学 位 論 文 の 要 旨

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学　位　論　文　名　Direct Exposure to Mild Heat Promotes Proliferation and Neuronal Differentiation of Neural Stem/Progenitor Cells *In Vitro*

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論 文 内 容 の 要 旨

INTRODUCTION

Heat acclimation is an adaptive physiological process that increases heat tolerance. Heat-acclimated animals exhibit various physiological changes, especially in the thermoregulatory and cardiovascular systems, such as enhanced sweating and cutaneous vasodilation, increased plasma volume, and reduced heart rate. In previous studies, we found that constant moderate heat exposure for 5 days increased neural stem/progenitor cells (NSCs/NPCs) proliferation in rat hypothalamus. In addition, we recently reported that inhibition of NSCs/NPCs proliferation by a mitotic blocker, cytosine arabinoside, decreased heat tolerance in rats. Thus, NSCs/NPCs proliferation and integration into hypothalamic neural circuitry may be important for acquired heat acclimation. However, the exact mechanisms of heat exposure-induced NSCs/NPCs proliferation in rat hypothalamus are unclear. To elucidate the mechanisms for heat acclimation, we investigated the effects of direct mild heat exposure on the proliferation and differentiation of cultured NSCs/NPCs *in vitro*.

MATERIALS AND METHODS

The NSCs/NPCs isolated from forebrain cortices of 14.5-day-old rat fetuses were propagated as neurospheres at either 37.0 °C (control) or 38.5 °C (mild heat exposure) for four days, and the effects on proliferation were investigated by MTS cell viability assay, measurement

of neurosphere diameter, 5-Bromo-2'-deoxyuridine (BrdU) pulse labeling and counting the total number of cells. The mRNA expressions of heat shock proteins (HSPs) and brain-derived neurotrophic factor (BDNF), cAMP response element-binding (CREB) protein and Akt phosphorylation levels, and intracellular reactive oxygen species (ROS) levels were analyzed using real time PCR, Western blotting and CM-H_2DCFDA assay, respectively.

All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines.

RESULTS AND DISCUSSION

Four days of continuous heat exposure increased viable cell number compared to the control temperature. In addition, the number of BrdU immunopositive (BrdU+) cells and percentage of BrdU+ cells to total cells were significantly higher at 38.5 °C. To investigate the mechanisms underlying increased NSC/NPC proliferation under heat exposure, we examined effects of heat on expression of HSPs, critical prosurvival (anti-apoptotic) proteins under cellular stress. Real-time PCR demonstrated that HSP27 mRNA level was increased to 9-folds in cells grown at 38.5 °C than at 37.0 °C. To explore the mechanisms for enhanced proliferation further, we examined whether heat exposure upregulates expression of BDNF. Mild heat exposure significantly increased BDNF mRNA level in NSCs/NPCs at all time points from day 1 to day 4 compared to the control temperature. It has been shown that BDNF expression is directly dependent on the phosphorylation of CREB. Thus, we assessed whether heat exposure also alters the level of p-CREB. In contrast to the damaging effects of ROS, there is evidence that in some systems, especially in NSCs/NPCs, nontoxic ROS levels can actually promote cell proliferation and survival. Thus, we measured intracellular ROS levels in NSCs/NPCs at the control culture temperature and under mild heat exposure. In heat-exposed cells, intracellular ROS levels were increased nearly 27% on day 1 compare to control cells and were still approximate 16% higher on day 4. BDNF and ROS, both induced by mild heat exposure, have been reported to activate Akt in NSCs/NPCs. In addition, HSP27 has been suggested to be important for sustained Akt activity. Thus, we examined whether mild heat exposure changes Akt phosphorylation (activation status) in NSCs/NPCs. The level of phosphorylated Akt did not change upon heat exposure on day 1, but continuous mild heat exposure significantly increased p-Akt levels on day 4. Moreover, treatment with LY294002, a PI3K inhibitor, abolished the effects of heat exposure on NSC/NPC proliferation. Furthermore, heat exposure under differentiation conditions increased the proportion of cells positive for Tuj1, a neuronal marker. These findings suggest that mild heat exposure increases NSC/NPC proliferation, possibly through activation of the Akt pathway, and also enhances neuronal differentiation.

CONCLUSION

Mild heat enhanced the proliferation and neural differentiation of NSCs/NPCs *in vitro*. This enhanced NSC/NPC proliferation may be mediated by Akt activation through upregulation of BDNF and HSP27 as well as by slight elevation of intracellular ROS. Direct effects of temperature on NSCs/NPCs may be one of the mechanisms involved in hypothalamic neurogenesis in heat-acclimated rats. Such heat-induced neurogenesis could possibly be adapted as an effective therapeutic strategy for neurodegenerative diseases.