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**MODULATION OF THE CYTOSOLIC ANDROGEN RECEPTOR
IN STRIATED MUSCLE BY SEX STEROIDS**

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ABSTRACT

We studied the influence of orchietomy (GDX) and steroid administration on the level of the cytosolic androgen receptor in the rat levator ani muscle and in rat skeletal muscles (tibialis anterior and extensor digitorum longus). Androgen receptor binding to muscle cytosol was measured using [³H]-methyltrienolone (R1881) as ligand, 100-fold molar excess unlabeled R1881 to assess non-specific binding, and 500-fold molar excess of triamcinolone acetonide to prevent binding to glucocorticoid and progestin receptors. Bound and free ligand were separated by column chromatography with Sephadex G-75. In levator ani muscles from intact animals (controls), maximum R1881 binding (B_{max}) determined by Scatchard analysis was 2.5 fmol/mg protein (K_d = 0.68 nM). Thirty days after GDX, B_{max} increased to 500% control with no significant change in K_d (0.96 nM). Using saturating levels of R1881, B_{max} was increased to 280% control at 12 hours post GDX, 700% at 14 days, 478% at 30 days, and 133% at 44 days with respect to controls. The increase in receptor binding was blocked by cycloheximide. Administration of Silastic capsules containing testosterone propionate (TP) at 30 days post-GDX resulted in R1881 binding at the control level at 44 days. Surprisingly, administration of estradiol-17β (E₂) at 30 days post-GDX resulted in increased (480%) R1881 binding. Thus, E₂ may cause induction of the cytosolic androgen receptor in levator ani muscle from GDX rats; alternatively, the rate of receptor degradation may be altered. R1881 binding by skeletal muscle cytosol was increased 139% at 12 h, 212% at day 14, 220% at day 30 and 158% at day 44 with respect to control. Administration of TP at 30 days caused R1881 binding to return to control by day 44, whereas E₂ was without influence. The differences in response of levator ani and skeletal muscle receptors may account for the differential effects of sex steroids on these muscle types.

INTRODUCTION

Androgenic hormones have marked effects on growth (1, 2) and metabolic activity (3-6) of striated muscle. The mechanism of androgen action on muscle is probably via a cytosolic androgen receptor, which has recently been identified in striated muscle (7-11). This receptor has been shown to have similar physiochemical properties to androgen receptors in prostate gland and kidney (12, 13).

The properties of the cytosolic androgen receptor might be expected to influence the sensitivity of muscle to hormone (14). If so, this would reveal an important physiological mechanism for regulation of androgen hormone action. In an attempt to determine the factors that are important to regulation of the androgen receptor in muscle, we manipulated the hormonal milieu of rats and measured maximum binding and ligand affinity. The results to be described reveal a striking effect of gonadectomy and hormone administration on androgen receptor binding.

Methods

Rats

Adult male Sprague Dawley rats weighing 150-250 g were purchased from Charles River Breeding Labs., Wilmington, Mass. Orchiectomy (GDX) was performed via an abdominal incision under ether anesthesia. Two types of muscle were examined: 1) the levator ani muscle, a perineal muscle that is dependent upon androgens for growth and that has a relatively high level of androgen receptors (10, 15), and 2) the extensor digitorum longus and tibialis anterior muscles, characteristic fast twitch skeletal muscles.

Chemicals

[³H] R1881 ([³H]-methyltrienolone) and unlabeled R1881 were purchased from New England Nuclear. All other chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO).

Hormone Administration

Steroids were administered by subcutaneous implantation of Dow-Corning Silastic medical grade tubing (outer diameter, 0.125 inches; inner diameter, 0.062 inches) filled with crystalline (17) or dissolved steroid (16). Estradiol-17 β was dissolved in sesame oil (150 μ g/mg) and administered in 20 mm lengths. These estradiol-containing Silastic capsules have been shown to deliver hormone levels of 15-20 pg/ml over 5 days (19). Crystalline testosterone propionate was packed into Silastic tubing and implanted in 30 mm lengths to produce physiologic levels (17). Capsules were changed after 7 days to ensure continuous delivery of hormone. Identical surgical procedures were performed on control rats without implantation of capsules.

Protocols

Binding parameters and time-course of receptor changes following castration

Rats were killed at 0, 12 h, 14 d, 30 d and 44 d after castration. Specific binding parameters (apparent K_d , B_{max}) of cytosolic androgen receptor were determined in levator ani and skeletal muscles by Scatchard (18) analysis using 0.5-10.0 nM [3 H] R1881 in the 0 and 30 day castrates. Receptor number was determined by using a saturating concentration of [3 H] R1881 (8 nM for levator ani muscle, apparent K_d 0.68 nM, and 5 nM for skeletal muscle) at all time periods. The saturating concentration is at the plateau of a graph of bound vs log free ligand concentration, as recommended by Klotz (19), and therefore represents a reliable estimate of B_{max} . A typical isotherm for R1881 specific binding in rat levator ani muscle cytosol is given in an earlier report from this laboratory (10).

Effect of cycloheximide

At the time of castration, rats received an i.p. injection of 50 mg/kg cycloheximide dissolved in 0.9% NaCl. The animals were decapitated after 6 h, and muscles were removed for receptor binding assays.

Effect of hormone administration

Animals were castrated, and after 30 days they were implanted with subcutaneous Silastic capsules of either testosterone propionate or estradiol-17 β for an additional 14 days. Controls were killed 44 days after castration.

Assays

Androgen receptor - After rats were decapitated, muscles were dissected, weighed, placed on ice, and minced with scissors. All subsequent operations were carried out in the cold room (0-4 $^{\circ}$). The levator ani muscle was diluted (4:1, w/v) in buffer [Tris (50 mM), EDTA (0.1 mM), dithiothreitol (0.25 mM) and glycerol (10% v/v), pH 7.4] and homogenized by hand with a Tenbroeck homogenizer (Bellco Glass Co.). Skeletal muscle was frozen and pulverized in liquid nitrogen using a mortar and pestle. Skeletal muscle powders were occasionally stored at -80 $^{\circ}$ C, with no loss of receptor binding capacity. For receptor assay, the powders were diluted (2:1, w/v) in buffer and were homogenized using a Polytron (Brinkman-Sybron) for 3 x 5 sec bursts at setting 5.

The androgen receptor assay was performed as described previously (10, 11). Triamcinolone acetonide (500-fold molar excess) was included in the incubation mixture to prevent binding to progestin and glucocorticoid receptors (20, 21). Separation of bound from free ligand was accomplished by column (18 x 0.5 cm) chromatography using Sephadex G-75. Ten 0.8 ml fractions, eluted with homogenization buffer, were collected from each column.

Protein was determined by the method of Lowry et al. (22) using crystalline bovine serum albumin as standard. DNA was determined by the method of Burton (23) using calf thymus DNA as standard.

Statistics

Binding data of muscle from 0 and 30 day castrated rats were analyzed according to Scatchard (18). Equality of slopes was tested by analysis of

covariance (24). Statistical comparisons were made using t-tests and Tukey's post test (25).

Results

Binding parameters and time-course of receptor changes following castration

In levator ani muscles from gonadally intact animals (controls), apparent maximum R1881 binding (B_{max}) determined by Scatchard analysis was 2.5 fmol/mg protein (apparent K_d = 0.68 nM) (Fig. 1). Thirty days after GDX, apparent B_{max} increased to 500% control with no significant change in apparent K_d (Fig. 1).

Using saturating levels of [3H] R1881, apparent B_{max} (fmols/mg protein) in levator ani muscle increased to 280% control at 12 h post gonadectomy, 700% at 14 days, 478% at 30 days and 133% control at 44 days (Fig. 2). Although the magnitude of the change was smaller, skeletal muscle showed a similar trend; R1881 binding was increased 139% at 12 h, 212% at day 14, 220% at day 30 and 158% at day 44 with respect to controls (Fig. 3). Changes in receptor level were also demonstrated in both muscle types when data were expressed in terms of fmols/mg DNA (Figs. 2 and 3).

Effect of cycloheximide

[3H] R1881 binding in the levator ani muscle increased by 56% ($p < 0.01$) 6 h after orchiectomy. Simultaneous injection of cycloheximide blocked this increase in apparent B_{max} (Table I). Cycloheximide also blocked the acute increase in receptor binding in skeletal muscle (Table I).

Effect of hormone administration

Administration of estradiol-17 β at 30 days post-castration resulted in a striking increase in [3H] R1881 specific binding at day 44 in the levator ani muscle (Fig. 4). An identical result was seen with diethylstilbestrol (not shown). Administration of testosterone propionate was without effect (Fig. 4). In contrast, administration of estradiol-17 β had no effect on receptor

binding in skeletal muscle, and testosterone reduced binding (Fig. 5).

Discussion

The present study demonstrates that modification of the levels of sex steroids can alter the content of androgen receptors of rat striated muscle. Both the estradiol- β and testosterone-containing implants we employed have been shown to deliver physiological levels of steroids (16, 17). These data demonstrate that 1) cytosolic androgen receptor levels increase after orchietomy in both levator ani muscle and skeletal muscle; 2) the acute increase in receptor is blocked by an inhibitor of protein synthesis; and, 3) administration of estradiol-17 β to castrated animals increases receptor binding in levator ani muscle but not in skeletal muscle.

We are in agreement with previous experiments showing an increase in cytosolic androgen receptor in rat skeletal muscle after gonadectomy (8, 26). This is in contrast to the result of Michael and Baulieu (9), who reported a decrease in receptor level in the quadriceps muscles for up to 9 days after castration. Our data further demonstrate an increase of receptor after castration that is of greater magnitude in the levator ani muscle than in skeletal muscle. This response is consistent with the exquisite hormone sensitivity of the levator ani muscle.

The return of cytosolic receptor binding to the control level in the levator ani muscle in 30 days, but not in skeletal muscle, may be due to proteolysis involved in the pronounced atrophy of the levator ani muscle after castration.

To elucidate further the mechanism of the increase in receptor binding, we injected cycloheximide, an inhibitor of protein synthesis, at the time of gonadectomy. Cycloheximide was effective in blocking the rise in [3 H] R1881 binding in levator ani and skeletal muscles. Therefore, the acute increase in cytosolic androgen receptor levels may reflect de novo receptor synthesis.

Contribution of other factors, e.g., 1) increased availability of receptor for binding with [³H] R1881 by removal of endogenous hormone, 2) movement of nuclear receptor into the cytosolic pool, or, 3) decreased degradation of receptor, cannot be excluded. Indeed, Cidlowski and Muldoon (27) showed that a similar rise of estradiol receptor was partly owing to receptor synthesis and partly owing to nuclear recycling. That the acute rise in receptor level following castration may depend on protein synthesis suggests that testosterone down-regulates its own receptor, although movement into the nuclear pool cannot be ruled out. This is reinforced by the demonstration that, in skeletal muscle (Fig. 5), physiologic amounts of testosterone reduced the castrate receptor level to the control level in skeletal muscle.

The present results are consistent with recent observations from this laboratory that androgens and estrogens may act synergistically to enhance glucose 6-phosphate dehydrogenase activity in the levator ani muscle (28). These data suggest that estradiol can induce cytosolic androgen receptors in muscle. The increased cytosolic receptor can then interact with androgen, translocate to the nucleus, and interact with the genome to influence enzyme activity. The data also provide an explanation for the estrogen-mediated increase in protein deposition in muscle of farm animals (29, 30).

Increases in cytosolic androgen receptor levels after estrogen treatment have also been shown in dog prostate gland (31) and in the chick oviduct (32); other examples of receptor regulation have been reviewed (33). Further studies will be needed to determine whether this effect of estrogen is due to an interaction with specific estrogen receptors, which have been identified in striated muscle (34).

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Figure Legends

Figure 1 - Scatchard analysis of the effect of gonadectomy on [³H] R1881 specific binding in rat levator ani muscle. Apparent K_d was unaffected by castration.

Figure 2 - Time-course of effect of gonadectomy on [³H] R1881 specific binding in rat levator ani muscle. Data are means \pm sem of 6-8 determinations. *Significantly different from control, $p < 0.01$.

Figure 3 - Time-course of effect of gonadectomy on [³H] R1881 specific binding in rat extensor digitorum longus and tibialis anterior muscles. Data are means \pm sem of 6-10 determinations. *Significantly different from control, $p < 0.01$.

Figure 4 - Effect of sex steroids on [³H] R1881 specific binding in rat levator ani muscle following gonadectomy (GDX). Thirty days after GDX one group of rats received Silastic implants of estradiol-17 β (E_2) for an additional 14 days. A second group of GDX rats received Silastic implants containing testosterone propionate (TP). Data are means \pm sem of 6-8 determinations. *Significantly different from control, $p < 0.01$.

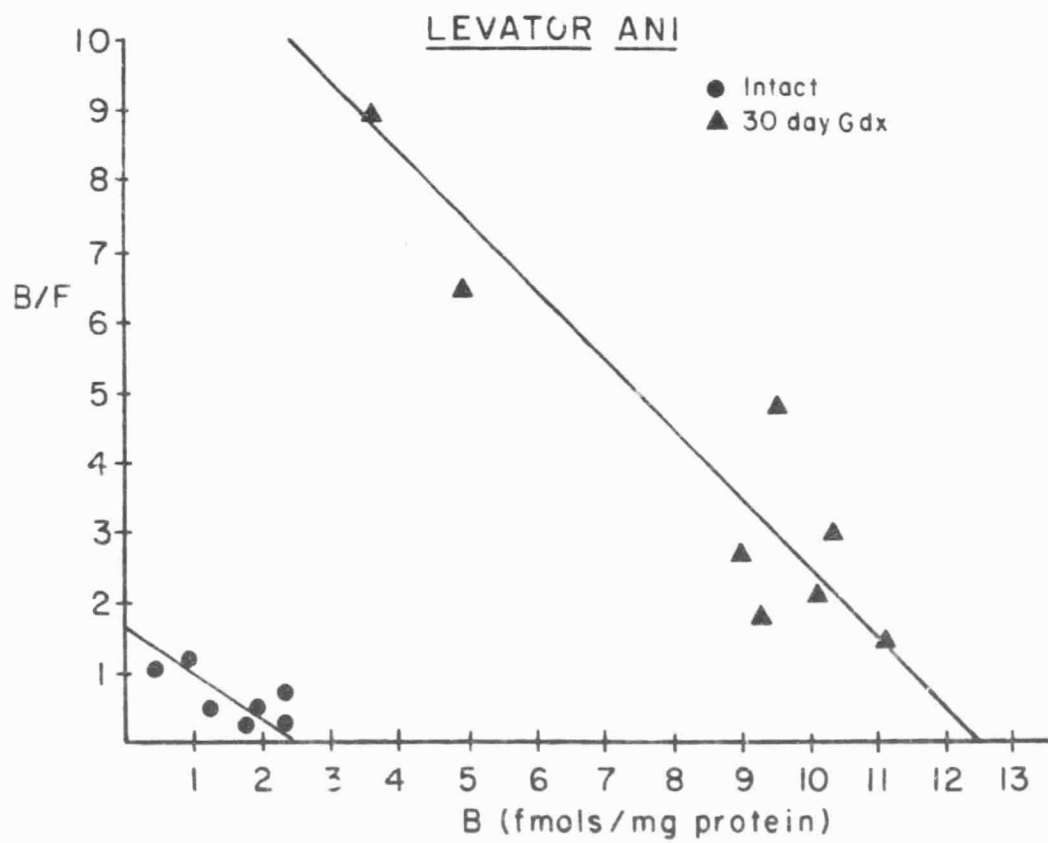
Figure 5 - Effect of sex steroids on [³H] R1881 specific binding in rat skeletal muscles following gonadectomy (GDX). Thirty days after GDX one group of rats received Silastic implants of estradiol-17 β (E_2) for an additional 14 days. A second group of 30-day GDX rats received Silastic implants containing testosterone propionate (TP). Data are means \pm sem of 8-10 determinations. *Significantly different from control, $p < 0.01$.

TABLE I

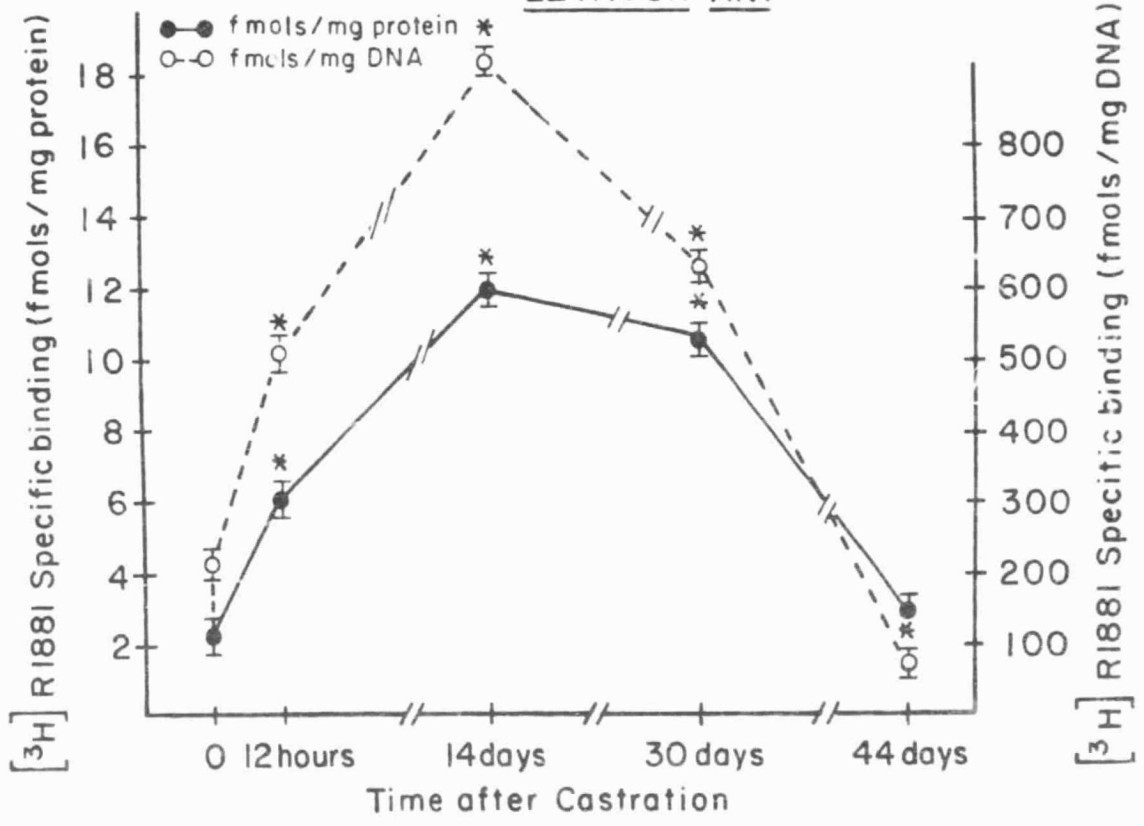
Effect of Cycloheximide on the Castration-Mediated Increase
in [³H] R1881 Specific Binding in Rat Levator Ani Muscle

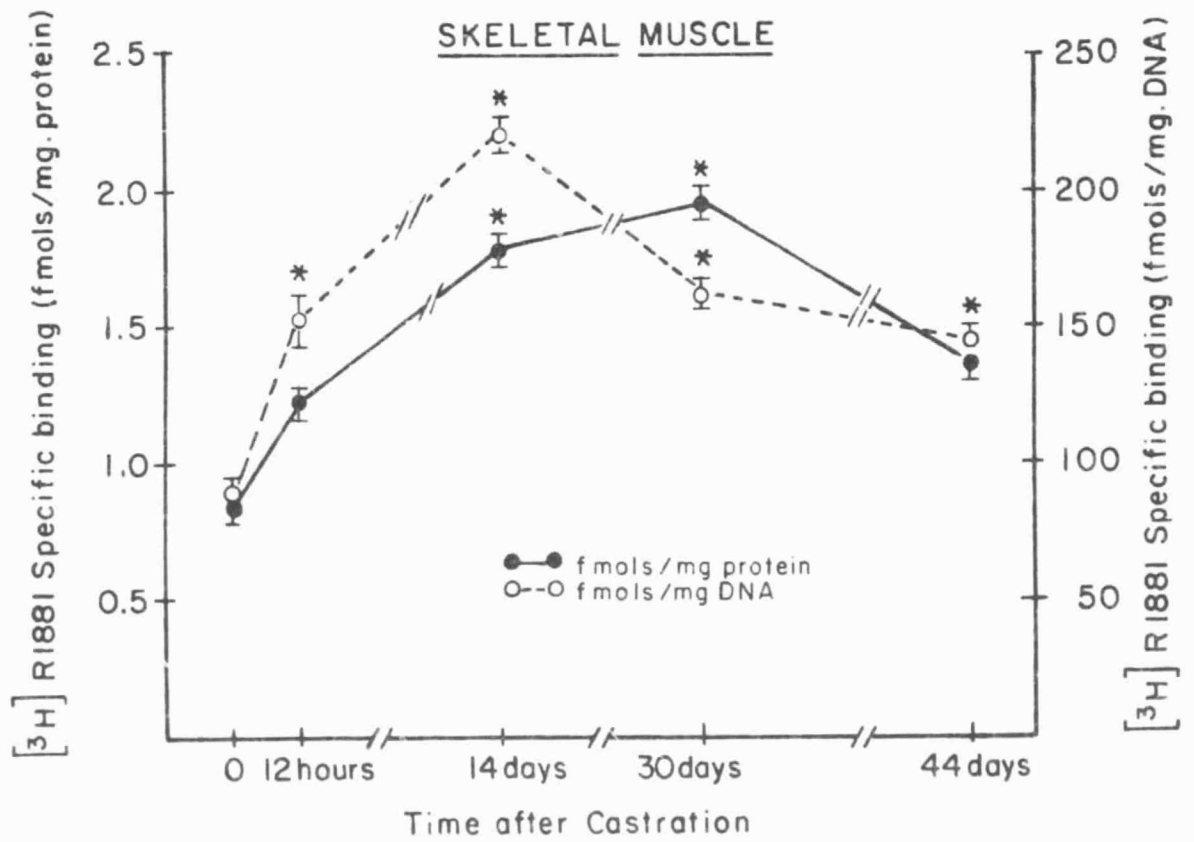
Treatment	Levator Ani Muscle	Skeletal Muscle
	fmols/mg protein	fmols/mg protein
None	4.35 ± 0.2 (3)	0.83 ± 0.09
Castration	6.62 ± 0.8 (3)*	1.25 ± 0.15*
Castration + cycloheximide	2.72 ± 0.5 (4)*	0.95 ± 0.06

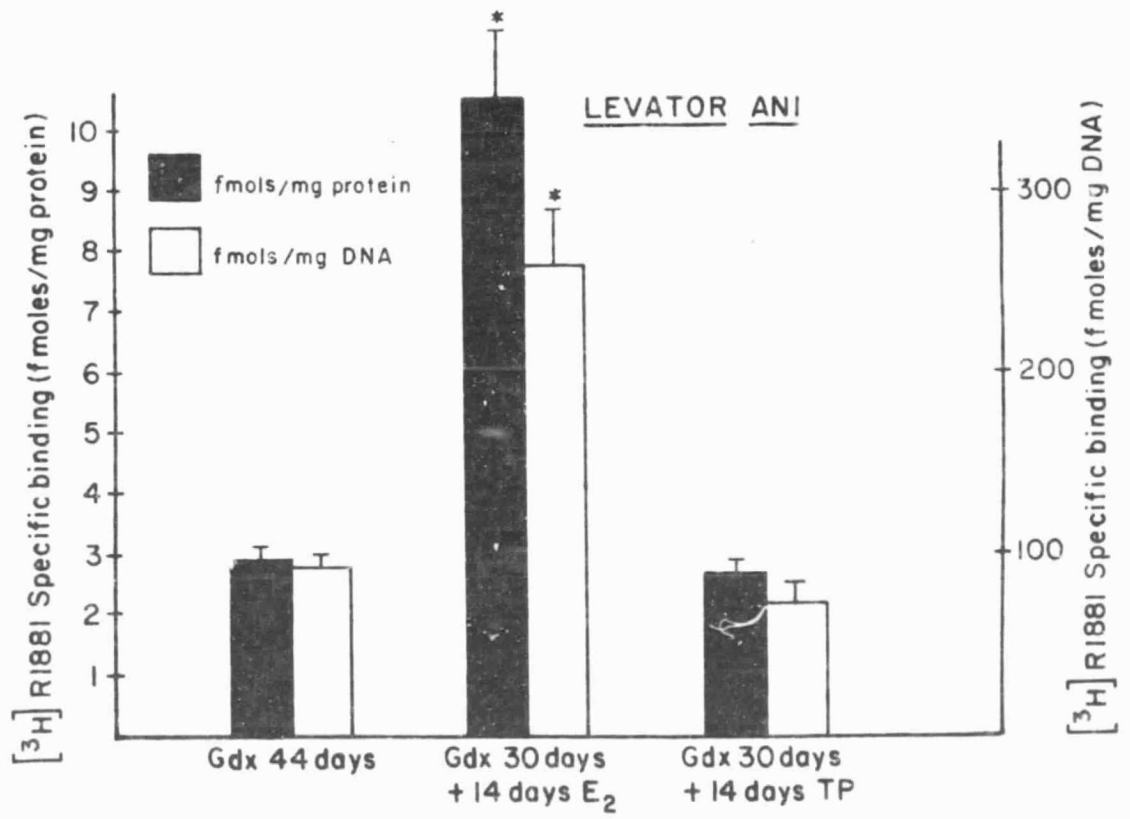
*Significantly different from control, $p < 0.05$. Rats were castrated for 6 hours. Cycloheximide (50 mg/kg) was injected (i.p.) at the time of castration. Data are means ± sem. The number of determinations is in parentheses.



LEVATOR ANI







SKELETAL MUSCLE

