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English Translated

Myoanabolic steroids and selective androgen receptor modulators: mechanism of action and perspectives

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Interest in anabolic steroids has been renewed in the last decade with the discovery of tissue-selective androgen receptor modulators exhibiting high myotrophic and small androgenic activity. An explanation put forward by us in 1982 for the mechanism of the preferential myotrophic effect of nandrolone (19-nortestosterone) exploits the fundamental difference between the 5α-reductase concentrations in skeletal muscle and androgenic target tissue. In androgenic tissue, testosterone is converted to the more potent 5α-dihydrotestosterone whereas nandrolone is converted to a less potent derivative. As 5α-reduction is negligible in skeletal muscle, this explains why nandrolone shows a greater myotrophic to androgenic ratio when compared with testosterone. Anabolic steroids that do not undergo 5α-reduction exert myotrophic–androgenic dissociation because their effect in androgenic tissues is not amplified by 5α-reduction. Tissue selectivity by receptor modulators may be achieved by inducing specific conformational changes of the androgen receptor that affect its interaction with transcriptional coregulators. Anabolic activity is mediated by the stimulation of ribosomal RNA synthesis therefore regulation of this synthesis by anabolic steroids would deserve detailed studies.

Keywords: anabolic steroids, androgen receptor modulators, mechanism of action, 5α-reductase, transcriptional coregulators, androgen receptor, myotrophic effect, anabolic therapy

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Abbreviations

AF = activation function; ARE = androgenic responsive element; DBD = DNA binding domain; DHT = 5α-dihydrotestosterone; DNA = deoxyribonucleic acid; HDL = high density lipoprotein; LBD = ligand binding domain; LDL = low density lipoprotein; LH = luteinizing hormone; M/A = myotrophic/androgenic; mRNA = messenger RNA; MT = 17-methyltestosterone; NTD = N-terminal domain; RNA = ribonucleic acid; rRNA = ribosome RNA; SARM = selective androgen receptor modulator; SERM = selective estrogen receptor modulator

Nearly thirty years ago, we explained the marked dissociation of the myotrophic and androgenic effects of 19-nortestosterone (nandrolone) [1, 2, 3, 4]. In our other papers [5], we also explained the myo-anabolic preference of 17-methyltestosterone (methandienone), a relatively low myotrophic–androgenic (hereinafter M/A) dissociation. For the interpretation of the M/A dissociation, this was the first conceptual explanation that has received a positive echo in the literature [6, 7, 8, 9, 10, 11], and is often quoted not only in print but also in electronic (internet) literature. Personally, it became the subject of my defended doctoral dissertation in 1987 [12], and was detailed in a small monograph published in 1990 [13]. Looking at the references published in the Citation Index, there is a renewed interest in the mode of action of nandrolone in the last 10 years (Figure 1). This is due to the appearance of selective androgen receptor modulators (SARMs) with myo-anabolic activity, which has led to a reassessment of the anabolic effects induced by androgen receptor mediation.

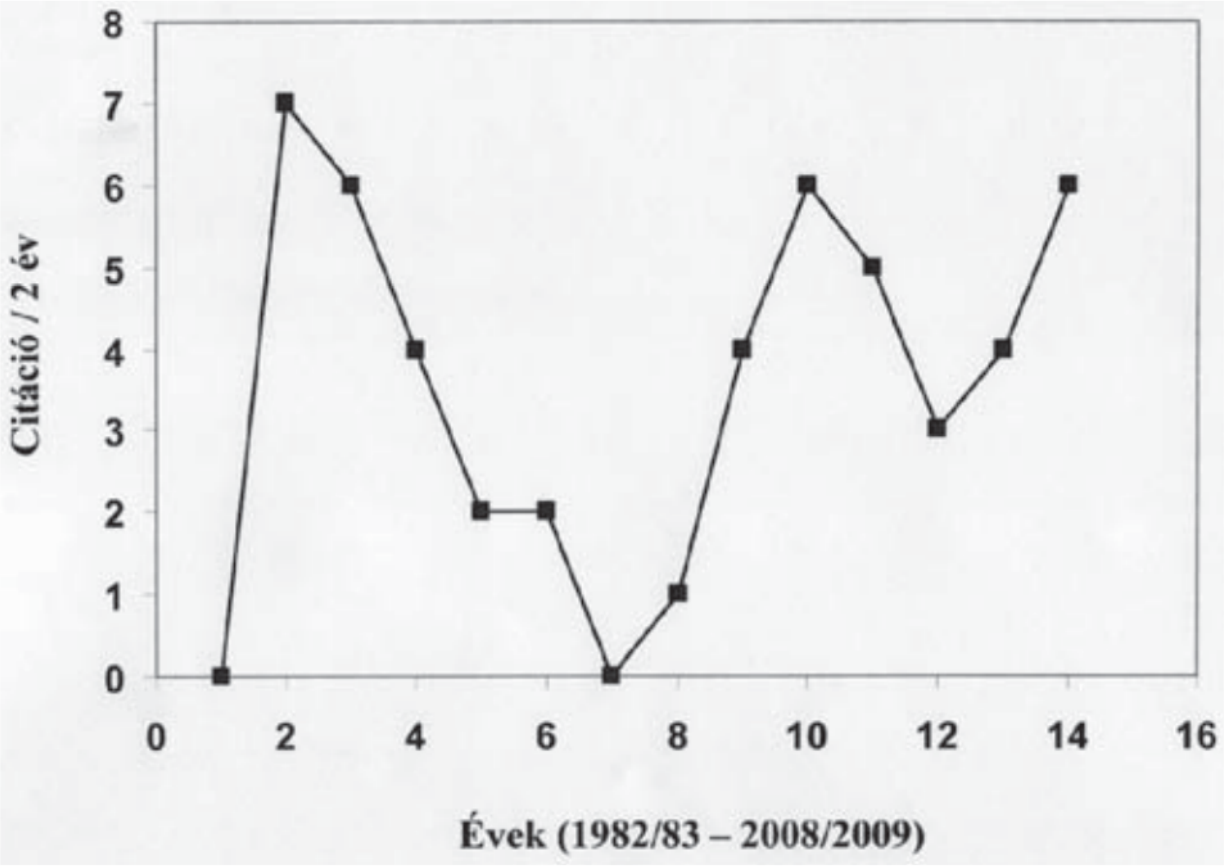

Figure 1. A Journal of Steroid Biochemistry 1982, 17, 653-660. page, the Citation Index cites 50 references (of which 48 are foreign links). The graph shows the time distribution of the biennial citations.

We explained the extremely high M/A dissociation effect of nandrolone by the tissue-specific distribution of 5α-reductase. In addition to this mechanism, attention has recently turned to another potential tissue selectivity based on the tissue-specific distribution of proteins (co-regulators, transcription factors) that interact with the active androgen receptor in the nucleus of the target organ [14, 15]. The following overview seeks to discuss the two modes of action side by side, summarizing the relevant findings, highlighting the pharmacological problems and perspectives.

For the quantitative evaluation of the M/A dissociation under the influence of anabolic steroids, a widely used test was developed by Hershberger et al [16] following Eisenberg and Gordan. This was based on the difference in weight gain between anabolic steroid treatment of levator ani muscle (myotrophic effect) and ventral prostate or seminal vesicles (androgenic effect) in castrated or immature rats compared to testosterone treatment. By dividing the myotrophic/androgenic effect quotient for the anabolic steroid by the myotrophic/androgenic effect quotient for testosterone, the M/A dissociation is obtained. When the weight gain of the organs is measured at a section of the weight gain curve where the weight increases proportionally to the dose of steroid used for treatment, the resulting dissociation is called the M/A index. Let's see a fictitious example for calculating the M/A index.

1. Nandrolone treatment resulted in a 50 mg increase in levator ani muscle mass and 25 mg in the seminal vesicle weight.

2. Testosterone treatment results in a 20 mg increase in levator ani muscle mass and 100 mg in the seminal vesicle weight.

Index value is 50/25:20/100 = 10.

The index can also be calculated by dividing the myotrophic effect of nandrolone by the myotrophic effect of testosterone (this is the so-called myotrophic ratio, in our example: 50/20 = 2.5), then dividing the androgenic effect of nandrolone by the androgenic effect of testosterone, in our example, 25/100 = 0.25), and finally the quotient of myotrophic ratio to the androgenic ratio gives the index value (i.e. 2.5/0.25 = 10).

The Hershberger test is simple to carry out but, by its nature, is not intended to be informative in its scientific accuracy. This is also reflected in the fact that the index values obtained for this same steroid in this test and published in the literature differ significantly. Therefore, in our experiments, we used a more accurate procedure: we measured the increase in the ratio of levator ani muscle to seminal vesicle RNA/DNA after treatment of castrated rats with different doses of nandrolone and testosterone phenylpropionate, i.e., dose–response curves. By graphically analyzing dose–response curves, the effect of treatment was characterized by accurate myotrophic and androgenic ratios independent of the dose used for treatment [2].

The treatment was adjusted to maintain a constant presence of nandrolone and testosterone in the blood, so that the effect of steroids was mainly determined by their affinity for the androgen receptor, the concentration of the steroid–androgen receptor complex resulting from different cellular steroids was not influenced by depletion (the so-called retention effect). The results obtained showed a nandrolone myotrophic ratio of 3.0 and an androgenic ratio of 0.4, thus the M/A index was 3/0.4 = 7.5 [2].

Our experiments have also shown that episodic steroid levels (e.g., steroid blood levels of 4–5 hours daily) can be increased to up to 30 because nandrolone and its metabolites are much less retinal bound to the androgen receptor, such as 5α-dihydrotestosterone (DHT) formed from testosterone, which significantly reduces the androgen ratio.

Our experiments have also shown that episodic steroid levels (e.g., steroid blood levels of 4-5 hours daily) can increase the M/A index up to 30 because nandrolone and its metabolites are relatively much less bound to the androgen receptor in the seminal vesicle than 5α-dihydrotestosterone (DHT) formed from testosterone, which significantly reduces the androgenic ratio.

The RNA/DNA ratio is a parameter that is directly linked biochemically to the protein accumulation effect of anabolic steroids [17, 18, 19]. The change in this ratio reflects the quantitative change in the amount of ribosomes accumulated in the cells, with 85–90% of the total cellular RNA being ribosomal RNA (rRNA). Each messenger ribosome linked to messenger RNA (mRNA) carries a progressively elongated polypeptide chain (protein that is produced) during protein synthesis, that is, a function of the cell's protein synthesis capacity, protein anabolic activity, and cellular ribosome content. In different organs, but also in different muscles, the rate of rRNA synthesis and ribosome production is variably androgen-dependent. The androgen-dependent organs of the prostate, seminal vesicles, and levator ani muscle are atrophic in the absence of testosterone. In rats, the RNA/DNA ratio of the prostate, seminal vesicles, and levator ani muscle is rapidly and dramatically decreased after castration. As a result of androgenic substitution, the ratio in the prostate and seminal vesicles is increased by a maximum of 5–7 times, while in the levator ani muscle about 3 times [2, 13, 20].

There appears to be a relationship between the degree of androgen dependence of the RNA/DNA ratio and the androgen receptor concentration in the organ. Rat prostate and seminal vesicles are distinguished by their high concentration of androgen receptors with high androgen dependence on the rate of rRNA synthesis. The rat levator ani muscle contains an order of magnitude less androgen, other skeletal muscles an order of magnitude less. Their androgen dependence is proportionally lower, the levator ani muscle is still an androgen-dependent organ, and only other testosterone concentrations in other muscles exert myotrophic effects [21].

The androgen receptor plays a central role in mediating the effects of the natural male sex hormone testosterone on androgen-dependent and androgen-responsive target cells. In this respect, there is no difference between the ancillary male gonads (prostate, seminal vesicles) and the skeletal muscles (including the levator ani muscle). However, in the mechanism of action of testosterone, active metabolism in the target cell is very important, and in this respect, a distinction must be made between the prostate or seminal vesicle type and the levator ani type androgenic mechanisms.

Prostate and seminal vesicles are predominantly converted to DHT by the enzyme testosterone 5α-reductase (Fig. 2), and this steroid is bound by the androgen receptor by nearly an order of magnitude greater affinity than testosterone. This metabolism significantly increases the testosterone sensitivity of androgen target cells, allowing for a complete androgen response even at lower testosterone concentrations. DHT is only very slowly dissociated from the androgen receptor, and therefore retains or retains in the target cells, i.e. it can be detected even when testosterone is no longer present in the circulation. The inactivation and elimination of DHT from the cell after enzymatic reductions occurs mainly in the form of androstanediols (or their glucuronides) [13].

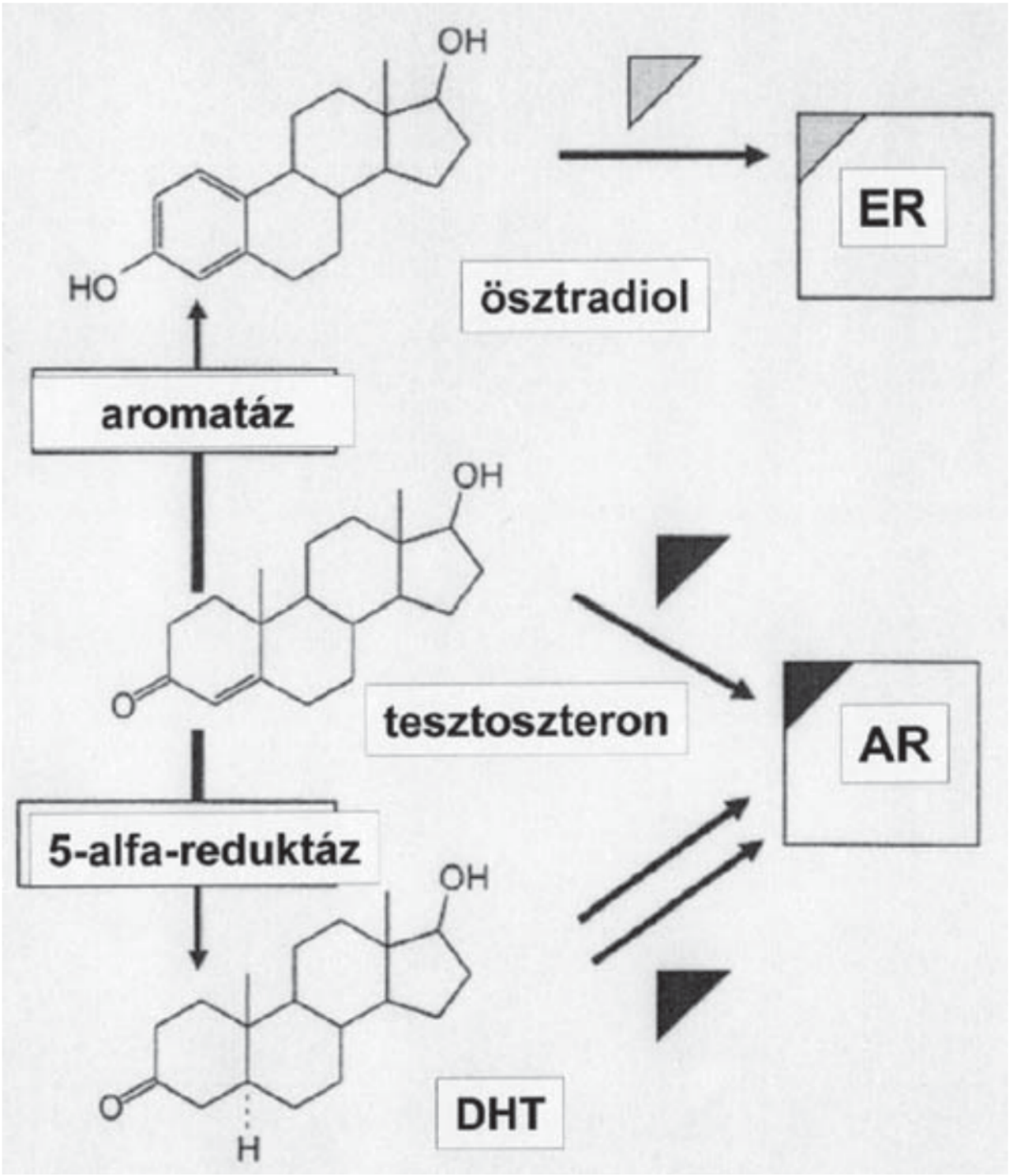

Figure 2. Active metabolism of testosterone. On the one hand, it is converted to 5α-dihydrotestosterone (DHT), which binds more closely to the androgen receptor (AR) than testosterone (the difference in binding is illustrated by the number of arrows). Testosterone can also be converted to estradiol and thus regulates in some target cells, but this is not responsible for the overall anabolic effect.

Levator ani muscle 5α-reductase activity is minimal or undetectable relative to the accessory sex glands, and there is no difference between levator ani muscle and other skeletal muscles. In the striated muscles,

testosterone itself forms a complex with the androgen receptor, and this complex exerts a gene regulatory effect. Muscle testosterone sensitivity, as measured by the hormone concentration required for the maximal effect on organ weight or RNA/DNA ratio (50%), is therefore significantly lower than that of the seminal vesicle or the prostate. This is therefore consistent with the higher affinity of the androgen receptor for DHT than for testosterone. It should be emphasized that, by acting on the ani muscle of the castrated rat as well as the accessory sex glands of the male rat, both the DHT–androgen receptor and the testosterone–androgen receptor complex can elicit a complete androgen response. Thus, for example, higher concentrations of testosterone have an effect on the prostate in the presence of 5α-reductase inhibitors [22]. Exogenously injected DHT would also be myotrophic, but it is not because the muscle's high activity of 3α- and -3β-hydroxysteroid dehydrogenase inactivates it almost immediately by converting it to androstanediols.

When we investigated how nandrolone behaves in the accessory sex glands and the levator ani muscle, we obtained results that shed light on the explanation of the M/A dissociation in nandrolone. We have shown that nandrolone is the same substrate for 5α-reductase as testosterone (Figure 3), however the resulting 5α-dihydronandrolone (DHN) binds to the androgen receptor with no greater affinity but about 8 times less affinity than DHT, meaning that most of the nandrolone is converted into a more inactive derivative in the seminal vesicles [3, 4, 13]. This undoubtedly explains the reduced "androgenic" effect of nandrolone (if we call it the seminal vesicle mechanism of action).

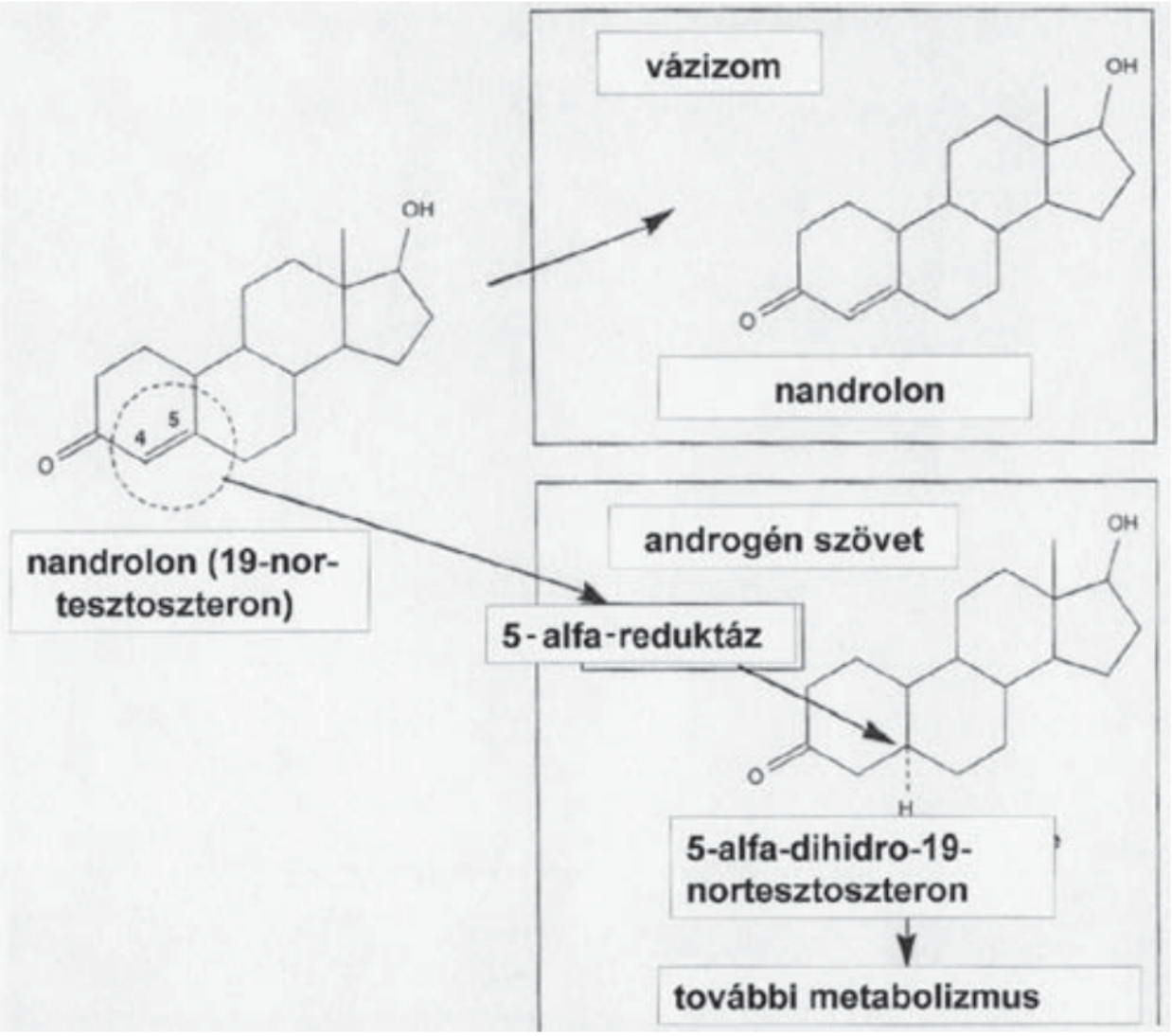

Figure 3. Nandrolone is not metabolized to any significant extent in skeletal muscle, whereas it is 5α-reduced in androgen tissues (seminal vesicles, prostate) just as testosterone, except that nandrolone loses its potency while testosterone 5α-reduction results in a more efficient androgen.

In levator ani muscle, nandrolone is not 5α-reduced (Figure 3), its myotrophic effect is determined by its affinity for the androgen receptor relative to testosterone. Our own measurements with seminal vesicle cytosol and nuclear androgen receptor showed 2- to 4-fold, and measurements of androgen receptor in skeletal muscle by Gustafsson et al. showed 3.1-fold, of affinity for nandrolone over testosterone [23]. This is very consistent with the myotrophic quotient we have defined for nandrolone and described above. But what is the relationship between the relative androgen activity (= 0.42) of nandrolone and the formation of androgen receptor–steroid complexes?

In response, seminal vesicle tissue fragments were incubated at 37 °C in the presence of increasing concentrations of radiolabeled testosterone, unlabeled testosterone, and nandrolone, and concentrations of DHT bound to the cytosol and nuclear receptors were measured. A portion of the labeled DHT was displaced by unlabeled testosterone DHT and nandrolone from the androgen receptor, and the relative

affinity of the nandrolone added to the medium for testosterone was determined after linearization in the experiments. Relative affinity was calculated for nandrolone and testosterone, since they were added during in vivo treatment, so the relative affinity and relative in vivo effect became comparable. This ratio was 0.44 (cytosolic androgen receptor) and 0.4 (nuclear androgen receptor), which was in good agreement with nandrolone relative androgenic activity. The credibility of the result was enhanced by the fact that very structurally similar testosterone and nandrolone undergo very similar metabolism both in vivo and in vitro [2, 3, 4, 13].

These experiments have been very useful in interpreting the dissociation of nandrolone M/A: they have shown a close correlation between the in vivo effect of nandrolone and its affinity for the androgen receptor at body temperature under conditions where steroid metabolism may occur. The results from seminal vesicles and levator ani muscle could be explained in one way: nandrolone binds to the androgen receptor with a higher affinity than testosterone, but the same chemical conversion (5α-reduction) that increases testosterone receptor affinity, significantly reduces the receptor affinity of nandrolone.

The M/A dissociation is thus due to the fact that the enzyme responsible for the chemical transformation, 5α-reductase, has an uneven organ distribution: it is abundant in the male accessory sex glands but not (or barely) in the skeletal muscle. At first, it could be stated (which later became generally accepted) that no specific anabolic receptor is required, and the androgen receptor plays a key role in mediating the effects of anabolic steroids.

17-Methyltestosterone (MT) is as potent an androgen as testosterone. The 17-methyl group protects the 17β-hydroxyl group, which is critically important for bioactivity, from first-pass oxidation in the liver, so MT is also effective when administered orally. A double bond was introduced into the MT A ring to give them the notorious anabolic steroid called "infamous" methandienone (MT 1), which was obviously also an orally effective anabolic, which facilitated its abuse. We have shown that methandienone is not a substrate for 5α-reductase [5], so it was worth characterizing it as an anabolic steroid. Methandienone showed little M/A dissociation relative to MT of up to 2.1, and also had significantly lower affinity for androgen receptor (also for 5α-reduced MT and worse for MT). Methandienone is thus a prototype of anabolic steroids that do not lose their efficiency in metabolic glands but are not activated by 5α-reduction and therefore exhibit a slight myotrophic–androgenic dissociation. Because of its low affinity for the androgen receptor, high doses of methandienone are required for a high myotrophic effect, which makes it more likely that side effects will occur. The most severe of these is hepatotoxicity specific to 17-alkylated steroids, which has led to the removal of both MT and methandienone from the authorized drugs.

The nandrolone decanoate ester is marketed by the Organon company (Deca-Durabolin, nandrolone is gradually released from the ester), which is the best-known and most widely used anabolic steroid, and company researchers have made significant efforts to elucidate the mechanism of the preferential myotrophic effect of nandrolone. The solution we proposed in an article published in 1982 was not thought of, at least as evidenced by the results of our own research in 1985, which confirmed ours and referred to our article 3 years earlier, acknowledging our priority, in the abstract of their paper [8]. At the symposium on "Anabolic Steroids in the '80s" held in the fall of 1984, sponsored by Organon (published in the literature in 1985), our findings have already received special attention [6, 7].

The androgen receptor

The structure of the androgen receptor is now well known [11, 24, 25], a protein of about 100 kDa, which binds androgenic steroids to a portion of the apolar "pocket" forming ligand binding domain (LBD). (Ligands are smaller molecules that specifically bind to proteins, in this case steroids and their analogues, and domains are major portions of the protein that specialize in specific functions.) The androgen receptor is in the cytoplasm. Binding of androgenic steroids (which means activation of the receptor) alters the structure (conformation) of the receptor, resulting in the transformation (transformation) of the receptor: on the one hand, the "protective" proteins are released and the specific paired surface regions of the androgen receptor form homodimers. Homodimerization stabilizes the DNA binding site structure of the androgen receptor and increases its affinity for the DNA binding site. A further consequence is that the homodimeric structure is translocated from the cytoplasm to the nucleus and binds to two identical hexanucleotide sequences (6 specific nucleotides in sequence) through its two surface-binding DNA binding domains (DBDs) (Figure 4). The two binding sequences on the DNA are located on two different strands of the DNA duplex, with their hexanucleotides in the reverse order (so-called palindrome sequences), with a distance of 3 nucleotides.

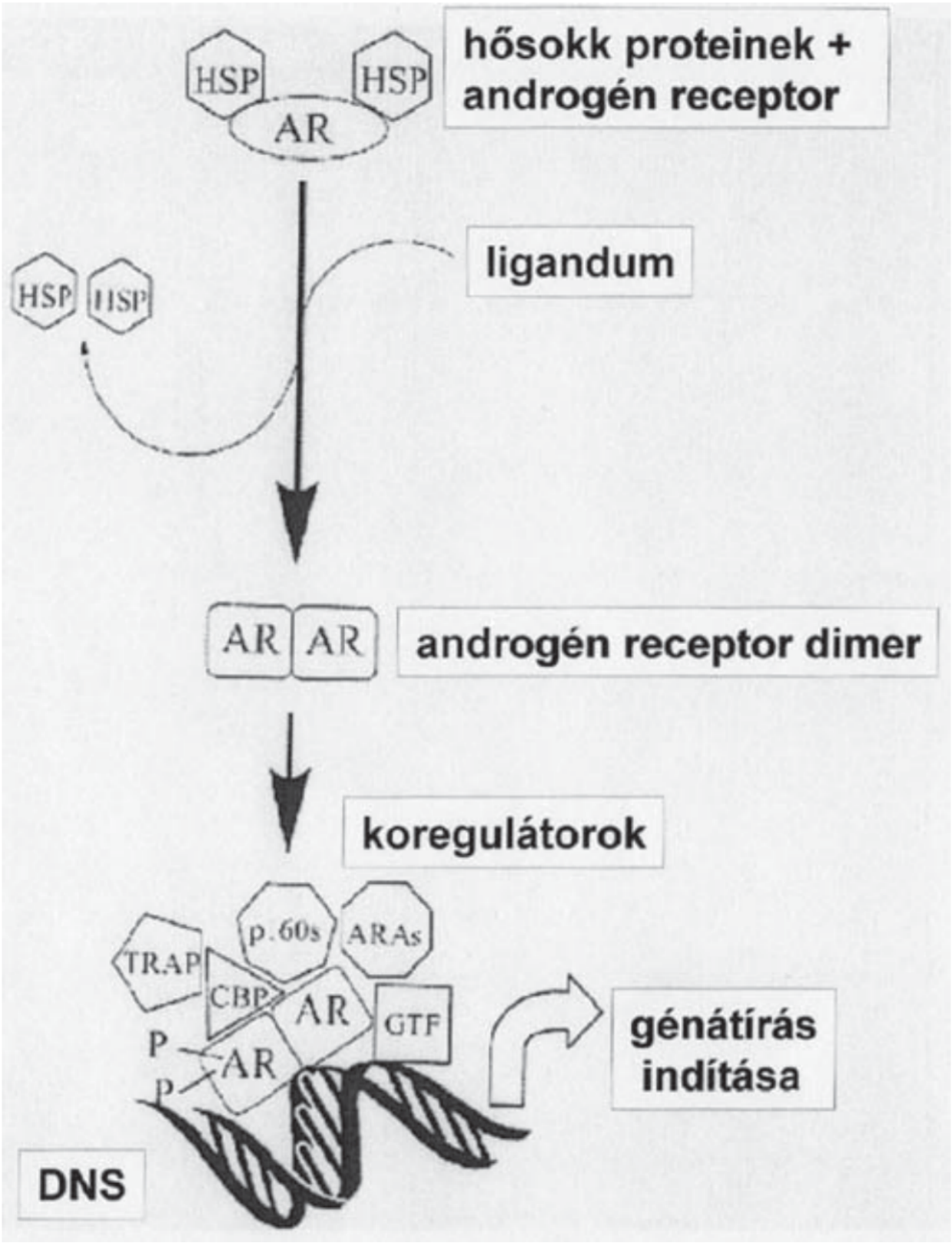

Figure 4. Outline of the mechanism of action of the androgen receptor.

Each subunit of the androgen receptor homodimer thus has 1 DNA binding motif, which binds the androgen receptor to a portion of the DNA containing said sequences, briefly called the androgen responsive element (ARE). ARE is located next to the androgen-regulated gene in the so-called promoter region. It also binds RNA polymerase, which carries out transcription of the gene, that is, RNA synthesis. The androgen receptor specifically promotes the binding of RNA polymerase to the promoter, thereby accelerating gene transcription (i.e., DNA-directed RNA synthesis). In this effect, the androgen receptor is not involved alone, but without it, gene transcription is slowed down or stopped. But what do we know about the role of the androgen receptor in this process?

In addition to the basic mechanism of transcription (RNA polymerase, general transcription factors), the simultaneous presence of several transcription factors is required for gene transcription to start as many times per unit of time as possible. For the activation of androgen-regulated genes, the activated androgen receptor must first bind to the promoter. Therefore, the androgen receptor is a protein that specifically binds a given DNA sequence, a ligand-activated transcription factor (in short, a transactivating factor). Activating function (AF) regions are located on the surface of the androgen receptor. Two such regions are distinguished, the AF-1 containing the N-terminal domain (NTD) and the AF-2 located on the LBD. At the androgen receptor, the AF-1 region serves to collect coactivator proteins in a specific order through protein-protein interactions and to activate gene transcription with them. However, this requires the involvement of AF-2, whereby the protein surfaces formed by the action of the bound steroid promote the interaction of coactivators and the presentation of the resulting protein complexes to the AF-1 region. Coactivators promote the interaction of the androgen receptor with various transcription factors. The protein complex (Figure 4) forms a bridge between the androgen receptor and RNA polymerase and forms the stable RNA polymerase promoter initiation complex required to initiate gene transcription. Upon initiation of gene transcription, the androgen receptor dissociates from the promoter, and activation of the next RNA polymerase requires the activation of another activated androgen receptor.

The latest era in the research and development of anabolic and other selective androgen receptor ligands has been initiated by experimental results suggesting that various steroid and nonsteroidal ligands can activate and inhibit tissue-specific transcription. In the former case, tissue-specific coactivators are involved, in the latter tissue-specific co-activators are implicated [24].

Androgenic and anabolic steroids, tissue specific activation of the androgen receptor, antiandrogens

Androgen steroid binding is known to induce a change in the structure of the androgen receptor. However, the question is whether all synthetic and physiological anabolic–androgenic steroid binding results in the same conformation change, i.e. whether this change is somewhat dependent on the steroid structure. Namely, the various conformations can select between different coactivators, co-depressors and transcription factors (hereinafter referred to as "gene transfer factors"), and they can work only where the necessary factors are available. If the gene transduction factors involved in androgen regulation show tissue selectivity, then tissue-specific androgen receptor ligands should be sought. This opportunity has led to a renewed interest in the mechanism of action of anabolic steroids and to a whole new area of pharmacological research on selective androgen receptor modulators (SARM). SARMs are mostly nonsteroidal, but there are also known steroids [24].

Tissue-specific androgenic steroid activity is also exemplified for testosterone and DHT. For example, urogenital differentiation of the sinus (external genitalia, scrotum, prostate) occurs by DHT action, and testosterone alone is not sufficient [26].

Recently, more and more studies have been carried out into cells containing DNA fragments (such as plasmids) containing the androgen receptor code and the androgen responsive element (promoter). (The operation is called transfection, and since two different pieces of DNA were introduced together: cotransfection.) After the androgen-responsive promoter, a gene was introduced into the DNA that produced a highly measurable enzyme. Such cotransfected cells are useful for studying the gene regulatory effects of androgen receptor complexes of various androgenic–anabolic steroids. In such cells, anabolic steroids that bind poorly to the androgen receptor also show spectacular and gene-dependent gene activation, which has been shown to vary depending on the structure of the androgen-responsive promoter [27]. All this favors the idea of tissue-specific gene transduction factors and ligand-specific androgen receptor site structure, the problem being that rRNA promoters essential for the mechanism of action of anabolic steroids have not yet been investigated.

Antiandrogens are steroidal (e.g., cyproterone acetate) and nonsteroidal (e.g., hydroxyflutamide) compounds that bind to the androgen ligand site of the androgen receptor but do not induce transformation and activation of the androgen receptor, but prevent androgenic steroid (DHT, testosterone, nandrolone) androgen receptor binding and androgen cell responses [13]. Antiandrogens bind to the LBD region of the androgen receptor without sinking into the ligand-binding pocket and therefore dissociate relatively quickly from the androgen receptor. Androgenic steroids bound to the androgen receptor are covered by a helix of LBD as a result of a change in the structure of the LBD, which does not occur when antiandrogens bind. However, modification of the structure of hydroxyflutamide unexpectedly led to an androgen agonist that exhibited tissue-selective pharmacological activity and initiated the study of SARMs (selective androgen receptor modulators) [24].

Nonsteroidal, tissue-selective androgen receptor modulators [SARM: (tissue)-selective androgen receptor modulators]

The first nonsteroidal receptor modulators were known in association with the estrogen receptor. These are collectively referred to as SERMs by the English term selective estrogen receptor modulators. The first such estrogen receptor modulators were antiestrogens (tamoxifen, idoxifene, droloxifene), followed by raloxifene, which retained the osteogenic and cardiovascular benefits of estrogens, but did not act as an estrogen in the uterus and instead behaved like an antiestrogen. What is exciting about the binding of raloxifene and estradiol to the estrogen receptor is that the nonsteroidal raloxifene produces a different receptor structure than the steroid hormone. This was confirmed by crystallizing the ligand-binding domain (LBD) of the receptor after binding to the two compounds and comparing the crystal structure [28].

The discovery of SARMs began in the 1970s with the discovery of the androgen receptor nonsteroidal antiandrogen, the propionamide derivative flutamide. The active metabolite of flutamide, hydroxyflutamide, has been found to be capable of binding to the androgen receptor. In the ‘80s, bicalutamide, which had better pharmacokinetic properties, was synthesized starting from hydroxyflutamide. When the sulfonyl group in bicalutamide was replaced by a thioether and the fluorine substituent at the para-position was replaced by an acetamide group (Figure 5), the androgen antagonist compound, when tested in vitro in cytoskeletal cells, unexpectedly became an androgen agonist

propionamide derivative, more specifically, thioacetolutamide [29, 30, 31, 32]. However, due to the rapid oxidation of the thioether group (sulfoxide, sulfone formation), this derivative is inactivated in vivo with a half-life of about 25 minutes, thus not exhibiting the expected agonist activity and therefore the thioether group has been replaced by the metabolically inert ether group. The resulting compound (Figure 5) showed androgen receptor-dependent androgen agonist activity in vivo, but also proved to be tissue selective, affecting levator ani muscle 3–4 times more selectively than seminal vesicles or prostate (myotrophic–androgenic dissociation = 3–4). In addition, its effect on muscle was 3 times greater than that on the gonads, so it was partially androgenic at full anabolic effect. Such nonsteroidal compounds, which are capable of separating certain androgen receptor-mediated effects (e.g., anabolic and androgenic), have been defined as tissue-selective androgen receptor modulators (SARMs) by analogy with SERMs.

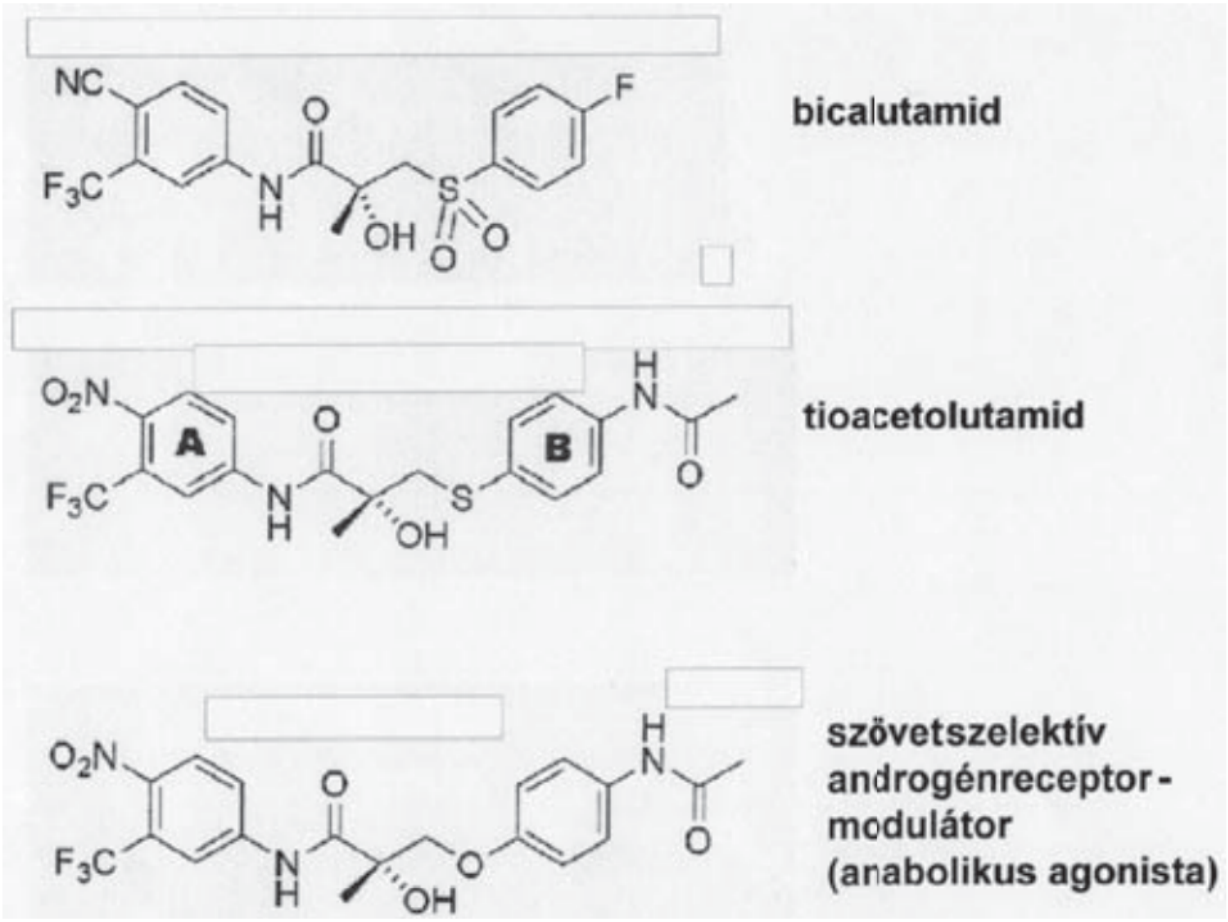

Figure 5. Chemical modifications from antiandrogen bicalutamide to in vivo androgenic SARM.

An effective myoanabolic SARM is rapidly and completely absorbed after oral administration, is active at low doses (i.e., has high affinity for the androgen receptor) and has a short elimination half-life of less than 1 day. Medical applications include all conditions and diseases associated with skeletal muscle mass and bone loss (sarcopenia and osteopenia). They may be of particular use in preventing sarcopenia and osteoporosis in the elderly and thereby improving quality of life. Importantly, they have virtually no effect on the prostate, do not virilize skin formulas, do not increase hematocrit values, and do not induce voice deepening or hirsutism in women [24]. On the other hand, LH secretion of the pituitary gland is inhibited and serum LDL/HDL ratio is brought to the lower end of the cardiovascular risk range. SARM treatment

may be indicated in cases of AIDS, carcinoma, kidney disease, septic conditions, and burns in which cachexia occurs. An arylpropionamide SARM called ostarine is already in clinical trials. In the first test, 60 elderly men and 60 postmenopausal women were given 3 mg/day of ostarine for 3 months. Favorable experiences include weight gain (1.4 kg compared to placebo), improvement in body strength and physical performance, decreased fasting blood glucose and insulin levels, improved insulin resistance, and decreased body fat [24].

Research on SARMs in the United States has been accelerated by the results of two small research teams since 1998. Today, major pharmaceutical companies have also entered, including Bristol-Myers Squibb (BMS), GlaxoSmithKline, Eli Lilly, and Merck. BMS has already achieved breakthrough success: in 2007, it was reported that their SARM compound (BMS-564929, Figure 6), in vivo, was 80 times more selective for levator ani muscle than for prostate compared to testosterone propionate. In the case of LH suppression, the effect on the levator ani is 9 times more selective. This SARM is already in clinical testing [35].

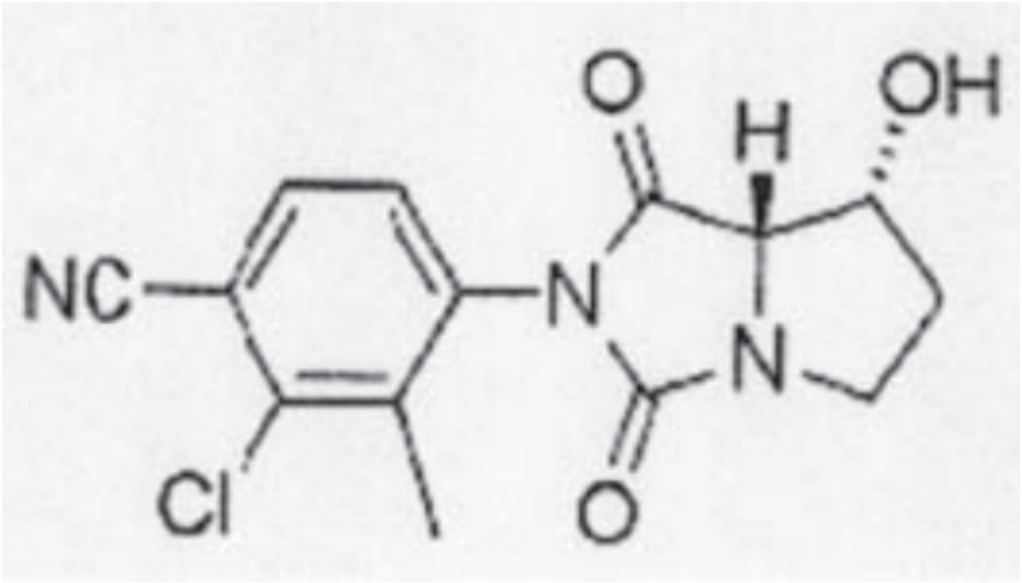

Figure 6. Clinically tested SARM compound for Bristol-Myers Squibb.

Currently, a variety of structured organic ring compounds are being tested, and the number of SARMs is steadily increasing, not only are myoanabolic and osteoanabolic SARMs, but also antiandrogenic and anticontraceptive SARMs are being planned and sought. The selection of possible compounds is facilitated by X-ray crystallographic research. The androgenic steroid binding crystal structure of the androgen receptor ligand-binding domain (LBD) was described in 2000 [36] and the first SARM (a bicalutamide derivative) binding androgen receptor LBD structure in 2005 [37]. However, the SARM-binding LBD structure and the androgen agonist steroid-binding LBD structure (as compared to SERMs) showed no spatial differences [38], and this has been true for all SARM–LBD complexes since then [24]. However, crystallization has clarified the way in which SARMs bind to the androgen receptor, allowing the design of further SARMs. It is hypothesized that SARMs and androgen agonist steroids will otherwise influence the reconstituted androgen receptor conformation. On the other hand, it should be remembered that, unlike the estrogenic steroid–estrogen receptor complex, the co-regulator proteins in the androgenic steroid–androgen receptor complex are primarily located at the AF-1 site in the N-terminal

region rather than the AF-2 site in the LBD, therefore, the active androgen receptor would require knowledge of the N-terminal crystal structure, but we do not yet have it.

The explanation of tissue selectivity in the effect of SARMs is an exciting question, and the answer to this question can only be expected given the experimental results obtained so far. Accordingly, myoanabolic SAR binding to the androgen receptor induces a spatial structure of the receptor capable of collecting and efficiently mobilizing co-activators and transcription factors in skeletal muscle cells on androgen receptor responsive (primarily rRNA) promoters. In contrast, this spatial structure is not, or is not, fully capable of activating androgen receptor-dependent gene transcription in prostate and other androgen-dependent organs, for example because it can bind co-activators instead of co-activators. Thus, this explanation is fundamentally related to the acceptance that cells of different tissues have different tissue-specific coactivators, co-depressors, and transcription factors that interact with the SARM–androgen receptor complex in the skeletal muscle differently from the adjoining male sex glands.

With the advent of SARMs, a new chapter in the research of androgen receptor-dependent myoanabolic and osteoanabolic drugs has opened in the last ten years. The SARM concept is promising because it raises the possibility that certain physiological effects of the androgenic hormone can be pharmacologically selectively influenced, thus opening the way to expanding the arsenal of medical therapy.

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