

IN PRESS  
Accepted 12/4/86

ORIGINAL PAGE IS  
OF POOR QUALITY

#191/86  
J. Neurol. Sci.  
(Revised)

Evaluation of the Endogenous Glucocorticoid Hypothesis of Denervation Atrophy

Masaaki Konagaya\*, Yoko Konagaya\* and Stephen R. Max

Department of Neurology  
University of Maryland  
School of Medicine  
Baltimore, MD 21201  
U.S.A.

NAG 2-100  
IN-51-CR  
146721  
13P

Key Words: denervation, atrophy, glucocorticoid, antiglucocorticoid, glutamine synthetase, glucose 6-phosphate dehydrogenase, choline acetyltransferase, muscle

Send proofs to:

Stephen R. Max, Ph.D.  
Department of Neurology  
University of Maryland  
School of Medicine  
Baltimore, MD 21201 U.S.A.  
(301) 528-3436

RECEIVED  
AIAA  
LIBRARY  
87 MAR 16 08:00

\*Permanent address:

Department of Neurology  
Nara Medical University,  
840 Shijo-cho, Kashihara  
Nara 634, Japan

(NASA-CR-182848) EVALUATION OF THE  
ENDOGENOUS GLUCCORTICCID HYPOTHEISIS OF  
DENERVATION ATRCPHY (Maryland Univ.) - 13 p  
CSCL 06B

N88-24157

Unclas  
G3/51 0146721

## SUMMARY

We studied the effects of oral administration of RU38486, a potent and selective glucocorticoid antagonist, on muscle weight, non-collagen protein content, and selected enzyme activities (choline acetyltransferase, glucose 6-phosphate dehydrogenase, and glutamine synthetase) following denervation of rat skeletal muscle. Neither decreases in muscle weight, protein content, and choline acetyltransferase activity, nor increases in the activities of glucose 6-phosphate dehydrogenase and glutamine synthetase were affected by RU38486. These data do not support the hypothesis that denervation atrophy results from enhanced sensitivity of muscle to endogenous glucocorticoids.

## INTRODUCTION

The mechanism by which denervation causes atrophy of skeletal muscle is not known. DuBois and Almon (1980, 1981) proposed an intriguing hypothesis, according to which enhanced sensitivity of muscle to endogenous glucocorticoids might play a role in the promotion of denervation atrophy. This hypothesis was based upon the demonstration of strikingly increased cytoplasmic glucocorticoid receptor binding after denervation (reviewed in Karpati, 1984). Indeed, DuBois and Almon noted enhanced cytoplasmic glucocorticoid receptor binding in a number of other causes of atrophy including disuse (1980), murine (1984b) and avian (1982) dystrophies, and atrophy of the levator ani muscle following orchiectomy (1984a); they suggested that atrophy, irrespective of cause, is related to glucocorticoid actions. It is well-established that exogenous glucocorticoids can cause pronounced muscle atrophy (Koski et al., 1974; Kelly et al., 1986; Konagaya et al., 1986a). The hypothesis of DuBois and Almon is therefore of interest and warrants investigation, especially because of the possibility of treating denervated muscles with glucocorticoid antagonists. We (Konagaya et al., 1986a) were able to prevent, to a significant extent, glucocorticoid-mediated muscle atrophy in the rat using RU38486 (Philibert, 1984), a potent and selective glucocorticoid antagonist. Support for the hypothesis of DuBois and Almon was provided by the ability of RU38486 to prevent, to a significant extent, gonadectomy-mediated atrophy of the levator ani muscle (Konagaya and Max, 1986). We have now used RU38486 to assess whether chronic blockade of glucocorticoid receptors can influence the course of muscle atrophy following denervation. We assessed choline acetyltransferase and glucose 6-phosphate dehydrogenase activities because they are pre- and post-synaptic biochemical indices of muscle denervation (Wagner and Max, 1979; Max et al., 1981; Max et

al., 1982). We studied glutamine synthetase because 1) its activity is enhanced following denervation (Konagaya et al., 1986b), 2) it is involved in mobilization of amino acids from muscle proteins (Goldberg and Chang, 1979), and 3) it may be an important index of muscle wasting (Max et al., 1986; Max and Silbergeld, 1986). Further, glutamine synthetase is induced in muscle by glucocorticoids (Smith et al., 1984; King et al., 1983; Max et al., 1986).

#### MATERIALS AND METHODS

Adult, male rats (Crl:CD (SD) BR Strain, Charles River Breeding Laboratories, Wilmington, MA) weighing 200 g were used in all experiments. They were given Purina Rodent Laboratory Chow (#5001, Ralston-Purina, St. Louis, MO) and water ad libitum, and they were maintained on a lighting schedule of 12 h of light and 12 h of darkness. Denervation was accomplished under ether anesthesia by unilateral section of the sciatic nerve at the hip. RU38486 (11 $\beta$ -(4-dimethylaminophenyl)17 $\beta$ -hydroxy-17 $\alpha$ -(prop-1-ynyl)estra-4, 9-dien-3-one; Roussel - UCLAF - Paris, France) was given p.o. at 50 mg/kg beginning the day before denervation and every day thereafter until the termination of the experiment. This regimen has been shown to cause blockade of muscle glucocorticoid receptors (Konagaya et al., 1986). At 2 and 7 days following denervation, rats were decapitated and denervated and contralateral control extensor digitorum longus and plantaris muscles were removed and weighed. The extensor digitorum longus muscle was assayed for choline acetyltransferase (Max et al., 1982; Fonnum, 1975) and glucose 6-phosphate dehydrogenase activities (Max et al., 1981; Schaerf et al., 1982) as described. Plantaris muscles were used for determination of glutamine synthetase activity as described (Rowe, 1985; Smith et al., 1984), using 5 mM glutamate as substrate. Both muscles were used to provide sufficient material for the assays.

ORIGINAL PAGE IS  
OF POOR QUALITY

Non-collagen protein was assayed as described (Rifenberick et al., 1974). Protein in supernatant fractions was determined by the method of Lowry et al. (1951).

Statistical analyses were made with a paired t-test.

## RESULTS

As expected, denervated muscles lost weight. Seven days after neurotomy, denervated extensor digitorum longus muscles weighed 70% of the contralateral control muscle, while denervated plantaris muscles weighed 75% of the contralateral control muscle (Table 1). Losses of muscle wet weight were reflected in corresponding losses of non-collagen protein, (Table 1). Glucose 6-phosphate dehydrogenase activity increased 2-fold in denervated extensor digitorum longus muscles, as expected (Wagner and Max, 1979) (Table 2). Choline acetyltransferase activity in extensor digitorum longus muscles declined to about 25% of control (Table 2). Glutamine synthetase activity in plantaris muscles increased 5.5-fold 8 days after denervation (Table 1). None of these changes was altered by administration of RU38486 (Tables 1 and 2).

## DISCUSSION

The results described above reveal no effect of chronic blockade of glucocorticoid receptors on four denervation-mediated events. Choline acetyltransferase, a marker of presynaptic integrity (Max et al., 1981) decreased, glucose 6-phosphate dehydrogenase (Max et al., 1981) and glutamine synthetase (Konagaya et al., 1986b), biochemical markers of post-synaptic (i.e., muscle) integrity increased, and muscle weight and protein content decreased. Concurrent treatment with RU38486 had no effect on the progression of these alterations following denervation. These data do not support the hypothesis that denervation-mediated muscular atrophy results from receptor-mediated glucocorticoid action (Karpati, 1984). Tremblay et al. (1986)

compared denervation atrophy in adrenalectomized rats with controls; they found no effect of adrenalectomy in denervation atrophy. Thus, we have confirmed and extended the conclusions of Tremblay et al. (1986) by using a potent glucocorticoid receptor blocker, RU38486, and by measuring the biochemical indices noted above.

We consider glutamine synthetase to be a valuable marker in the present work because its activity is enhanced in muscle following denervation (Konagaya et al., 1986b) or administration of glucocorticoids (Smith et al., 1984; King et al., 1983; Max et al., 1986). That the denervation effect is not blocked by RU38486 (Table 1), whereas that of glucocorticoids is blocked by this compound (Max et al., 1986; Max et al. - 1987) suggests two different mechanisms for the increases. The present results suggest that endogenous glucocorticoids do not cause the increase in glutamine synthetase activity following denervation. Thus, glucocorticoid- and denervation-mediated muscle atrophy may be subserved via distinct mechanisms. However, glucocorticoid hormones may be involved in enhanced glutamine synthetase activity in soleus muscles from tail-casted, hind-limb suspended rats (Jaspers et al., 1986), because adrenalectomy abolished differences in muscle glutamine synthetase between normal and suspended rats. Jaspers and Tischler (1986) concluded that elevation of circulating glucocorticoids is unlikely to be solely responsible for muscle atrophy secondary to reduced activity, but that catabolic levels of glucocorticoids could alter the response of muscle to unloading. RU38486 has been shown to be effective in treating a patient with Cushing's syndrome (Nieman et al., 1985). Unfortunately, it seems not to be effective in preventing denervation-mediated effects on muscle. The significance of the interesting increase in glucocorticoid receptor binding in denervated and disused muscles (DuBois and Almon, 1980, 1981) warrants further exploration.

## ACKNOWLEDGEMENTS

We thank Drs. B.H. Sohmer and A. Mattia for helpful comments, Ms. Marilyn Wennes for technical assistance and Ms. B. Pasko for preparation of the typescript. RU38486 was the generous gift of Dr. R. Deraedt, Roussel-UCLAF (Paris, France). This work was supported in part by grants from NASA (NAG 2-100), NIH (HD 16956), and the Bressler and Chancellor's Funds of the University of Maryland.

## REFERENCES

- DuBois, D.C. and R.R. Almon (1980) Disuse atrophy of skeletal muscle is associated with an increase in number of glucocorticoid receptors. Endocrinology 107:1649-1651.
- DuBois, D.C. and R.R. Almon (1981) A possible role for glucocorticoids in denervation atrophy. Muscle Nerve 4:370-373.
- DuBois, D.C. and R.R. Almon (1982) The chicken dystrophic model: Does hypersensitivity to glucocorticoids cause atrophy? Exp. Neurol. 75:555-565.
- DuBois, D.C. and R.R. Almon (1984a) Perineal muscles: Possible androgen regulation of glucocorticoid receptor sites in the rat. J. Endocr. 102:225-229.
- DuBois, D.C. and R.R. Almon (1984b) Increased content of glucocorticoid receptors in mouse muscular dystrophy. Endocrine Res. 10:3-10.
- Fonnum, F. (1975) A rapid radiochemical method for the determination of choline acetyltransferase. J. Neurochem. 24:407-409.
- Goldberg, A.L. and T.W. Chang. (1979) Regulation and significance of amino acid metabolism in skeletal muscle. Fed. Proc. 37:2301-2307.
- Jaspers, S.R., S. Jacob and M. Tischler (1986) Metabolism of amino acids by the atrophied soleus of tail-casted, suspended rats. Metabolism 35:215-223.
- Jaspers, S.R. and Tischler, M.E. (1986) Role of glucocorticoids in the response of rat leg muscles to reduced activity. Muscle Nerve 9:554-561.
- Karpati, G. (1984) Denervation and disuse atrophy of skeletal muscles - Involvement of endogenous glucocorticoid hormones? Trends Neurosci. 7:61-62.

ORIGINAL PAGE IS  
OF POOR QUALITY



- Kelly, J., J.A. McGrath, D.F. Goldspink, and M.J. Cullen (1986) A morphological/biochemical study on the actions of corticosteroids on rat skeletal muscle. Muscle Nerve 9:1-10.
- King, P.A., L. Goldstein and E.A. Newsholme (1983) Glutamine synthetase activity of muscle in acidosis. Biochem. J. 216:523-525.
- Konagaya, M., P.A. Bernard and S.R. Max (1986a) Blockade of glucocorticoid receptor binding and inhibition of dexamethasone-induced muscle atrophy in the rat by RU38486, a potent glucocorticoid antagonist. Endocrinology 119:375-380.
- Konagaya, M., Y. Konagaya and S.R. Max (1986b) Neural control of glutamine synthetase activity in rat skeletal muscle. Soc. Neurosci. Abstr. 12:1107.
- Konagaya, M. and S.R. Max (1986) A possible role for endogenous glucocorticoids in orchietomy-induced atrophy of the rat levator ani muscle: Studies with RU38486, a potent and selective antiglucocorticoid. J. Steroid Biochem., in press.
- Koski, C.L., D.H. Rifkin, and S.R. Max (1974) Oxidative metabolism of skeletal muscle in steroid atrophy. Arch. Neurol. 31:407-410.
- Lowry, O.H., N.S. Rosebrough, A.L. Farr and R.J. Randall (1951) Protein determination with the folin phenol reagent. J. Biol. Chem. 193:265-275.
- Max, S.R. and E.K. Silbergeld (1986) Skeletal muscle glucocorticoid receptor and glutamine synthetase activity in the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. In press.
- Max, S.R., S.S. Deshpande, and E.X. Albuquerque (1982) Neural regulation of muscle glucose 6-phosphate dehydrogenase: Effect of batrachotoxin and tetrodotoxin. J. Neurochem. 38:386-391.

- Max, S.R., M. Konagaya, Y. Konagaya, J.W. Thomas, C. Baumer, and L. Vitkovic (1987) Dexamethasone regulates glutamine synthetase expression in rat skeletal muscles. Fed. Proc., in press.
- Max, S.R., J.W. Thomas, C. Banner, L. Vitkovic, M. Konagaya and Y. Konagaya (1986) Glucocorticoid receptor-mediated induction of glutamine synthetase in skeletal muscle cells in vitro. Endocrinology, submitted for publication.
- Max, S.R., J.L. Young and R.F. Mayer (1981) Neural regulation of glucose 6-phosphate dehydrogenase in rat muscle: Effect of denervation and reinnervation. Brain Res. 220:131-138.
- Nieman, L.K., G.P. Chrousos, C. Kellner, I.M. Spitz, B.C. Nisula, G.B. Cutlery, G.R. Merriam, C.W. Barden, and D.L. Loriaux (1985) Successful treatment of Cushing's syndrome with the glucocorticoid antagonist RU486. J. Clin. Endocrinol Metab. 61:535-540.
- Philibert, D. (1984) RU38486: An original, multifaceted antihormone in vivo. In: Agarwal, M.K. (ed.) Adrenal Steroid Antagonism, Walter de Gruyter and Co., Berlin, pp 1-25.
- Rifenberick, D.H., C.L. Koski and S.R. Max (1974) Metabolic studies of muscle regeneration. Exp. Neurol. 45:527-540.
- Rowe, W.B. (1985) Glutamine synthetase from muscle. Methods Enzymol. 113:199-212.
- Schaerf, F.W., T. Patz and S.R. Max (1982) Estrogen modulates neural control of muscle glucose 6-phosphate dehydrogenase. J. Neurochem. 38:1765-1767.
- Smith, R.J., S. Larson, S.E. Stred and R.P. Durschlag (1984) Regulation of glutamine synthetase and glutaminase activities in cultured skeletal muscle cells. J. Cell Physiol. 120:197-203.

- Tremblay, R.R., M.A. Ho-Kim, C. Champagne, J. Gagnor and J.Y. Dube (1986)  
Variations in glucocorticoid receptors in intact or denervated muscles:  
Lack of cause-effect relationship with muscle atrophy in the rat. J.  
Receptor Res. 6:183-193.
- Wagner, K.R. and S.R. Max (1979) Neurotrophic regulation of glucose 6-  
phosphate dehydrogenase in rat skeletal muscle. Brain Res. 170:572-576.

TABLE 1

EFFECT OF DENERVATION AND RU38486 ON MUSCLE WEIGHT AND NON-COLLAGEN PROTEIN CONTENT.

Treatment	Muscle Weight (mg)		Non-Collagen Protein (mg/muscle)	
	Extensor Digitorum Longus	Plantaris	Extensor Digitorum Longus	Plantaris
	Control	135.0 ± 31.5	230.0 ± 25.8	24.41 ± 2.92
Denervated	94.8 ± 5.4*	172.9 ± 21.4*	16.26 ± 2.10*	29.63 ± 2.45*
Control + RU38486	132.9 ± 12.5	242.9 ± 19.8	22.71 ± 2.05	43.97 ± 4.39
Denervated + RU38486	100.0 ± 5.8**	181.4 ± 15.7**	15.54 ± 1.44**	27.00 ± 4.37**

Data are means ± SD, n = 7. Rats were decapitated and muscles removed 8 days post-denervation. RU38486 was given orally at 50 mg/kg.

\*Significantly different from untreated contralateral control,  $p < 0.01$ .

\*\*Significantly different from contralateral control + RU38486,  $p < 0.01$ .

There were no significant differences between untreated and RU38486-treated groups. Experimental procedures are described in the text.

TABLE 2.  
EFFECT OF DENERVATION AND RU38486 ON GLUCOSE 6-PHOSPHATE DEHYDROGENASE, GLUTAMINE SYNTHETASE, AND CHOLINE ACETYLTRANSFERASE ACTIVITIES.

	Glucose 6-Phosphate Dehydrogenase (nmol/min/mg protein)	Glutamine Synthetase (nmol/h/mg protein)	Choline Acetyltransferase (pmol/min/mg non-collagen protein)
Control	1.50 ± 0.13	5.4 ± 0.8	11.99 ± 1.11
Denervated	3.27 ± 0.50*	29.8 ± 11.5*	2.97 ± 1.25*
Control + RU38486	1.64 ± 0.22	5.8 ± 1.6	11.31 ± 1.25
Denervated + RU38486	3.30 ± 0.38**	29.0 ± 9.1**	2.99 ± 1.25**

Data are means ± SD, n = 7. Rats were decapitated and muscles removed 8 days post-denervation. Glucose 6-phosphate dehydrogenase and choline acetyltransferase activities were assayed in extensor digitorum muscles. Glutamine synthetase activity was assayed in plantaris muscles. RU38486 was given orally at 50 mg/kg.

\*Significantly different from untreated contralateral control, p < 0.01;

\*\*Significantly different from contralateral control + RU38486, p < 0.01.

There were no significant differences between untreated and RU38486-treated groups.

Experimental procedures are described in the text.