with a negative shift in the voltage dependence of the channel activation by both β_1- and β_2-adrenoceptor stimulation mediated via the adenylyl

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。
２．※印の欄には記入しないこと。

cyclase (AC)-cAMP-PKA signaling pathway and also potentiated by α_1-adrenoceptor activation through the PKC signaling pathway; (3) separately from the β_2- and α_1-adrenergic pathways, norepinephrine more strongly potentiated the firing rate of action potentials in PVC through the β_1-adrenergic pathway, whereas it is noteworthy that the pharmacological activation of β_1- or β_2-adrenergic receptor by their selective agonists increased the firing rate to a similar degree; (4) the blocking potency of I_{Ks} inhibitor was higher under β-adrenoceptor-stimulated conditions than under basal conditions in the presence of the I_{Ks} selective inhibitor HMR-1556; and (5) the I_{Ks} current density was significantly higher in PVC than in left atrial myocytes.

Discussion: Our study clearly demonstrated that I_{Ks} protein was expressed and located on the cell membrane, and the electrophysiological experiments showed that the current kinetics of I_{Ks} in PVC were similar to those recorded from cardiac myocytes, although the current density was higher in PVC than left atrial myocytes.
Moreover, the present study is the first to demonstrate that the stimulatory action of both β_1- and β_2-adrenoceptors on I_{Ks} in guinea pig PVC was mediated by the AC-cAMP-PKA signaling pathway. The I_{Ks} current amplitude in PVC is more effectively potentiated by β_2-adrenoceptor stimulation than by β_1-adrenoceptor stimulation. Under heart failure conditions, the expression of β_1-adrenoceptor is down-regulated, whereas that of β_2-adrenoceptor is preserved. In addition, Selective blockade of β_1-adrenoceptor potentiates a positive inotropic effect to catecholamine mediated via the β_2-adrenoceptor in human atrial myocytes. Furthermore, the dominant subtype of β-adrenoceptors in the lungs is β_2-adrenoceptor, and the distribution between β_1- and β_2-adrenoceptor was shown to be around 22:78 in guinea pigs and 30:70 in humans, respectively. These previous reports may explain the stronger potentiation of I_{Ks} in PVC induced by β_2-adrenoceptor activation. More importantly, blockade of I_{Ks} is more effective after β-adrenoceptors stimulation than in control settings, indicating the critical modulation of I_{Ks} in PVC automaticity when sympathetic tone is promoted.

Conclusion: The present study showed that an elevated sympathetic tone is accompanied by an enhancement of voltage-dependent outward K^+ current through I_{Ks} in guinea pig PVC. Taken together with previous observations, our results form an important electrophysiological basis for insight into the regulatory mechanisms for ion channels in PVC under physiological conditions and during sympathetic stimulation.

別紙様式９（課程博士・論文博士共用）

学位論文審査の結果の要旨

整理番号	935	氏　名	MI XINYA
論文審査委員			

（学位論文審査の結果の要旨）※明朝体１１ポイント、６００字以内で作成のこと

本論文では、肺静脈内の心筋細胞（pulmonary vein cardiomyocyte: PVC）に着目し、その緩徐活性化遅延整流 K^+（I_{Ks}）チャネルの電気的特性、特にβ1、β2、α1アドレナリン受容体刺激が I_{Ks} 電流に及ぼす影響について、パッチクランプ法などを用いて検討を行い、以下の点を明らかにした。

1）PVC の細胞膜上に機能的 I_{Ks} チャネル（*KCNQ1*）が発現している。
2）β1、β2 刺激はアデニルシクラーゼ-cAMP-PKA 経路を介して I_{Ks} 電流を増強する。
3）α1 刺激は PKC 経路を介して、I_{Ks} 電流を増強する。
4）I_{Ks} チャネル阻害薬（HMR-1556）は、PVC の自動能（活動電位発生頻度）を抑制するが、その抑制効果はβ刺激下において、より増強される。

本論文は、PVC に発現する I_{Ks} チャネルの電気的特性について新たな知見を与えたものであり、また最終試験として論文内容に関連した試問を実施したところ合格と判断されたので、博士（医学）の学位論文に値するものと認められた。

（総字数 448 字）

（令和　4年　1月　25 日）