A SPECIFIC EFFECT OF GLUCOCORTICOIDS IN DECREASING NEUTRAL RIBONUCLEASE II IN SKELETAL MUSCLE[1]

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Received March 25,1976

Summary. Neutral ribonuclease II (EC 3.1.4.22) activity decreases to as little as 20% of its normal level in skeletal muscle of mice within several hours to several days after administration of any of a variety of glucocorticoids. The magnitude of the change varies directly with dose and duration of treatment. Nonglucocorticoid steroids fail to lower ribonuclease activity. The decrease in neutral ribonuclease II levels specifically induced by glucocorticoids may play an important role in the substantial elevation of intracellular RNA concentration known to be an early effect of these hormones.

Neutral ribonuclease II, one of two "bulk" RNases commonly found in tissues, has a specificity and properties similar to those of the well-known bovine pancreatic RNase (1). In certain tissues, including muscle and liver, an excess of a protein inhibitor of the enzyme occurs such that there is little of the latter in a free, active form. We have described an alteration of this pattern in the muscle of dystrophic mice (1-3) and patients with Duchenne muscular dystrophy (4) in which there is an increase of RNase activity to the point where it substantially exceeds the level of inhibitor present. We have suggested that this may play a key role in the altered nucleic acid and protein turnover characteristic of the diseased muscle. A wide variety of physiologic perturbations of muscle have generally had the effect of increasing RNase II levels (1, 3, 5, and 6). Here we report that glucocorticoids have a potent and specific effect in decreasing neutral RNase II activity in skeletal muscle of mice.

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[1]This work was supported by grant number NS10105 from the National Institute of Neurological and Communicative Disorders and Stroke.
[2]Recipient of a Summer Scholarship from the Muscular Dystrophy Association.

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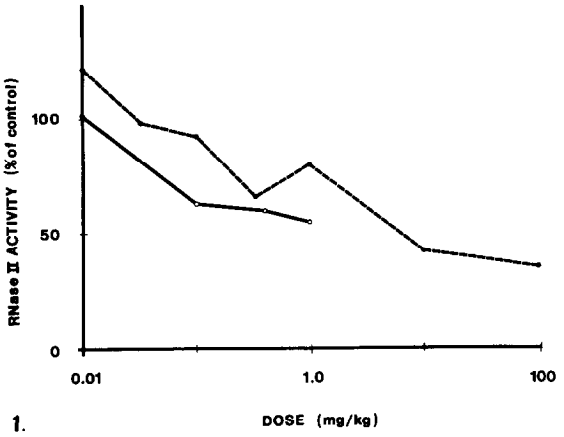

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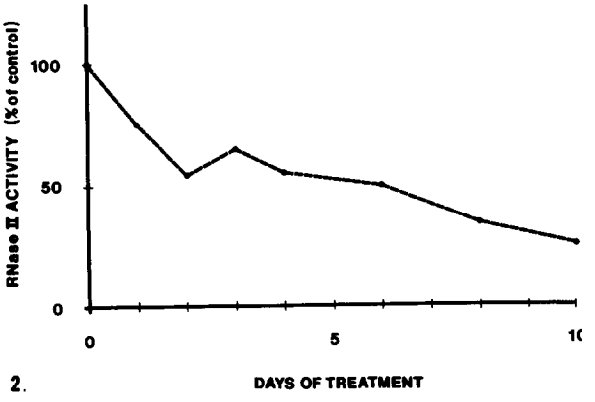

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Figure 1. Effect of glucocorticoid dose on depression of RNase II activity. Experimental protocol was exactly as described in Table 1 and Methods. Dashed line, triamcinolone acetonide; solid line, dexamethasone sodium phosphate. The curves are from separate studies.

Figure 2. Time course of depression of RNase II activity with administration of triamcinolone acetonide. Experimental protocol was exactly as described in Table 1 and Methods. Triamcinolone acetonide daily dose was 5 mg/kg body weight. Each point is for one experimental animal compared to one control killed the same day. The mean of the seven control values for activity is 97 units per gm wet weight of muscle with a standard deviation of ± 15 units. The RNase unit is defined in references 5 and 7.

Methods. Albino male mice 35-40 days old were given daily intraperitoneal injections (0.1 cc) of either 0.9% NaCl (controls) or preparations of various steroids. Animals were allowed free access to food and water and periodically were killed by concussion 24 hours following the last injection. Gastrocnemius muscle was excised and homogenized with 8-10 volumes of 300 mM KCl-1 mM Na_4EDTA. Centrifugation at 37,000 g for 15 min at 0° yielded a supernate in which RNase II was assayed at 45° using an RNA preparation and buffered cadmium precipitating procedure developed in our laboratory (7). RNase II inhibitor was assayed by our general method (5) modified by the use of free RNase II prepared from mouse skeletal muscle. Two other neutral hydrolases were measured: RNase I (7) and N-acetyl-L-phenylalanine ethyl esterase (8).

Results and Discussion. The data of Table 1 indicate that the administration of glucocorticoids results in decreased levels of total RNase II activity in skeletal muscle of mice. In this and other experiments, the effect was seen with every glucocorticoid tested. Injection vehicles were without effect. The depression of RNase activity becomes greater with increased dose of glucocorticoids as illustrated in Figure 1. The response is rapid and progressive over a period of days (Figure 2). RNase activity has

Table 1. Steroid effects on muscle RNase II activity

Steroid	Dose (mg/kg)	RNase units/gm (% of control)
Glucocorticoids:		
Hydrocortisone acetate	10	35
Triamcinolone acetonide	2	39
Cortisone acetate	10	56
Dexamethasone sodium phosphate	0.4	60
Prednisolone sodium succinate	2.5	83
Mineralocorticoids:		
Fludrocortisone acetate	0.3	69
Deoxycorticosterone acetate	12.5	139
Others:		
Estrone	7.5	82
Testosterone	10	82
17-α-methyl-testosterone	10	97
Progesterone	10	103
Stigmasterol	10	110
Cholesterol	10	96

Results are for total RNase II activity/gm wet weight of muscle expressed as % of activity in control muscle. Measurements were made 24 hours following the last of two daily injections with an amount of steroid per kg of body weight as indicated. Data are for single animals collected from separate studies run on three different days and in each case the value is compared to one or two control animals for that day. An indication of the absolute values for enzyme activity and of the variation among controls is presented in the legend to Figure 2.

never leveled off at less than 20% of control values no matter what glucocorticoid, dosage schedule, or length of treatment has been employed. The effect on RNase II is relatively specific; no appreciable changes in RNase I or N-acetyl-L-phenylalanine ethyl esterase activities or muscle to body weight ratio occur in short term experiments such as those described above.

Depression of RNase II activity is a specific effect of glucocorticoids. Other steroids (Table 1) do not cause any consistent depression of a magnitude comparable to that elicited by all glucocorticoids. The decrease with fludrocortisone may be due to its known glucocorticoid activity (9). Dose-response experiments like those of Figure 1 show that, unlike glucocorticoids, nonglucocorticoids do not cause progressive decreases in RNase activity below the values given in Table 1.

Free RNase II activity, which normally is a small fraction of the total, also decreases with glucocorticoid treatment; levels of total RNase inhibitor are relatively unaffected.

In view of the abnormally high levels of RNase II which are characteristic of murine and Duchenne muscular dystrophies (1-4), investigation of glucocorticoids as therapeutic agents in these diseases is indicated by the results described here.

In some of the experiments reported above, RNase II levels in liver also were measured and found to be substantially depressed by glucocorticoids. Thus, depression of RNase II levels may be a widespread, specific, and perhaps key element in the mechanism of glucocorticoid hormone action. It well may contribute to the increase in RNA levels which is a prominent effect of glucocorticoids in certain tissues and which precedes the induction of specific enzymes therein (10).

Comparison of our results for glucocorticoid effects on tissue RNase levels to results in the literature is difficult. No others report on muscle. Identification of the RNase activities actually assayed is very difficult. Frequently, mixtures of several activities were measured; given the conditions of assay and tissue preparation, varying combinations of "acid" RNase, RNase I and free RNase II, total RNase II, and other activities would be measured. With these and other variable bases for comparison, the bulk of the relevant studies may be summarized as follows. With one exception (11), RNase activity of lymphoid or tumor tissue has been reported to be elevated following glucocorticoid treatment (12-18). Because of large changes in lymphoid tissue weight, some of the increase reported simply may have been due to persistence of RNase while the general tissue mass declined or due to change in the cell-type composition. RNase activity has been reported to increase also in liver after glucocorticoid treatment (11). A few studies, usually with liver, have indicated that glucocorticoids may cause little if any change in RNase activity (13), or modest decreases in

either RNase activity levels (13, 19-21) or ribonucleolysis (22).

Our results are of particular interest with regard to a recent report (23) which presents evidence consistent with a role for estrogen in the regulation of RNase activity in the uterus. The authors suggest the possible identity of RNase II inhibitor and β-estradiol receptor protein. We are aware of no evidence that is more than tenuous with respect to the identity of RNase inhibitor and any steroid receptor protein.

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