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BRAIN RESEARCH

Research Report

Failure of estradiol to ameliorate global ischemia-induced CA1 sector injury in middle-aged female gerbils

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ABSTRACT

Global forebrain ischemia arising from brief occlusion of the carotid arteries in gerbils produces selective hippocampal CA1 neuronal loss. Pre-treatment with 17β-estradiol ameliorates, in part, ischemia-induced damage in young animals. Because stroke and cardiac arrest are more likely to occur among elderly individuals, neuroprotective studies in older animals have compelling clinical relevance. We investigated whether estradiol would attenuate ischemia-induced hippocampal neuronal injury in middle-aged (12–14 months) male, intact female, ovariectomized (OVX) female and OVX females treated for 14 days with estradiol. Core temperature telemetry probes were also implanted at the time that estradiol was initiated. Ischemia was induced by bilateral occlusion of the common carotid arteries (5 min), during which time skull temperature was maintained under normothermic conditions. Estradiol blocked the modest spontaneous hyperthermia that normally follows ischemia. However, all four groups exhibited substantial neuronal cell loss in the CA1, assessed at 7 after ischemia. These findings indicate that estradiol pre-treatment under conditions that produce neuroprotection in young animals does not protect against ischemia-induced CA1 cell loss in middle-aged female gerbils.

1. Introduction

Transient global ischemia, arising from cardiac arrest, open heart surgery or profuse bleeding, in humans or induced experimentally in animals, such as gerbils and rats via bilateral carotid artery occlusion, results in selective, delayed neuronal death, particularly of pyramidal neurons in the highly vulnerable CA1 area of the hippocampus (Kirino, 1982; Pulsinelli et al., 1982). Whereas pyramidal neurons in the hippocampal CA1 are particularly vulnerable, inhibitory interneurons survive. Histological evidence of CA1 pyramidal neuron degeneration is not observed until approximately 2–4 days after global ischemia in rats and gerbils (Kirino, 1982; Pulsinelli et al., 1982). The ischemia-induced loss of CA1 cells can be attenuated or delayed with a variety of neuroprotective strategies including pharmacological therapies that aim to alleviate the deleterious molecular cascades involved in ischemia-induced neuronal death such as glutamate receptor

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antagonists, free radical scavengers and thrombolytic agents (for a review, see Hossmann, 2006).

Estradiol-17β, the primary estrogen secreted by the ovaries, has been widely studied as a potential neuroprotectant against cerebral ischemia (for reviews, see Wise et al., 2001; Yang et al., 2003). Estrogens afford neuroprotection in experimental models of global and focal ischemia (Alkayed et al., 2000; Jover et al., 2002; Plahta et al., 2004) and ameliorate the cognitive deficits associated with ischemic cell death (Gulinello et al., 2006; Plamondon et al., 2006). Early studies show that young (3–6 months) female rodents sustain less damage than their male counterparts in both global (Hall et al., 1991; Hurn and Macrae, 2000) and focal (Alkayed et al., 1998; Wise et al., 2001) ischemia models. It was postulated that these sex differences were due, at least in part, to lower levels of estradiol in the male *vs.* female animals. This notion was supported by the demonstration that ovariectomy of young female rats, resulting in a deficiency of estradiol, abolishes differences between the sexes in the susceptibility of the animals to ischemic insults (Alkayed et al., 1998).

In recent years, the neuroprotective effect of estradiol pre-treatment has been widely demonstrated in a variety of experimental stroke models including global (Chen et al., 1998; He et al., 2002; Jover et al., 2002; Koh et al., 2006; Plahta et al., 2004; Shughrue and Merchenthaler, 2003; Sudo et al., 1997) and focal ischemia (Rusa et al., 1999; Shi et al., 2001), as well as hemorrhagic stroke (Auriat et al., 2005; Nakamura et al., 2005) in rodents. Although these studies convincingly show that estradiol is an effective treatment against experimentally induced stroke, all of these studies investigated its neuroprotective properties using young animals (<6 months). This is problematic because for both men and women, the risk of stroke increases with age (>55) and in women, this risk increases post-menopause (Cheung et al., 2004). To be more clinically relevant, neuroprotection studies should examine whether a particular treatment is also effective in older animals (Stroke Therapy Academic Industry Roundtable (STAIR) 1999). Outcomes of ischemia may also differ between young and old animals (Duverger and MacKenzie, 1988; Futrell et al., 1991; Sutherland et al., 1996; Yao et al., 1991). For instance, older animals exhibit age-related changes in the brain that may affect not only their vulnerability to ischemic insults, but also their responsiveness to a particular treatment (Sutherland et al., 1996; Yao et al., 1991). Only a few studies have assessed the neuroprotective effect of estradiol using older (9–16 months) female animals (Alkayed et al., 2000; Dubal and Wise, 2001; Toung et al., 2004; Wise and Dubal, 2000). In all of these studies, pre-treatment (7–14 days) with estradiol significantly reduced brain injury following middle cerebral artery occlusion (MCAO).

To date, no study has assessed whether estradiol confers protection against transient global ischemia in older (12–14 months) animals. The purpose of the present study was to ascertain whether estradiol at a dose that has previously been found to be effective in young male (Jover et al., 2002; Plahta et al., 2004) and female gerbils (Jover-Mengual et al., 2007; Koh et al., 2006; Kondo et al., 1997) would also confer protection in older female gerbils. Specifically, we investigated whether a 14-day pre-treatment with estradiol would attenuate ischemia-induced cell loss in ovariectomized (OVX), middle-aged female gerbils.

2. Results

2.1. Estradiol serum levels

There was a significant main effect of group [$F(3,20)=41.9$, $p<0.0001$]. Mean (±SEM) estradiol serum levels were significantly higher in the OVX gerbils implanted with estradiol pellets (477.8±61.4 pg/ml) compared to the INTACT female (23.2±7.4 pg/ml), OVX female (8.7±2.4 pg/ml) and MALE (14.4±5.6 pg/ml) gerbils (all $p<0.01$). Serum levels for the INTACT, OVX and MALE gerbils did not differ.

2.2. Temperature and activity

Baseline core temperature (T_{core}) of all animals in all groups was monitored 24 h prior to ischemia and ranged from 36.9 °C to 37.2 °C. Hourly averages of the first 12 h of baseline and the first 12 h of post-ischemic T_{core} were calculated and used for statistical analyses. There were significant main effects of group [$F(3,35)=3.5$, $p<0.03$] and time [$F(12,420)=33.3$, $p<0.0001$]. There was also a significant group×time interaction [$F(36,420)=5.4$, $p<0.002$]. Post hoc tests first revealed that the MALE, INTACT female and OVX female gerbils exhibited significant differences in T_{core} post-ischemia during the first 3 h post-ischemia compared to their baseline temperatures ($p<0.001$). As shown in Fig. 1, these gerbils exhibited spontaneous hyperthermia (T_{core} ranging from 38 to 39 °C) that lasted ≈3 h following ischemia. However, OVX gerbils with estradiol pellets did not exhibit hyperthermia after ischemia; indeed these gerbils were significantly cooler than were the other 3 groups for the first 7 h post-ischemia (Fisher LSD, $p<0.01$).

Baseline locomotor activity levels were also collected 24 h prior to ischemia. Similar to temperature, hourly averages of the first 12 h of baseline were used in the statistical analyses. For post-ischemic activity data, blocks of 6 h were averaged over 48 h and then used for statistical analysis. Activity levels did not differ significantly among groups at either baseline or

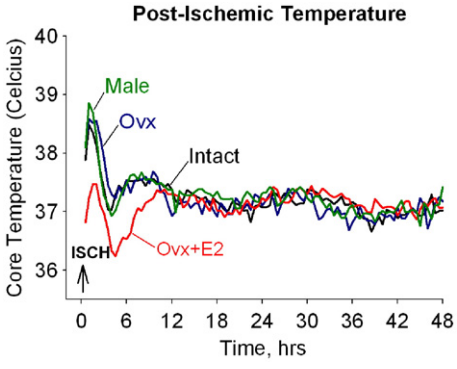

Fig. 1 – Mean T_{core} (°C) following ischemia. Data are shown for the first 48 h as subsequent temperatures were quite similar. Both the INTACT ($n=10$) and MALE ($n=8$) groups exhibited post-ischemic hyperthermia. Estradiol pre-treatment abolished spontaneous post-ischemic hyperthermia ($n=10$).