TEACHING EDITORIAL

Radiochemical Probes for Steroid Hormone Receptors

Steroid hormone receptors are thermolabile, specific, high-affinity binding proteins that are necessary for steroid hormone action at the cellular level in steroid hormone-responsive cells. According to our present understanding of the mechanism of steroid hormone action, circulating steroids are first taken up by target cells and then bind to specific cytoplasmic receptor molecules, forming hormone-receptor complexes. These complexes undergo an activation step and are translocated into the nucleus where the activated receptor-hormone complexes interact with cell chromatin, the final result being quantitative or qualitative alterations in specific gene transcription. The newly synthesized RNA undergoes processing, is transported to the cytoplasm, and translated into protein, eventually bringing about changes in the cell phenotype. This sequence of events is presented to demonstrate the complexity of hormone action and should be considered in the final interpretation of the results obtained with receptor assays. Thus, in this long chain of interactions, proof of the presence of specific receptors is not necessarily identical with proof of steroid responsiveness, since there exist several possible points for interruption, which might prevent hormone responsiveness.

It has been approximately two decades since radiolabeled steroid hormones were first synthesized and used for the identification of target-tissue receptors (*1*,*2*). Since that time, research on steroid receptors has been very active, and there have been many attempts to develop new and useful probes for receptor analysis. The main objectives have been (a) to develop steroid analogs that modify or block hormonal responses, (b) to synthesize derivatives that will react covalently with receptors as affinity-labeling agents, and (c) to prepare steroids that contain various signaling probes of particular advantage to the specific detection or localization of receptors. In this last objective, both fluorescent and radioactive derivatives have been considered.

Although these synthetic efforts offer new and important tools for basic research, probably their potential clinical utility has been the greater impetus to development. Most of the pioneering studies have concerned the detection of estrogen receptors that are valuable tumor markers in human breast cancers and are also potential sites for fertility control. About two-thirds of all primary breast cancers contain detectable estrogen receptors but only about half of all metastatic lesions from breast cancers are estrogen-receptor positive (*3*). One-third of benign breast lesions also contain estrogen receptors. Approximately 50% of human breast cancers that contain estrogen receptors will respond to additive or ablative forms of endocrine therapy, whereas less than 10% of receptor-negative tumors respond to such therapy (*4*). For this reason, the analysis of estrogen receptors in breast tumor biopsies or surgical specimens has become a routine procedure. In many cases, progesterone receptors are also measured, since their presence strengthens the predictions for positive endocrine therapy. In addition, androgen and glucocorticoid receptors have been detected in about 30% and 50% of breast tumors, respectively (*5*). The relationship between response to endocrine therapy and the presence of these hormone receptors, however, is not clearly established. These receptor assays involve the measurement of specific binding by radioactive hormone in tissue extracts, and although they are quite reliable, they are somewhat difficult to perform accurately, particularly when the tissue sample is small.

Recent attempts have been made to use fluorescent estrogen analogs, which would allow a histological analysis of receptor distribution in small tissue samples. This approach holds some promise, but the accuracy or specificity of the fluorescence methods used thus far remain uncertain (*6*).

Another approach has been to use more highly radiolabeled steroids that would improve assay sensitivity for small tissue samples and may also be used in a noninvasive procedure for the imaging of receptor-positive tissues in vivo. Four promising studies on this subject using radiohalogenated estrogens are reported in this issue (*7–10*). Although somewhat surprising, it is clear that the proper positioning of iodine or bromine on the steroid causes very little hindrance to the specific,

high-affinity interaction with receptors. In addition to its radioactive specific activity and receptor binding properties, however, other features with regard to the practical application of the ligand must also be considered. The ease of synthesis and the stability of the product are important if widespread application is ever to be achieved. In this respect the synthesis of E-17α-[^{125}I]iodovinylestradiol by Hanson et al. (*10*) is very attractive. Another consideration is that the degree of nonspecific or nonreceptor interactions must be minimal, and Dr. Katzenellenbogen and his colleagues have probably utilized the most formalized approach regarding this aspect. They have characterized ligands according to their "binding selectivity index," which is a ratio of specific-to-nonspecific binding affinities (*11*). Also, their observations that the specifically bound isotope can be readily washed out by administering unlabeled hormone offers a reliable means for testing specificity in vivo.

Naturally, the release and relocation of isotope and the extent and location of maximal exposure must be at acceptable levels. The present reports demonstrate concentration of halogenated steroids throughout the excretion routes and localization of iodine in the thyroid. These expected results do not appear to be a health hazard; however, they do place limits on the imaging of target tissues and tumors. The preliminary imaging studies by McElvany et al. (*8*) are encouraging, but a more thorough analysis of the sensitivity and reproducibility of the imaging methods are still needed. As noted by the authors, it should be possible to improve the imaging by use of other radionuclides such as I-131, Br-75, or F-18. Also, imaging with Br-77 might be improved by use of the newer derivative, 16α-[^{77}Br] bromo-11β-methoxyestradiol-17β, which is retained in target tissues for longer periods of time than are other estrogens (*9*). Thus, imaging might be performed at a later time when background radiation is lower. The ability to image tumors that are targets for estrogen (or other steroids) would probably have little impact as diagnostic tests for breast cancer, or metastases from it, because of the low sensitivity and specificity one can expect from interpretation of the tissue data presently available in the literature. Images may be helpful, however, in identifying areas for tissue biopsies, since breast cancer is pathologically heterogeneous and, in addition, is composed of malignant cells that infiltrate into normal stroma. Incorrect sampling for biopsy is an occasional cause for a false-negative result in the tissue-receptor assay. Quantitation of uptake conceivably could be attempted and correlated with estrogen-receptor concentrations obtained with other assays. Whether this radiopharmaceutical could be developed for use as a therapeutic agent is only speculative at this time.

Overall, these reports offer much encouragement toward the development of more sensitive and versatile methods for steroid-receptor detection. One can expect future improvements both in the probes to be used and in the detection methodology. Although now attention is focused on the estrogens, one can expect that similar studies on other steroid hormones will follow.

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REFERENCES

1. GLASCOCK RF, HOEKSTRA WG: Selective accumulation of tritium-labeled hexoestrol by the reproductive organs of immature female goats and sheep. *Biochem J* 72:673-682, 1959
2. JENSEN EV, JACOBSON HI: Fate of steroid estrogens in target tissues. In *Biological Activities of Steroids in Relation to Cancer*. Pincus G, Volmer EP, Eds. New York, Academic Press, 1960, pp 161-178
3. ALLEGRA JC, LIPPMAN ME, GREEN L, et al: Estrogen receptor values in patients with benign breast disease. *Cancer* 44:228-231, 1979
4. *Estrogen Receptors in Human Breast Cancer*. McGuire WL, Carbone PP, Volmer EP, Eds. New York, Academic Press, 1975, pp 1-7
5. ALLEGRA JC, LIPPMAN ME, THOMPSON EB, et al: Distribution, frequency, and quantitative analysis of estrogen progesterone, androgen and glucocorticoid receptors in human breast cancer. *Cancer Res* 39:1447-1454, 1979
6. CHAMNESS GC, MERCER WD, MCGUIRE WL: Are histochemical methods for estrogen receptor valid? *J Histochem Cytochem* 28:792-798, 1980
7. MCELVANY KD, CARLSON KE, WELCH MJ, et al: In vivo comparison of 16α-[^{77}Br]bromoestradiol-17β and 16α-[^{125}I]iodoestradiol-17β. *J Nucl Med* 23:420-424, 1982
8. MCELVANY KD, KATZENELLENBOGEN JA, SHAFER KE, et al: 16α-[^{77}Br]Bromoestradiol: Dosimetry and preliminary clinical studies. *J Nucl Med* 23:425-430, 1982

9. KATZENELLENBOGEN JA, MCELVANY KD, SENDEROFF SG, et al: 16α-[^{77}Br]bromo-11β-methoxyestradiol-17β: A gamma-emitting estrogen imaging agent with high uptake and retention by target organs. *J Nucl Med* 23:411-419, 1982
10. HANSON RN, SEITZ DE, BOTARRO JC: E-17α-[^{125}I]iodovinylestradiol: An estrogen-receptor-seeking radiopharmaceutical. *J Nucl Med* 23:431-436, 1982
11. KATZENELLENBOGEN JA, HEIMAN DF, CARLSON KE, et al: In vitro and in vivo steroid receptor assays in the design of estrogen radiopharmaceuticals. In *Receptor Binding Radiopharmaceuticals*. Eckelman WC, Ed. Boca Raton, FL, Chemical Rubber Co., in press