REVIEW

Estrogen receptor β – a new dimension in estrogen mechanism of action

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Introduction

The cloning of estrogen receptor β (ERβ) has provided the first example of a steroid hormone receptor existing as two isoforms, each of which is encoded by a separate gene (Kuiper *et al.* 1996). The finding of ERβ was met with great surprise, as the existence of more than one estrogen receptor had been actively denied for several decades. Attempts to clone a second estrogen receptor on the basis of sequence homology to the classical estrogen receptor (ERα) were not successful, although this approach did lead to the identification of two orphan receptors, namely estrogen receptor-related receptors (ERR) 1 and 2 (Giguere *et al.* 1988). However, none of these ER-related orphan receptors binds estrogens and it is still unclear whether they require specific ligands in order to be activated.

ERβ seems to be a most important factor in the mechanism of action of estrogen, and it is expressed in many tissues, including the central nervous system, the cardiovascular system, the immune system, the urogenital tract, the gastrointestinal tract, the kidneys and the lungs (Arts *et al.* 1997, Enmark *et al.* 1997, Kuiper *et al.* 1997, 1998*a*, *c*, Lindner *et al.* 1998, Österlund *et al.* 1998). Although ERβ is also expressed in the mammary gland, it appears that ERα is an important estrogen receptor in this particular tissue, and in the uterus also there seems to be much more ERα present than ERβ (Fig. 1). This dominant role of ERα in the uterus probably explains why it was the first cloned estrogen receptor, as most purification and cloning attempts were based on uterine tissue. As will be referred to below, it is now obvious that ERβ plays an important role in the physiology of several tissues, and it cannot be excluded that it is the more generally expressed estrogen receptor, whereas ERα dominates in some few specific tissues and is mainly involved in reproductive events. Obviously, these differences in tissue distribution are of extreme importance from the pharmaceutical point of view, as hormone replacement therapy in postmenopausal women is such an increasingly significant health issue.

Although the DNA-binding domains of ERα and ERβ show a high degree of homology (only three amino acids differ), the ligand-binding domain shows only 59% homology (Fig. 2). Closer inspection indicates that it should be possible to develop ERα- and ERβ-specific ligands. In view of the facts presented above, it is not unreasonable to assume that it might be possible to develop ERβ-specific estrogen agonists targeting the central nervous system, the urogenital tract, the cardiovascular system and bone, but leaving the mammary gland and uterus relatively unaffected. In this way, much of the current controversy concerning estrogen treatment of postmenopausal women might be resolved (Barkhem *et al.* 1998, Gustafsson 1998*a*,*b*, Kuiper *et al.* 1998*b*, Nilsson *et al.* 1998).

Biological roles of estrogen receptors

ERβ is localized on human chromosome 14, in contrast to ERα, which sits on chromosome 6 (Enmark *et al.* 1997). ERα and ERβ thus represent two separate gene products and share a relationship to one another that is similar to those between, for instance, the glucocorticoid receptor and the mineralocorticoid receptor, or the glucocorticoid receptor and the progesterone receptor, which show a homology between their ligand-binding domains that corresponds to those of ERα and ERβ.

It appears quite clear today that ERβ has biological roles that are distinct from those of ERα. Recently, knock-out mice deficient in ERβ (BERKOs) have been produced and, as will be discussed more in detail below, these animals show a quite distinct phenotype when compared with that of ERα −/− mice (Krege *et al.* 1998). Furthermore, examples are accumulating of different respective modes of regulation of ERα and ERβ. For instance, in vascular tissue, where both ERα and ERβ appear to be expressed, experimental denudation involving removal of the endothelial cell layer leading to smooth muscle cell proliferation is accompanied by a huge increase (up to 80-fold) of ERβ expression in smooth muscle cells and endothelial cells, whereas the expression of ERα in these cells is unaffected (Lindner *et al.* 1998). In this particular case, it may be speculated that ERβ mediates

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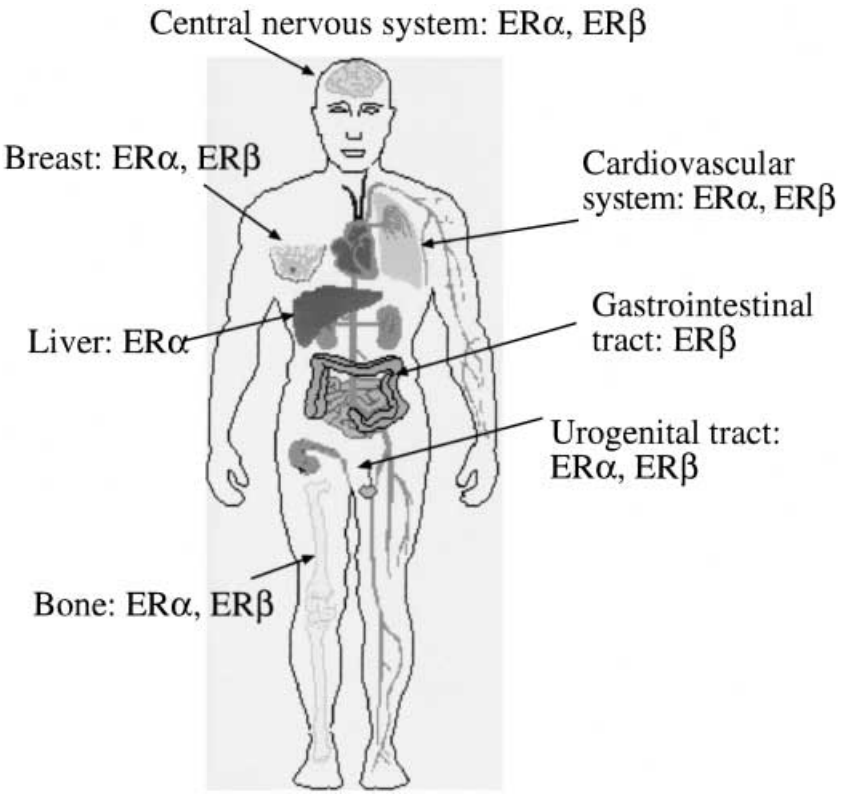

Figure 1 Overall distribution of ERα and ERβ in different tissues.

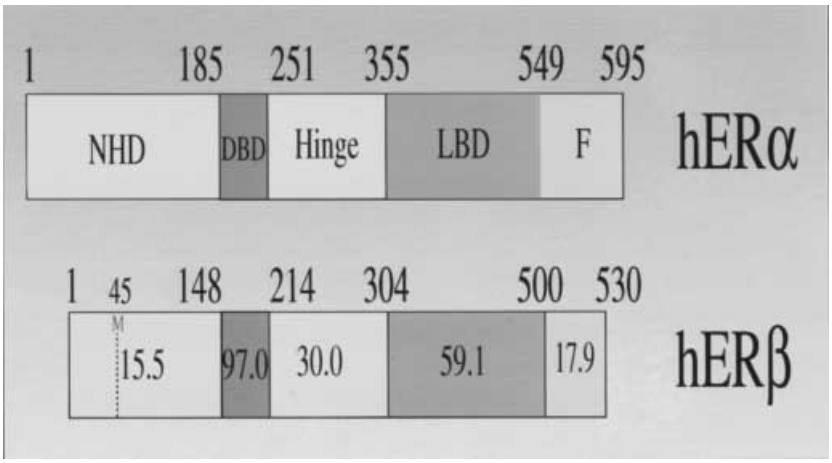

Figure 2 Comparison of the primary structures of ERα and ERβ, respectively. The figures above the receptor representations indicate the number of amino acids, with number 1 being the most N-terminal. The numbers within the ERβ receptor represent the degree of homology (%) between respective domains in the two receptors.

the protective effect of estrogens on vascular lesions involving inhibition of smooth muscle cell proliferation. These results are in good agreement with previous experiments on ERα −/− mice showing that the vascular protective effect of estrogens in experimental vascular lesions is unchanged despite the absence of ERα (Iafrati *et al.* 1997). Needless to say, it will be very exciting to perform similar experiments on the ERβ −/− mice to investigate further the possible vascular protective role of ERβ.

ERβ in cancer

Another example of differential regulation of ERβ and ERα is seen after neonatal estrogenization of male rats. This treatment, which is known to result in greater susceptibility to estrogen-induced carcinogenesis of the urogenital tract in rodents, leads to increased expression of ERα, but decreased expression of ERβ, in ventral prostates from adult animals (Prins *et al.* 1998). This opposite mode of regulation might possibly indicate that the ratio of ERα to ERβ might be of importance in determining the susceptibility of a tissue to estrogen-induced carcinogenesis. If so, this would be another example of ERβ exerting a protective role against disease. A third such example might possibly be afforded by ERβ in colonic tissue: several recent epidemiological reports seem to indicate that estrogens given to postmenopausal women might protect against cancer of the colon (Franceschi & La Vecchia 1999).

One might ask what is the mechanism behind this alleged protective effect of ERβ against cell proliferation and carcinogenesis. One possible clue is that ERβ has been found to be involved in mediating tamoxifen induction of quinone reductase in MCF-7 breast cancer cells, via its interaction with an antioxidant response element in the 5′ flank of the quinone reductase gene (Montano *et al.* 1998). These results are in good agreement with earlier data from our laboratory showing that estradiol antagonists such as tamoxifen actually work as agonists via an AP-1 element (which has a certain resemblance to an antioxidant response element) in front of a reporter gene (Paech *et al.* 1997). Taken together with information in the literature that both tamoxifen and certain estrogens such as phytoestrogens may work as antioxidants, these results might indicate that ERβ is involved in the control of antioxidant-regulated genes, the products of which are known to control the concentrations of free radicals and reactive oxygen in the cell. Future experiments will obviously be necessary to determine whether this notion is valid or not. Interestingly, it was recently reported (Hu *et al.* 1998) that expression of ERβ could be induced in chemical-carcinogen-transformed human breast epithelial cells, the more transformed cells showing greater levels of ERβ expression. Obviously, these results could be interpreted in at least two ways: either in support of the above notion that ERβ represents a cellular defense mechanism against proliferation, or indicating the ERβ may contribute to the initiation and progression of neoplastic transformation. Again, future experiments should clarify this issue.

ERβ in the urogenital tract

Another tissue with a predominant expression of ERβ is the urogenital tract. Both the transitional epithelium in the bladder, and the epithelium in the urethera, the seminal vesicles, the prostate and the kidney pelvis express significant quantities of ERβ mRNA (Hess *et al.* 1997, Sharpe 1998). The expression of ERα in these tissues is much less pronounced, and it may be speculated that the alleviating effect of hormone replacement therapy on urinary incontinence in women might be mediated via ERβ. Not only urinary tract epithelium, but also smooth muscle cells, and nerve cells in local ganglia, contain ERβ (unpublished observations), and the micturition function in rodents is known to be under the influence of estrogen. Again, these ideas are open to experimentation using the recently generated ERβ − / − mouse model.

Central nervous system

In the central nervous system, estrogens are well known to cause a plethora of effects, and it is obvious that estrogen signaling in the brain is of paramount importance for control of reproduction, including sexual behavior. In collaboration with Istvan Merchenthaler's laboratory at Wyeth-Ayerst, we have reported that ERβ is the major estrogen receptor in the olfactory lobe, cortex and cerebellum (Kuiper *et al.* 1998*c*). In the hypothalamus, both ERα and ERβ are expressed, and in some cases they appear to be present in one and the same cell. Under these conditions, ERα and ERβ are known to heterodimerize (Pettersson *et al.* 1997), and it cannot be excluded that the ERα–ERβ heterodimer has different biological effects than the respective homodimers. In the hippocampus, ERβ seems to be the major ER expressed. On the basis of these findings, it may be concluded that ERβ is of potentially great significance in brain function, and it will be of particular interest to study the ERβ − / − mice from the point of view of behavior, not only in the context of reproduction, but also with reference to, for example, cognition and memory.

Xenoestrogens and ERβ

As alluded to above, phytoestrogens appear to have a greater affinity for ERβ than for ERα (Kuiper *et al.* 1998*b*). We have also been interested in studying the relationship between other xenoestrogens and ERβ, namely estrogenic endocrine disruptors, which currently attract a great deal of interest both from the scientific community and from the public. Examples of such environmental contaminants are hydroxylated metabolites of polychlorinated biphenyls (PCBs), methoxychlor, biphenol, and many others. Using both *in vitro* receptor-binding studies with recombinant ERα and ERβ, cellular assays with stably transfected ERα or ERβ, and ER-dependent reporter genes, we were able to show that ERβ has about the same affinity for these chemical agents as ERα (Kuiper *et al.* 1998*b*). The reason why ERβ may be of particular significance in this context is that it appears to

be expressed at significant levels in testis, more specifically in gonocytes, spermatogonia and spermatocytes. Lower levels are also found in Sertoli cells. ERα, although also expressed in the testis, does not appear to be expressed in spermatocytes to the same extent as ERβ; this preferential expression of ERβ in spermatocytes of various developmental stages may suggest that endocrine disruptors with an affinity for ERβ might find their way into precursors of sperm and cause disturbances in their function (Saunders *et al.* 1998, our unpublished observations).

Molecular mechanisms of action

Another line of research in our laboratory is to compare the molecular mechanisms of action of ERα and ERβ. The two receptor isoforms show quite significant differences in their N-terminal domains, and it is to be expected that the two receptors might interact with different sets of proteins. It has become apparent that many of the nuclear receptor coregulators interacting with the AF-2 domain of various receptors interact equally well with ERα and ERβ. Examples of such coregulators are RIP 140, TIF-2, SRC-1 and SHP (short heterodimer partner) (Seol *et al.* 1998, Johansson *et al.* 1999, our unpublished observations). In contrast, very recent data seem to suggest that the novel coregulator, TRAP 220, shows significant differences in its interactions with ERα and ERβ (our unpublished observations). We are currently attempting to understand the biological implications of this interesting difference.

The BERKO mouse

As mentioned above, we have recently managed to develop mice with a deleted ERβ gene (Krege *et al.* 1998). This breakthrough makes it possible to study various biological roles of ERβ *in vivo*. The most obvious phenotype of the so-called BERKOs is a significantly reduced fertility in female $-/-$ mice, resulting in a reduced number of litters that are also of smaller size than usual. The ovaries show signs of follicular arrest and anovulation, in addition to increased growth of stroma. It is obvious that ERβ is important for normally functioning ovaries. These findings are in excellent agreement with the very significant expression of ERβ at both the mRNA and protein levels in the granulosa cells of ovarian follicles. At the beginning of the estrus cycle in particular, ERβ expression is high but, after the LH surge, ERβ is rapidly downregulated (Byers *et al.* 1997). It can also be shown *in vitro* that gonadtropins downregulate ERβ in cultured primary granulosa cells. The ratio between ERα and ERβ in the ovary is about 1 : 9.

Another quite conspicuous phenotypic characteristic of BERKO animals of both sexes is that the bladder epithelium, the epithelium of the dorsal prostate, the coagulation glands and the urethra show signs of hyperproliferation, with large variations in nuclear size and multilayered epithelial cells. In the prostate, the changes are somewhat reminiscent of benign prostatic hyperplasia in man (our unpublished observations). It would therefore seem as if growth control of these tissues is impaired, and it may thus be inferred that ERβ is important for growth control of the epithelium in the urogenital tract. Interestingly, this concept complements our suggestion, put forward above, that ERβ may have some kind of protective role against hyperproliferation and carcinogenesis.

We are currently continuing our exciting characterization of the phenotypic characteristics of BERKO animals, with particular reference to the cardiovascular system, bone, the immune system, sexually differentiated liver metabolism, and reproductive and non-reproductive behavior.

Conclusion

In conclusion, ERβ appears to have many important biological roles in the context of estrogen action, and future studies will show whether our hypothesis that it may have an important role in protection against hyperproliferation and carcinogenesis is of relevance. If this is the case, ERβ might be of paramount importance in, for instance, control of the growth of breast cancer.

Summary

Cloning of the novel estrogen receptor β (ERβ) has led to a paradigm shift in our understanding of estrogen action. The concept of a second estrogen receptor with somewhat different ligand binding specificity than the previously known receptor (ERα) opens up the possibility for development of novel estrogenic drugs that might distinguish between the two receptors. This could be of importance in treatment of various diseases, as the two receptors seem to be differentially expressed in several tissues. ERβ appears to be very important in the central nervous system, bone, lung, urogenital tract, cardiovascular system, ovary, testis, kidney and colon. The physiological function of ERβ is under intense study but certain results indicate that ERα and ERβ have different or even opposite biological actions.

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