RISPERIDONE TREATMENT AFTER TRANSIENT ISCHEMIA INDUCES HYPOTHERMIA VIA 5-HT2A ANTAGONISM AND PROVIDES NEUROPROTECTION IN THE GERBIL HIPPOCAMPUS BY DECREASING OXIDATIVE STRESS

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Introduction

Neuroprotective compounds provide protection in models of ischemic stroke, however, none have shown efficacy in clinical trials. Body temperature influences outcome of ischemic stroke, and hypothermia is one of the most promising neuroprotective therapies, as assessed by the Stroke Therapy Academic Industry Roundtable criteria. Although precise mechanism of hypothermic neuroprotection in ischemic stroke is not known, hypothermia may act upon multiple pathways to ultimately prevent neuronal death. It has been tried to find drugs including atypical antipsychotic agents with hypothermic effects to treat ischemic stroke. The drugs reduce temperature instantly when a patient is picked up by emergency personnel, are already approved for patient treatment and used anywhere independent of sophisticated cooling equipment, and may prolong therapeutic time window for other treatments. Thus, atypical antipsychotic agent-induced hypothermia may help to overcome brain injury following ischemic stroke. For several decades, risperidone (RIS) has widely been used for treatment of schizophrenia as a selective monoaminergic antagonist with high-affinity for serotonin type 2 (5-HT2A) receptor and dopamine type 2 (D2) receptor in the limbic system. In addition, RIS-induced hypothermia has been reported. It remains possible that RIS contains neuroprotection against ischemic stroke via hypothermia.

Experimental purpose

This study determined whether neuroprotective effect of RIS is hypothermia as a post-conditioning stimulus for inducing ischemic tolerance and elucidated RIS-induced hypothermic mechanisms. For this purpose, we intended (1) to compare effects of RIS on transient ischemic damage between ischemic animals without regulating body temperature and with maintaining body temperature; (2) to study effects of RIS against ischemia-induced oxidative stress; (3) to investigate roles of central 5-HT2A and D2 receptors in body temperature regulation in RIS-treated animals.

Materials and Methods

Experimental animals

- Mongolian gerbil (male, 6 months of age)
- 1) Vehicle-sham group
- 2) Vehicle-ischemia group
- 3) Risperidone (RIS)-sham group
- 4) RIS-ischemia group

Immunohistochemistry

Mouse anti - NeuN, GFAP, 4-HNE
Rabbit anti - Iba-1
Sheep anti - SOD1, SOD2
Goat anti - 8-OHdG

Histofluorescence

Fluoro-Jade B (F-J B)
Dihydroethidium (DHE)

Western blot

Sheep anti - SOD1, SOD2

BCCAO operation

Tissue processing for histology

Results

Fig. 1. Effects of RIS against TI injury under UBT condition. (A) Changes in body temperature under UBT condition for 12 h after TI. Body temperature is significantly low in the TI+10 mg/kg RIS group compared to the TI+vehicle group. White arrows indicate times of RIS treatment. The bars indicate the means $\pm$ SEM. (B) Effects of RIS on NeuN+ and F-J B+ cells in the CA1 under UBT condition after TI. In the TI+vehicle group, a few NeuN+ cells (arrows) are shown in the stratum pyramidale (SP) 5 days after TI; however, the distribution of NeuN+ cells in the TI+RIS group is similar to that in the sham+vehicle group. In the TI+vehicle group, many F-J B+ cells (asterisk) are detected in the SP 5 days after TI; however, F-J B+ cells are hardly detected in the TI+RIS group.

CA1, cornu ammonis 1; CA3, cornu ammonis 3; DG, dentate gyrus, SO, stratum oriens; SR, stratum radiatum. Scale bar = 50 μ m. Note histograms of quantitative analyses of NeuN+ and F-J B+ cells in all the groups, as shown C and D. The bars indicate the means $\pm$ SEM (* p < 0.05 vs. sham+vehicle group; # p < 0.05 vs. TI+vehicle group).

Fig. 2. Effects of RIS on GFAP+ astrocytes (A) and Iba-1+ microglia (B) in the CA1 after TI under UBT condition. GFAP+ astrocytes (arrows in A) and Iba-1+ microglia (arrows in B) are markedly increased in the TI+vehicle group 5 days after TI; however, their immunoreactivity in the TI+RIS group is significantly lower than that in the TI+vehicle group. SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum. Scale bar = 50 μ m. Quantitative analyses of GFAP+ (C) and Iba-1+ cells (D). The bars indicate the means $\pm$ SEM (* p < 0.05 vs. sham+vehicle group; # p < 0.05 vs. TI+vehicle group).

Fig. 3. Effects of RIS against TI injury under CBT condition. (A) Changes in body temperature under CBT condition for 12 h after TI. Body temperature is slightly low in the TI+10 mg/kg RIS group compared to the TI+5 mg/kg RIS group. White arrows indicate times of RIS treatment. The bars indicate the means $\pm$ SEM. (B) Effect of RIS (10 mg/kg) on NeuN+, F-J B+, GFAP+ and Iba-1+ cells in the CA1 under CBT after TI 5 days after TI. In the TI+RIS group, a few NeuN+ neurons (arrows) and many F-J B+ cells (asterisk) are shown in the stratum pyramidale (SP) at 5 days after TI. GFAP+ astrocytes (arrows) and Iba-1+ microglia (asterisk) are markedly increased in the TI+RIS group under CBT 5 days after TI. SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum. Scale bar = 50 μ m.

Fig. 4. Effects of RIS on superoxide anion production after TI under UBT and CBT conditions. (A) Changes of DHE immunofluorescence in the CA1 after TI under UBT and CBT conditions. DHE immunofluorescence is significantly increased in CA1 pyramidal neurons of the stratum pyramidale (SP, asterisks) in the TI+vehicle group under UBT and TI+RIS group under CBT at 1 day after TI; however, DHE immunofluorescence is not significantly increased in the SP (arrows) of the TI+RIS group under UBT condition.

SO, stratum oriens; SR, stratum radiatum. Scale bar = 50 μ m. (B) Note analyses of DHE immunofluorescence in the SP. A ratio of the ROD is calibrated as %, with the sham+vehicle group designated as 100%. The bars indicate the means $\pm$ SEM (* p < 0.05 vs. sham+vehicle group under UBT condition; # p < 0.05 vs. TI+vehicle group under UBT condition; † p < 0.05 vs. TI+RIS group under UBT condition).

Fig. 5. Effects of RIS on 8-OHdG and 4-HNE immunoreactivities after TI under UBT and CBT conditions. Representative images of immunohistochemistry for 8-OHdG (A) and 4-HNE (B) in the hippocampal CA1 after TI under UBT and CBT conditions. Under UBT condition, 8-OHdG and 4-HNE immunoreactivities in the TI+vehicle group are significantly increased in CA1 pyramidal neurons of the stratum pyramidale (SP, asterisks) in the TI+vehicle group under UBT and TI+RIS group under CBT at 1 day after TI; however, the immunoreactivities are significantly low (arrows) in the TI+RIS group under UBT. Scale bar = 50 μ m. Note analyses of 8-OHdG (C) and 4-HNE (D) immunoreactivity in the SP. A ratio of the ROD is calibrated as %, with the sham+vehicle group designated as 100%. The bars indicate the means $\pm$ SEM (* p < 0.05 vs. sham+vehicle group under UBT condition; # p < 0.05 vs. TI+vehicle group under UBT condition; † p < 0.05 vs. TI+RIS group under UBT condition).

Fig. 6. Effects of RIS on levels and immunoreactivity of SOD2 protein after TI under UBT and CBT conditions. (A) Western blot analysis of SOD2 protein in the hippocampus after TI under UBT and CBT conditions. Note that SOD2 levels are maintained in the TI+RIS group under UBT after TI. (B) Quantitative analysis of SOD2 protein in the hippocampus. Protein expression is normalized to β -actin. The bars indicate the means $\pm$ SEM (* p < 0.05 vs. sham+vehicle group under UBT condition; # p < 0.05 vs. TI+vehicle group under UBT condition; † p < 0.05 vs. TI+RIS group under UBT condition). (C) Representative images of immunohistochemistry for SOD2 in the hippocampal CA1

after TI under UBT and CBT conditions. SOD2 immunoreactivity is significantly decreased in CA1 pyramidal neurons of the stratum pyramidale (SP, asterisks) in the TI+vehicle group under UBT and TI+RIS group under CBT at 1 day after TI; however, the immunoreactivity is maintained (arrows) in the TI+RIS group under UBT. SO, stratum oriens; SR, stratum radiatum. Scale bar = 50 μ m. (D) Quantitative analysis of SOD2 immunoreactivity in CA1 pyramidal neurons. A ratio of ROD is calibrated as %, with the sham+vehicle group designated as 100%. The bars indicate the means $\pm$ SEM (* p < 0.05 vs. sham+vehicle group under UBT condition; # p < 0.05 vs. TI+vehicle group under UBT condition; † p < 0.05 vs. TI+RIS group under UBT condition).

Fig. 7. Effects of endogenous 5-HT2A- and D2- receptors on RIS-induced hypothermia. Hypothermia was defined as a reduction of at least 2°C below baseline (normothermia). NBOH-2C-CN hydrochloride does not alter body temperature, but bromocriptine mesylate significant decreased body temperature at 80 min after the administration, and (±)-DOI hydrochloride slightly increases body temperature at 60 min after the administration. Namely,

RIS-induced hypothermia is interrupted following the administration of NBOH-2C-CN hydrochloride and (±)-DOI hydrochloride. The bars indicate the means $\pm$ SEM. (* p < 0.05 vs. sham group; # p < 0.05 vs. RIS group).

Conclusion

In conclusion, RIS-induced hypothermia protected neurons from TI, and the neuroprotection might be associated with attenuation of glial activation and maintenance of antioxidants following hypothermia, showing that the hypothermia was abolished by NBOH-2C-CN hydrochloride treatment. These suggest that 5-HT2A receptor is involved in RIS-mediated reduction of body temperature and RIS-induced hypothermia as a post-conditioning stimulus might be given to stroke patients immediately at pick-up for hospitalization.