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Hyperthermic preconditioning severely accelerates neuronal damage in the gerbil ischemic hippocampal dentate gyrus via decreasing SODs expressions

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ABSTRACT

It is well known that neurons in the dentate gyrus (DG) of the hippocampus are resistant to short period of ischemia. Hyperthermia is a proven risk factor for cerebral ischemia and can produce more extensive brain damage related with mortality rates. The aim of this study was to examine the effect of hyperthermic conditioning (H) on neuronal death, gliosis and expressions of SODs as anti-oxidative enzymes in the gerbil DG following 5 min-transient cerebral ischemia. The animals were randomly assigned to 4 groups: 1) (N + sham)-group was given sham-operation with normothermia (N); 2) (N + ischemia)-group was given 5 min-transient ischemia with N; 3) (H + sham)-group was given sham-operation with H; and 4) (H + ischemia)-group was given 5 min-transient cerebral ischemia with H. H (39 ± 0.5 °C) was induced by subjecting the animals to a heating pad for 30 min before and during the operation. In the (N + ischemia)-groups, a significant neuronal death was observed in the polymorphic layer (PL) from 1 day after ischemia-reperfusion. In the (H + ischemia)-groups, neuronal death was also observed in the PL from 1 day post-ischemia; the degree of the neuronal death was severer than that in the (N + ischemia)-groups. In addition, we examined the gliosis of astrocytes and microglia using anti-glial fibrillary acidic protein (GFAP) and anti- ionized calcium-binding adapter molecule 1 (Iba-1). $GFAP^+$ and $Iba\text{-}1^+$ glial cells were much more activated in the (H + ischemia)-groups than those in the (N + ischemia)-groups. On the other hand, immunoreactivities and levels of SOD1 rather than SOD2 were significantly lower in the (H + ischemia)-groups than those in the (N + ischemia)-groups. In brief, on the basis of our findings, we suggest that cerebral ischemic insult with hyperthermic conditioning brings up severer neuronal damage and gliosis in the polymorphic layer through reducing SOD1 expression rather than SOD2 expression in the DG.

1. Introduction

Several clinical studies have evaluated body temperature in relation to stroke severity, infarct size, mortality and outcome [1]. Body temperature is a major factor in neuronal survival/death after cerebral ischemia; hypothermia is neuroprotective and hyperthermia is damaging [2]. Clinical data have confirmed that mild hyperthermia enlarges cerebral infarction and worsens the outcome of ischemic damage in ischemic stroke patients [3]. Hyperthermia has been also shown to display detrimental effects in some animal models of cerebral ischemia, such as global transient ischemia [4] and focal permanent ischemia [5] and focal transient ischemia [6,7]. However, in all of them, its influence is higher for transient ischemia than for permanent one [7]. It has been proposed that the release of neurotoxic excitatory neurotransmitters and reactive oxygen species, calcium influx into neurons and the dysfunction of vascular permeability might be mechanisms through which hyperthermia leads to tissue injury [8–10]. However, exact mechanisms are not yet fully understood.

The hippocampus morphologically consists of several distinct subregions, the cornu ammonis 1–4 (CA1–CA4) and dentate gyrus (DG),

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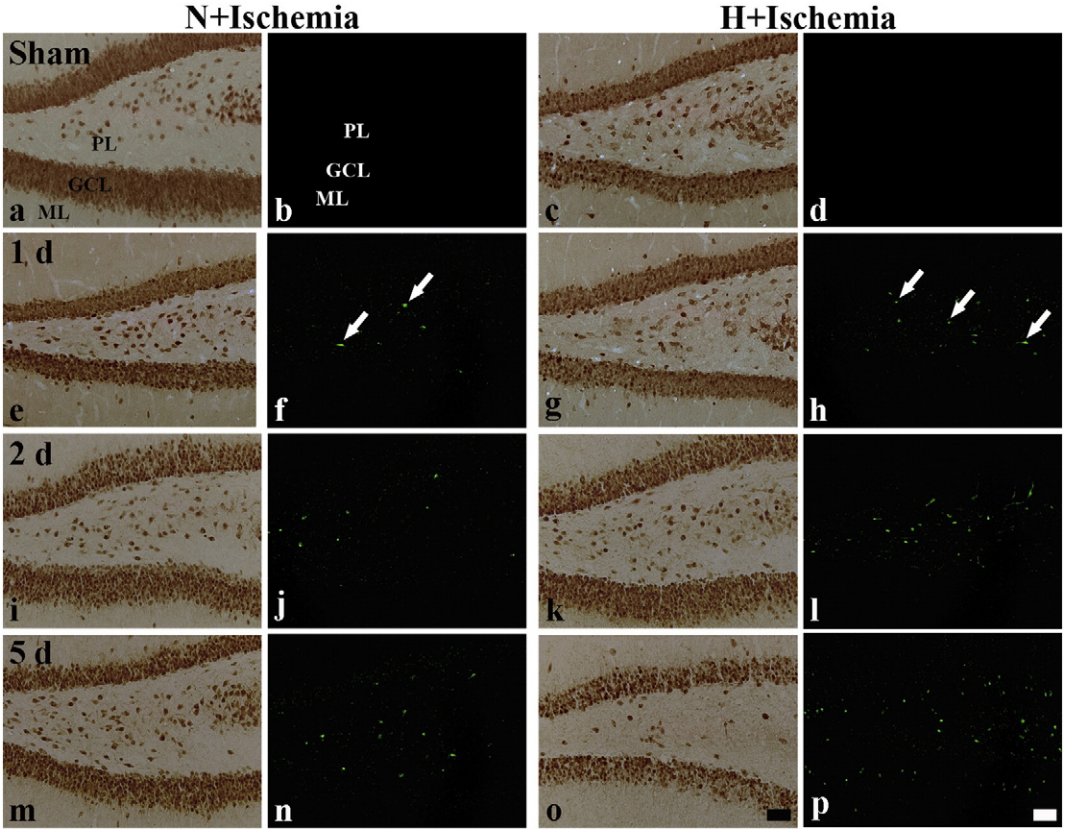

Fig. 1. NeuN immunohistochemistry (1st and 3rd columns) and F-J B histofluorescence staining (2nd and 4th columns) in the dentate gyrus of the (N + ischemia)- (left two columns) and (H + ischemia)- (right two columns) groups 1 (e–h), 2 (i–l) and 5 days (m–p) after ischemia-reperfusion. A distinct loss of NeuN$^+$ neurons is shown in the polymorphic layer (PL) of the (H + ischemia)-group at 5 days post-ischemia. A few F-J B$^+$ cells (arrows) are detected only in the PL of the (N + ischemia)-group from 1 day post-ischemia. In the (H + ischemia)-groups, F-J B$^+$ cells (arrows) in the PL are much more than those in the (N + ischemia)-group. ML; molecular layer, GCL; granular cell layer. Scale bar = 50 μm.

which may differ in a large number of properties including cell type, function, connectivity and vulnerability to various insults [11,12]. Pyramidal neurons of the gerbil hippocampus, particularly, those in the CA1 region, are the most vulnerable to transient cerebral ischemia, and their neuronal death is termed "delayed neuronal death (DND)" because neuronal death occurs very slowly from 4 to 5 days following 5 min of transient cerebral ischemia [13].

On the other hand, neurons in the hippocampal dentate gyrus are resistant to ischemic insults [11,14]. However, some researchers demonstrated that some neurons in the polymorphic layer of the dentate gyrus were damaged following transient ischemia [15,16]. Furthermore, we recently reported that, using Fluoro-Jade B (a marker for neuronal degeneration) histofluorescence staining, neuronal degeneration/death in the polymorphic layer of the dentate gyrus occurred earlier than that in the CA1 region following transient cerebral ischemia in gerbils [11].

However, no studies regarding neuronal damage/death in the ischemic dentate gyrus with hyperthermic condition have been reported, although hyperthermia displays detrimental effects in some animal models of cerebral ischemic insults, as we described above. Therefore, in the present study, we examined whether hyperthermic condition before and during the ischemic insult is associated with the degree of neuronal damage, expressions of the antioxidant enzymes SOD1 and SOD2 and glial changes in the gerbil hippocampal dentate gyrus following transient cerebral ischemia.

2. Materials and methods

2.1. Experimental animals

We used male Mongolian gerbils (*Meriones unguiculatus*) obtained from the Experimental Animal Center, Kangwon National University, Chunchon, South Korea. Gerbils were used at 24 weeks (Body weight 65–75 g) of age and were maintained in pathogen-free conditions under controlled temperature (23 °C) and humidity (60%). All the experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Kangwon University and adhered to guidelines that are in compliance with the current international laws and policies (Guide for the Care and Use of Laboratory Animals, The National Academies Press, 8th Ed., 2011).

2.2. Animal groups

Experimental animals were divided into four groups ($n = 14$ at each point in time per group): (1) (normothermia (N) + sham)-group, which was normothermic (37 ± 0.2 °C) and given no ischemia

Table 1
Changes in the mean number of F-J B$^+$ cells in the dentate gyrus in the (N + ischemia)- and (H + ischemia)-groups.

Groups	Layer	Sham	Time after ischemia-reperfusion		
			1 day	2 days	5 days
N + Ischemia	ML	0	0	0	0
	GCL	0	0	0	0
	PL	0	6.9 ± 2.7*	7.8 ± 2.5*	10.3 ± 3.1*
H + Ischemia	ML	0	0	0	0.23 ± 0.29
	GCL	0	0	0.15 ± 0.18	27.1 ± 7.1$^{*\#\dagger}$
	PL	0	15 ± 4.4*†	19.2 ± 3.6*†	20.4 ± 5.9*†

The mean number of F-J B$^+$ cells is counted in a 250 × 250 μm square of the PL after ischemia-reperfusion ($n = 7$ at each point in time per group; $^*P < 0.05$, significantly different from the corresponding (N + sham)-group, $^\#P < 0.05$, significantly different from the respective pre-time point group, $^\dagger P < 0.05$, significantly different from the respective N + Ischemia-group. GCL, granule cell layer; ML, molecular layer; PL, polymorphic layer.