氏　　名	謝　宇
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学位論文題目	Ca2+/calmodulin potentiates IKs in sinoatrial node cells by activating Ca2+/calmodulin-dependent protein kinase II. (Ca2+/カルモジュリンによるモルモット洞房結節細胞の IKs の調節)
審査委員	主査　教授　前川　聡
	副査　教授　遠山　育夫
	副査　教授　小島　秀人

別紙様式３

論 文 内 容 要 旨

※整理番号	732	氏名（ふりがな）	謝 宇（シャ ウ）
学位論文題目	Ca^{2+}/calmodulin potentiates I_{Ks} in sinoatrial node cells by activating Ca^{2+}/calmodulin-dependent protein kinase II (Ca^{2+}/カルモジュリンによるモルモット洞房結節細胞の I_{Ks} の調節)		

Aim of the study:

Sinoatrial (SA) node cells have automaticity and function as physiological pacemakers of the mammalian heart, which is generated and maintained by multiple ionic currents. The slow component of the delayed rectifier K^+current (I_{Ks}) is a major outward current, responsible for the repolarization of pacemaker action potential and also play a crucial role in determining the automaticity. Intracellular Ca^{2+}, a fundamental modulator of electrical activities in cardiac myocytes, interacts with calmodulin (CaM) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) to regulate various cardiac ion channels. In the guinea pig ventricular cells, I_{Ks} has been shown to be a Ca^{2+}-sensitive current, but there are conflicting data on the Ca^{2+} or Ca^{2+}/CaM sensitivity of the KCNQ1 or KCNQ1/KCNE1 current in different cell lines (KCNQ1 gene encodes the pore- forming α subunit, which associates KCNE1 to produce I_{Ks} the channel). The present study was designed to examine the possible regulation of I_{Ks} by Ca^{2+} or Ca^{2+}/CaM and clarify the involvement of CaMKII-dependent mechanism in this process.

Methods:

1. SA node cell preparation: Single SA node cells were obtained from the heart of adult Hartley guinea pigs using an enzymatic dissociation procedure. The isolated cells were stored at 4 °C in the high-K^+, low-Cl^- Kraftbrühe (KB) solution for later use within 8 h.

2. Whole-cell patch-clamp recording: I_{Ks} was elicited by depolarizing test pulses given from a holding potential of −50 mV (Na^+ current was then inactivated) to various levels, and $I_{Ca,L}$ and I_{Kr} were blocked by nisoldipine (0.4 μM) and E-4031 (5 μM), respectively. SA node cells were constantly perfused with extracellular solution at 36 ± 1 °C. Data for voltage dependence of I_{Ks} activation was fitted to a Boltzmann equation: $I_{Ks,\,tail} = I_{Ks,\,tail,\,max}/(1+\exp((V_{1/2} - V_m)/k))$, where $I_{Ks,\,tail,\,max}$ is the fitted maximal tail current amplitude, $V_{1/2}$ is the half-maximal voltage, V_m is the test potential, and k is the slope factor. The deactivation kinetics was determined by a two exponential fit of I_{Ks} tail current trace.

3. Action potential recording: Spontaneous action potentials were recorded in normal Tyrode solution with amphotericin B (100 μg/ml) added in the pipette solution, and the measurements were started 5-10 min after seal formation.

4. Solutions and chemicals: To examine the sensitivity of I_{Ks} to Ca^{2+}, the pipette solution containing lower Ca^{2+} concentration (pCa 10, Ca(-)) was compared with control (pCa 7, Ca(+)), as well as with BAPTA-AM, a membrane-permeable calcium fast chelator. The inhibitors, AIP, KN-93 together with its analog KN-92 were used to test the involvement of CaMKII in Ca^{2+}/CaM potentiated I_{Ks}. A specific I_{Ks} inhibitor, HMR1556, was applied to clarify role of I_{Ks} in pacemaker activity.

（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で印字すること。

２．※印の欄には記入しないこと。

Results:

1. I_{Ks} in SA node cells is sensitive to intracellular Ca^{2+}: By the different dialysis of the cell interior, Ca (-) pipette solution typically reached the maximum reduction ~5 min after rupture of the patch membrane. However, in a separate control set, the normalized tail current with Ca (+) solution (1.01 ± 0.025, n = 25) was significantly larger than in cells dialyzed with Ca (−) solution (0.68 ± 0.045, n = 19). The current densities were 3.67 ± 0.39 and 7.42 ± 0.76 pA/pF in cells dialyzed with Ca (−) and Ca (+) pipette solutions, respectively. Moreover, under Ca (+) conditions, a reduction in I_{Ks} was detected immediately after extracellular perfusion with 10 μM BAPTA-AM. These results suggest that cytosolic Ca^{2+} is critical for maintaining the basal activity of I_{Ks} in native SA node cells.

2. CaM increases I_{Ks} in a Ca^{2+}-dependent manner: Since CaM is a major mediator in Ca^{2+}-dependent modulation of ion channels we investigated the possible involvement of CaM modulation in the Ca^{2+} sensitivity of I_{Ks}. Dialysis of CaM (400 nM) with Ca (+) pipette solution significantly increased I_{Ks} (~ 42 % increase) compared with the initial value obtained shortly (≤ ~ 40 s) after intracellular perfusion and induced a significant left shift in the voltage dependence of channel activation. In contrast, CaM could not potentiate I_{Ks} in cells dialyzed with Ca (-) pipette solution, suggesting the stimulatory action of CaM on I_{Ks} is Ca^{2+} dependent.

3. Involvement of CaMKII signaling in Ca^{2+}/CaM-induced I_{Ks} activation: Next, we examined the role of CaMKII in the regulation of the Ca^{2+}/CaM-induced I_{Ks} increase using CaMKII inhibitors KN- 93 and AIP. The I_{Ks} was only markedly reduced by 60.0 ± 3.7 % after treatment with KN-93 but not with its analog KN-92. However, KN-93 resulted in weaker I_{Ks} inhibition (20.5 ± 4.7 %) with Ca (−) solution, suggesting that the CaMKII action on I_{Ks} potentiation is also Ca^{2+} dependent. KN-93 performed a same result in the voltage-clamp protocol simulating spontaneous action potentials. Similar results were obtained in dialysis with AIP. Furthermore, when pretreated with KN-93 before rupture patch membrane, dialysis CaM with Ca (+) solution could not increase I_{Ks}, indicating that the increase of I_{Ks} elicited by Ca^{2+}/CaM is mediated via the CaMKII signaling pathway. The conclusion was strengthened by the observation of a significant increase in I_{Ks} after direct dialysis of CaMKII delta.

4. Effect of CaMKII on spontaneous action potentials in guinea pig SA node cells: Finally, we examined the effects of CaMKII on spontaneous action potentials. Extracellular application of KN-93 gradually slowed the repolarizing phase of the action potential and spontaneous excitation was almost abolished by the exposure to KN-93 for ~ 2 min. Also, AIP (permeable type) almost completely arrested the pacemaker activity. Collectively, these data suggest that the basal CaMKII pathway plays an essential role in modulation of pacemaker activity in guinea pig SA node cells. A specific I_{Ks} inhibitor, HMR1556, produced depolarization of the MDP and reduction in firing rate, suggesting tthat he function of I_{Ks} is critically involved in regulation of spontaneous excitation.

Discussion

In the present study, we identified that intracellular Ca^{2+} regulated I_{Ks} and blocked the phenomenon of I_{Ks} decline. Exogenously applied CaM potentiated I_{Ks} in a Ca^{2+}- dependent manner. CaM also changed the channel gating by producing a significant left shift in the voltage dependence of channel activation, which may due to the binding between CaM and KCNQ1 according to recent reports. Most importantly, the inhibitors of CaMKII abolished Ca^{2+}/CaM-induced I_{Ks} increase, suggesting the involvement of CaMKII, which may directly phosphorylate I_{Ks} channel and mediate the Ca^{2+}/CaM-induced enhancement of I_{Ks}.

Conclusion

Ca^{2+}/CaM modulates I_{Ks} in SA node cells of guinea pig through activation of the CaMKII signaling pathway.

別紙様式８（課程・論文博士共用）

学位論文審査の結果の要旨

整理番号	７３２	氏　名	謝　宇
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。）

心筋ペースメーカーや心室筋において遅延整流性カリウム電流の Slow Component (Iks)は、活動電位の再分極において重要な役割を果たし、種々の情報伝達経路によって制御されている。本研究は、モルモットの洞房結節細胞において Whole cell patch clamp 法を用いて、Ca^{2+}/ calmodulin (CaM)の関与について検討を行い、以下の点を明らかにした。

１）Iks の電流密度は、細胞内の低 Ca 濃度に比し、高 Ca 濃度状態で増大した。
２）細胞内 CaM (400nM) 投与は、ガラス電極内液 Ca 存在時に著明に増加した。
３）特異的 CaMKII 阻害剤である autocamtide- 2 inhibitory peptide (500 nM)および KN-93(1 μM)は、高濃度 Ca 存在下で Iks 活性を抑制した。
４）KN-93 の前処理により CaM による Iks 活性は消失した。

以上の結果より、モルモットの洞房結節細胞において、Ca^{2+}/CaM は CaMKII を介して Iks を活性化し、CaMKII の増強効果が生理的あるいは病態生理的な状態における洞房結節の自律性の調節に関与している可能性が示唆された。

本論文は、遅延整流性カリウム電流の Iks の Ca^{2+}/ CaM による制御について新しい知見を与えたものであり、最終試験として論文内容に関連した試問を受け合格したので、博士（医学）の学位論文に値するものと認められた。

（総字数 598 字）

（平成 27 年　1 月 27 日　）