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学位論文題目	Putative binding sites for arachidonic acid on the human cardiac Kv1.5 channel （ヒト心筋 Kv1.5 カリウムチャネルにおけるアラキドン酸の作用部位）
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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	Putative binding sites for arachidonic acid on a human cardiac Kv1.5 channel （ヒト心筋 Kv1.5 カリウムチャネルにおけるアラキドン酸の作用部位）		

Backgroud:Arachidonic acid (AA) is a cis-polyunsaturated fatty acid ubiquitously present in the plasma membrane. In response to extracellular signals, AA can be released from membrane phospholipids via G-protein- or Ca^{2+}-mediated activation of phospholipase. Some previous results suggest that AA is known to modify ion channel activities in various cell types including cardiac myocytes.

In the human atrium, the molecular component, which underlies the ultra-rapid delayed rectifier potassium current (I_{Kur}), is the human Kv1.5 channel (hKv1.5). The channel plays an important role in the repolarization of atrial action potential, but not ventricle. Previous studies have shown that several residues located near the outer pore region, the base of selectivity filter and the transmembrane segments (S6 domain) of hKv1.5 could provide crucial binding sites for pharmacological inhibitors.

Objective:An early study has described that relatively high concentration of AA (30 μM) resulted in a marked current inhibition of the mouse Kv1.5 (mKv1.5) and concluded a probable direct action of AA on mKv1.5 channel without involvement of AA metabolites. However, the molecular mechanism underlying this channel inhibition has not been fully delineated. Therefore, my study was designed to investigate the modulation of hKv1.5 channel by AA under the different conditions and determined the possible binding sites of AA.

Materials and Methods

Site-directed mutagenesis and transfection

Full-length complementary DNA (cDNA) of hKv1.5 channel was subcloned into the mammalian expression vector pcDNA3.

Site-directed mutagenesis was applied to introduce eleven single point mutations into hKv1.5 cDNA by using Quikchange-II-XL Kit .WT and mutant cDNA were transiently transfected into CHO cells together with GFP using Lipofectamine,

（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。

Patch-clamp recordings

After transfection for 48 h incubation at 37 °C, the GFP-positive cell was used for the patch-clamp study. Whole-cell membrane currents or macroscopic currents were recorded with an EPC-8 patch-clamp amplifier (HEKA). The hKv1.5 whole-cell membrane currents were elicited by applying 300-ms depolarizing steps from a holding potential of −80 mV to various voltage steps.

Data analysis

All data were expressed as mean ± S.E.M. Statistical comparisons between two groups were analyzed using Student's *t*-test. Comparisons among multiple groups were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett' post hoc test and a p-value <0.05 was considered significant.

Results

hKv1.5 current was minimally affected at the onset of depolarization but was progressively reduced during depolarization by the presence of AA, suggesting that AA acts as an open channel blocker (IC_{50} of 6.1±0.6 μM; n=10). AA itself affected the channel at the extracellular sites independently of its metabolic and signalling pathways. The blocking effect of AA was attenuated at pH 8.0 but not at pH 6.4. The blocking action of AA developed rather rapidly by coexpression of Kvβ1.3. The degree of current block by AA was significantly attenuated in H463C, T480A, R487V, I502A, I508A, V512A and V516A, but not in H463C, A501V and L510A mutants of hKv1.5 channel. Docking simulation predicted that H463, T480, R487, I508, V512 and V516 are potentially accessible for interaction with AA.

Discussion

AA acts on hKv1.5 as an open channel blocker, which is supported by the following AA minimally affects the initial current levels at the onset of depolarization but progressively reduced the current during the depolarizing steps

The present study also found that while T480A decreases the sensitivity to AA inhibition, mutation of its neighbor amino acid T479 enhances AA inhibition (Figure 6). Although the precise mechanism remains to be fully understood, there might be some positive steric effects of T479A mutation on the neighboring T480 or on other potent AA binding sites.

Previous studies have shown that I502, I508 V512 and V516 are important for mediating the blocking action of various drugs on hKv1.5,.Similarly,R487 and H463 are also important for the blocking actions of protons,drugs.Based on the crystal structure of Kv1.2, T480, I508, V512 and V516 are predicted to face toward the inner cavity of the channel

.whereas I502 is positioned away from the central cavity of the pore and forms a hydrophobic interface with the adjacent S5 domain.The present docking simulation study predicted that H463, T480, R487, I508, V512 and V516, but not I502, interact with AA.The mutation of I502 affects the blocking actions of compounds through conformational changes of the pore.hydrophobic moieties of the compound protrude into hydrophobic subunit interfaces and thereby approach the I502 residue.

About the effect of PH on Kv1.5.It is generally accepted that the environmental pH affects the degree of hydrophobicity in AA. In addition, the activation of hKv1.5 channels is sensitive to changes in external pH.These pH-dependent changes in properties of both AA and hKv1.5 might underlie the attenuation of the inhibition of hKv1.5 by AA in an alkaline pH of 8.0.

It is probable that AA-induced inhibition of hKv1.5 could prolong APD and ERP in human atrium and thereby mediate the anti-arrhythmic effect of AA. However, APD critically depends on a delicate balance between several inward and outward currents, and AA has various effects on other ionic currents that regulate APD in the heart,AA therefore could produce complicated effects on overall atrial excitability

Conclusion, the present study provides experimental evidence to support that several amino acids in the pore domain (H463, T480, R487, I502, I508, V512 and V516) confer the AA sensitivity of hKv1.5. This information may help investigate the molecular determinants for the action of other polyunsaturated fatty acids on hKv1.5 and provide new direction for future development of selective blockers of hKv1.5.

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

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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。）

心筋細胞のアラキドン酸は、イオンチャネルの活性制御に関わっているとの報告があるものの、不明な点も多く残されている。本研究では、アラキドン酸とヒト心房筋に発現するカリウムチャネルKv1.5との関連について検討を行い、以下の点を明らかにした。

１）ヒトKv1.5をCHO細胞に発現させ、パッチクランプ法で膜電流を測定したところ、アラキドン酸によりKv1.5の脱分極が抑制されたことから、アラキドン酸はこのチャネルの開口を阻害する作用があった。
２）アラキドン酸の代謝物ではなく、アラキドン酸自身がKv1.5の細胞外部位に影響を及ぼしていた。
３）アラキドン酸のKv1.5に対する阻害効果は細胞外液がアルカリ性では抑制された。
４）アラキドン酸のKv1.5に対する阻害効果は、Kv1.5のH463C、T480A、R487V、I502A、I508A、V512AおよびV516A変異体で有意に減弱した。
５）コンピュータシミュレーションにより、Kv1.5のH463、T480、R487、I508、V512およびV516が、アラキドン酸の結合可能部位として示された。

本論文は、ヒト心筋カリウムチャネルKv1.5の活性制御機構にお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580字）

（平成２８年　８月２９日）