Ultrastructural analysis of estrogen receptor immunoreactive neurons in the medial preoptic area of the female rat brain*

Zs. Liposits1 **, I. Kalló1, C.W. Coen2, W.K. Paull3, and B. Flerkó1

1 Department of Anatomy, University Medical School, Szigeti u. 12, H-7643 Pécs, Hungary
2 Department of Anatomy and Human Biology, King's College, London, UK
3 Department of Anatomy and Neurobiology, University of Missouri-Columbia, Columbia, MO 65212, USA

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Summary. Neurons of the medial preoptic area were studied in the brain of the female rat by means of ultrastructural immunocytochemistry using a monoclonal antibody generated against purified estrogen receptor (ER), in order to delineate the morphological correlates of estrogen feedback mechanisms. In addition to the preoptic area, the bed nucleus of the stria terminalis, the arcuate and ventromedial nuclei of the hypothalamus exhibited an intense labelling for estrogen receptor. At the light microscopic level, the cell nuclei were immunoreactive. No major alterations were detected in the ER expression of medial preoptic neurons sampled during the estrous cycle, but proestrous rats did exhibit a slightly increased intensity of staining. At the ultrastructural level, the ER immunoreactivity was primarily confined to the nuclei and associated with the chromatin. Long term steroid deprivation elicited by either ovarectomy or ovariectomy plus adrenalectomy resulted in a marked intensity of nuclear labelling. This pattern was not influenced by acute estradiol replacement.

These morphological data indicate that neurons of the medial preoptic area have the capacity to detect estrogens via receptor mechanisms and that changes in the level of the circulating ligand are manifested in an alteration in the staining for the estrogen receptor. The study also supports the revised concept of estrogen receptor action by demonstrating the presence of receptors in the nuclei of the cells, whether or not they are occupied by their ligand.

Introduction

It had been postulated in one of our laboratories (Flerkó and Szentágothai 1957) that neuronal structures of the hypothalamus are capable of sensing estrogens. During the past 30 years, several lines of experimental data have emerged to support the view that estrogen has an action within the central nervous system. Steroid autoradiography performed following intravenous infusion of [3H]-estradiol revealed several loci within diencephalic and limbic structures of the brain that accumulated the tracer (Pfaff 1968; Stumpf 1968; Pfaff and Keiner 1973). The axonal projections (Morell and Pfaff 1982) and chemical identity (Heritage et al. 1977; Morell et al. 1985; Fuchs et al. 1984) of some of these steroid concentrating neurons have also been reported. Recently, monoclonal antibodies have been generated against the purified estrogen receptor (Greene et al. 1980; Monchamont and Parikh, 1984; Miller et al. 1982) and were used for immunomorphological characterization of structures that contain estrogen receptors in a variety of normal and pathological peripheral tissues (King and Greene 1984; Andersen et al. 1988; Tse and Goldfarb 1988; Soler and Aoki 1989). Estrogen receptor immunoreactivity has also been localized in different cell populations of the rat brain (Sar and Parikh 1986; Cintra et al. 1986).

From an endocrine point of view, the feedback sites for estrogens and the relationships of estrogen sensing neurons with other neuronal networks involved in the central regulation of gonadal function have been a focus of interest. Because luteinizing hormone-releasing hormone (LH-RH)-synthesizing neurons located within the preoptic area have been reported to lack estrogen binding capacity (Shivers et al. 1983), it is believed that other neurons, acting as transducing neurons, mediate the humoral signal of estrogens. The medial preoptic area is a region that binds estrogens and may relay the feedback actions of this hormone thus contributing to the induction of the surge release of luteinizing hormone (LH) (Goodman 1978a). Recent studies have emphasized the significance of these neurons in this function by demonstrating the suppression of spontaneous LH surges (Petersen and Barraclough 1988) and alterations in hypothalamic LHRH mRNA and LHRH content (Petersen et al. 1989) in estrogen treated ovariectomized rats that had received antiestrogen microimplants in the medial preoptic area.

The present work was primarily concerned with the ultrastructural characterization of the estrogen receptor-containing neurons of the medial preoptic area. One of the objectives in studying these cells has been to elucidate whether the ultrastructural localization of estrogen receptors after various endocrine manipulations would provide support for the "two-step" model of estrogen action (Jensen and DeSombrer 1973), or favor the theory of exclusive nuclear residence of both unoccupied and occupied receptors (Gorski et al. 1984; King and Greene 1984; Welshons et al. 1984).

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** To whom reprint requests should be sent
Materials and methods

Animals. This study was carried out on adult female Wistar rats (N = 40). Vaginal smears were taken daily and animals exhibiting only regular 4-day ovarian cycles were used. The rats were kept under standard environmental conditions (lights on from 05:00 h to 17:00, temperature 25 °C, relative humidity 60%, water and food ad libitum). They were divided into three main experimental groups: intact, ovariectomized (OVX) and both ovariectomized and adrenalectomized (OVX/ADX). Bilateral removal of the gonads and adrenal glands was performed 3 weeks prior to sacrifice under Nembutal anaesthesia (40 mg/100 g body wt. i.p.), using a dorsal approach. The drinking water of OVX/ADX animals was supplemented with 0.9% sodium chloride. Three rats from both the OVX and OVX/ADX groups were injected intraperitoneally with 17β-estradiol (1 µg/100 g body wt.) 1 h before fixation.

Fixation and section preparation. The animals were always anesthetized (Nembutal) at noon and perfused through the ascending aorta, first with 30–50 ml of cold phosphate buffered saline (PBS; pH: 7.4; 420 mmol) followed by 400–500 ml of buffered picric acid-formaldehyde solution (Zamboni and Martino 1967) containing 0.02% glutaraldehyde. After fixation the brains were immediately removed from the skull and 30-µm-thick coronal sections were cut from the prosencephalon on a Lancer vibratome. Brains prepared for ultrastructural examination, were post-fixed for additional 2 h in the same fixative.

Immunocytochemical labelling. Preembedding immunocytochemical techniques were used for the morphological detection of estrogen receptor (ER)-immunoreactive elements. For recognition of the estrogen receptor, a rat monoclonal antibody (H225p-g) raised against purified human estrogen receptors (Greene et al. 1980; Miller et al. 1982) was utilized (donation of Abbott Laboratories, Chicago, IL, USA). The sections were incubated for 36–48 h in the primary antibody (1:200 dilution, i.e. 11 μg/ml) and then for 1–2 h in both the bridging antibody (Biotin-SP-AffiniPure goat anti-rat IgG (H+L), 1:200 dilution; Jackson Immunoresearch Laboratories, West Grove, PA, USA) and the marker enzyme solution (Peroxidase conjugated Streptavidin, 1:100 dilution, Jackson Immunoresearch Laboratories, West Grove, PA, USA). The antigen-antibody complexes were visualized according to the method of Streit and Reubi (1977). In some instances, the diaminobenzidine chromogen was amplified by the silver-gold intensification technique of Gallays (1982), as previously reported for ultrastructural studies (Liposits et al. 1984).

The characterization of the monoclonal antibody against human estrogen receptor has been published elsewhere (Greene et al. 1980; Miller et al. 1982). Preabsorption of this ER-antibody with purified estrogen receptors has been shown to block the immunostaining (Cintra et al. 1986). Omission of the primary antibody from the staining protocol resulted in reaction-free sections. Sections prepared from animals exhibiting different stages of the estrus cycle were processed together in order to make comparable analysis. Most of the sections were mounted on glass slides, coverslipped and examined with a Nikon Biophot microscope. For ultrastructural analysis, the sections were osmicated and embedded in Epon resin. The ultrathin sections were placed on Formvar coated single resin. The ultrathin sections were placed on Formvar coated single

Results

In intact animals, estrogen receptor (ER)-immunoreactivity (IR) was intensely expressed in neurons located throughout the rostro-caudal extent of the medial preoptic area (Fig. 1a). The immunoreaction was associated with the cell nuclei of the neurons. Estrogen receptor positive loci were not present in the cytoplasm. The neighboring bed nucleus of the stria terminalis (BNST) also contained ER-IR cells (Fig. 1b). The dorso-lateral aspect of the preoptic area and the ventro-medial compartment of the BNST were “bridged” by a continuous population of ER-containing cells. Another prosencephalic locus of neurons that expressed estrogen receptor immunoreactivity was demonstrated in the arcuate nucleus (Fig. 1c). Similar to neurons of the preoptic nucleus, nuclear localization of the receptor was characteristic for this region. The ventromedial nucleus also contained a substantial number of ER-immunopositive neurons that were distributed mainly in its ventro-lateral compartment (Fig. 1d).

Sections of the preoptic area, obtained from female rats demonstrating various stages of the gonadal cycle and processed under the same conditions, indicated that receptor immunoreactivity is exhibited throughout the cycle. Samples taken from proestrous animals showed a slightly more intense staining (Fig. 1e) than those obtained from estrous (Fig. 1f) and diestrous (Fig. 1g) rats. Evidence for a cytoplasmic location of estrogen receptors was not found in any of these animals.

In order to amplify the sensitivity of the diaminobenzidine chromogen based immunocytochemical technique, silver-gold postintensification was carried out on the immunolabelled sections that were processed for ultrastructural examination. Use of this sensitive technique failed to detect a significant cytoplasmic distribution of the estrogen receptor in neurons of the preoptic area. An exclusive nuclear localization of ER was observed in both thick vibratome (Fig. 2a and b) and semithin (Fig. 2c) sections. In accord with the light microscopic observations, the silver-gold intensified diaminobenzidine chromogen was confined to the cell nuclei showing a homogenous distribution throughout the karyoplasm (Fig. 2d). At higher power receptor immunopositive loci were seen associated with the nuclear chromatin (Fig. 2e). Occasionally, a small number of antigenic sites were detected in the perinuclear cytoplasm. These were, however, negligible compared to those in the nucleus.

In both the chronic ovariectomized (OVX) (Fig. 3a) and ovariectomized plus adrenalectomized (OVX/ADX) (Fig. 3d) animal groups, the estrogen receptor was localized in the cell nuclei. The labelling was more intense than that observed in intact control female rats at any stage of the cycle. The acute administration of estradiol at a dose of 1 µg/100 g body wt., 1 h before sacrifice, failed to alter either the pattern or intensity of the staining (Fig. 3b and 3c).

Fig. 1a-g. Expression of estrogen receptor (ER) immunoreactivity in different prosencephalic regions of the intact female rat brain. a Medium power photomicrograph demonstrates intensely labelled neurons located in the medial preoptic area (MPOA). × 70. b Scattered ER-immunopositive cells in the bed nucleus of the stria terminalis. The anterior commissure (CA) is indicated by dashed line. × 140. c Association of ER-immunoreactivity with the cell nuclei of neurons in the arcuate nucleus. × 280. d A cluster of estrogen receptor-IR neurons located in the ventro-lateral part of the ventromedial nucleus. × 70. e-g Identical regions of the medial preoptic area obtained from proestrous (e), estrous (f) and diestrous (g) animals and immunoostained for ER immunoreactivity under standardized conditions. Note, the higher density of estrogen receptor expressing neurons and slightly increased staining intensity in the proestrous rat. e × 70; f × 70; g × 70