Nucleotide Sequence Analysis of Protein Kinase Ca and Low Affinity Nerve Growth Factor Receptor Genes in SHRSP. Kiyomitsu Nemoto*, Tatsuya Hagiwara*, Haruaki Kageyama*, Isao Tomita*, Takashi Ueyama**, Masahiko Ikeda***, and Takako Tomita***. *School of Pharm. Sci. ***Graduate School of Health Sci., Univ. of Shizuoka, 52-1 Yada, Shizuoka 422. **Dept. Medicine, Wakayama Medical College, Wakayama 640.

Objective:

Peripheral sympathetic hyperinnervation and hyperactivation found in SHR suggest that there might be a structural abnormality of receptors for nerve growth factor (NGF) which is a unique trophic factor for sympathetic neurons. In addition, there is defective protein (p47) phosphorylation in platelets from SHRSP, and protein kinase Ca (PKCa) which mediates the phosphorylation has less sensitivity to Ca^{2+} in SHRSP platelets, compared with that in age-matched WKY platelets. These findings imply a possibility of structural changes in PKCa gene in SHRSP. Both PKCa gene, and low affinity NGF receptor (LNGFR) gene encoding one of receptors for NGF, locate nearby the specified region on chromosome 10 which is linked to blood pressure in a cross between SHRSP and WKY (Hilbert et al.). Therefore, nucleotide sequences of these genes in SHRSP and WKY were investigated in this study to see if there are mutations related to hypertension.

Design and Methods:

Total RNAs were prepared from brain basal ganglia of 8-week-old SHRSP and WKY. The cDNAs were constructed from the prepared SHRSP and WKY RNAs by using reverse transcriptase. Then several fragments were amplified by polymerase chain reaction (PCR) with rat PKCa or LNGFR sequence-specific oligonucleotide primers. Amplified fragments were cloned and nucleotide sequences were determined by a dideoxy sequencing method.

Results:

No differences in PKCa nucleotide sequences were found between SHRSP and WKY.

The single nucleotide change which generates an amino acid substitution was observed in the LNGFR gene in SHRSP. This mutation (G to A) located on the region encoding the signal peptide of LNGFR, changing a alanine (GCC) to a threonine (ACC).

Discussion and Conclusion:

No mutation found in SHRSP PKCa gene, led us to an conclusion that less sensitivity to Ca^{2+} of PKCa in SHRSP platelets does not result from the genetic change in PKCa gene but by the changes of any other factors modifying the activity.

In LNGFR gene of SHRSP, a single nucleotide mutation was detected. This mutation generates an amino acid substitution in signal peptide region of this gene product which plays an important role for the LNGFR maturation and expression on the cell surface. This structural change of the signal peptide induced by this amino acid substitution might induce changes in LNGFR biological activities such as a quantitative change of the expression on the cell surface leading to abnormal peripheral sympathetic nervous system in SHRSP. Thus, the detection of the mutated LNGFR gene from SHRSP would provide useful informations to search for the promising candidates of the causative genes related to hypertension.