7. S. D. Blair, J. Nanchahal, C. M. Backhouse, et al., Lancet, 483 (1987).
8. R. A. Clark, The Molecular and Cellular Biology of Wound Repair, R. A. Clark and P. M. Henson (eds.), New York (1988), pp. 3-33.
9. D. F. Kennedy and W. Cliff, Pathology, **11**, 207 (1979).
10. M. L. Zhang, Z. Chang, X. Han, and M. Zhu, Burns, **12**, 540 (1986).

MECHANISMS OF THE THYMOLYTIC EFFECT OF METHANDROSTENOLONE

N. S. Chermnykh, A. S. Dukhanin, Yu. A. Andreev, and Ch. T. Karymshakova

UDC 615.357:577.175.624].015.4:612.438].076.9

Key Words: thymocytes; receptors; anabolic steroids; thymus.

Anabolic steroids, testosterone derivatives widely used in clinical practice, give rise to a number of side effects, including depression of activity of the T system of immunity. Some authors [1, 6, 7] have observed that anabolic steroids cause a decrease in weight of the thymus. However, the mechanism of the thymolytic action of the androgenic-anabolic steroids, by contrast with that of the glucocorticoids [3, 4], remains virtually unstudied.

The aim of this investigation was to study the effect of administration of methanodrostenolone for 7 days on protein biosynthesis in the rat thymus and the character of its interaction with the thymus cytosol and thymocytes.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred male rats weighing 160-200 g. Bilateral orchidectomy was performed 5 days before the experiment began. Methandrostenolone (MA, USSR product; equivalent Metandienone) was injected intraperitoneally in a dose of 10 mg/kg (the dose exhibiting maximal anabolic activity of MA in animals [2]) in the form of a solution in aqueous alcohol, in a volume of 0.2 ml. The intensity of protein biosynthesis was investigated by the use of a radioactive indicator [2]. The character of binding of ^{3}H-MA with the soluble fraction of thymus homogenate was determined by the following method: thymus glands were homogenized in TDM buffer (10 mM Tris-HCl, 0.5 mM dithiothreitol, 10 mM sodium molybdate, pH 7.8) and the supernatant was obtained at 10,000*g*. To 0.2 ml of supernatant 20 μl of a solution of ^{3}H-MA was added to final concentrations of between 10^{-8} and $5 \cdot 10^{-7}$ M. Samples were incubated for 2 h on an ice bath, after which 1 ml of a suspension of dextran-coated carbon was added to each sample, which was then shaken and allowed to stand for 10 min on the ice bath. After subsequent centrifugation at 5000*g* for 10 min 0.2 ml of supernatant was taken for radiometry and the quantity of bound ^{3}H-MA was expressed in cpm/mg protein. To determine specific binding of MA, incubation was carried out in the presence of a 500-fold excess of unlabeled MA. The difference between binding of ^{3}H-MA in the absence and presence of an excess of unlabeled MA having been calculated, parameters of MA binding by the thymus cytosol were determined with the aid of standard mathematical software for the EMG 666/B computer (Hungary). Ligand specificity of MA receptors was studied by competitive analysis of binding of ^{3}H-MA and of other unlabeled steroids (testosterone, estradiol, cortisol) by the cytosol.

Chromatography of ^{3}H-MA-receptor complexes was carried out by the method in [8]. To measure binding of ^{3}H-MA by isolated thymocytes, 20 μl of ^{3}H-MA (final concentrations in the incubation medium 10^{-8}-$5 \cdot 10^{-7}$ M) was added to the suspen

Department of Molecular Pharmacology and Radiobiology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR M. D. Mashkovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 5, pp. 475-477, May, 1990. Original article submitted December 14, 1987.

TABLE 1. Effect of Administration of MA (10 mg/kg) for 7 Days on Parameters of Protein Metabolism in Thymus of Intact and Castrated Rats ($M \pm m$)

Experimental conditions	Weight of organ		Protein content		Incorporation of ^{14}C leucine into protein, cpm/mg protein
	mg	per cent of body weight	mg	per cent of weight of organ	
Intact rates					
Control	335±26	0,22±0,03	52,7±9,9	15,7±2,3	1086±103
MA	323±30	0,19±0,02	51,1±5,4	15,8±1,4	986±88
Castrated rats					
Control	410±21	0,17±0,01	72,8±9,5	18,2±2,4	932±79
MA	299±34*	0,12±0,01*	32,7±8,7*	10,9±1,9*	627±54*

Legend. Mean result of six independent experiments are given. *$p < 0.05$, difference from control statistically significant.

sion of thymocytes ($1 \cdot 10^8$ cells/ml). The samples were incubated in the absence and in the presence of a 500-fold excess of unlabeled MA for 2 h at 4°C. Next, 10 ml of Hanks' solution (pH 7.4, 4°C) was added to 100 μl of the cell suspension and the mixture centrifuged at 2000*g* for 5 min. The supernatant was poured off and 0.3 ml of ethanol added to the residue to extract labeled MA. After 1 h the samples were recentrifuged and their radioactivity measured. For radiometry of the samples an SL-30 liquid scintillation radiometer was used.

EXPERIMENTAL RESULTS

The study of the effect of administration of MA for 7 days on the weight of the thymus, its protein content, and incorporation of labeled leucine into proteins of the thymus showed (Table 1) no change in the test parameters compared with the control. The weight of the thymus and its total protein content in castrated rats were not increased, and the percentage protein content of the thymus also was unchanged. After administration of MA the weight of the thymus fell to the level recorded in intact animals and this was accompanied by a decrease in the protein content in the gland and a decrease in the intensity of incorporation of labeled leucine into proteins. The results of the investigation indicate inhibition of protein synthesis by MA in the thymus of the castrated rats. Counting the number of thymocytes leaving the organ showed that the thymus of the castrated rats contained $(1.35 \pm 0.25) \times 10^9$ cells. Administration of MA to the castrated animals caused the number of thymocytes to fall to $(0.8 \pm 0.2) \times 10^9$ cells.

It can be postulated on the basis of our observations and data in the literature [5] that the action of MA on the thymus is due to its androgenic activity. To test this hypothesis we studied the character of binding of labeled MA by thymus cytosol and by thymocytes and determined its ligand specificity. The following parameters of binding of MA by thymus cytosol of intact rats were found experimentally: dissociation constants (K_d) = 27 and 10^{-9} M, number of binding sites (N) = 101 ± 12 fmoles/mg protein, whereas in castrated rats the corresponding values were $K_d = 9.1 \cdot 10^{-9}$ M and N = 122 ± 18 fmoles/mg protein. Subsequent competitive analysis of binding of ^{3}H-MA by thymus cytosol in the presence of different steroids enabled its type to be established (Table 2). The most actively labeled MA was displaced from its complex with the receptors by unlabeled MA and testosterone, whereas cortisol and estradiol, in a 100-fold excess, reduced the specific binding of labeled MA by thymus cytosol only by half. To determine the concentration of the hormone at which specific binding was inhibited by 50% (IC_{50}), the results of competitive analysis were plotted between semilogarithmic coordinates. Values of IC_{50} are given in the same table. The results showed that the binding capacity of sites interacting with ^{3}H-MA decreases in the following order: MA ≥ testosterone >> estradiol > cortisol.

Steroid-receptor complexes can exist in two basic states: initial (inactivated) and activated, in which its ability to bind with cell nuclei and with DNA rises sharply. These two forms of complex can be separated by chromatography on DEAE-cellulose [8]. To test the hypothesis that MA receptors are classical androgen receptors, we subjected the ^{3}H-MA-receptor complexes to chromatography on DEAE-cellulose. The elution profile of MA-receptor complexes in the composition of thymus cytosol, which was incubated with 90 nM ^{3}H-MA at 0°C for 2 h (a) or at 37°C for 30 min (b), is shown in Fig. 1. With the use of activated thymus cytosol (b) two fractions of hormone-receptor complexes were obtained by chromatography on DEAE-cellulose: I) weakly bound with DEAE-cellulose, leaving the column during elution with 50 mM KCl, and II) firmly bound, leaving during elution with 200 mM KCl. Fraction I, weakly adsorbed on the anion-exchange resin, corresponds to the activated

TABLE 2. Specific Binding of ^{3}H-MA by Thymus Cytosol of Rats in Presence of Various Steroid Hormones ($M \pm m$)

Hormone	Amount of bond ^{3}H-MA, cpm/mg protein			IC_{50}
	concentration of unlabeled steroid			
	$7{,}5 \cdot 10^{-8}$ M	$2{,}5 \cdot 10^{-7}$ M	$2{,}5 \cdot 10^{-6}$ M	
MA	374±81	103±33	—	$6{,}3 \cdot 10^{-8}$ M
Testosterone	607±94	88±26	—	$8{,}9 \cdot 10^{-8}$ M
Cortisol	980±72	760±49	606±60	10^{-5} M
Estradiol	1080±109	701±55	514±45	$2{,}5 \cdot 10^{-6}$ M

Legend. Specific binding of ^{3}H-MA ($2.5 \cdot 10^{-8}$ M) in absence of unlabeled hormones of 993 ± 185 cpm/mg cytosol protein. Men values from four independent experiments are given.

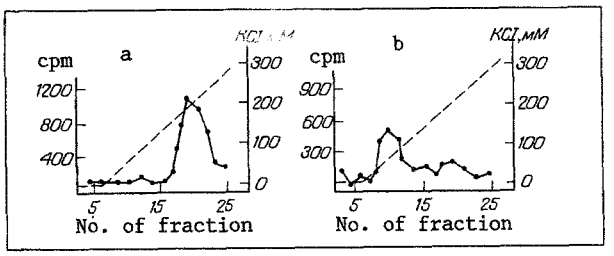

Fig. 1. Chromatography of cytosol ^{3}H-MA receptor complexes on DEAE-cellulose. a) Thymus cytosol incubated with 90 nM ^{3}H-MA at 0°C for 2 h, b) at 37°C for 30 min. Broken line indicates gradient of TDM buffer containing KCl.

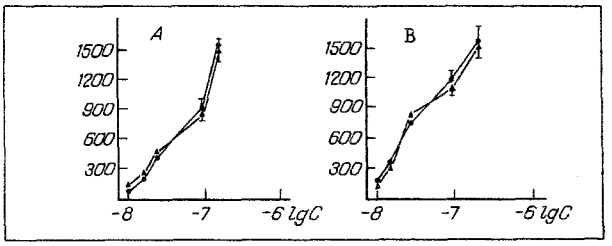

Fig. 2. Binding of ^{3}H-MA with thymocytes of intact (A) and castrated rats (B). Ordinate, binding of ^{3}H-MA by thymocytes (in cpm/10^7 cells) in absence of unlabeled MA (circles) and in presence of 500-fold excess of MA (triangles). C) concentration of labeled MA in incubation medium (in M).

form of MA-receptor complex, whereas fraction II corresponds to its initial, inactivated form. During elution of cytosol preparations obtained after incubation with ^{3}H-MA at 0°C, the MA-receptor complexes leave the column of DEAE-cellulose in one peak in the zone of elution of the inactivated form of the complex (Fig. 1a). Similar results, but during chromatography of androgen-receptor complexes isolated from rat thymus cytosol, were obtained in [7]. Thus analysis of the results of competitive binding and chromatography and their comparison with information in the literature indicate that MA receptors found in the cytosol of rat thymus are androgen receptors.

To determine the precise location of MA receptors in the thymus, binding of ^{3}H-MA by isolated thymocytes of intact and castrated rats was studied. The results in Fig. 2 showed that binding of ^{3}H-MA by thymocytes in the absence and in the presence of an excess of unlabeled hormone does not differ over the whole range of concentrations. Thus specific binding sites for MA are not found in thymocytes. The possibility cannot be ruled out that they are located in the epithelial cells of the thymus. Other workers [5, 7] reached similar conclusions when they showed that androgen receptors, detected in the cytosol of

a homogenate of whole thymus, do not belong to the lymphoid cells of the gland. These workers also showed that in vitro thymocytes do not exhibit sensitivity for androgens.

We concluded that the thymolytic action of androgenic-anabolic steroids is not the result of their direct effect on thymocytes, but is realized indirectly by endogenous factors controlling metabolic processes in lymphoid tissue.

LITERATURE CITED

1. V. G. Zaryan, V. Shilov, and V. Falomeev, Some Urgent Problems in Biology and Medicine [in Russian], Moscow (1968), pp. 77-78.
2. R. D. Seifulla, N. S. Chermnykh, and P. V. Sergeev, Farmakol. Toksikol., No. 1, 57 (1974).
3. P. V. Sergeev, T. G. Pukhal'skaya, V. N. Bol'shev, and N. S. Chermnykh, Farmakol. Toksikol., No. 6, 101 (1982).
4. N. S. Chermnykh, Farmakol. Toksikol., No. 1, 109 (1980).
5. A. McGruden and W. H. Stimson, Thymus, **3**, No. 2, 105 (1981).
6. P. Pearce, B. Khalid, and W. Funder, Endocrinology, **109**, No. 4, 1073 (1981).
7. N. Sato, S. Kyakumoto, R. Kurokava, and M. Oto, Biochem. Int., **13**, No. 1, 15 (1986).
8. T. J. Schmidt and G. Litwack, Physiol. Rev., **62**, No. 1, 1131 (1983).

EFFECT OF UHF RADIATION AND EXPOSURE TO COLD ON DESTRUCTION AND REPAIR OF REGENERATING BONE IN DOGS

N. A. Plotnikov, N. Z. Spiridonova, and G. I. Lavrishcheva

UDC 616.71-003.93-02:[615.846+615.832.9]-07

Key Words: UHF radiation; exposure to cold; regenerating bone.

Local cryosurgical destruction combined with physical and chemical factors, namely laser therapy and ultrasound, is used in organ-conserving operations of a sufficiently radical nature in oncology [2, 3, 5]. The mechanism of action of ultrahigh frequencies (UHF) is not always the same, but most investigators [1, 4] assess its effect as potentiating the destructive action of cold. Under the influence of UHF microwaves the thermophysical characteristics of biological tissue are modified, and this leads to a greater increase in the volume of cryonecrosis. Morphological changes in bone tissue after exposure to cold and in combination with UHF radiation have not been studied. We have investigated the character of prophylactic changes in tissues during cryosurgery, alone and in conjunction with preliminary UHF irradiation, with attention to the character of repair processes. The experimental model was mechanical injury to the mandible inflicted by a drill, allowing the proliferative reaction of cells of osteogenic tissue, which simulates to some degree the changes of neoplastic cell proliferation, to be studied.

EXPERIMENTAL METHOD

Altogether 36 experiments (four series) were carried out on the mandible of 18 mongrel dogs, bilaterally. The operations were performed under hexobarbital anesthesia. Series I (control group) involved mechanical injury to the body of the mandible without any additional procedures; in series I the bone wound was irradiated by UHF radiation 3 days after its formation; in series III the bone wound was treated cryosurgically 3 days after its formation; in series IV cryosurgery was preceded by UHF irradiation; 3 days after formation of the bone wound also.

M. F. Vladimirskii Moscow Regional Clinical Research Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 5, pp. 478-481, May, 1990. Original article submitted March 3, 1989.

0007-4888/90/0005-0634$12.50