# A POSSIBLE ROLE FOR ENDOGENOUS GLUCOCORTICOIDS IN ORCHIECTOMY-INDUCED ATROPHY OF THE RAT LEVATOR ANI MUSCLE: STUDIES WITH RU38486, A POTENT AND SELECTIVE ANTIGLUCOCORTICOID

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SUMMARY

We employed RU38486, a potent and selective antiglucocorticoid, to study a possible role for endogenous glucocorticoids in atrophy of the levator ani muscle secondary to castration of male rats. RU38486 was shown to block [$^3$H] triamcinolone acetonide binding to cytosol from levator ani muscle. Daily oral administration of RU38486 to castrated rats partially prevented atrophy of the levator ani muscle, as well as a decrease in RNA concentration. In a control group receiving RU38486 alone, the levator ani underwent significant (20%) hypertrophy. Administration of exogenous dexamethasone also caused pronounced atrophy of the levator ani muscle. This atrophy was prevented, to a significant degree, by simultaneous oral administration of RU38486. It is concluded that endogenous glucocorticoids, the actions of which are blocked by RU38486, may be involved in regulation of the mass of the levator ani muscle in intact rats.

## INTRODUCTION

The levator ani muscle of the rat is hormone dependent; it undergoes atrophy following orchiectomy, and it hypertrophies when male rats are given testosterone [1]. The muscle is present in female rats at birth. Shortly thereafter, unless the animals are given androgens, the muscle disappears [2, 3]. An obvious hormonal regulator of the size of this muscle is testosterone. Recently, however, DuBois and Almon reported that castration of male rats caused a two-fold increase in the cytosolic glucocorticoid receptor in this muscle (together with the bulbocavernosus muscle) [4]. DuBois and Almon also demonstrated increases in cytoplasmic glucocorticoid receptor binding in skeletal muscle following denervation [5], disuse [6], and in murine [7] or avian muscular dystrophy [8]. Based on these results, it was hypothesized that endogenous glucocorticoids may be involved in a fundamental manner in muscle atrophy in these diverse conditions [9]. Thus, maintenance of the mass of the levator ani muscle, and, possibly, "ordinary" skeletal muscle, may involve a balance between the anabolic actions of androgens and the catabolic actions of glucocorticoids. We tested this hypothesis by assessing the effects of a potent and selective antiglucocorticoid, RU38486, on dexamethasone- and orchiectomy-induced atrophy of the rat levator ani muscle.

## EXPERIMENTAL

Adult male rats of the Crl:CD(SD)BR strain (Charles River Breeding Laboratories, Boston, MA), weighing 184 ± 9 grams (orchiectomy study) or 157 ± 6 grams (dexamethasone study) were used in all experiments. They were maintained in a 12h on:12h off light cycle, and they were fed Purina Lab Chow #5001 and water ad libitum. Bilateral orchiectomy was carried out by the abdominal route under ether anesthesia. Dexamethasone was injected

subcutaneously at 5 mg/kg. RU38486 was administered orally at 50 mg/kg in an aqueous vehicle comprising carboxymethylcellulose (0.25%) and polysorbate 80 (0.20%) (10).

Glucocorticoid receptor binding in gastrocnemius muscle was assessed using [$^3$H] triamcinolone acetonide (9α-fluoro-11β-16α, 17α,21 tetrahydroxy-pregn-1,4-diene-3, 20-dione-16,17-acetonide, TA, specific activity 30 Ci/mmol). Minced muscle was homogenized in a polytron homogenizer in ice-cold buffer (5 ml/g tissue) comprising 25 mM $Na_2$ $HPO_4$, 1.5 mM EDTA, 10% (v/v), glycerol, 2 mM dithiothreitol, 10 mM sodium molybdate and 1 mM phenylmethylsulfonyl fluoride, pH 7.2. The homogenate was centrifuged for 60 min at 110,000 x g in a Sorvall OTD-50 preparative ultracentrifuge; the resulting supernate was designated the cytosol. Receptor binding to the cytosol fraction was assessed at a single saturating concentration (15 nM) of TA. Portions (0.4 ml) of the cytosol were incubated in duplicate with 0.1 ml of [$^3$H] TA in buffer in either the presence or absence of a 200-fold molar excess of unlabelled TA. Incubation was carried out for 20 h at 0-4°C. These conditions permit optimal exchange in rat skeletal muscle cytosol [11]. Hydroxylapatite was employed to separate bound and unbound ligand [12-14]. Ethanol-extracted hydroxylapatite (2 ml) was added to 16 ml Liquiscint aqueous fluor (National Diagnostics, Somerville, NJ), and radioactivity was determined at 32% efficiency in a Beckman LS-235 liquid scintillation spectrometer. Specific binding was defined as the difference in the value of binding in the absence and in the presence of excess unlabelled steroid.

Non-collagen protein was determined as described [15] using the protein assay of Lowry et al. [16].

RNA and DNA were determined according to Fleck and Munro [17].

Statistical analyses were made by analysis of variance and Dunnett's multiple comparisons test [18].

## RESULTS

As reported for skeletal muscle [19], RU38486 blocks [$^3$H]-TA specific binding to cytosolic glucocorticoid receptors in levator ani muscle in vivo (Fig. 1). Receptor blockade remained complete for 12 h after oral administration of RU38486. By 24 h after RU38486 administration receptor binding returned to the control value (Fig. 1). The regimen described above for RU38486 was effective to a significant extent in preventing muscular atrophy secondary to daily dexamethasone injections or to castration. Fig. 2 shows levator ani muscle wet weights from control, dexamethasone-treated, dexamethasone + RU38486-treated, and RU38486-treated rats. These were expressed as % body weight [20], because body weight changes following dexamethasone administration [19]. Dexamethasone caused about a 50% loss of levator ani muscle wet weight in 8 days of daily injections. In dexamethasone + RU38486-treated rats, the loss of muscle wet weight was significantly less. Surprisingly, in the group treated with RU38486 alone, the levator ani muscle wet weight was significantly (20%) larger than muscle from the untreated group (Fig. 2). Similar changes were noted in the RNA content of levator ani muscles. In Table I, we see that neither non-collagen protein concentration nor DNA concentration changed significantly in the dexamethasone-treated rats. There was, however, a significant loss of RNA in the dexamethasone-treated group. This effect was partly ameliorated by RU38486 (Fig. 2).

Hypertrophy caused by RU38486 suggested that endogenous glucocorticoids might influence levator ani muscle weight, since RU38486 is a

antiandrogen [10]. Indeed, RU38486 was able to prevent, to a significant degree, atrophy of the levator ani muscle secondary to castration (Fig. 3). Eight days after orchiectomy, levator ani wet weight was about 40% of the control value. Administration of RU38486, beginning the day before castration and continuing daily thereafter, prevented this atrophy to a marked extent (Fig. 3). Table II shows RNA, DNA, and non-collagen protein data for castrated rats. Again the DNA and non-collagen protein concentration of the muscles did not decrease. However, there was RNA loss, which was partially prevented by RU38486 (Table II).

## DISCUSSION

The data presented above indicate that RU38486 can prevent, to a significant degree, atrophy of the levator ani muscle secondary to exogenous glucocorticoid administration or following gonadectomy. In the first instance, the data confirm our results on hind-limb muscles (gastrocnemius, plantaris, extensor digitorum longus) [19]. Since the levator ani muscle is composed homogeneously of type II muscle fibers, it was expected that it would be sensitive to glucocorticoid administration, and that the resulting atrophy would be retarded by RU38486. The surprising result of this experiment was that RU38486 alone caused a 20% increase in the wet weight of this muscle. Because RU38486 is a weak antiandrogen [10], and because there is no evidence for the presence of a progestin receptor in muscle [S.R. Max, unpublished observations; 21, 22], it is likely that the antiglucocorticoid activity of RU38486 was responsible for this effect. Thus, maintenance of the mass of the levator ani muscle may not be dependent exclusively on the presence of androgens; endogenous glucocorticoids also might be involved. This hypothesis is supported by the data of Fig 3 and Table 1. In this experiment RU38486 was able to prevent, to a significant extent, atrophy of the levator ani muscle

secondary to gonadectomy. If the sole action of RU38486 is to block glucocorticoid receptors, as appears to be the case, then regulation of the mass of the levator ani muscle depends upon a balance between anabolic and catabolic hormones. If the balance is upset by relative androgen excess, the muscle hypertrophies. If the balance is upset by relative glucocorticoid excess, the muscle atrophies.

As noted in the tables, the RNA content of the atrophying levator ani muscles decreased. This result is in contrast to our finding with skeletal muscle [19], in which dexamethasone administration caused no significant decrease in RNA concentration. Others [23, 24] have reported that glucocorticoid-induced muscle atrophy is accompanied by decreased RNA content. The loss of RNA in rat levator ani muscle was prevented by RU38486.

These data provide a novel perspective on muscular atrophy, and they suggest possible clinical applications of glucocorticoid antagonists.

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FIGURE LEGENDS

Figure 1 - Time-course of blockade of [$^3$H] triamcinolone acetonide binding to the cytosolic glucocorticoid receptor from rat levator ani muscle. Experimental procedures are described in the text.

Figure 2 - Effect of dexamethasone (5 mg/kg/day, subcutaneous) and RU38486 (50 mg/kg/day, oral) on levator ani muscle wet weight as % body weight. V = vehicle, dex = dexamethasone. Experimental procedures are described in the text. Data are means ± SD. *Significantly different from V, $p < 0.001$.

Figure 3 - Effect of RU38486 on wet weight of rat levator ani muscle following castration (GDX). V, vehicle. Experimental procedures are described in the text. *Significantly different from V, $p < 0.01$; δ, significantly different from GDX, $p < 0.001$.

Table I

Effect of Dexamethasone

| | Non-collagen protein (mg/g wet w.) | RNA* (mg/g wet w.) | DNA ( mg/g wet w.) | RNA/DNA |
|---|---|---|---|---|
| Control | 175 ± 20 | 1.57 ± 0.18 | 2.43 ± 0.34 | 0.66 ± 0.17 |
| Dexamethasone | 194 ± 24 | 1.20 ± 0.15* | 2.52 ± 0.55 | 0.50 ± 0.14 |
| Dexamethasone + RU38486 | 191 ± 28 | 1.35 ± 0.09 | 2.09 ± 0.58 | 0.69 ± 0.21 |
| RU38486 | 172 ± 17 | 1.69 ± 0.20** | 2.64 ± 0.60 | 0.65 ± 0.14 |

*Significantly different from control, $p < 0.0025$.

**Significantly different from dexamethasone, dexamethasone + RU38486, $p < 0.025$.

Table II

Effect of Orchiectomy and RU38486 on Non-Collagen Protein, RNA and DNA of Rat Levator Ani Muscle

| | Non-collagen protein (mg/g wet w.) | RNA (mg/g wet w.) | DNA (mg/g wet w.) | RNA/DNA |
|---|---|---|---|---|
| Control | 171 ± 14 | 1.41 ± 0.07 | 1.90 ± 0.25 | 0.75 ± 0.9 |
| Orchiectomy | 173 ± 22 | 1.17 ± 0.15* | 2.14 ± 0.45 | 0.57 ± 0.15 |
| Orchiectomy + RU38486 | 184 ± 12 | 1.36 ± 0.13** | 2.26 ± 0.53 | 0.63 ± 0.16 |

*Significantly different from control and orchiectomy + RU38486, $p < 0.0025$.

**Significantly less than orchiectomy, $p < 0.01$