Evidence for the Colocalization of Estrogen Receptor-β mRNA and Estrogen Receptor-α Immunoreactivity in Neurons of the Rat Forebrain

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Abstract Estrogen receptor β (ERβ) mRNA is expressed in several rat brain regions where ERα is abundant. In vitro studies have shown that ERα and ERβ can heterodimerize and that the activity of this complex may be different than an ERα or ERβ homodimeric complex. The purpose of the present study was to ascertain if ERα and ERβ are co-expressed by certain neuronal populations using a double label in situ hybridization/immunocytochemistry method. The results revealed that neurons in the bed nucleus of the stria terminalis, medial amygdala and preoptic area contain both ERs, with the vast majority of the neurons being double labeled. In other brain regions including the arcuate nucleus, cortical amygdaloid nucleus and ventromedial nucleus, only a few double-labeled cells were detected, while neurons in the paraventricular nucleus, supraoptic nucleus, and cerebral cortex expressed only ERβ mRNA. The results of these double label experiments provide the first evidence that ERα and ERβ coexist in neurons under in vivo conditions and suggest that estrogens may differentially modulate the activity of certain neuronal populations depending on whether the cells expresses ERα, ERβ or both ERs.

Introduction

Estrogen receptor-β (ERβ), a novel nuclear estrogen receptor, was recently isolated from the rat prostate and shown to have a high degree of sequence homology with ER (ERα, 1). In vitro studies have shown that the efficacy and affinity of estradiol for ERα and ERβ are comparable and that estrogens were capable of activating gene transcription in cells transfected with ERβ and an estrogen response element-reporter construct (1,2). Interestingly, Puech et al., (3) showed that under certain in vitro condition some estrogens behave differently on the two ERs. This observation and the finding that the two ERs heterodimerize (4,5) suggests that the activity of estrogenic compounds may depend, in part, on whether a cell contains ERα, ERβ or both ERs. ERβ mRNA has been detected in a variety of tissues including the prostate, ovary, pituitary and brain (6, 7). In situ hybridization histochemistry studies have demonstrated that ERβ mRNA is expressed in specific regions of the rodent brain, areas where ERα is expressed as well as areas where ERα is sparse or absent (8). In particular, ERα and ERβ mRNA are both abundant and share similar topographical distribution in the preoptic area, bed nucleus of the stria terminals, and medial amygdala (8). The purpose of the present studies was to ascertain whether ERβ mRNA and ERα protein are localized within the same neurons using a double label in situ hybridization/immunocytochemistry technique.

Materials and Methods

Sixty-day-old Sprague-Dawley rats (n=5), housed in the Wyeth-Ayerst animal care facility (AAALAC certified) with a 12-h light/ dark photoperiod and free access to tap water and food, were ovariectomized for 7 days, deeply anesthetized and transcardially perfused with 1% and then 45% paraformaldehyde. Vibratome sections (35μm) from the hypothalamus (Bregma -0.25 - -4.3) were collected in PBS containing 300U/ml of Heparin, transferred to mesh baskets (Ted Pella, cat. #4592), rinsed in PBS and then processed for in situ hybridization (8) with the dehydration step ending in PBS. The processed sections were then transferred to 24-well cell culture plates (2-3 sections/ well) containing 300μl of the probe (a cocktail of 35S-UTP-labeled ERβ-285/ ERβ-558; 4x106 DPM/ probe/ well; see 8) -50% formamide hybridization mix and incubated overnight at 55°C. The sections were then transferred back to the baskets, rinsed (2xSSC/ 10mM Dithiothreitol), treated with RNase A (20μg/ml) and washed at 67°C in 0.1xSSC to remove nonspecific label. The sections were then transferred to PBS, treated with 10% normal donkey serum and incubated overnight at 4°C with a rabbit polyclonal ERα antisera (FMS-ER7; raised in rabbit against the last 21 aa of ERα; 13ng/ml). The sections were washed, incubated with biotinolized donkey anti-rabbit serum (Jackson; 1:1500) and the immunoreactivity visualized with a standard ABC method. After the DAB reaction, sections were mounted on slides and dipped in NTB2 nuclear emulsion. The slides were exposed for 2-4 weeks, photographically processed and coverslipped. The percentage of cells containing ERα, ERβ or both ERs was determined from 5 sections from each region evaluated.

Results and Discussion

The results of these studies have demonstrated that ERβ mRNA is colocalized with ERα in specific regions of the female rat brain (Table 1). The highest degree of colocalization was seen in the posterior subdivisions of the bed nucleus of the stria terminalis (BNST; Figs. 1A-C), medial nucleus of the amygdala (Me; Fig. 2A) and periventricular preoptic nucleus (Pe; Fig. 1E). In these

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Figure 1: Representative photomicrographs depicting the localization of ERβ mRNA (grains) and ERα immunoreactivity (gray nuclei) in the rat bed nucleus of the stria terminalis (A-C) and medial preoptic area (A-B,D-E) by *in situ* hybridization and immunocytochemistry. Note the similar topography of ERα staining (A) and ERβ mRNA (B) in the bed nucleus/preoptic area of the brain and colocalization of the ERs in many of the neurons in these regions (C,E). Abbreviations: BNST; bed nucleus of the stria terminalis; LV, lateral ventricle; POA, medial preoptic area. Asterisks indicate third ventricle.
Figure 2: Representative photomicrographs depicting the localization of ERβ mRNA (grains) and ERα immunoreactivity (gray nuclei) in the rat medial amygdala (A) and arcuate (B,C) and ventromedial (B, D) nuclei of the hypothalamus by in situ hybridization and immunocytochemistry. Note the high degree of colocalization of the ERs in the amygdala (A), while only a few of the ERα containing neurons in the arcuate and ventromedial nuclei (B) contain ERβ mRNA (C-D; arrows). Abbreviations: ARC, arcuate nucleus; ot, optic tract; VMN, ventromedial nucleus. Asterisks indicate third ventricle.
regions, the majority of cells contain both ERs, although a number of cells clearly express only ERα or ERβ. In the bed nucleus of the stria terminalis, most single labeled cells contained ERβ mRNA only (Fig. 1C), while in the periventricular preoptic nucleus (Figs. 1D-E) and medial amygdala (Fig. 2A), ERα-immunoreactive neurons were more abundant. Double-labeled cells were also seen throughout the rostral (MPNr, Fig. 1D) to caudal (MPNe, Fig. 1A) extent of the medial preoptic nucleus and area, but the number of cells that express only ERα was equal to or greater than the number of cells co-expressing both ERs. There was also a tendency for the number of double-labeled cells to decrease at the more caudal preoptic levels. In addition, a few double-labeled cells were also detected in the other subnuclei of the bed nucleus of the stria terminalis, arcuate (Fig. 2B-C) and caudal ventromedial (Figs. 2B-D) nuclei of the hypothalamus and cortical amygdaloid nuclei. Only ERα was detected in the periventricular nucleus and subformical organ, while ERβ mRNA was exclusively expressed in the paraventricular and supraoptic nuclei and cerebral cortex. A previous study compared the distribution of ERα and ERβ mRNA in adjacent sections from the rat brain and showed that in several brain regions, such as the preoptic area, bed nucleus of the stria terminalis and medial amygdala, both the number and distribution of labeled cells were very similar (8). The results of the present studies have demonstrated that ERα and ERβ are co-expressed in neurons that are restricted to a few regions of the rat brain. The posterior subdivisions of bed nucleus of the stria terminalis and medial amygdala had the highest degree of co-expression, which may reflect their similar ontogeny and anatomical structure. Numerous double labeled cells were also seen in the periventricular preoptic area, medial preoptic nucleus and preoptic area. The restricted distribution of cells that express both ERs suggests that ERβ is not merely a backup receptor for ERα. In fact, the presence of both ERs may enable the cell to respond differently to estrogen action when compared with cells that express ERα or ERβ alone. For example, a previous in situ hybridization study showed that the levels of ERα mRNA change over the estrous cycle, with the pattern of change being different in the preoptic area as compared with the arcuate and ventromedial nuclei (9). Since ERα and ERβ are both expressed in the preoptic area, including many of the same neurons and ERβ is sparse in the arcuate and ventromedial nuclei, the differential regulation of ERα mRNA may reflect this difference in ER distribution.

In vitro studies have shown that ERα and ERβ are capable of forming both homodimers and heterodimers (4,5). This observation and the finding that the activity of the ERs may depend on the ligand and DNA response element (3), indicates that the activity of an ER heterodimer complex may be completely different than either homodimer. In vivo, the physiological relevance of an ER heterodimer complex may be limited, since ERα and ERβ are generally localized in different brain regions and in different peripheral tissues (6,7). However, in tissues such as the bone, pituitary and in certain brain regions where both ERs are present, the putative formation of heterodimers may have a profound effect on the cellular response to 17β-estradiol and provide a substrate for the actions of tissue-selective estrogens.

References
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