

氏 名 (本 籍)	Dimitar Petrov Zankov (ブルガリア)
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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 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)
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

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	<p>Angiotensin II Potentiates the Slow Component of Delayed Rectifier K⁺ Current via the AT₁ Receptor in Guinea Pig Atrial Myocytes Zankov D.P., Omatsu-Kanbe M., Isono T., Toyoda F., Ding W., Matsuura H., Horie M.</p> <p>【 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 】</p>		
<p>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 (AT₁) and type 2 (AT₂) 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.</p> <p>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.</p> <p>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>I_{Ks}</i>) 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>I_{Ca,L}</i> and <i>I_{Kr}</i>. Action potentials were recorded at rate of 0.2 Hz after suprathreshold current pulses of 2- to 3-ms duration applied via the glass electrode in the current clamp mode.</p> <p>Results Bath application of Ang II increased the amplitude of <i>I_{Ks}</i> (measurements were on the tail current magnitude) with EC₅₀ equal to 6.16 nmol/L. Even more effective was</p>			

- (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。

Sar¹-Ang II – a stable Ang II analogue with higher affinity to AT₁ receptor. The maximal effect was 60.8±6.8% and 100.7±16.4% for Ang II and Sar¹-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 AT₁ receptor agonists.

*I*_{Ks} enhancement was blocked by the specific AT₁ receptor antagonist valsartan (1 μmol/L) and was markedly attenuated by inclusion of nonhydrolyzable GDP analogue GDPβS (2 mmol/L) in the internal solution, indicating an involvement of G proteins coupled to AT₁ 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 AT₁ 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 AT₁ receptor stimulation thus excluding another intracellular pathway responsible for the *I*_{Ks} modulation.

Sar¹-Ang II markedly shortened action potential duration at 90% of repolarization (APD₉₀) from 113.1±8.8 to 63.1±5.8 ms (*n* = 10). Subsequent extracellular administration of 1 μmol/L valsartan partially recovered the APD₉₀ to 88.1±7.0 ms. The resting membrane potential and action potential amplitude remained unchanged during exposure to Sar¹-Ang II.

Conclusions The slow component of the delayed rectifier K⁺ current of guinea pig atrium was stimulated by activation of AT₁ receptor coupled to G protein – phospholipase C – protein kinase C intracellular pathway.

The potentiation of *I*_{Ks} via AT₁ 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
<p>(学位論文審査の結果の要旨)</p> <p>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。</p> <p>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、GDP β S、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。</p> <p>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-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。</p>			
(平成 19 年 2 月 1 日)			