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Muscle-Bound? A Tissue-Selective Nonsteroidal Androgen Receptor Modulator

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Human male sexual development and function require testosterone (T), the major circulating form of androgen secreted by the testes, and conversion of T in peripheral male accessory sex organs to dihydrotestosterone (DHT), a more potent androgen. The functional effects of T and DHT are mediated by high-affinity binding to the androgen receptor (AR), a ligand-activated transcription factor that interacts with DNA, coregulators, and the transcriptional machinery of chromatin.

Of the two active androgens, T predominates in muscle and DHT in prostate, appearing to provide proof-of-principle that tissue-selective ligands can be identified for AR (1). However, differences in tissue levels of 5α-reductase, the enzyme that converts T to DHT (Fig. 1), to a large extent account for tissue androgen levels. Furthermore, there is no compelling evidence that T and DHT regulate different sets of genes. Rather, more rapid binding kinetics contribute to T being a weaker androgen, requiring approximately 10-fold higher levels than DHT for the same transcriptional response (2, 3). Nevertheless, myotrophic synthetic steroid derivatives discovered over the past several decades are being used to promote anabolic effects on protein metabolism (4 – 8).

More recently, the pharmaceutical industry has been actively screening nonsteroidal ligands for anabolic activity with the intent to minimize androgenic effects in prostate and liver toxicity associated with steroid derivatives (9). Structural studies have revealed a remarkable flexibility of amino acid side chains lining the ligand binding pocket that enable steroid receptors to accommodate a wide range of chemical classes, despite an apparent rigid backbone core structure of the ligand binding domain. Among the clinically more useful receptor selective modulators is tamoxifen, an estrogen receptor antagonist used in the treatment of breast cancer (10).

It is therefore of interest that Ostrowski and co-workers at Bristol-Myers Squibb (1) have demonstrated muscle-selective effects of a high-affinity, orally active, nonsteroidal synthetic ligand, BMS-564929. Their assays in castrated rats indicate that BMS-564929 has a strikingly greater dose-dependent effect in the bulbocavernosus levator ani muscle than in prostate. The backbone structure of the BMS-564929-bound AR ligand binding domain is indistinguishable from the DHT-bound crystal structure. Like DHT, BMS-564929 makes

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hydrogen bond contacts to several key residues in the binding pocket required for high-affinity specific binding.

The mechanism for the apparent muscle selectivity of BMS-564929 awaits further study. One possibility is tissue-selective metabolism. *In vivo* hydroxylation of nonsteroidal ligands can alter the binding affinity for AR (11). Alternatively, greater activity in muscle may result from ligand-dependent, tissue-selective recruitment of coactivators to the AR transcription complex. AR activation function 1 (AF1), a predominant activation region located in the AR NH_2-terminal domain (Fig. 2), is largely insensitive to ligand-specific effects beyond the requirements for agonist-induced AR nuclear transport and DNA binding (12). In contrast, activation function 2 (AF2) in the ligand binding domain lies close to the bound ligand, well positioned to respond to ligand-induced structural changes required for its interaction with α-helical motifs. AR AF2 serves as an androgen-dependent interaction site for F*XX*LF and W*XX*LF motifs present in AR that mediate the AR NH_2- and carboxyl-terminal N/C interaction (13–16), F*XX*LF motifs in coregulatory proteins (17, 18), and as yet unidentified cofactors (18–22) and L*XX*LL motifs of SRC/p160 coactivators (Fig. 2) (17).

Crystal structures of the agonist-bound AR ligand binding domain bound with an L*XX*LL or F*XX*LF motif peptide confirm the close structural relationship between bound ligand and AF2 (23). Structural communication from the receptor interior to the exposed AF2 surface is evident from naturally occurring loss-of-function AR gene mutations that cause the androgen insensitivity syndrome (24, 25) and gain-of-function AR gene mutations in prostate cancer that increase AR F*XX*LF motif binding and coactivator recruitment (24). Steroid or nonsteroid ligand binding can induce subtle changes in van der Waals contacts and hydrogen bonding pathways (26) that specify motif binding at the AF2 surface and contribute to tissue-selective AR transcriptional activity. Indeed, Ostrowski *et al*. report amino acid contacts of BMS-564929 that differ from DHT and presumably specify tissue-selective interactions of AR with an as yet unidentified muscle cell-specific AR coregulator.

What are the caveats? Human muscle mass increases in response to T or DHT with increased myogenesis (29 –31). However, rat levator ani muscle, a frequently studied model of androgen effects in muscle because of higher AR levels compared with most skeletal muscle (27), may not necessarily be indicative of general anabolic activity (8, 28). Furthermore, if BMS-564929 is not readily metabolized and excreted, prolonged use could have undesirable effects on other organ systems including the prostate or, as shown for BMS-564929, suppression of LH in classical androgen-dependent feedback inhibition of the hypothalamic-pituitary axis. Evidence that the muscle-selective effects of BMS-564929 are inhibited by classical AR antagonists such as hydroxyflutamide and ca-sodex would provide support for a direct action through the AR.

Whether the beneficial effects of BMS-564929 aimed at improving body strength, sex drive, cognition, and overall well-being in men with hypogonadism or age-related decline in T levels outweigh adverse effects awaits the results of ongoing clinical trials. Nevertheless, in a broader sense, the development of tissue-selective nuclear receptor modulators may provide new pharmaceutical agents for treating disease and improving the quality of life.

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Abbreviations

AF1	Activation function 1
AF2	activation function 2
AR	androgen receptor
DHT	dihydrotestosterone
T	testosterone

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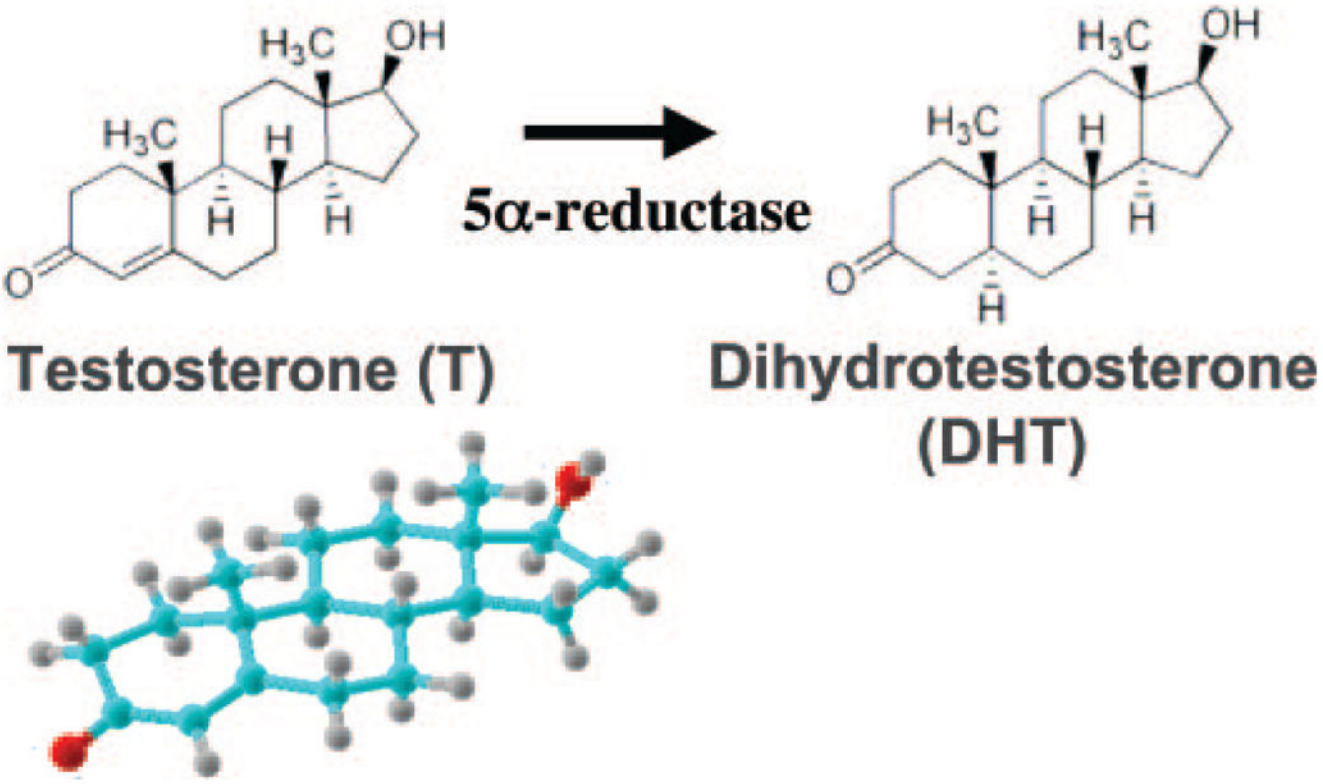

FIG. 1.

Physiologically active forms of androgen. In the male, T is produced by the testes and secreted into the blood. DHT, a more potent androgen, is produced by peripheral conversion from T by 5*α*-reductase. Levels of 5*α*-reductase are low in muscle, making T the predominant androgen, and higher in prostate where DHT predominates. The model of T was obtained from http://chemistry.umeche.maine.edu/CHY132/Ster-stat.html.

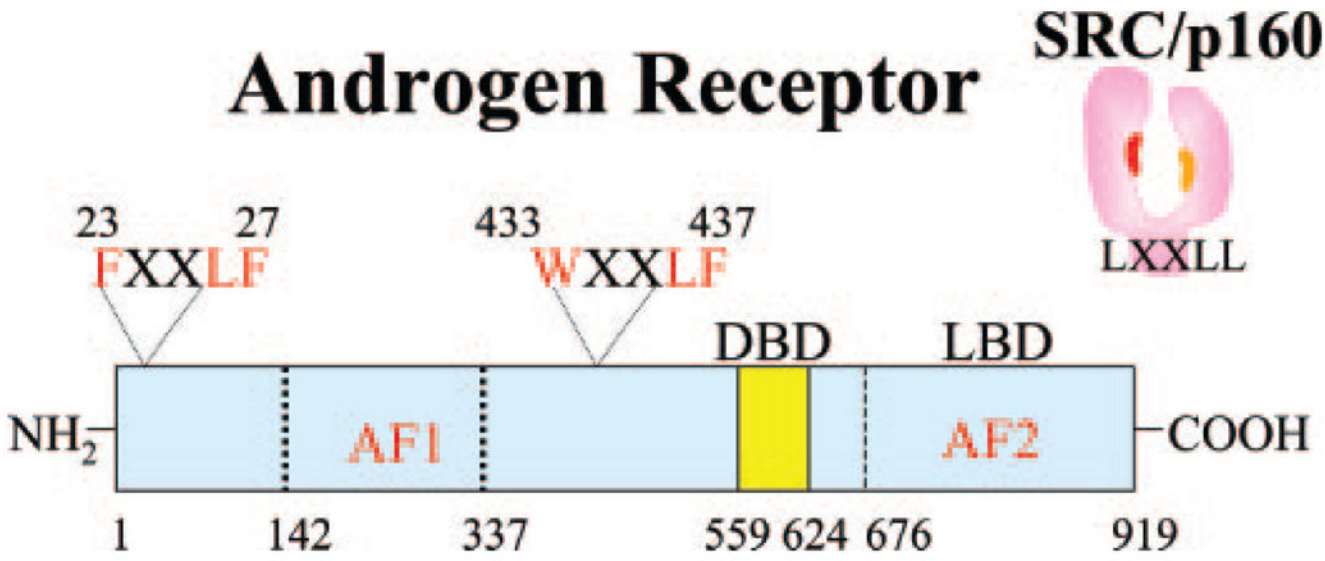

FIG. 2.

Human AR domain structure and an interacting SRC/p160 coactivator. AR contains a DNA binding domain (DBD), ligand binding domain (LBD), and NH_2-terminal region. A predominant AF1 region is in the NH_2-terminal domain. AF2 is a hydrophobic surface in the LBD that interacts in an androgen-dependent manner with the AR NH_2-terminal F*XX*LF and W*XX*LF motifs. AR AF2 also serves as an interaction site for the L*XX*LL motifs in AR coregulator proteins such as the SRC/p160 coactivators (*right*, SRC/p160).