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Mechanism of Anabolic Androgenic Steroid Action

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The mechanism of AAS action in an organism includes a succesive interaction with transport, receptor and enzyme binding proteins. The characteristics of these proteins interacting with various AAS may essentially differ. A diverse efficiency of the same AAS demonstrated in experiment may depend on absolute values and the ratio of hormone agent towards all three types of specific binding proteins. Skeletal muscle was the tissue type of choice on which the effect of AAS was studied. For comparison we used the tissues of male sex glands. Testosterone and its derivatives actively participate in the regulation of their metabolism. Such an approach had three reasons. First, skeletal muscle constitutes the major mass of an organism and has a number of metabolic pecularities which differ from other tissues. When moving from a state of rest to carrying out a specific function (muscular contraction), systematic intensification of intracellular metabolism using the existing enzymic system is possible. The high intensity of metabolism in skeletal muscles during physical exercise (which are different according to their character and intensity) remains during the first hours of rest postexercise. One can use such a model for studying the influence of intracellular metabolism on the velocity of passing a hormonal signal into a muscular cell. Various AAS were used as a hormone. Second, the empirical evidence of AAS application in cattle breeding for increasing animal muscular mass demonstrates the existence of some molecular mechanism that ties these events together. Last, until recently skeletal muscle as a target tissue for AAS has not been considered one of the possible sites of action for this group of steroid hormones due to the lack of information on the presence of an indispensable receptor apparatus. Some rare works which appeared in the 1970s were considered to be highly questionable and for a long time they were practically ignored by the preparation of reviews and monographs. Fourteen years have passed since the publication of Mainwaring's fundamental book that discovered the principal mechanisms of action of androgens in an organism. The skepticism and doubts of this author about the possibility of specific AAS binding in muscular tissue have not been confirmed. On the contrary, the experimental data obtained in our laboratory give convincing evidence as to the presence of a specific binding of various AAS by receptor proteins in cytoplasm and skeletal muscle nuclei. In addition, it was shown that there is an interaction of such a hormone-receptor complex with unique sites of DNA and an intensification of the synthesis of contractile proteins. By using ^{3}H AAS in the method of receptor binding determination,

the presence of androgen receptors in cytoplasm and muscular tissue nuclei of different experimental animals was seen. These results were confirmed and expanded in a series of investigations carried out in the laboratories of Gustaffson, Max, Tremblay and others in the past five years.

Before attempting an analysis of intracellular AAS metabolism it is necessary to address a number of problems which are associated with hormone transport in the organism. It is an important starting point for realizing AAS action.

This group of steroid hormones, in physiological concentrations, has a pronounced effect on certain tissues and the capability to induce alterations of metabolic processes within them. The tissues or cells selectively responding to a steroid or some other hormones are usually called target tissues, or target cells while other tissues are usually called hormone-resistant. AAS possess a large spectrum of action which is manifested both in different kinds of tissues and in a variety of regulatory pathways in cells of the same type.

The trend, the duration, and the nature of metabolic effects caused by steroid hormones depend upon the structure of the hormone and phenotypical features of the target organs.

Each hormone, including AAS, selectively affects specific tissues in an organism and causes a metabolic alteration that allows an examination of the action as a specific hormonal effect. On the other hand, the variety of metabolic alterations and the heterogenity of target tissues which leads to the multiplicity of the hormonal effect should be taken into account. Therefore, AAS may be considered to be a steroid hormone possessing a specific multiplicity of action.

In order to accomplish their function in an organism, AAS need to be delivered to the target organ. In every organism there exist the following kinds of proteins which are necessary for interaction with steroid hormones: transporter, receptor and enzymes. Each group of proteins accomplishes a certain function and is situated in certain organs and liquids.

AAS, like steroid hormones, are transported as complexes with binding proteins in the organism by blood. The binding of hormones in blood depends on their affinity for binding proteins and on the concentration of these proteins. With the binding of steroids, the proteins in the blood protect them against premature breakdown and against exessive quantities of free hormones in the blood. This results in a regulation of their concentration.

Androgens, estrogens, and AAS form complexes with testosterone-estradiol binding globulin (TEBG) in human blood. Another transport protein, found in epididymis liquid, was named androgen binding protein (ABP). Hence, in organic fluids, there are two specific transport proteins which form dissociative complexes with AAS:TEBG in blood and ABP in epididymis fluid.

Additionally, the plasma albumin of blood, which binds low molecular substances, can bind steroids. Steroid hormones can appear in blood in three states: free, bound to TEBG, and bound to albumin. Though the affinity of TEBG for steroid hormones is 1000 times higher than that of albumin, the quantity of albumin in blood is 1000 times greater than that of

TEBG. The binding index (the ratio of binding capability to the dissociation constant) of TEBG specific transport protein remains within the limits of the albumin binding index, and the steroid hormones can be equally distributed in blood between both binding proteins. Such a conclusion is confirmed by direct measurements of testosterone and the distribution of its active metabolite in blood. In men 45% of testosterone is bound to TEBG in blood, 53% is bound to albumin, and only 2% of testosterone is in a free state. In women these values are 62, 37 and 1% respectively.

A reversible binding to specific transport proteins circulating with AAS blood selectively restricts their migration to target organs. Various androgens, their active metabolites, and AAS compete for the transport protein binding sites. They are able to displace a familiar hormone from the complex with protein. In such conditions the concentration of a free hormone in blood increases and hence increases the possibility for its penetrating into the cell and its subsequent binding to cytoplasmic androgen receptors.

Hence, a distinguishing feature of androgen binding transport proteins appeares to be an active participation in the processes of androgens and AAS transport and their distribution among the organs as well as their control of metabolic processes which are associated with manifesting specific multiple hormonal effects.

The ideas about the succession of events in the transmission of a hormone signal into the cell have recently been evolving. For steroid hormones this process is associated with the presence of system of receptors inside the cell. The proteins that bind to a steroid hormone and deliver it to the place of active participation in cell metabolism appear to be the receptors of steroid hormones. A molecule of the receptor can be considered to be the first molecular element which recognizes a specific extracellular signal. Then, by means of a selective binding in the chain of intracellular reactions, a hormone response is formed.

All androgen receptors are found inside the cell and according to their location they can be divided into cytoplasmic and nuclear receptors. The experiments using AAS marked by tritium clearly showed the potential of these hormones in various tissues primarily sex organs, liver, kidneys, skeletal muscle and heart. Over a long period of time either steroid or its active metabolite accumulates in these tissues.

In the process of studying these androgen receptors in animal tissues, some principal factors typical for their normal functioning in a cell were revealed. Among them one can note such properties of receptor as structural and steric specificity, limited capacity and saturation, hormone specificity and high affinity. A high velocity of receptor metabolism and their high modificative variability stipulated by the processes of phosphorylation, methylation and acetylation of receptor molecules was also revealed. It also was shown that receptors may be repeatedly used, which suggests the existence of a closed receptor cycle.

The most basic method of identification of androgen receptors is based on their capability to selectively, and their relative strength, bind to androgens and their metabolites. By analyzing the androgen-receptor interaction it becomes evident that this interaction is

reversible and that the binding kinetics is determined by means of the laws of active masses. During the hormone and receptor interaction, a hormone-receptor complex is formed.

The dependence of the hormone-receptor complex formation on steroid concentration is most often subjected to an analysis by drawing a Scatchard plot. The experiments show that while investigating cytoplasmic and nuclear androgen receptors, two types of hormone and protein interaction occur: a non-specific interaction stipulated by the presence of ballast proteins (it is characterized by a low affinity and unlimited capacity) and a specific binding to receptor proteins based on a selective interaction with high affinity. To determine the level of nonspecific binding in the total quantity of androgen binding ratio activity, one usually adds 100 to 1000 units of unlabeled hormone.

A wide dispersion on the Scatchard plot for the experimental findings in the investigations on the interaction of various steroids with receptors is, to a certain degree, conditioned by a sufficient homogenity of binding sites. However, it is possible to have several hormone-binding centers which differ in their affinity for the hormone.

According to existing theories, the receptors in cells are in one of two states: free or bound to a hormone. By determining the receptor binding ability, one usually can determine a free receptor fraction unoccupied by the hormone. To measure the total quantity of receptors in a cell, it is necessary to release the occupied receptors from the hormone, that is to carry out a dissociation of existing hormone-receptor complexes. There are several methods for separating hormone from its receptor, but unfortunately each of these methods has certain limitations. First, there is a rise of incubation temperature (up to 30) which not only causes the dissociation of hormone-receptor complexes, but also leads to an inactivation of the receptor as occurs in skeletal muscles. Second, the use of specific reagents accelerate the process of hormone-receptor complex dissociation. Third, there is the use of the ligand interchange method.

The results from this laboratory show that after a single injection of 19-nortestosterone into white rats, an appreciable alteration in the content of androgen receptors was observed in skeletal muscle cytosol (Figure 1).

As shown in Figure 1 after injecting a hormone into an animal the number of free cytoplasmic androgen receptors rapidly decreases and in 30 to 60 minutes it is only 15 to 20% of the initial level. These alterations in the content of free androgen receptors are associated with the formation of hormone-receptor complexes due to the appearance of 19-nortestosterone molecules in cytoplasm and a further translocation of these complexes to the muscular cell nucleus. Two hours after injecting 19-nortestosterone, a gradual increase of AAS receptor binding in cytoplasm occurs and reaches maximum values by the fourth hour. The rise in the number of free cytoplasmic androgen receptors during 2 to 4 hours is associated with the migration of the receptors from the nucleus back into cytoplasm. Such conclusion is based on the measured values of 19-nortestosterone binding by nuclei from skeletal muscle, which was at maximum 2 hours after the hormone injection. Hence, a

single injection of 19-nortestosterone into an organism leads to the change in the number of free androgen receptors in skeletal muscle cytoplasm with the formation of a hormone-receptor complex and with its further translocation to a cellular nucleus.

By comparing the degree of affinity of various AAS for androgen receptors in skeletal muscle cytoplasm it was shown that 19-nortestosterone is bound to the receptor three times more strongly than testosterone is. The absence of the enzyme 5a-reductase in skeletal muscle leads to the rise in myotrophic activity in a series of AAS, primarily 19-nortestosterone and its derivatives.

Hence, in the cytoplasm of various tissues, there are group of receptor proteins which selectively interact with androgens and AAS. The process of hormone-receptor interaction has a reversible charater. It may be represented by a number of kinetic parameters. The concentration of binding sites of cytoplasmic androgen receptors has a limited capacity and makes up only 2 to 4 fmol/mg of protein. One of the most important characteristics of cytoplasmic androgen receptor binding sites is a stereospecificity of their affinity for certain hormones, that is the ability of receptors to selectively bind separate androgens, active metabolites and AAS, so that the androgen receptor of the prostate has a higher affinity for dihydrotestosterone than for testosterone. For the translocation of a cytoplasmic hormone receptor complex to a nucleus, an activation of this complex, or of its conversion to a form in which it is able to be bound to structural components of cell nuclei is needed.

The translocation of a hormone-receptor complex from cytosol to nuclei under the influence of hormones appears to be a general property of steroid hormone receptors; this property seems to be common for all the target organs and hormones. The principal biological significance of the translocation process is the fact that the interaction between the steroid-receptor complex and chromatin causes a modification of gene transcription and leads to the synthesis of hormone controlled proteins. The properties of the receptor are changed by steroid binding in such a way that it gains affinity for the nuclei. These alterations in a receptor molecule are called "activation". The hormone binding to a receptor and the hormone activation are not simultaneous, but rather successive events. It may be demonstrated by the fact if target cells are incubated with a steroid at a low temperature, a steroid-receptor complex is formed in cytoplasm and it remains here for a relatively long period of time before it is bound to nuclei. Under the influence of high temperatures, the complex develops the ability to interact with cellular nucleic membranes and may be translocated from cytoplasm to nucleus.

When hormonal information passes to the nucleus, the receptor may appear in at least two states: inactive and active. When studying hormone-receptor complexes, the alteration of a receptor molecule structure was demonstrated. Although they were assigned the terms "activation" and "transformation", they are often used in the same sense. There also exists the possibility of differentiating these notions and connecting them with certain receptor physical-chemical properties. The term "transformation" of a receptor is tightly associated

with the alterations of a molecular mass and the receptor molecular sizes that may be determined from different methods (sedimentation constant, the velocity of migration during electrophoresis or by electrofocusing). The term "activation" of a receptor can be defined as a conformational modification of receptor structure that leads to the appearance of the ability to bind to cellular nucleic components, primarily to chromatin. It appears to be an indispensable stage of cytoplasmic hormone-receptor complex translocation to cell nucleus.

The results of determining androgens and AAS nuclear binding in skeletal muscles is given in table 1. One can see that skeletal muscle nuclei have the ability to bind androgens; however, to date there has been no experimental evidence to support this view and some skepticism has been expressed about the possibility of identifying androgen nuclear binding in skeletal tissues.

While studying the mechanism of AAS receptor binding in skeletal muscles, it was considered whether there exists the possibility of such a hormone-receptor complex transport to nuclei and their interaction with intracellular structures.

The following series of experiments shows that according to extraction with salt solutions of various concentrations, nuclear androgen receptors from rat skeletal muscles may be divided into three fractions. The first, easily released, fraction of receptors is isolated by the concentration of 0.25 M KCl and it probably represents the receptors which are mainly free from hormones. The second receptor fraction is extracted from the nuclei with a solution of 0.4 M KCl and represents slowly dissociating hormone-receptor complexes extracted from chromatin.

The third fraction of androgen receptors dissolves with a solution of 1.0 M KCl during extraction. If the first two androgen receptor fractions, according to their localization, may be attributed with certainly to receptor complexes interacting with chromatin, then the receptors which are insoluble during salt extraction might be bound to the nuclear matrix.

In spite of considerable difficulties that are inevitably caused from the interpretation of the data obtained in the experiments with nuclear androgen receptors, these findings nevertheless indicate that

1. Androgen receptors may be in a free or bound to a hormone state in the nucleus, and
2. Receptor molecules have functional groups or centers which allow binding to DNA, RNA, nonhistone proteins of chromatin, and nuclear matrix.

The functional necessity of such a heterogeneous receptor location in the nucleus has not been clearly established and needs special study.

To estimate the receptor cycle in skeletal muscles by saturating an organism with AAS, we investigated temporal alterations of cytoplasmic and nuclear 19-nortestosterone binding after injecting a physiological dose of hormone. The results of the 19-nortestosterone binding in cytoplasm and skeletal muscle nuclei, as well as its content in rats' blood, are given in Figure 2.

After a hormone injection, the number of free cytoplasmic receptors rapidly reduces to 15 to 20% of the initial level. Such a decrease in the number of receptors is associated with their interaction with 19-nortestosterone and the translocation of hormone-receptor complexes to the nucleus. In 2 hours, an increase in receptor binding is observed and in 4 hours it twice exceeds the initial level. Such an increase in the number of cytoplasmic receptors is probably due to their return to cytoplasm. The maximum value of 19-nortestosterone nuclear binding was observed 2 hours after injection of the hormone. The change of 19-nortestosterone concentration in the blood has an impulsive character, with a maximum of 30 min after an injection. Four hours later the hormone concentration in the blood is practically reduced to zero. The findings obtained show that after a single injection of AAS-19-nortestosterone into the organism in skeletal muscles, there occurs a hormone binding to a receptor, the translocation of hormone-receptor complex to the nuclei, and the subsequent return to cytoplasm. The receptor cycle is completed 4 to 6 hours before free receptors migrate to the cytoplasm. It also should bs noted that in this series of investigations we determined only the fractions of free androgen receptors. To have more precise characteristics of the receptor cycle it is necessary to know the total quantity of androgens (both free and occupied by a hormone).

Summing up the problems discussed in this part it should be noted that we have accumulated a large quantity of experimental facts which demonstrate a scheme of biological processes ensuring the participation of receptors in the transmission of a hormonal signal into a cell.

The receptor cycle includes the following successive stages:

1. Transport of hormone to a cell.
2. Receptor activation, receptor phosphorylation, hormone-receptor complex formation.
3. Hormone-receptor complex translocation to the nucleus.
4. Hormone-receptor complex interaction with chromatin acceptor sites and hormone signal transmission.
5. Hormone-receptor complex release from chromatin, receptor dephosphorylation, dissociation to free receptor and hormone.
6. Transport of free receptor to cytoplasm, receptor splitting by cytoplasmic proteases.
7. Synthesis of new receptor molecules in cytoplasm.

The sequence of these possible events for the androgen receptor binding AAS in skeletal muscles is given in Figure 3. Today only the first step in studying the mechanism of AAS action has been taken. However, by now there can be no doubt that the receptors appear to be one of the main links in the mechanism which ensures the realization of the hormonal signal at the level of gene expression. Experimental data on the high degree of correlation between the presence of receptors and the reactivation of tissues and between hormone affinity for androgen receptors and its biological effectiveness are the basis for the hypothesis of the important role of receptors in the realization of AAS hormonal effects.

Summing up the basic alterations in metabolism which are observed at early stages of AAS action, one could note the following events:

1. Rapid stimulation of the activity of nuclear RNA polymerases and RNA synthesis.
2. Ability of RNA, extracted from the cells and worked up with steroid hormones, to cause some hormonal responses in hormone deficient cells.
3. Prevention of the effect of steroids by inhibitors of RNA and protein synthesis.
4 . Induction of proteins including enzymes.

According to the character of alterations caused by the metabolism of skeletal muscles, a number of common traits may be observed in the action of AAS and physical loads. Generally they can be represented as an active effect on the process of protein synthesis. A comparison of separate indices of metabolism is by analogy justified up to a certain limit, but it does not help to explain the molecular mechanism of action of these two factors Therefore, experiments with simultaneous action on AAS skeletal muscle and systematic function are needed. In our laboratory we carried out a prolonged experiment where rats performed intensive strength exercises for 84 days and were injected with 0.3 mg/kg of methandienone (2 courses, each for 21 days). The finding demonstrated an intensification of anabolic processes and the stimulation of basic metabolic pathways ensuring an increase in muscular protein synthesis. We also investigated the velocity at which ^{14}C-orotic acid was incorporated into RNA fractions, ^{4}C-leucine into muscular proteins and amino acids, as well as the activity of cytochrome oxidase and aspartate aminotransferase and the content of glycogen, ATP, CP and lactic acid in skeletal muscle. The findings showed that the functional activity of skeletal muscle determined the intensity of the development of anabolic processes following exercise. It is of considerable importance for the manifestation of hormone action that the dose exceeds a certain threshold value. Our experiments showed an increase in muscular tissue radioactivity, by injection of ^{14}C-leucine and ^{14}C-orotic acid into the animals which had received methandienone following exercise, which testifies to the hormone effect on the mechanism of substance transport regulation to skeletal muscles. As stated above, AAS injection into an organism causes a rise in DNA-dependent RNA polymerase I activity in skeletal muscle. It seems reasonable to hypothesize that AAS use during systematic muscular activity is also reflected by this enzyme activity. The data obtained on the rise of RNA polymerase activity in the nuclei of rat skeletal muscle subjected to exercise training confirms this supposition (Table 2). One can see that exercise training induces a rise in enzyme activity by 47%. After four injections of nandrolone-decanoate during the training process, RNA polymerase activity rose by 93% in comparison with the control. The kinetics of this reaction confirmed the fact that the increase in RNA synthesis in skeletal muscle was associated with a rise in RTNA polymerase activity. A rise in the activity of cytochrome-C oxidase and aspartate aminotransferase parallel with the acceleration of the incorporation of ^{4}C-leucine into myofibrillar and sarcoplasmic proteins

testifies to the activation of protein synthesis processes in muscle under the influence of AAS.

The data obtained give an indication of the presence of many factors that can determine the development of methandienone anabolic action in skeletal muscle by an intensive muscular activity. One may consider that the intensification of skeletal muscle metabolism during systematic physical activity increases the sensitivity of muscular tissue to methandienone, and the threshold values of hormone effective doses prove to be lower than those under the usual regime.

The participation of AAS in metabolic regulation is not limited to sex organs and skeletal muscles. These steroid hormones actively regulate metabolism in other various organs and tissues of an organism. The plurality of AAS hormonal effects which regulate separate reactions of carbohydrate, lipid, and protein metabolism should be noted. By estimating the AAS anabolic action, the major effect is on the process of protein synthesis; however, these steroid hormones also considerably intensify lipid metabolism in the organism. AAS regulated the activity of separate enzyme reactions accelerating the oxidation of fatty acids. It should be taken into consideration that in addition to such diverse positive effects, the systematic use of AAS leads to a disturbance in the function of the organism's endocrine system. First of all, it alters the content of endogenous androgens themselves. A sharp inhibition of testosterone synthesis in Leyding cells is one of the most striking examples of such endocrine alterations which is associated with the presence of AAS. The basic enzymes responsible for testosterone synthesis in mammals are concentrated in endoplasnatic reticulum and mitochondria of Leydig cells.

Attempts were made to bind the alterations in the level of testosterone secretion with those of ultrastructure and Leydig cell volume. It turned out that the alteration of testosterone secretion was closely associated with only one morphological index of these cells, the volume of smooth endoplasmic reticulum. By injecting gonadotrophin into an organism, growth of smooth endoplasmatic reticuluum occurs and the amount of testosterone synthesized increases. Conversely, by injecting AAS, some morphological alterations occur that lead to the diminution of smooth endoplasmatic reticulum and the decrease of testosterone synthesis. The inhibition of testosterone synthesis is accompanied in turn, a change in concentration of this hormone in other androgen dependent tissues. The change of androgen concentration was clearly demonstrated in the experiments which used a systematic method of training animals (Table 3).

The use of AAS nandrolone-phenylpropionate during such training regimens (1 mg/kg of mass/day, 8 times for 28-day training cycle) led to a decrease in the level of testosterone in blood, skeletal muscle and heart muscle. This phenomenon was demonstrated in experiments on animals of different gender and was linked with the inhibition oi androgen synthesis by the use of AAS. A possible mechanism regulation the level of androgen in tissue is associated with the presence of androgen receptors, which prove to be occupied

chiefly by AAS and their metabolites. It excludes the androgen hormones from the receptor sphere and, according to the mechanism of reverse bond, it inhibits the processes of their synthesis.

Now let us consider some possible molecular mechanisms of the specific effect of AAS on metabolism in muscular tissues and the mechanisms of these regulatory processes.

All the principal stages of steroid interaction that are typical for the other target organs, primarily for androgens may be seen by the effects of intracellular AAS in skeletal muscle. Therefore, any potential mechanism of AAS participation in the regulation of metabolism should include the description of wellknown notions of the succession of events which involve androgens. The differences may be as subtle as the method of injection of AAS into the organism and blood.

The order of temporary events occuring in skeletal muscle after 19-nortestosterone injection is given in Table 4. The regulation of a molecular mechanism of AAS interaction with proteins in muscular tissue includes several successive stages during which the change in concentration of the hormone itself, or the substance interacting with it, is reflected by the specific physiological effect of the hormone. Intracellular AAS binding by transport proteins in blood may be considered to be the first stage of the regulation (Fig.4). In the target cell itself there are no less than six stages in which the regulatory mechanism is possible:

1. AAS binding to a receptor in the cytoplasm;
2. Hormone-receptor complex translocation to the nucleus
3. Hormone-receptor complex binding to nonhistone proteins of chromatin;
4. Hormone-receptor complex release from chromatin;
5. Hormone-receptor complex dissociation nucleus;
6. Biotransformation of AAS and its metabolities.

One can imagine that quantitative changes in the expression of certain, principally specialized, genes, which control the complicated multistage process of protein biosynthesis in a living cell, are the basis oi the mechanism of AAS action.

Study of molecular mechanisms of the hormonal regulation of gene action shows that AAS exert their basic influence on transcription only in the form of hormone-receptor complexes and the association of these complexes with DNA-specific sites in chromatin. At the same time, it is quite possible that the character of the interaction of the hormone-receptor complex is also dependent on higher levels of DNA and DNP structure and on the general organization of chromatin.

By examining the ultimate effects of AAS, it should be taken into consideration that anabolic and androgenic activities which are attributed to these steroids only differ in classification signs and not because these signs or properties concern the steroids. The androgenic effect of AAS in an organism differs from the anabolic effect of the same hormones only according to their localization in tissues and organs and not in their

molecularbiological nature. The general molecular mechanism of hormonal regulation of a specific gene activity is the basis of anabolic and androgenic effects of AAS. The specific functions of AAS in an organism are versatile and widely represented in intracellular metabolism of various organs and tissues. AAS positively, but to varying degrees, influence the growth of many tissues. The action of AAS is accomplished through the enzymic apparatus of a target tissue. The concentration of androgenic receptors in tissues and the reactivity of the tissue towards hormone action may change under the influence of AAS. Quantitative changes in the concentration of androgen receptors and the sensitivity of the tissues to AAS as well as their dependence on sex, age, and functional state, are shown. Active participation of AAS in the regulation of the processes of adaptation of an organism to muscular activity of a different trend was revealed.

The experimental material made it possible to offer the sequence of biochemical processes that ensures the passage of an AAS hormonal signal into the cell in the form of a closed receptor cycle. One can distinguish three peculiarities of the receptor cycle action based on new experimental facts. In recent years more and more data have accumulated showing that the phosphorylation of proteins is a part of a general mechanism of regulation which ties the hormonal and nervous signals with the processes of intracellular metabolism. This concept is based on the possibility that different cellular functions are controlled by protein kinases and protein phosphotases. This has gradually been confirmed by various examples of intracellular regulation including the activation of steroid hormone receptors. It was shown that the process of intracellular receptor activation occurs in the presence of protein kinase and is accompanied by the phosphorylation of a receptor molecule with the participation of ATP. Therefore the process of the interaction of a hormone with a receptor inside the cell is stipulated by the presence of ATP and the state of energy metabolism. The second peculiarity involves the opposite process, that of receptor inactivation, being connected with the dephosphorylation of a receptor molecule in the nucleus under the action of protein phosphatase. The participation of the processes of receptor protein phosphorylation as an indispensable condition for receptor conversion to an activated state in the presence of a hormone tightly associates the processes of hormonal and intracellular autonomic regulation to a common biochemical mechanism of metabolic control. The third peculiarity shows the possibility of the existence of a closed receptor cycle in the cell nucleus. The presence of free receptors, hormones, protein kinases and protein phosphatases not only in the cytoplasm but also directly in the cell nuclei creates the necessary conditions for the formation of a hormone-receptor complex.

The experiments carried out by different authors show that the intensity and the character of AAS metabolism depend on the modification of their chemical structure as well as on the enzymic systems of human and experimental animals ensuring the catalysis of the transitions. In the first stage of the biotransformation, AAS participates in the reactions of oxidation, reduction, and hydrolysis. Later on there occurs the formation of more polar

hydrophilic metabolites in the reactions of conjugation, with sulphuric and glucuronic acids, which are excreted from the organism in the same form. The basic processes of AAS biotransformation are catalyzed with various enzymic systems which are concentrated in the endoplasmic membranes of liver hepatocytes. Due to the variety of AAS chemical structures essential differences in the degree of interaction with blood transportproteins and receptor protein target tissues make use examine the common molecular mechanism of AAS action with a certain amount of prudence. The multiplicity of methods of AAS hormonal effects is supposed to be due to a receptor functional heterogenity. The possibility of the existence of receptors which differ in a number of physical-chemical properties has been confirmed experimentally. The appearance of active metabolites with a higher affinity for androgen receptors in the process of AAS biotransformation may also lead to the ultimate hormonal effect. The phenomenon of the multiplicity of an endocrinic regulation may be examined as alternative means of AAS action in muscular tissue. It is manifested in the metabolic control of each tissue with the hormone complex. The effect of several AAS on the level of homone growth is a good example. Recently our notions about the basic functions of AAS and metabolism in an organism have become more precise and extensive. However, the participation of AAS in the regulation of intracellular metabolism ensuring the development, the growth, and the reduction of a cell remains one of the major problems which is of interest to many specialists. It is evident that new information on the molecular mechanisms of steroid hormones including AAS will emerge in the near future.

TABLE I
The Concentration of Nuclear Androgen Receptor Binding Sites in Skeletal Muscles

Nuclei components	K_d(n*M*)	B_{max} (fmol/mg DNA)
Intact nuclei	11.2	24
0.4 *M* KCl extract	9.9	55
Nuclear matrix	2–3	56

TABLE 2
Effect of Training and Nandrolone-Decanoate on Nuclear RNA Polymerase Activity of Rat Skeletal Muscles

Experimental conditions	pmol ^{3}H-UMP/mg DNA ± SD
Control	73 ± 5
Training	107 ± 9
Training with nandrolone-decanoate	141 ± 9

TABLE 3
Testosterone Content in Rat Organs (pg/g wet wt ± SD)

Tissue or fluid	Nontrained	Trained	Trained with hormone taking
Blood	118.0 ± 22.7	64.0 ± 7.8	73.0 ± 5.3
Skeletal muscles	200.0 ± 34.3	100.6 ± 12.6	37.9 ± 5.8
Heart	235.0 ± 26.9	127.0 ± 19.5	64.4 ± 6.3

TABLE 4
Temporary Distribution of Events in Skeletal Muscle After 19-Nortestosterone Injection

Processes	Time (hr)
19-Nortestosterone binding to a receptor and transport to nucleus	0.1
mRNA synthesis	0.5
pRNA synthesis	2.0
Sarcoplasmic protein synthesis	5.0
Myofibrillar protein synthesis	10.0

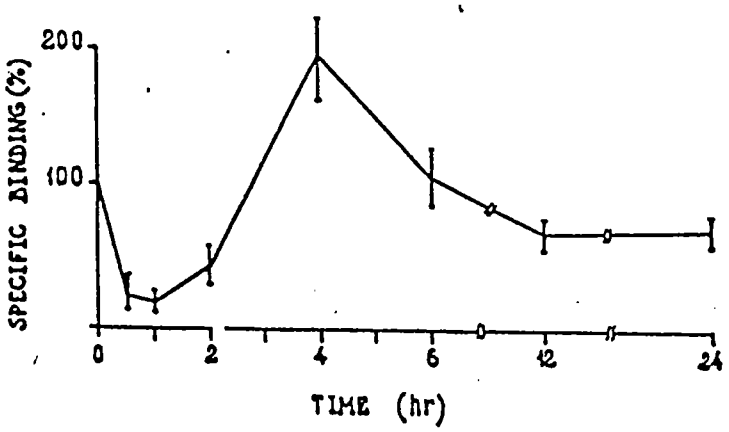

FIGURE I. 19-Nortestosterone binding in rat skeletal muscle cytosol.

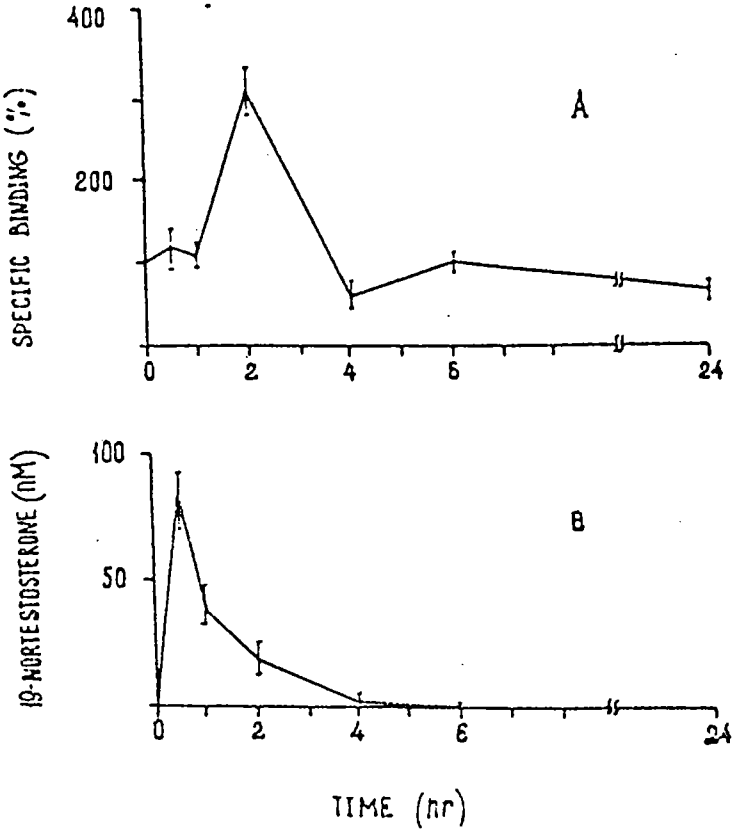

FIGURE 2. Binding of ^{3}H-19-nortestosterone in rat skeletal muscle nuclei (A) and its concentration in blood (B) after hormone injection.

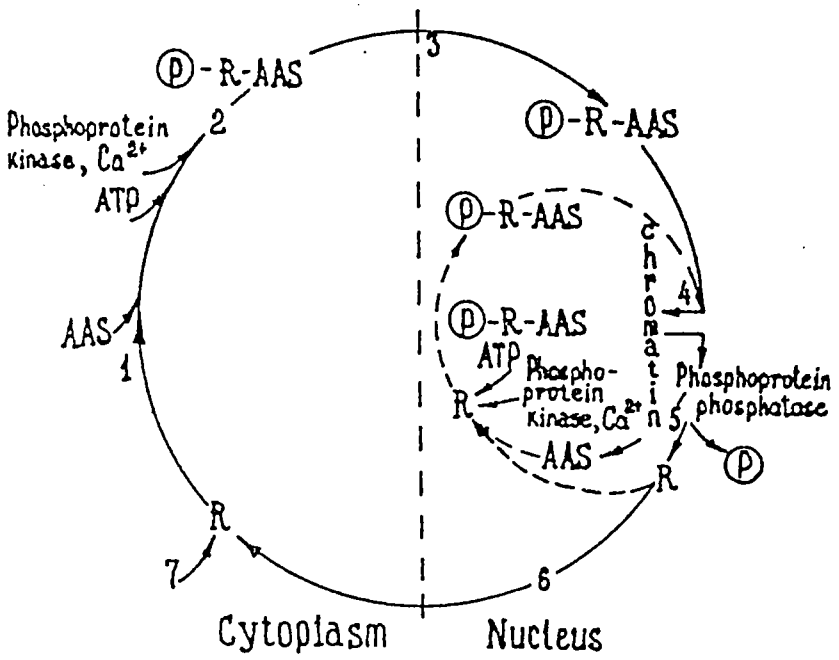

FIGURE 3. Scheme of androgen receptors intracellular cycle in skeletal muscles. R, androgen receptor; P–R, activated receptor; P–R–AAS, hormone-receptor complex.

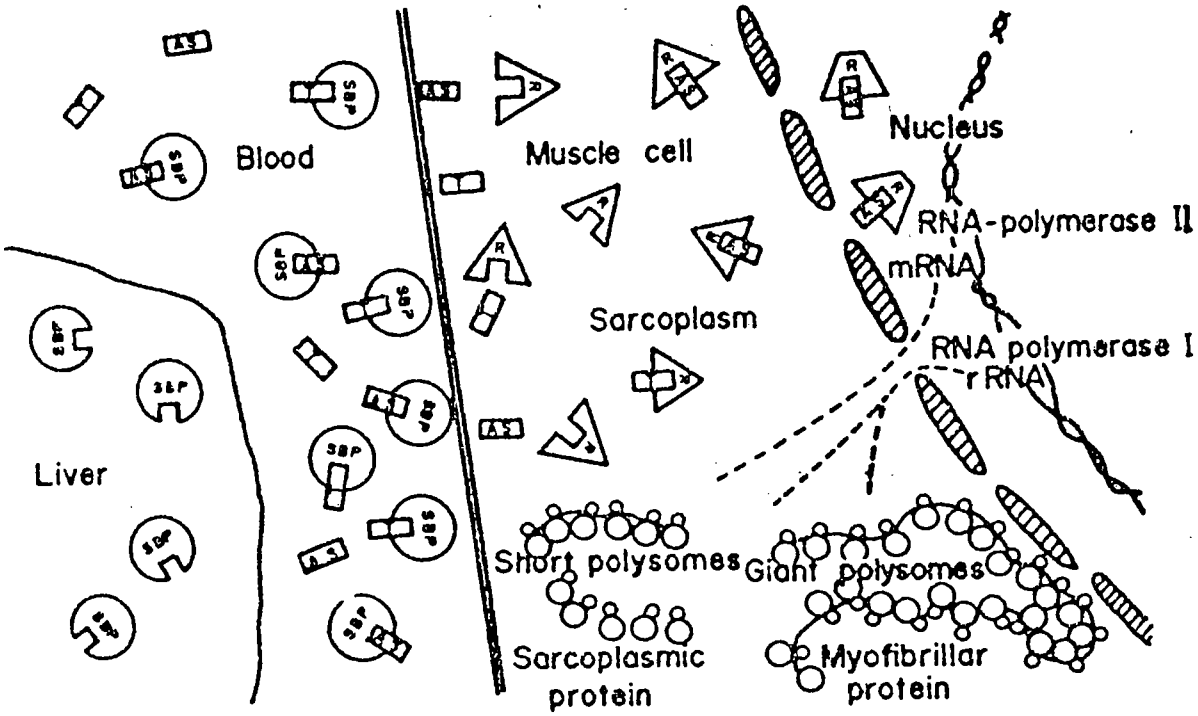

FIGURE 4. A possible model of AAS action in skeletal muscle.