THE EFFECTS OF 17β ESTRADIOL, 17α ESTRADIOL AND PROGESTERONE ON OXIDATIVE STRESS BIOMARKERS IN OVARIECTOMIZED FEMALE RAT BRAIN SUBJECTED TO GLOBAL CEREBRAL ISCHEMIA

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Running Title: ESTROGENS AND PROGESTERONE IN GLOBAL CEREBRAL ISCHEMIA

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ABSTRACT

Neuroprotective effects of estrogens and progesterone have been widely studied in various experimental models. The present study was designed to compare possible neuroprotective effects of 17α estradiol, 17β estradiol, and progesterone on oxidative stress in rats subjected to global cerebral ischemia. Global cerebral ischemia was induced in ovariectomized female rats by four vessel occlusion for 10 min. Following 72 hr of reperfusion, levels of malondialdehyde (MDA, oxidative stress marker), and reduced glutathione (GSH, major endogenous antioxidant) were assessed in hippocampus, striatum and cortex of rats treated with either 17α estradiol, 17β estradiol, progesterone or estradiol + progesterone beforehand. Steroid administration ameliorated ischemia-induced decrease in GSH and increase in MDA levels. Our data offers additional evidence that estrogens and progesterone or combination of two exert a remarkable neuroprotective effect reducing oxidative stress.

Key words: estradiol, global cerebral ischemia, progesterone, oxidative stress.

INTRODUCTION

It has been demonstrated in *in vivo* and *in vitro* studies that estrogen and progesterone are neuroprotective in models of acute neuronal stress and neurodegeneration (Amantea *et al*. 2005). Several mechanisms are conceivable for neuroprotection by estrogen: a genomic estrogen receptor-mediated pathway that involves gene transcription; a non-genomic signaling pathway associated with activation of signaling molecules such as mitogen-activated protein kinase and/or phosphatidylinositol-3-kinase/protein kinase B; and an antioxidant free-radical scavenging pathway (Garcia-Segura *et al*. 2001). One important protective mechanism of estradiol is through its antioxidant action. Estrogen suppresses lipid peroxidation induced by amyloid β-peptide and ferrous sulfate (Kii *et al*. 2005) and attenuates

cell death caused by oxidative stress in hippocampal cell line (Vedder *et al*.1999). The mechanism for the antioxidative action of the estrogen is proposed to reduce the elevation of intracellular Ca^{2+} concentration, which is a major element in the development of ischemic damage by reactive oxygen species (Behl *et al*.1995). Several members of the estrogen family are potent antioxidants with their phenolic structure in the steroid A ring being responsible for the inhibition of iron catalyzed-lipid peroxidation (Kii *et al*. 2005).

Progesterone also possesses neuroprotective properties following cerebral ischemia as well as traumatic brain injury (Morali *et al*. 2005, Roof and Hall 2000). Progesterone may reduce oxidative stress by its membrane stabilizing effect as well (Roof and Hall 2000).

A number of studies have investigated the effect of 17β estradiol and progesterone or combined treatment (Murphy *et al*. 2002, Toung *et al*. 2004) against I/R injury. Since estrogen and progesterone have antioxidant potencies, we aimed to test hypothesis that gonadal steroids reduces ischemic neuronal damage by lowering oxidative stress, which is associated with decreased lipid peroxidation.

MATERIAL and METHODS

Thirty six adult female Wistar rats (4-6 months old, 200-250 g) were used. Animals were bilaterally ovariectomized under sodium thiopenthal (60 mg/kg, i.p.) anesthesia to eliminate endogenous estradiol and progesterone production. Ovariectomies were performed at 4-6 weeks prior to the study. All protocols followed in the study were approved by the animal care and use committee at the Zonguldak Karaelmas University Medical School.

Cerebral ischemia was generated by the method of four vessel occlusion for 10 min. Six groups were designed: 1) sham-operated control group (*n*=6), subjected to all surgical procedures except occlusion; 2) I/R control group treated with vehicle (*n*=6); 3) I/R group treated with 17α estradiol (*n*=6); 4) I/R group treated with 17β estradiol (*n*=6); 5) I/R group treated with progesterone (*n*=6); 6) I/R group treated with 17β estradiol + progesterone (*n*=6).

The treatment paradigm was based on previously published studies (Santizo *et al*. 2000). Estradiol (i.p., 0.1 mg/kg) and progesterone (i.p., 10 mg/kg), prepared in DMSO, was given daily for 1 week preceding the study, whereas control animals were given vehicle only. Additional doses of drugs or vehicle were administered at 24, 48, and 72 h of reperfusion. Animals were sacrificed at the end of 72 h reperfusion period; then, brains were quickly removed followed by careful dissection of hippocampus, striatum, and cortex in ice-cold saline. MDA was measured using a method described by Casini *et al*. 1986. Based on modified Ellman method, GSH content of the brain tissue samples was also measured (Aykaç *et al*. 1985).

RESULTS

I/R caused significant increase in the MDA content of the tissue samples from cortex, hippocampus, and striatum (Table 1). Administration of steroids except 17α estradiol provided remarkable attenuation of the elevated MDA content in striatum and hippocampus, bringing them back to control levels. Among groups treated with steroids were no statistically meaningful differences. In cortex, however, treatment with all steroids was effective in reducing MDA level.

The treatment with steroids ameliorated I/R-induced decrease in GSH levels in hippocampus (Table 1). In cortex, GSH content of sham control group was statistically indifferent from that of 17β estradiol group. I/R treated with 17α estradiol was not statistically different from I/R control in terms of GSH level, while the combined group was significantly different not only from I/R control but also from sham control. In striatum, only 17α estradiol and progesterone treated groups were statistically different from I/R control.

DISCUSSION

We observed that gonadal steroids appeared to show regional effect on lipid peroxidation and GSH homeostasis in rat brain. For instance, MDA levels of hippocampus and striatum decreased significantly in all groups except for 17α estradiol-treated animals, relative to I/R group. Yet, the levels in cortex were lowered in all groups. Acute or chronic treatment with 17β estradiol in cerebral ischemia model has been shown to be protective. Moreover, the protection has also been observed in animals given the purportedly receptor inactive 17α estradiol. Although only investigated in a handful of studies, 17α estradiol is shown to attenuate brain lipid peroxidation and induce sustained activation of the survival promoting protein kinases (McClean and Nunez 2008). We observed in hippocampus and striatum that 17α estradiol was not as effective on reducing lipid peroxidation as 17β estradiol. These findings appear to be consistent with a non-genomic process and might be viewed as evidence of antioxidant actions of estrogens, as some investigators have suggested (Vedder *et al.* 1999). On the other hand, estrogen related neuroprotection, to some degree, may involve interactions with classic receptors (Singer *et al*. 1996). Furthermore, the discrepancy in cortex in respect to two species of estradiol may be explained by variations among regional responses of rat brain. Nevertheless, further studies are required to delineate more clearly how estradiol mediates the neuroprotection in brain.

We showed that the combined treatment reduced lipid peroxidation in all brain tissues. The mechanism by which progesterone provides protection from ischemic brain injury may be through its free radical scavenger action. Our finding is consistent with other reports in that progesterone alone protects hippocampal neurons against different experimental brain injury models (Ciriza *et al*. 2006). However, we observed no any additive effect on reducing MDA levels and GSH metabolism in the combined treatment group. This is consistent with previous experiments showing estrogen and progesterone combination has no additive effect

on reducing ischemic brain injury (Toung *et al*. 2004). An explanation may be related to that progesterone could modify the impact of estradiol in hippocampus (Galea *et al*. 2006).

GSH is an endogenous antioxidant scavenging free radicals and protecting against oxidative stress. Preserving GSH-mediated antioxidant defense is critical for cell survival (Anderson *et al.* 2004). GSH content was significantly preserved in hippocampus of all the treatment groups. In cortex, treatments with 17β estradiol and 17β estradiol + progesterone alleviated I/R-induced decrease in GSH, whereas, in striatum, 17α estradiol or progesterone administration was effective to protect GSH levels. It is well known that sex steroid hormones modulate the enzyme activities in the antioxidant defense system (Pajovic *et al*. 2003). In the present study, 17β estradiol, its non-estrogenic stereoisomer 17α estradiol, and progesterone protect GSH depletion in hippocampus, suggesting a non-receptor mechanism. However, 17α estradiol failed to preserve GSH content in cortex. This might be explained by 17α estradiol induced consumption of GSH in cortical tissues. However, this consumption may not effective in reducing lipid peroxidation.

Data of the present study provided an additional evidence for a possibility that the neuroprotective effects of estradiol and progesterone are mediated, at least in part, through reduction of oxidative stress and restoration of GSH levels in transient global cerebral ischemia. The beneficial effects of estrogens in the brain including the lipid peroxidation-inhibiting actions may even result in therapeutic consequences, as suggested by recent studies on the clinical potential of estrogens in neurodegenerative diseases (Simpkins and Dykens 2008).

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GROUPS	**MDA nmol/g**			**GSH μmol/g**		
	Striatum	Hippocampus	Cortex	Striatum	Hippocampus	Cortex
Sham Control	166±34	157±19	153±21	2,41±0,18	2,27±0,1	1,35±0,09
I/R Control	351±29*	248±13*	313±20*	1,45±0,15*	1,66±0,09*	0,51±0,06*
I/R + 17α estradiol	248±15	206±18	201±8≠	2,32±0,04≠	2,51±0,2≠	0,67±0,07*
I/R + 17β estradiol	207±3≠	175±15≠	168±5≠	1,74±0,11	2,3±0,1≠	1,29±0,12≠
I/R + Progesterone	178±13≠	133±5≠	147±9≠	2,19±0,04≠	2,25±0,16≠	0,88±0,08≠
I/R + 17βestradiol + progesterone	176±36≠	156±23≠	143,24≠	1,61±0,22	2,38±0,03≠	1,46±0,17*≠

Table 1. The effect of gonadal steroids on MDA content of cortex, hippocampus, and striatum in ovariectomized rats subjected to global cerebral ischemia. Data is expressed as mean ± SE (n=6). *P<0.05 vs. sham control, ≠P<0.05 vs. I/R control.