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BRAIN RESEARCH

Short Communication

A decrease of cell proliferation by hypothermia in the hippocampus of the neonatal rat

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ABSTRACT

Hypothermia is a potential therapy for cerebral hypoxic ischemic injury of not only adults but also neonates. However, the side effects of hypothermia in the developing brain, where a massive amount of neurogenesis occurs, remain unclear. We investigated the proliferation of neural progenitor cells by systemic application of the thymidine analog 5-bromodeoxyuridine (BrdU) in neonatal rats in a severe hypothermic environment. The rat pups were divided into two groups, a hypothermia group (30 °C: $n=10$) and a normothermia group (37 °C: $n=10$). After the pups were placed for 21 h in each environment, 100 mg/kg/day of BrdU was injected intraperitoneally to label dividing cells, and then the pups were sacrificed at 24 h. We examined the number of BrdU-labeled cells in the subventricular zone of the periventricle and the subgranular zone of the dentate gyrus. In the hypothermic environment, BrdU-labeled cells significantly decreased in number in the dentate gyrus, but not in the periventricular region. Thus, the severe hypothermic environment induced a decrease of neurogenesis in the neonatal rat. These observations are noteworthy regarding clinical hypothermia therapy following cerebral hypoxic ischemic injury during the perinatal period.

Severe perinatal asphyxia remains an important cause of brain injury in newborn infants, with a high risk for behavioral and neurological deficits and death (Berger and Garnier, 1999). At present, there are no treatments for such asphyxia that have proven to be effective and the emphasis thus far has been on supportive therapy. A potent neuroprotective effect of hypothermia following cerebral hypoxic ischemic injury has been reported in various animal models. Although hypothermia may be the most effective current method of neuroprotection, the other effects of hypothermia on the developing brain remain unclear.

In the meantime, since the pioneering studies of Altman (Altman and Das, 1967) and Kaplan (Kaplan and Hinds, 1977), neurogenesis in the brain has generated a great deal of interest and research effort. Persistent neurogenesis occurs in discrete regions of the adult mammalian brain (Altman and Das, 1965), including the human brain (Eriksson et al., 1998). Adult neurogenesis is observed in some limited regions: the subventricular zone (SVZ) of the lateral ventricle (Doetsch et al., 1999; Johansson et al., 1999) and subgranular zone (SGZ) of the hippocampus dentate gyrus (Altman and Das, 1967; Eriksson et al., 1998; Johansson et al., 1999). Therapy involving

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neurogenesis is a new strategy for adult brain disorders. Recently, it has been shown that postnatal neurogenesis may be modulated by diverse factors, including an enriched environment (Kempermann et al., 1997), stress (Gould et al., 1998; Tanapat et al., 2001) and hormones (Cameron and Gould, 1994; Cameron and McKay, 1999; Gould and Gross, 2002). Despite important advances made in this field, information concerning how thermal factors influence neurogenesis in the developing brain of neonates, including mammals, is still scarce.

The purpose of the present study was to demonstrate that severe hypothermia modulates the neurogenesis in the brain of the neonatal rat. Here we used the 7-day-old rat, which is roughly equivalent to a full-term infant in terms of brain growth (Hagberg et al., 1997). We evaluated the activity of the cell proliferation with bromodeoxyuridine (BrdU), which is broadly used in neuroscience to study adult neurogenesis (Gould and Gross, 2002), to label the proliferating cells. We also examined the neuronal phenotype expression in the newly generated cells using double-immunohistochemistry for BrdU and Doublecortin (DCX), which is known to be a useful marker for neuroblasts (Francis et al., 1999; Nacher et al., 2001).

The rectal temperature of the rat pups remained rather constant and stayed at about 0.5 °C above the water bath temperature for 24 h. The nuclei of newly generated cells labeled with BrdU were irregularly shaped, well delineated, and densely stained (Fig. 1). In the SVZ, the BrdU-labeled cells were observed adjacent to the ependyma in both groups (Figs. 1A, B). In the SGZ, the BrdU-labeled cells were seen at the border between the granule cell layer and hilus region (Figs. 1C, D). Immunohistochemistry of BrdU in the SGZ area showed that the hypothermia group was a significant difference in the number or distribution of BrdU-labeled cells compared to the normothermia group (Figs. 1C, D). The number of BrdU-labeled cells is shown in Fig. 1E. The number of BrdU-labeled cells in the SVZ was not significantly different between the normothermia and the hypothermia group. However, in the SGZ, the number of BrdU-labeled cells in the hypothermia group was significantly reduced (by 51.0%) compared to that in the normothermia group ($p=0.001$, t score $=5.34$).

The double-immunohistochemistry for BrdU and DCX in all areas examined 3 days after the BrdU injection (10th postnatal day) showed that the majority of the BrdU-labeled cells colocalized with DCX (Fig. 2). The colocalization of BrdU and DCX was observed in both the hypothermia and normothermia groups (data not shown). BrdU-labeled postmitotic cells expressed the neuroblastic antigenic phenotype on the 10th postnatal day.

This study shows that a severe hypothermic environment induces a decrease of neurogenesis in the neonatal rat. To the best of our knowledge, this is the first such demonstration in a mammal.

In poikilothermal animals, temperature influences neurogenesis. Ramirez et al. investigated the effects of temperature and photoperiod on the regenerative neurogenetic activity of the medial cortex in lizards. They found that a long (summer)

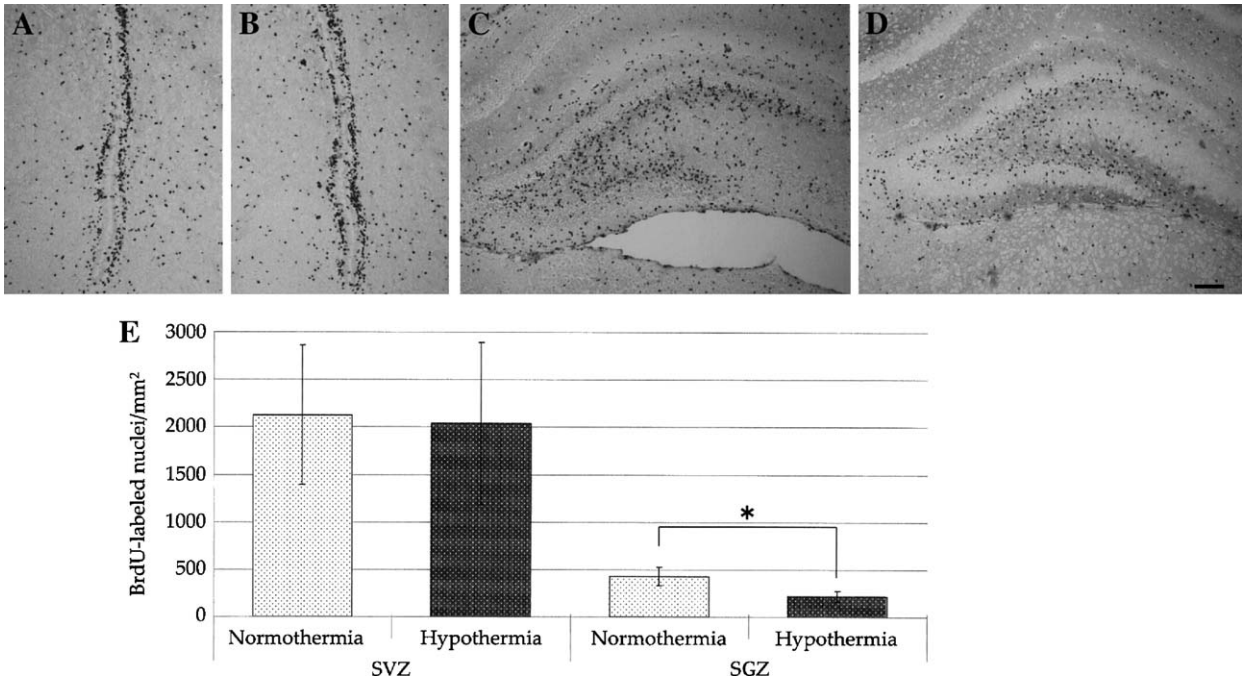

Fig. 1 – Bromodeoxyuridine (BrdU)-labeled cells in the normothermic environment (A, C) and in the hypothermic environment (B, D). Effects on temperature on neurogenesis in the subventricular zone (SVZ) and the subgranular zone (SGZ) (E). All rats were given BrdU (100 mg/kg/day, i.p.) before sacrifice. Note the dramatic decline in the number of mitotically active cells in the SGZ of neonatal rats in the hypothermic environment compared to the normothermic environment. There was a significant reduction of the BrdU-labeled cells in the SGZ of the hypothermia group. There was, however, no difference in SVZ. (A, B) the subventricular zone (SVZ); (C, D) the subgranular zone (SGZ). All specimens were photographed at 100×. Scale bar, 100 μm. (E) Comparison of BrdU-labeled cells. *Probability value < 0.01. Values are presented as means; error bar, 1SD.