Neuroscience 136 (2005) 135–146

8-OXOGUANINE DNA GLYCOSYLASE, BUT NOT Kin17 PROTEIN, IS TRANSLOCATED AND DIFFERENTIALLY REGULATED BY ESTROGENS IN RAT BRAIN CELLS

S. ARANEDA,[a]* S. PELLOUX,[b] J. P. RADICELLA,[c] J. ANGULO,[d] K. KITAHAMA,[a] K. GYSLING,[e] AND M. I. FORRAY[e]

[a]*Physiologie Intégrative, Cellulaire et Moléculaire, UMR5123 CNRS/ UCB Lyon 1. Bat Raphaël Dubois, Campus La Doua, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France*

[b]*Centre Commun de Quantimétrie, Inserm EMIU 0226, UCBL, 8 Avenue Rockefeller, 69373 Lyon Cedex 08, France*

[c]*Département de Radiobiologie et Radiopathologie, UMR217 CNRS Commissariat à l'Energie Atomique, 92265 Fontenay-aux-Roses, France*

[d]*Laboratoire de Génétique de la Radiosensibilité, Département de Radiobiologie et Radiopathologie, Commissariat à l'Energie Atomique, 60-68 Av. Général Leclerc, 92265 Fontenay-aux-Roses, France*

[e]*Department of Cell and Molecular Biology, Faculty of Biological Sciences, Catholic University of Chile, Santiago, Chile*

Abstract—8-oxoguanine DNA glycosylase and Kin17 are proteins widely distributed and phylogenetically conserved in the CNS. 8-oxoguanine DNA glycosylase is a DNA repair enzyme that excises 7,8-dihydro-8-oxoguanine present in DNA damaged by oxidative stress. Kin17 protein is involved in DNA repair and illegitimate recombination in eukaryotic cells. The present study evaluates the effect of ovarian hormones on the expression of both proteins in the magnocellular paraventricular nucleus of the hypothalamus and the bed nucleus of the stria terminalis in female and male rat brains.

In the paraventricular nucleus, ovariectomy induced a significant decrease in the number of 8-oxoguanine DNA glycosylase-positive nuclei as well as in their relative fluorescent intensity as compared with ovariectomized-estradiol treated and proestrous groups. Confocal microscopy observation demonstrated that oxoguanine DNA glycosylase protein is located in the Hoechst-dyed nuclei and cytoplasm in male and ovariectomized rats. Surprisingly, following estradiol administration to ovariectomized and proestrous rats, the 8-oxoguanine DNA glycosylase immunolabeling was observed in the nucleolus, the cytoplasm and the dendrites of cells, while Kin17 protein was always localized in the cell nuclei.

In the bed nucleus of the stria terminalis, the number of 8-oxoguanine DNA glycosylase-positive nuclei during proestrous was significantly lower than the number obtained in males and ovariectomized rats and similar to the number of ovariectomized-estradiol-treated groups. In contrast to these observations, no significant differences were observed in the expression of kin17 protein. Our results suggest that estrogens differentially regulate the expression of 8-oxoguanine DNA glycosylase, but not that of Kin17 protein, in specific regions of the rat brain and that estradiol can translocate the 8-oxoguanine DNA glycosylase protein within nuclei and to other subcellular compartments. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: estrogen, DNA repair proteins, PVN, stria terminalis.

Repair of DNA damage ensures cell survival and is guaranteed by well-known mechanisms of nucleotide (or base) excision repair (Arai et al., 1997; Brooks et al., 1996; Kisby et al., 1999; Lin et al., 2000) or non-homologous end-joining pathways (Gao et al., 1998; Takata et al., 1998). In the CNS of mammals, we have reported the presence of two repair-recombination proteins in the cell nuclei of neurons and glial cells: the 8-oxoguanine DNA glycosylase (Ogg1) and the Kin17 proteins (Araneda et al., 1997, 2001, Mermet et al., 1998, Ros et al., 1999; Verjat et al., 2000). The *hOGG1* gene, mapped to chromosome 3p26.2 (Arai et al., 1997; Ishida et al., 1999a,b; Radicella et al., 1997; Roldan-Arjona et al., 1997) encodes two isoforms: α-hOgg1 nuclear (37 kDa) and β-hOgg1 mithocondrial (44 kDa) proteins (Nishioka et al., 1999). Both human and rodent Ogg1 proteins are DNA glycosylases that preferentially repair the 8-oxoguanine in the non-transcribed DNA, but not in the transcribed DNA sequence (Le Page et al., 2000). This mutagenic adduct is generated by free radicals issued by cellular metabolism (Arai et al., 1997; Radicella et al., 1997, Demple and Harrison, 1994; Takao et al., 1998) and it has been associated with human diseases such as cancer (Hollenbach et al., 1999; Audebert et al., 2000), neurological disorders and aging (Kisby et al., 1999, Lovell et al., 2000). Overexpression of Ogg1 is correlated with the repair of oxidative DNA damage (Chevillard et al., 1998; Hollenbach et al., 1999). The promoter region of the *OGG1* gene presents a CpG island and can be silenced by DNA methylation (Dhenaut et al., 2000).

Kin17 is a nuclear protein widely conserved during brain phylogeny (Araneda et al., 2001, Mermet et al., 1998). It shares an epitope with the RecA protein, which is known as a recombination-repair enzyme (Sancar et al., 1980) implied in the SOS inducible system of *Escherichia coli* (Little and Mount, 1982). Kin17 gene, located in human chromosome 2 (Angulo et al., 1989, 1991; Tissier et al., 1995), is up-regulated following the DNA damage

*Corresponding author. Tel: +33-472-43-12-21; fax: +33-472-43-11-72. E-mail address: araneda@univ-lyon1.fr (S. Araneda).

Abbreviations: BNST, bed nucleus of the stria terminalis; DAB-Ni, 3,3′-diaminobenzidine and nickel sulfate; EB, estradiol benzoate; ER, estrogen receptor; ERE, estrogen response element; GNS, goat normal serum; ICC, immunocytochemistry; ID, integrated density; Ogg1, 8-oxoguanine DNA glycosylase; OVX-30, 30-day ovariectomized rats; OVX-30+EB, ovariectomized rats+estradiol injection; PB, phosphate buffer; PVN, paraventricular nucleus.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.06.080

produced by UV-C or ionizing radiation (Biard et al., 1997; Kannouche et al., 1998). The Kin17 protein binds to curved DNA at a hot spot of illegitimate recombination in eukaryotic chromosomes (Millot et al., 1992; Mazin et al., 1994a,b) and can control gene expression by substitution of a bacterial transcriptional regulator called H-NS (Timchenko et al., 1996). Recently, Rappsilber et al. (2002), using proteomic analysis, reported that Kin17 is a component of the spliceosome, which forms part of the multicomponent RNA-protein complex (Kannouche and Angulo, 1999), suggesting a role in the maturation of primary RNA.

Estrogens control the expression of proteins through the regulation of target gene expression via estrogen receptors (ER) (Shughrue et al., 1992): the ER-α (Walter et al., 1985) and the ER-β (Kuiper et al., 1996). Both receptors interact with estrogen response elements (ERE) in DNA to initiate changes in RNA transcription (Loven et al., 2001; Hall et al., 2002; Hall and McDonnell, 1999; Sanchez et al., 2002). Both ER-α and ER-β mRNA and protein are distributed with different intensity in specific regions of the brain (Laflamme et al., 1998; Shughrue et al., 1997). The ER-β was observed preferentially in supraoptic, paraventricular (PVN), suprachiasmatic and tuberal hypothalamic nuclei, zona incerta and ventral tegmental area; the ER-α was detected in the ventromedial hypothalamus and the subfornical nuclei (Shughrue et al., 1997) and both forms of ER mRNA were observed in the bed nucleus of the stria terminalis (BNST), medial and cortical amygdala nuclei, preoptic area, lateral habenula, periaqueductal gray, parabrachial nuclei, locus coeruleus and nucleus of solitary tract.

A link has recently been reported between DNA repair protein and estradiol: 17B estradiol attenuates nucleotide excision repair in human keratinocytes (Evans et al., 2003). Cross-talk mechanisms between DNA repair proteins and ERs have also been described: BRCA1 protein, which is implicated in double-strand break repair, regulates the activity of ER-α (Fan et al., 2001); thymine-DNA glycosylase is a coactivator for ER-α (Chen et al., 1999); MMS19 factor, a human homologue of DNA repair TFIIH transcription factor is an specific AF-1 coactivator of ER-α (Wu et al., 2001); and a modified form of the human *O*(6)-methylguanine-DNA methyltransferase represses ER-α mediated transcription by inhibiting the binding of ER to its coactivators (Teo et al., 2001).

In order to study the effect of ovarian hormones on the expression of Ogg1 and Kin17 proteins in the rat brain, we performed an immunocytochemical quantification of immunolabeled nuclei in the PVN subdivisions and the BNST of four experimental groups: a) male rats, b) female rats in proestrous, c) 30 days after ovariectomy (OVX-30), and 4) ovariectomized rats having received a single estradiol injection (OVX-30+estradiol benzoate (EB)).

Our results indicate that, in specific brain regions, ovarian hormones can translocate and differentially regulate the expression of Ogg1 protein, while the expression of Kin17 protein does not seem to be affected.

EXPERIMENTAL PROCEDURES

Experimental animals

Female and male Sprague–Dawley rats weighing 200–300 g were used. Food and water were available *ad libitum* and rats were maintained on a 14-h light, 10-h dark schedule (lights on between 07:00 and 21:00 h). Three experimental groups of females were used: 1) Rats in estrous which were selected by vaginal smear examination of animals presenting at least four 4-day consecutive cycles. 2) OVX-30+EB group: Female rats (200–240 g) were bilaterally ovariectomized under deep chloral hydrate anesthesia (400 mg/kg body weight, i.p.). Thirty days after ovariectomy, groups of rats weighing 270–320 g were intramuscularly injected at 11:00 h with EB (10 μg/kg in corn oil, i.m.) and killed 28 h later. To assess estradiol plasma levels obtained after this EB treatment, a parallel group of ovariectomized rats was subjected to the same EB injection protocol. Twenty eight hours after the EB injection, estradiol plasma levels were 58.0±9.1 pg/ml (n=3). 3) Control OVX-30 rats: Female were bilaterally ovariectomized and 30 days after were injected with the vehicle (corn oil) and killed as above. The procedures involving the animals and their care were conducted in accord with institutional guidelines, which comply with the national and international laws and policy guidelines (French Decree No. 87848). The minimal numbers of animals were used to achieve statistical significance, and special attention was made to minimize animal suffering.

Brain fixation and sectioning

Animals were anesthetized (pentobarbital, 0.1 ml/100 g of weight, i.p.) and fixed by transcardial perfusion of phosphate buffer (PB 0.1 M; pH=7.4) containing 3% paraformaldehyde. Brains were dissected out and postfixed in the same fixative overnight. Then they were cryoprotected in PB containing 20% sucrose, frozen and stored at −80 °C. Thirty micrometer thick coronal sections were collected at the level of the BNST (0.4–0.2 mm rostro-caudal level from bregma (Paxinos and Watson, 1986) and of the PVN of the hypothalamus (1.8–1.2 mm rostro-caudal level from bregma) by using a cryostat (Reichert-Jung, Heilderberg, Germany).

Immunocytochemistry (ICC) and confocal analysis

Free-floating slices were H_2O_2 treated (0.3%, 20 min), rinsed and then pre-incubated with 2% goat normal serum (GNS, Dako, Copenhagen, Denmark) for 30 min. Ogg1 protein was detected by cross-reaction with rabbit anti-hOgg1 antibodies (Hollenbach et al., 1999), which were obtained against the whole Ogg1 protein. This primary antibody was diluted 1:4000 in PBS containing 0.2% Triton X100 (Sigma Chemical Co., St. Louis, MO, USA) and 2% GNS. Kin17 protein was immuno-detected by cross-reaction with rabbit anti-Kin17 antibodies (diluted 1:4000) (Miccoli et al., 2002).

After 3 days of incubation at 6 °C, the sections were treated with either biotinylated goat anti-rabbit immunoglobulin (1:1000 for 12 h) and avidin–biotin–HRP complex (1:1000 for 1 h, ABC reagent, Vector Laboratories, Burlingame, CA, USA) or Cy^{TM3}-conjugated affini-Pure goat anti-rabbit IgG secondary antibodies (Jackson Immuno Research Laboratories, Inc., USA) diluted 1:500 in PBS. In the case of peroxidase (HRP) reaction, a blue dark stain was obtained using 3,3′-diaminobenzidine and nickel sulfate (DAB-Ni). In order to perform quantification of the number of immunoreactive cells, the time of the DAB-Ni reaction was strictly controlled and the reaction was stopped using PB-Na azide 0.5%. The sections were mounted and viewed with a fluorescence or bright field illumination microscope (Leica, DMR, Wetzlar, Germany).

The immunostaining specificity of anti-Ogg1 or anti Kin17 antibodies was established in control brain sections by omitting the primary antibodies or by adsorption of Ogg1 or Kin17 antibod-