学 位 論 文 の 要 旨

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学 位 論 文 名 Effect of *p22phox* Depletion on Sympathetic Regulation of Blood Pressure in SHRSP: Evaluation in a New Congenic Strain

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論 文 内 容 の 要 旨

INTRODUCTION

Among diverse effects of reactive oxygen species (ROS) in the pathogenesis of hypertension, the regulatory role in the sympathetic nervous system attracted our attention since the sympathetic nervous system plays an important role in the pathogenesis of hypertension. Several studies indicated that an increased ROS level in the rostral ventrolateral medulla (RVLM), one of the most important regulatory centers of the sympathetic nerve activity, was observed in various hypertensive models including the spontaneously hypertensive rat (SHR) and the stroke-prone SHR (SHRSP). Among pathways producing ROS, NADPH oxidases (NOX) are known to play an important role in various pathophysiological processes including hypertension. In this study, we therefore hypothesized that NOX were responsible for the high level of ROS in RVLM in SHRSP, which might causally relate to the exaggerated sympathetic responsiveness in this model rat. To examine this hypothesis, we introduced a P22PHOX-depleted congenic SHRSP (called as SP.MES), that was established to harbor the null mutation in the *P22phox* gene of the Matsumoto Eosinophilic Shinshu rat (MES). P22PHOX is a membrane-bound subunit that is essential for the NOX activity, and therefore it was expected that the most of the NOX activity was depleted in SP.MES. Thus, we showed that the response to glutamate (Glu) injection into RVLM differed significantly between SP.MES and SHRSP, suggesting the key role of NOX in sympathetic regulation of blood pressure (BP) in SHRSP.

MATERIALS AND METHODS

Male rats at 11-12 weeks of age were used in the experiments. All the rats were fed the stroke-permissive diet (Funabashi Farm Co. Ltd, Chiba, Japan) and water *ad libitum*. All experimental protocols were approved by the local committee of animal research in Shimane University. The null mutation of *P22phox* in the MES rat was introduced in SHRSP by the speed congenial method. An established congenial strain, SP.MES, harbored a 1.4-Mbp chromosomal fragment of the MES rat including *P22phox* on the genetic background of SHRSP. In the RVLM, microinjection of Glu and other substances (tempol, losartan, apocynin, DETC etc.) were performed using a stereotaxic method, and change in BP was monitored with an intraarterial cannulated probe. Haematoxylin & Eosin (HE) staining was performed to locate the microinjection point labelled with India ink. ROS in the brainstem was measured with the lucigenin method and DHE staining. BP changes under cold stress at 4°C was monitored with the telemetry system. Norepinephrine was also measured in urine samples collected at 4 °C for 6 hours. Statistical analyses were performed either using the Bonferroni's post-hoc test, the Dunnett's post-hoc test or the Student's t test when they were appropriate. $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSIONS

Microinjection of Glu into RVLM elicited a greater increase of BP and heart rate (HR) in SHRSP when compared with SHR and WKY. Losartan, a blocker of the angiotensin II (Ang II) receptor type 1 (AT1R), as well as tempol, a ROS scavenger, reduced the exaggerated response in SHRSP, whereas little effects were observed in SHR and WKY. These results implied that the exaggerated response to Glu observed in SHRSP was due to activation of ROS production, which might be regulated by AT1R. Glu microinjection elicited a greater increase of BP and HR in SHRSP when compared with SP.MES. Losartan, tempol and apocynin (an inhibitor for NOXs) significantly reduced the response in SHRSP to the level of that in SP.MES. ROS production in the brainstem, quantified by the lucigenin method and DHE staining, were greater in SHRSP than in SHR, WKY and SP.MES. The response to cold stress was examined in SP.MES to evaluate a role of NOX in the stress response. Under the cold stress, increase in SBP and in urinary norepinephrine was significantly smaller in SP.MES than in SHRSP. These observations indicated that BP increase and sympathetic activation under cold stress was

attenuated in SP.MES. In addition, infusion of losartan into the lateral ventricle decreased the response to cold stress in SHRSP, suggesting an important role of Ang II in the stress response. Using the microinjection technique targeting RVLM, this study showed that response to Glu was greater in SHRSP than in WKY and SHR. As the difference was abolished with losartan or tempol, AT1R and ROS production seemed involved in the exaggerated response in SHRSP. Further, the evaluation in the P22PHOX-depleted SP.MES implied that exaggerated NOX activity was responsible for the enhanced response in SHRSP. Telemetry experiments also suggested that the NOX system as well as AT1R activation in the brain was likely to contribute to an exaggerated sympathetic response to cold stress in SHRSP.

Here, SP.MES provided a new insight into a putative pathophysiological role of NOX in the exaggerated sympathetic response observed in SHRSP. P22PHOX is a subunit essential for the activity of the NOX complex; among four major subtypes of NOX expressed in the cardiovascular system, three (i.e., NOX1, 2 and 4) are known to require P22PHOX for their activity. Accordingly, SP.MES was expected to have a low NOX activity, which was indeed shown in the lucigenin experiment and DHE staining. As shown above, comparison between SHRSP and SP.MES provided new evidence supporting the important role of the NOX system in the pathogenesis of hypertension in SHRSP, which might apply to human hypertension. Further studies are warranted in humans to clarify the pathological roles of NOX in essential hypertension.

CONCLUSION

In conclusion, we showed that the response to Glu microinjection into RVLM was significantly greater in SHRSP than in SHR and WKY, which seemed to depend on a higher level of ROS in this strain. The observation in SP.MES suggested that the exaggerated response to Glu as well as high ROS level in SHRSP was due to P22PHOX-dependent NOX activity. As SP.MES is equivalent to SHRSP except lack of functional P22PHOX, this congenic strain is a useful model to study roles of the NOX system in hypertension and hypertensive organ damages when used in combination with SHRSP.

論文審査及び最終試験又は学力の確認の結果の要旨

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論文審査の結果の要旨

血圧調節において交感神経系が大きな役割を担っていることが知られている。本態性高血圧症のモデル動物であるstroke-prone spontaneously hypertensive rat (SHRSP)では、従来、交感神経活性の亢進があることが報告されており、その原因の一部に脳内での酸化ストレス亢進が示唆されている。申請者は、交感神経活性の調節に重要な吻側延髄腹外側野（RVLM）での酸化ストレスが高血圧発症に関与するかどうかを検討することを目的に、酸化ストレスを生成する酵素であるNADPH oxidaseの活性を失ったSHRSPを作成し、そのRVLMに注入した各種物質に対する反応を検討した。NADPH oxidaseの重要なコンポーネントである*p22phox*を欠損したSHRSP（以下SP.MESと呼ぶ）のRVLMに興奮性神経伝達物質であるglutamateを注入したときの血圧上昇はSHRSPに注入したときより有意に小さく、また、脳幹で測定した酸化ストレスのレベルはSP.MESでSHRSPより有意に低かった。更に、SHRSPにおける過剰な血圧上昇は、酸化ストレスのスカベンジャーやNADPH oxidase阻害剤にて抑制された。また、angiotensin II receptor type 1 (AT1R)阻害薬でもSHRSPの過剰な反応を抑えることができた。これらの検討から、SHRSPではRVLMにおいて、AT1RやNADPH oxidaseの関与による酸化ストレスの亢進がみられ、これが交感神経の過剰な反応に繋がっていることが示唆された。さらに、SHRSPでは寒冷ストレス下での血圧上昇がSP.MESに比べて高く、SHRSPでの血圧上昇がAT1R阻害薬にて抑制出来ることから、AT1Rと NADPH oxidase活性化による酸化ストレス亢進がSHRSPのストレス反応性亢進に関連していることが示唆された。本研究は交感神経系中枢における酸化ストレス亢進が高血圧発症に重要な役割を果たしていることを示唆する学術的価値の高い知見であると考えられた。

最終試験又は学力の確認の結果の要旨

申請者は、交感神経系中枢における酸化ストレス亢進が高血圧発症に重要な役割を果たしていることを示した。周辺の知識も豊富で学位授与に値すると判断した。（主査：田邊一明）

申請者は、重症な高血圧モデルラットを用い、AT1Rを介した一連の系による中枢性の交感神経系の過剰応答が高血圧発症の一要因である可能性を明らかにした。これは今後の高血圧治療の開発にも繋がる重要な知見である。公開審査でのプレゼンテーションや質疑応答も適切で関連知識も十分であり、学位授与に値すると判断した。（副査：紫藤　治）

申請者は、本態性高血圧症遺伝的モデル由来のコンジェニックラットを用いて、高血圧発症機序における交感神経系中枢内の酸化ストレス亢進の関与を導き出し得た。質疑応答も的確で、関連分野の知識も豊富であり、学位授与に値する。（副査：竹下治男）

（備考）要旨は、それぞれ４００字程度とする。