学 位 論 文 の 要 旨

氏名　大洲　光裕

学 位 論 文 名　Quantitative Analysis of Delayed Neuronal Death in the Hippocampal Subfields of SHRSP and SHR

発 表 雑 誌 名（巻，初頁～終頁，年）　Cellular and Molecular Neurobiology in press

著 者 名　Mitsuhiro Daisu, Toshihisa Hatta, Yasuko Sakurai-Yamashita, Toru Nabika, Kouzo Moritake

論 文 内 容 の 要 旨

INTRODUCTION

Transient forebrain ischemia and reperfusion (TIR) induces delayed neuronal death (DND) in the hippocampal CA1 subfield of the stroke-prone spontaneously hypertensive rat (SHRSP). The vulnerability to DND is potentially related to the genetic susceptibility to stroke in this strain. To elucidate the mechanism of DND in SHRSP, however, it is essential to establish a method for quantitative evaluation of DND, which is not available yet. In this regard, the present study was designed to quantify the severity of DND with the stereology. A quantitative comparison of DND between SHRSP and the spontaneously hypertensive rat (SHR) was performed for the first time in this study. In addition, the precise distribution of DND in CA1 was examined.

MATERIAL AND METHODS

Male SHRSPs and SHRs at 12 weeks of age were used in the experiment. These rats were

anesthesized with 2.5% halothane and the body temperature was maintained at 37.0±1.0℃ on a heating pad. The common carotid arteries were surgically exposed bilaterally, and occluded with aneurysmal clips for 10 minutes. After 10 minutes occlusion, the clips were removed to start the reperfusion. The brain was taken out seven days after the experiment of TIR, and was sliced into coronal sections. Serial coronal sections of 5 μm thick were then obtained throughout the whole CA region. The first section and one in every 40 sections were selected for counting the pyramidal cells.

Quantitative estimation of the number of viable pyramidal cells in the CA1 and CA2/3 subfields was performed based on the stereology with a random and systematic sampling. Microscopic images of the coronal sections were taken at 100× magnification with a digital CCD camera. The area of the pyramidal cell layer in CA1 and CA2/3 per section was measured using the Image J software. Grids (25×25 μm in the size) were superimposed on the images to cover the whole CA region using the Adobe Photoshop software. Viable pyramidal cells in the grids were counted manually in CA1 and CA2/3. The grids for counting were selected in a random and systematic manner. The average number of viable cells in a grid was obtained and the density per 1 μm^2 was calculated. The cell number in CA1 and CA2/3 on the each section was calculated by multiplying the mean cell density by the area of the region measured as described above. The sum of the cell numbers on the selected sections divided by a pickup ratio (one in every 40 sections) and multiplied by the revised coefficient (1/3) based on Abercrombie's method gives the total cell number in CA1 and in CA2/3.

RESULTS AND DISCUSSION

The transient ischemia and reperfusion significantly reduced the number of viable pyramidal cells in CA1 of SHRSP (61000±20100 in TIR vs. 128500±21900 in the sham-operation, $p<0.000001$ by Student's t-test), while no significant difference was observed in SHR (140300±30800 in TIR vs. 128200±16700 in the sham-operation, $p=0.35$). In CA2/3, the similar change was obtained in SHRSP, in spite that the difference was much smaller (101500±13400 in TIR vs. 123200±13500 in the sham-operation, $p=0.002$). In SHR, TIR tended to decease the cell number in CA2/3 though the difference was not significant (121300±23000 in TIR vs. 133900±10000 in the sham-operation, $p=0.18$).

Further analysis revealed a dorsal-ventral gradient in the distribution of DND in CA1 of SHRSP with the most severe change in the dorsal area. To test a possible 'labor-saving' method, we evaluated the difference between TIR and the sham-operation in the three consecutive histological sections, randomly selected from the dorsal one-third of CA1. The test was repeated 10 times and results implied that only three consecutive sections selected from the dorsal one-third of CA1 enables to estimate the severity of DND.

CONCLUSION

The quantitative measurement using the stereological method is useful in the precise evaluation of DND in SHRSP. This method, especially on the dorsal one-third portion of CA1, can be applied in the studies of effects of medical treatments on the 'ischemia/reperfusion' insult.