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.