氏　　名（本　籍）	Dimitar Petrov Zankov　（ブルガリア）
学 位 の 種 類	博　士　（医　学）
学 位 記 番 号	博　士　第５３４号
学位授与の要件	学位規則第４条第１項該当
学位授与年月日	平成１９年　3月２６日
学 位 論 文 題 目	Angiotensin II Potentiates the Slow Component of Delayed Rectifier K^+ Current via the AT_1 Receptor in Guinea Pig Atrial Myocytes (アンギオテンシンⅡは単離モルモット心房筋細胞においてAT1受容体刺激を介して緩徐活性型遅延整流性K＋電流を増大させる)
審　査　委　員	主査　教授　　岡　村　　富　夫
	副査　教授　　三　ツ　浪　　健　一
	副査　教授　　柏　木　　厚　典

別紙様式３

論　文　内　容　要　旨

※整理番号	539	（ふりがな） 氏　　名	ディミター ペトロフ ザンコフ Dimitar Petrov Zankov
学位論文題目	Angiotensin II Potentiates the Slow Component of Delayed Rectifier K^+ Current via the AT_1 Receptor in Guinea Pig Atrial Myocytes Zankov D.P., Omatsu-Kanbe M., Isono T., Toyoda F., Ding W., Matsuura H., Horie M. 【　アンギオテンシンIIは単離モルモット心房筋細胞においてAT_1受容体刺激を介して緩徐活性型遅延整流性K^+電流を増大させる　】		

Background The renin-angiotensin system (RAS) maintains cardiovascular homeostasis in physiological and diseased states like hypertension or heart failure. Octapeptide angiotensin II (Ang II) is the principle molecule mediating the regulatory effects of RAS on the cardiac and vascular morphology and function through the specific membrane receptors, namely Ang II type 1 (AT_1) and type 2 (AT_2) receptors. There are increasing number of reports demonstrating that RAS and Ang II also affect cardiac electrical activity. However, little information is available regarding immediate electrophysiological effects of Ang II on repolarizing K^+ currents in the heart atrium.

Methods Cardiac atrial myocytes from adult Hartely guinea pig were dissociated using Langendorff's perfusion method and collagenase, dissolved in Ca^{++}-free normal Tyrode's solution.

The cells were current- and voltage-clamped by the means of the standard whole-cell patch-clamp method. Slowly activating component of the delayed rectifier K^+ current (I_{Ks}) was elicited by depolarizing voltage steps from holding potential -50 mV to various test potentials under the condition of inactivation of Na^+ current by the holding potential and pharmacological blockade of $I_{Ca,L}$ and I_{Kr}. Action potentials were recorded at rate of 0.2 Hz after supratreshold current pulses of 2- to 3-ms duration applied via the glass electrode in the current clamp mode.

Results Bath application of Ang II increased the amplitude of I_{Ks} (measurements were on the tail current magnitude) with EC_{50} equal to 6.16 nmol/L. Even more effective was

（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。

Sar[1]-Ang II – a stable Ang II analogue with higher affinity to AT_1 receptor. The maximal effect was 60.8±6.8% and 100.7±16.4% for Ang II and Sar[1]-Ang II, respectively. The voltage dependence of I_{Ks} activation (assessed by comparison of the values of membrane potential at which the current was activated 50%) and kinetics of deactivation (evaluated by fitting the tail current decay to double exponential function) were not significantly affected by these AT_1 receptor agonists.

I_{Ks} enhancement was blocked by the specific AT_1 receptor antagonist valsartan (1 μmol/L) and was markedly attenuated by inclusion of nonhydrolizable GDP analogue GDPβS (2 mmol/L) in the internal solution, indicating an involvement of G proteins coupled to AT_1 receptor. The stimulatory effect was also significantly reduced by phospholipase C inhibitor compound 48/80 (100 μmol/L) and the protein kinase C inhibitors bisindolylmaleimid I (200 nmol/L) and H-7 (10 μmol/L), suggesting that AT_1 receptors act through phospholipase C – protein kinase C signaling cascade to potentiate I_{Ks}.

Pretreatment with protein kinase C activators phorbol 12-myristate 13-acetate (PMA, 300 nmol/L) or 1-oleoyl-2-acetyl-*sn*-glycerol (OAG, 20 μmol/L) almost completely abolished the effect of the following AT_1 receptor stimulation thus excluding another intracellular pathway responsible for the I_{Ks} modulation.

Sar[1]-Ang II markedly shortened action potential duration at 90% of repolarization (APD_{90}) from 113.1±8.8 to 63.1±5.8 ms (n = 10). Subsequent extracellular administration of 1 μmol/L valsartan partially recovered the APD_{90} to 88.1±7.0 ms. The resting membrane potential and action potential amplitude remained unchanged during exposure to Sar[1]-Ang II.

Conclusions The slow component of the delayed rectifier K+ current of guinea pig atrium was stimulated by activation of AT_1 receptor coupled to G protein – phospholipase C – protein kinase C intracellular pathway.

The potentiation of I_{Ks} via AT_1 receptor in atrial myocytes, accompanied by marked shortening of APD, might involve RAS in the pathogenesis of atrial fibrillation (AF) under pathophysiological conditions associated with elevated levels of Ang II.

学位論文審査の結果の要旨

整理番号	５３９	氏名	Dimitar Petrov Zankov

（学位論文審査の結果の要旨）

本研究は、循環調節に重要な役割を果たしているアンジオテンシン（Ang）IIの心筋作用を電気生理学的な観点から検討したものである。

全細胞パッチクランプ法を用いて、モルモット単離心房筋細胞の緩徐活性型遅延整流性K^+電流（I_{Ks}）を選択的に記録すると、Ang II は同電流を増大した。同増大作用は、選択的 AT_1 受容体拮抗薬、GDPβS、ホスホリパーゼC阻害薬、プロテインキナーゼC阻害薬で抑制されたが、カルシウムキレーターならびにプロテインキナーゼA阻害薬では抑制されなかった。さらに、AT_1 受容体刺激により活動電位持続時間（APD）が短縮した。他方、心室筋細胞ではこれらAng II による作用は観察されなかった。

本論文は、Ang II による心房筋 I_{Ks} 電流増大作用とその細胞内情報伝達機構を明らかにすると共に、APD 短縮による心房細動の発症にレニンーアンジオテンシン系亢進の関与を示唆したもので、博士（医学）の学位を授与するに値すると評価された。

（平成　19年　2月　1日）