The Search for a Gamma-Emitting Estrogenic Ligand

Despite nearly two decades of research since the presence of steroid receptors was first demonstrated, investigators have only recently recorded substantial progress in receptor purification methods and in understanding their fundamental molecular biology. Microgram quantities of the estrogen receptor have been purified both by conventional methods and by affinity chromatography and have been used to find that its molecular weight is about 200,000 and that it binds estrogens stereospecifically and with very high affinity but noncovalently. The initial step in the interaction of hormonal steroids with target tissues is the binding of hormone to soluble cytoplasmic estrogen receptors. This initial ligand-receptor complex then undergoes an "activation" process that transforms the complex to a species that translocates into the nucleus, becomes bound to chromatin, and causes increased transcription of DNA and resulting changes in cellular protein synthesis. Thus, the effect of the hormone on the cell is manifested.

What tissues contain receptors for steroid hormones, most particularly estrogen receptors? Initial tracer studies with [^{3}H]-estradiol by Jensen identified two different types of tissue uptake (*1,2*). In nontarget tissues, such as lung, stomach, spleen, and muscle, the maximum uptake occurred within a few minutes and was followed by a rapid decline. In contrast, hormonally responsive tissues, such as uterus, vagina, and pituitary (*3*), continued to incorporate tracer without significant washout for up to 5 hr and contain estrogen binding protein (also recently identified in the islets of the pancreas (*4*)).

Moderate success in imaging the adrenal cortex (*5*) and the prostate (*6*) has been achieved with gamma-emitting estrogens, and it js possible they may be the key to a better pancreatic scanning agent. The medical imaging application of labeled estrogens that has received the most attention, however, involves their use in imaging breast tumors. This approach is attractive because there are relatively few estrogen receptors in healthy breast tissue, but they are abundant in about half of the primary breast cancers (*7*). The in vitro measurement of estrogen content of breast tumor biopsy specimens has proven helpful in selecting treatment. In one study of endocrine therapy, a 73% remission rate was reported for patients whose breast cancers contained high concentrations of estrogen receptors, whereas only a 2% remission was observed in patients whose tumors lacked receptors (*7*). Thus a gamma-emitting estrogen could be useful in the scintigraphic detection of breast tumors and their metastases and in the prediction of the response of breast cancer to hormonal therapy. Although such a radiopharmaceutical would not replace current screening methods, if it detected lymph node involvement reliably, it could guide in the selection of the appropriate extent of surgery.

For 10 yr John Katzenellenbogen has been studying the basic chemistry of estrogen receptors by photoaffinity labeling, a technique for covalently labeling the binding sites of receptor proteins. Because of technical difficulties of aggregation and lability associated with purifying receptor proteins, it is often advantageous to label covalently specific binding sites using his technique in unpurified preparations. Such studies are more easily ascribed in vivo significance because the receptors are, of necessity, "impure." In contrast, in vitro competitive binding experiments involve some purification of the estrogen receptor. In this issue of the *Journal,* Katzenellenbogen (*8*) applies his experience in tracer investigations of estrogenic ligands to the problem of imaging receptor populations. His investigations are part of continuing research that involves many laboratories in the attempt to identify the ideal gamma-emitting estrogenic ligand (*5,6,9–12*).

There are several physiochemical properties that are necessary in molecules that bind to estrogen receptors. The word estrogen is generic for any substance, natural or synthetic, that exerts biological effects characteristic of the female sex hormone. The naturally occurring steroids are formed in the placenta, ovary, testes, and adrenal cortex from cholesterol through an intermediary, pregnenolone. The major naturally occurring estrogen is 17 β-estradiol, but estrone is also im-

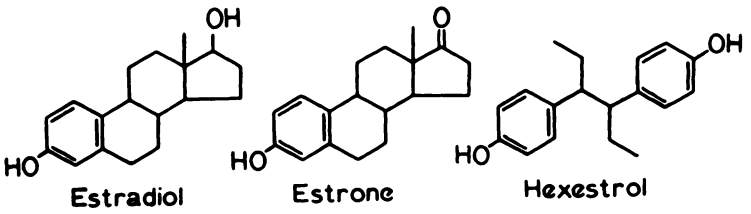

FIG. 1. Estrogenic structures.

portant and a series of synthetic estrogens have been synthesized based primarily on hexestrol, a nonsteroidal molecule with a geometry similar to estradiol (Fig. 1). In all cases the "A"-ring is aromatic, and the distance between the two oxygen atoms (on C3 and C17 of the steroids) is approximately equal.

In considering the rational development of a gamma-emitting estrogenic ligand, one must examine the molecular biological and the chemical consequences of introducing a label. Because of the very low concentration of receptors, the radiopharmaceutical must be of the highest specific activity achievable. At least 1000 Ci/millimole will probably be required for imaging humans, which theoretically can be achieved with a number of halogen radionuclides, including I-123, Br-77, and F-18. With decreasing size in this series, the carbon-halogen bond strength increases and steric perturbations decrease, thus fluorine would provide the most chemically advantageous label. Although aryl-halide bonds are less susceptible to breakdown than are alkyl halides, the position of the label has an even greater effect on the physiological activity of the molecule. The estrogen receptor is relatively intolerant of bulk substitutions at the A-ring (*13*), therefore, the first attempts to iodinate estradiol that involved the 2 and 4 positions were doomed to failure (*10,14,15*). The affinities of these compounds were only a few percent of that of underivatized estradiol when tested by in vitro competitive binding assays.

Because high affinity is the primary biochemical requirement for an effective estrogenic ligand tracer, Katzenellenbogen initially suggested the use of the nonsteroidal estrogen, hexestrol (*13*). This compound has a higher affinity than estradiol for the estrogen receptor, perhaps because its symmetry and conformational flexibility enable a substituent to adopt a number of alternative orientations in a binding site. In his article Katzenellenbogen (*8*) reports the affinity of four halogenated hexestrols, the fluoro-derivative labeled ortho- to the aromatic hydroxyl and on the terminal carbon of the hexane, and the bromo- and iodo- derivatives labeled on the hexane. Only the fluorinated hexestrols have a relative affinity for binding protein from lamb uterine cytosol that is higher than that for 17β-estradiol. The bromo- and iodo- derivatives have affinity constants about half that of estradiol and one fourth those of the fluorohexestrols.

Maximum affinity is not the sole biological quality that characterizes the ideal tracer estrogenic ligand. The subject article emphasizes the effects of structural modification and substitution on binding to other nonspecific, nonreceptor proteins capable of binding steroids and steroidal analogs. Because of the chemical similarities between the A-ring of estrogens and the hydroxyphenyl residue of thyroid hormones, there is some unavoidable cross-talk between the two receptor systems. Furthermore, estrogens exhibit a low affinity and nonspecific and nonsaturable binding to serum proteins such as albumin. Most of the radiopharmaceutical that is not bound to estrogen receptors in vivo is bound to nonreceptor binding proteins (rather than existing as a free ligand), and therefore constitutes the bulk of the background activity in in vivo estrogen tracer experiments. The affinity for nonspecific binding proteins appears related primarily to the ligand's lipophilicity. Thus, the more lipophilic iodo- and bromo- derivatives show uptake patterns that are much less selective than those of the fluorohexestrols (*8*). From the results (*8*), it is clear that either fluorohexestrol may be a useful radiopharmaceutical if it can be prepared with F-18 at sufficient specific activity, radiochemical purity, and in vivo stability.

Alternatively, Hochberg et al. (*16,17*) have recently reported a new approach to iodination of estradiol that renews the potential of this steroid estrogen as an imaging radiopharmaceutical. Their approach is to introduce iodine at C16 because substitution at this position would not block receptor binding. Various C16 and C6 iodo- derivatives of estradiol can displace estradiol from estrogen binding protein (*18*). The synthesis was achieved by nucleophilic displacement of bromine from 16β-bromoestradiol with $Na^{125}I$. The exchange proceeds with epimerization and the re-

sulting α-iodo-product was chromatographically separated from the unreacted β-bromo- compound. There was less nonspecific binding and higher uterine uptake for 16α-iodoestradiol than for tritiated estradiol. This research has been confirmed by Lee Lieberman and his colleagues and is reported as an abstract in this *Issue* (*19*). Their compound, 16α-iodoestradiol radiopharmaceutical, achieved impressively high uterus-to-blood and adrenal-to-blood ratios in rats and suggests the potential of 17β[16α-^{123}I]-iodoestradiol as a useful new imaging agent. It may even be that 16α-bromoestradiol labeled with Br-77 would have a higher stability, affinity, and specificity, and therefore be even more useful.

These two reports on gamma-emitting radiopharmaceuticals that bind with high affinity and specificity to estrogen receptors offer exciting potential for imaging breast and prostatic cancers and the adrenal gland. Both deserve to be studied further in animals and clinically evaluated and certainly will provide new impetus in this important class of receptor-specific radiopharmaceuticals.

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