ANDROGEN BINDING BY SKELETAL-MUSCLE NUCLEI

M. G. Stepanov and B. I. Fel'dkoren

UDC 612.352.3-06:612.621.31

KEY WORDS: skeletal muscles; andrògens; steroid hormones.

It has now been established that the effect of steroid hormones is mediated by interaction with cytoplasmic receptors and translocation of hormone—receptor complexes into the nuclei, and is realized through activation of the genetic apparatus of the target organ [1, 2, 5]. Studies of the effect of androgens on skeletal muscles have demonstrated the existence of cytoplasmic receptors for these hormones [6, 11] and activation of RNA synthesis [8]. Meanwhile the process of androgen binding by isolated skeletal muscle nuclei remains virtually unstudied.

The aim of this investigation was accordingly to study binding of androgens by isolated skeletal muscle nuclei and some properties of the nuclear hormone—receptor complex.

EXPERIMENTAL METHOD

Mature noninbred male rats weighing 200-250 g were used. To stimulate translocation of hormone—receptor complexes from the cytoplasm into the nucleus, the animals were given an injection of 19-nortestosterone in a dose of 2.5 mg/kg 1-1.5 h before decapitation. All subsequent operations were performed at 0°C. Nuclei were isolated from hind limb muscles by differential centrifugation in 2.2 M sucrose [8]. The purity of the nuclear preparation was verified under the microscope. The nuclei were suspended in buffer containing 50 mM Tris-HCl, pH 7.4; 25 mM KCl, 6 mM $MgCl_2$, 1.5 mM EDTA, 10 mM 2-mercaptoethanol, and 250 mM sucrose. The DNA concentration in the nuclear suspension was determined by the diphenylamine method [3]. The total androgen-binding ability of the nuclei was determined after incubation for 18 h with [6,7-$^3H_2$]-19-nortestosterone (1.1-1.3 GBq/mmole; or [1,2,6,7-$^3H_4$]-testosterone (3.3-4.0 GBq/mmole) in a concentration of 40 nM, and in experiments to determine the dissociation constant [9], in a concentration of between 1.5 and 50 nM. To determine nonspecific binding, a 500-fold excess of nonradioactive 19-nortestosterone was added to some of the samples. Receptors were extracted from the nuclei with 0.4 M KCl for 1 h. In some experiments the nuclei were extracted with 0.4 M KCl immediately after isolation and the extract was incubated with [^{3}H]-19-nortestosterone under the same conditions. The protein-bound and free steroid were separated on columns (75 × 10 mm) with Sephadex LH-20 (Pharmacia, Sweden). Radioactivity of the fraction of protein-bound steroid was determined on a Mark III liquid scintillation counter (Tracor, USA), with counting efficiency of 56-58%, and expressed in cpm. Specific binding was calculated as the difference between total and nonspecific binding, allowing for the specific activity of the [^{3}H]-19-nortestosterone preparation, and was expressed in femtomoles/mg nuclear DNA. In the experiments involving enzyme treatment, DNase I and pronase R (Sigma, USA) were used in concentrations of 0.5-1 mg/ml, ensuring sufficiently complete hydrolysis of the biopolymers. The enzyme was added at the following stages; 1) on incubation of the nuclear extract in 0.4 M KCl with [^{3}H]-19-nortestosterone; 2) during extraction of nuclei incubated with [^{3}H]-19-nortestosterone with 0.4 M KCl; 3) after extraction of nuclei incubated with [^{3}H]-19-nortestosterone, with 0.4 M KCl. Enzyme treatment continued for 30 min at 20°C and until the end of the corresponding stage at 0°C. All calculations were done on computers of the 21MX (Hewlett-Packard, USA) and Elektronika D3-28 type, using our own programs.

EXPERIMENTAL RESULTS

Investigation of dependence of nuclear binding of [^{3}H]-19-nortestosterone on temperature and incubation time showed that binding reached a maximum after 18 h of incubation at 0°C.

Leningrad Scientific-Research Institute of Physical Culture. (Presented by Academician of the Academy of Medical Sciences of the USSR V. G. Baranov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 5, pp. 606-608, May, 1984. Original article submitted February 7, 1983.

0007-4888/84/9705-0657$08.50

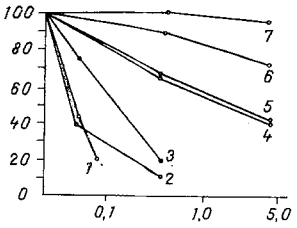

Fig. 1

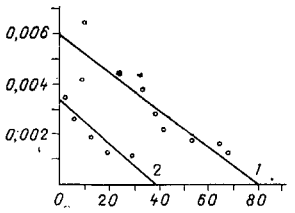

Fig. 2

Fig. 1. Binding of [^{3}H]-19-nortestosterone by skeletal muscle nuclei in presence of steroid competitors. Abscissa, concentration of competitor (in μM); ordinate, specific binding (in % of maximal). Competitors: 1) 19-nortestosterone, 2) 5-α-dihydrotestosterone, 3) testosterone, 4) progesterone, 5) 17-β-estradiol, 6) dexamethasone, 7) corticosterone.

Fig. 2. Determination of dissociation constant of nuclear androgen receptors. Abscissa, concentration of bound [^{3}H]-19-nortestosterone (in pM); ordinate, ratio of concentrations of bound and unbound [^{3}H]-19-nortestosterone. 1) Incubation of nuclei (K_α = 13.8 nM), 2) incubation of nuclear extract with 0.4 M KCl (K_α = 11.0 nM).

These conditions were adopted for future experiments. During incubation at 20-25°C this degree of binding could not be achieved. Its maximum, which was 60-65% of that at 0°C, was reached after incubation for 1-2 h; binding of the steroid subsequently decreased, and the higher the temperature, the more rapid the fall.

Specificity of androgen binding was established by incubation of the nuclei with [^{3}H]-19-nortestosterone in the presence of unlabeled steroids of different classes. The results of these experiments, illustrated in Fig. 1, are evidence that only androgens effectively inhibit binding of [^{3}H]-19-nortestosterone by skeletal muscle nuclei (5-α-dihydrotestosterone ≈ 19-nortestosterone > testosterone). The relative competitiveness of estradiol and progesterone was about 2%, and of corticosterone and dexamethasone under 1%. Similar results were obtained using [^{3}H]testosterone as the ligand.

To determine quantitative parameters of androgen binding by nuclei and their extract with 0.4 M KCl the usual method of incubation with increasing concentration of labeled ligand was used. Characteristic results of these experiments are given in Fig. 2. The linear form of the relationship between the ratio of concentrations of protein-bound steroid to free steroid on the concentration of bound steroid is evidence of the existence of only one type of binding site for androgens. Values of the dissociation constant obtained by incubation of nuclei and extract of nuclei in 0.4 M KCl with [^{3}H]-19-nortestosterone were 11.6 ± 2.5 and 9.9 ± 1.6 nM respectively (difference not significant). The high affinity, specificity, and the limited capacity of the binding sites are evidence of the receptor character of androgen binding by skeletal muscle nuclei. The dissociation constant of the nuclear androgen receptors in skeletal muscles was found to be higher than for cytoplasmic receptors [4, 6, 11], and lay within the range cited by different workers for nuclear steroid receptors in other tissues [1, 7, 10]. The number of binding sites of nuclear androgen receptors in skeletal muscles of animals injected with 19-nortestosterone varied within fairly wide limits, probably because of differences in the degree of translocation of hormone–receptor complexes from cytoplasm into nuclei. In experiments in which the animals did not receive 19-nortestosterone this value was 24.1 ± 1.7 fmoles/mg DNA or 13.7 ± 1.0 fmoles/g tissue. This is lower than for cytoplasmic androgen receptors in skeletal muscles [6, 11], and much lower than for nuclear and cytoplasmic receptors in target tissues for androgens belonging to the reproductive system [10, 12].

To determine the nature of nuclear androgen receptors in skeletal muscles, nuclei were treated with enzymes in the course of extraction with 0.4 M KCl or after extraction with 0.4 M KCl. The results of these experiments (Table 1) show that the receptors are protein in nature, for treatment with pronase at either stage led to considerable lowering of the level of bound [^{3}H]-19-nortestosterone.

TABLE 1. Effect of Treatment with Pronase and DNase on Specific Binding of [^{3}H]-19-Nortestosterone by Skeletal Muscle Nuclear Receptors (M ± m)

Conditions of enzyme treatment	Binding, % of control
Without treatment (control)	100.0 ± 8.3
Pronase during incubation of extract	15.4 ± 18.8
Pronase during extraction	0.0 ± 3.6
Pronase after extraction	17.0 ± 4.2
DNase during extraction, pronase after extraction	8.6 ± 4.4
DNase during incubation of extract	19.8 ± 5.5
DNase during extraction	146.2 ± 4.3
DNase after extraction	108.1 ± 14.6

The results of treatment with DNase suggest that DNA has a stabilizing action on nuclear androgen receptors, for removal of DNA during incubation of the extract with [^{3}H]-19-nortestosterone leads to a significant decrease in the quantity of protein-bound radioactivity, but during treatment of the extract after incubation of the nuclei with [^{3}H]-nortestosterone no such effect was observed. Treatment of the nuclei with DNase during extraction with 0.4 M KCl after incubation with [^{3}H]-19-nortestosterone significantly increased the number of receptors discovered. This fact may perhaps be connected with solubilization of receptors firmly bound with chromatin, unextractable under ordinary conditions by 0.4 M KCl [1, 2].

The results are thus evidence that skeletal muscle nuclei contain protein receptors characterized by specificity, high efficiency, and limited capacity of their binding sites for androgens.

LITERATURE CITED

1. W. Mainwaring, Mechanisms of Action of Androgens [Russian translation], Moscow (1979).
2. V. B. Rozen and A. N. Smirnov, Receptors and Steroid Hormones [in Russian], Moscow (1981).
3. K. Burton, Biochem. J., 62, 315 (1956).
4. M. A. Ho-Kim, R. R. Tremblay, and J. Y. Dubé, Endocrinology, 109, 1418 (1980).
5. S. Liao, Int. Rev. Cytol., 41, 87 (1975).
6. G. Michel and E.-E. Baulieu, Endocrinology, 107, 2088 (1980).
7. S. Onoyama and S. Ichii, Endocrinol. Jpn., 29, 349 (1982).
8. V. Rogozkin and B. Feldkoren, Med. Sci. Sports, 11, 345 (1979).
9. G. Scatchard, Ann. New York Acad. Sci., 51, 660 (1949).
10. D. A. N. Sireff and J. K. Grant, J. Endocrinol., 92, 95 (1982).
11. M. Snochowsky, E. Dahlberg, and J.-A. Gustaffson, Eur. J. Biochem., 111, 603 (1980).
12. W. W. Wright and A. I. Frankel, Endocrinology, 107, 314 (1980).