Role of ligand in oestrogen-receptor function

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Introduction

Oestrogens are able to regulate gene expression by binding to specific receptors that function as transcription factors [1-3]. Hormone binding is required initially for the dissociation of an inactive, oligomeric, receptor complex to allow receptor dimerization and high-affinity DNA binding [4, 5] and, secondly, to produce the full transcriptional activity of the receptor [6-9]. In view of the role of oestrogens as mitogens in certain types of cells and the observation that approximately 30% of breast cancer can be treated with endocrine therapy, a large number of anti-oestrogens have been developed as potential drugs. To date these anti-oestrogens appear to fall into two groups. One group, which includes the non-steroidal anti-oestrogen tamoxifen, also act as partial agonists while another group, which includes ICI 164384, appear to act predominantly as 'pure' anti-oestrogens [10, 11]. The molecular basis for the action of different ligands is now beginning to emerge and is the subject of this review.

Structure of the oestrogen receptor

The oestrogen receptor, in all species, has a molecular mass of approximately 66,000, with 595 amino acids in the human protein. The receptor is organized into three distinct structural domains on the basis of protease-digestion experiments and studies of chimeric proteins (Fig. 1). The hormone-binding domain comprises 250 amino acids of the C-terminal half of the receptor which is presumed to form a hydrophobic ligand-binding pocket [6]. The hormone-binding domain is also responsible for two other functions, protein dimerization and a transcriptional activation function (TAF) [5-9, 12]. The DNA-binding domain contains about 100 amino acids comprising two zinc ‘finger’ motifs which are responsible for target-gene specificity and high-affinity DNA binding [6]. In addition, this domain contains a basic region shown to be responsible for nuclear localization of the receptor [13]. The N-terminal domain contains sequences which are involved in a second TAF-function [6].

Role of oestrogen in receptor activation

The oestrogen receptor, as with other steroid receptors, is activated as a transcription factor by hormone binding. In the absence of steroid the oestrogen receptor exists as an inactive oligomeric complex containing the heat-shock protein hsp 90, which appears to inhibit DNA binding [4]. Oestrogen binding results in dissociation of the complex thereby allowing receptor dimerization and high-affinity DNA binding [5]. A region of the mouse oestrogen receptor has been identified which is essential for oestrogen binding and overlaps, but is not coincident with, a region involved in dimerization [12]. The importance of this region for hormone binding is supported by the observation that cysteine 530 in the human oestrogen receptor is covalently labelled by both the oestrogen, ketononestrol aziridine, and the anti-oestrogen, tamoxifen aziridine, indicating that this residue is in close proximity to the ligand-binding pocket [14]. The structure of the region is not known, but the mutagenesis data suggest that it does not resemble a leucine zipper or coiled-coil structure, or form part of a helix-loop-helix structure found in other dimeric transcription factors [15].

The oestrogen receptor binds to specific DNA sequences, termed oestrogen-response elements, which consist of inverted repeats of the sequence TGACC separated by three base pairs. The elements function as typical transcriptional enhancers with perfect inverted repeats being most active. The structure of the DNA-binding domain has been determined by n.m.r. spectroscopy [16]. The two zinc-finger motifs are found to be folded into a single domain. Amino acids near the first zinc coordination site, which are responsible for discrimi-
Functions associated with oestrogen-receptor action

Solid bars represent regions of the receptor responsible for a number of functions. Differences in the transcriptional activity of agonists, partial agonists and 'pure' antagonists are designated by a (+) or (−) and refer to information described in [21, 22].

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<th>TAF-1</th>
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- Nuclear localization
- Steroid binding
- Receptor dimerization
- DNA binding
- Transcriptional activation

Agonists e.g. oestradiol
Partial agonists e.g. tamoxifen
'Pure antagonists' e.g. ICI 164 384

Mechanisms of action of hormone antagonists

A group of anti-oestrogens including tamoxifen, that also act as agonists, appear to promote dimerization and high-affinity DNA binding, but fail to induce the formation of TAF-2 in the hormone-binding domain [7, 9; Fig. 1]. Chambon and his coworkers have proposed that the agonist effect of tamoxifen is derived from TAF-1 in the N-terminal domain which is constitutively active [21]. The potency of tamoxifen as an antagonist or as an agonist probably reflects the relative contributions of TAF-1 and TAF-2 to promoter activity. Accordingly, in cells where the responsive promoter depends primarily on TAF-2 tamoxifen would be an antagonist, whereas where the promoter depends on TAF-1 activity tamoxifen could be an agonist. ‘Pure anti-oestrogens’ such as ICI 164 384, on the other hand, seem to inhibit receptor dimerization [22]. This inhibition is probably caused by steric interference produced by the large 7α-alkylamide extension present in ICI 164 384 [23] since we have shown that the steroid-binding pocket is at or near the dimer interface [12]. We have now shown that ICI 164 384 markedly reduces the intracellular content of the oestrogen receptor by reducing its half-life and propose that this results from impaired receptor dimerization. As a consequence it appears that neither TAF-1 or TAF-2 are functional (Fig. 1).

Concluding remarks

Tamoxifen is currently used successfully for the treatment of hormone-dependent breast cancer, with approximately 30% of patients responding to treatment [10]. Since tamoxifen is not only an oestrogen antagonist but also a partial agonist, it is conceivable that a pure anti-oestrogen might be more effective. The initial choice of treatment might still be tamoxifen however, since few side-effects have been observed with the drug and pure anti-oestrogens might adversely affect conditions such...
as osteoporosis. Nevertheless, in time, tamoxifen-resistant tumours often arise in patients and some of these could benefit from treatment with an anti-oestrogen, the mechanism of action of which is different from that of tamoxifen. It is important to establish whether other anti-oestrogens function by one or other of the mechanisms suggested for tamoxifen and ICI 164384 or by another novel means. Clearly, a better understanding of the mechanism of hormone antagonists is likely to lead to more efficacious treatment not only of breast cancer but also other endocrine disorders.

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**Development of novel oestrogen-receptor antagonists**

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The discovery of non-steroidal anti-oestrogens stemmed from observations, dating back more than 50 years, that simple molecules like triphenylchloroethylene have stimulatory effects in classical bioassays of oestrogenic activity, for example the capacity to induce vaginal cornification and uterine growth in immature or ovariectomized rodents. Subsequently, it was shown that addition of a basic-ether side-chain to the triphenylethylene nucleus produced compounds, like clomiphene and tamoxifen, with anti-fertility activity. Agents of this kind were of great interest because of their potential use as contraceptives, and also for the treatment of malignant and benign diseases dependent on oestrogenic stimulation. Clinical studies failed to confirm useful contraceptive activity, but opened up other therapeutic applications including the treatment of breast cancer.

Clomiphene and tamoxifen were shown to have mixed oestrogen-agonist and antagonist activity. Thus, in the immature-rat-uterus bioassay, tamoxifen stimulates growth, but to a lower maximum extent than the natural hormone 17β-oestradiol; this is a classical partial-agonist effect. Correspondingly, when tamoxifen is administered together with 17β-oestradiol the uterotrophic action of the natural hormone is reduced in a dose-dependent manner, demonstrating anti-oestrogenic activity. The maximum antagonist effect in this assay is limited by the intrinsic agonist activity of...