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Dexamethasone Regulates Glutamine Synthetase Expression in Rat Skeletal Muscles

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ABSTRACT. We studied the regulation of glutamine synthetase by glucocorticoids in rat skeletal muscles. Administration of dexamethasone strikingly enhanced glutamine synthetase activity in plantaris and soleus muscles. The dexamethasone-mediated induction of glutamine synthetase activity was blocked to a significant extent by orally administered RU38486, a glucocorticoid antagonist, indicating the involvement of intracellular glucocorticoid receptors in the induction. Northern blot analysis revealed that dexamethasone-mediated enhancement of glutamine synthetase activity involves dramatically increased levels of glutamine synthetase mRNA. The induction of glutamine synthetase was selective in that glutaminase activity of soleus and plantaris muscles was not increased by dexamethasone. Furthermore, dexamethasone treatment resulted in only a small (15%) increase in glutamine synthetase activity in heart. Accordingly, there was only a slight (if any) change in glutamine synthetase mRNA level in this tissue. Thus, glucocorticoids regulate glutamine synthetase gene expression in rat muscles at the transcriptional level via interaction with intracellular receptors. Such regulation may be relevant to control of glutamine production by muscle and to mechanisms underlying glucocorticoid-induced muscle atrophy.

Glucocorticoids cause marked atrophy of skeletal muscle (1-4). This atrophy, which appears to involve intracellular glucocorticoid receptors (4), involves alterations in protein synthesis (2, 5-10) and, possibly, protein degradation (11-16). However, the molecular basis of glucocorticoid-mediated muscle atrophy is not understood. An important step toward understanding how glucocorticoids cause muscle atrophy is the study of genes that may be regulated to produce the catabolic response. Such a gene may be that for glutamine synthetase, the activity of which is enhanced in L6 muscle cells _in vitro_ and in rat muscles _in vivo_ following dexamethasone treatment (17-19). Skeletal muscle synthesizes and releases glutamine, which is a substrate for energy metabolism in a number of other tissues, including intestine, fibroblasts, and, possibly, brain (20 - review). Amino acids derived from degradation of muscle proteins (21) are the primary source of muscle glutamine; glutamine synthetase is the synthetic enzyme.

Recently, we demonstrated that dexamethasone caused a striking increase in the level of glutamine synthetase mRNA in L6 muscle cells in culture (19). However, an _in vitro_ system is valuable only insofar as it reflects events in the intact organism. Therefore, we examined the effects of dexamethasone on rat skeletal muscles _in vivo_. A major objective was to evaluate the hypothesis that increased glutamine synthetase is involved in the catabolic actions of glucocorticoids on muscle (19).

In this report, we provide evidence that glutamine synthetase activity in rat muscles _in vivo_ is increased by dexamethasone;

that the increase results from enhanced production of glutamine synthetase mRNA; and, that induction of glutamine synthetase is mediated via intracellular glucocorticoid receptors. By contrast, dexamethasone exerted minimal effects on glutamine synthetase activity and mRNA level in heart.

Materials and Methods

Materials

Male rats (crl:CD(SO)BR strain, Charles River Breeding Labs, Wilmington, Mass.) weighing 200-225 g were used. They were maintained on a schedule of 12 h light and 12 h darkness, and they were fed Purina Rodent Laboratory Chow (no. 5001, Ralston Purina, St. Louis, MO) and water *ad libitum*. Dexamethasone was administered s.c. at a doses of 5 mg/kg; RU38486 [11β -(4-dimethylaminophenyl) (17β-hydroxy-17α-(prop-1-ynyl estra-4,9-dien-3-one)] was administered p.o. at 50 mg/kg. The dexamethasone dose was selected because it is effective in eliciting muscle atrophy (e.g., 4). The dose of RU38486 was selected because of its ability to block muscle glucocorticoid receptors and glucocorticoid-mediated muscle atrophy *in vivo* (4). RU38486 was a gift of Roussel-UCLAF (Paris, France). L-glutamic acid-[U-^{14}C], specific activity 200-250 mCi/mmol, and L-glutamine-[U-^{14}C], specific activity 200-250 mCi/mmol, were purchased from Research Products International (Mount Prospect, IN). The RNA probe vector pGEM-2, and the *in vitro* transcription system, Riboprobe, were from Promega Biotec (Madison, WI). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MI).

Enzyme Assays

Glutamine synthetase and glutaminase activities were assayed using the method of Rowe (22) as described by Smith et al. (17), except that the glutamate concentration was 5 mM in our glutamine synthetase assays. These assays separate product from substrate by ion-exchange chromatography. The substrates were glutamic acid [U-^{14}C] and glutamine-[U-^{14}C], for glutamine synthetase and glutaminase, respectively. Glutamine synthetase activity in rat muscles was completely inhibited by 10 μM L-methionine sulfoximine, a specific inhibitor of this enzyme (22).

Protein

This was determined according to Lowry et al. (23), using crystalline bovine serum albumin as standard.

mRNA (Northern Blot) Analysis

Soleus and plantaris muscles and heart were frozen and pulverized in liquid nitrogen in a mortar and pestle that had been treated with diethyl pyrocarbonate (24). The powders were stored at -70°C. Total cellular RNA was isolated from frozen powders of soleus, plantaris and heart muscles using the guanidine isothiocyanate procedure of Chirgwin et al. (25). RNA was fractionated by electrophoresis through agarose gels containing formaldehyde (26), transferred to nitrocellulose filters, and hybridized with a radioactive RNA probe by the method of Southern (27). The RNA probe was prepared by in vitro transcription (28) from an EcoRI/HindIII fragment of the Chinese hamster glutamine synthetase gene (29) subcloned into pGEM-2, a pUC-12-derived plasmid containing both SP6 and T7 promoters (28).

Statistical Analysis

This was performed using analysis of variance and Dunnett's multiple comparisons test (30) or Student's t-test (Table 1).

Results and Discussion

Dexamethasone caused a striking increase in glutamine synthetase activity in rat soleus and plantaris muscles. The time-course of the effect of dexamethasone on glutamine synthetase activity in rat soleus and plantaris muscles is shown in Fig. 1. Glutamine synthetase activity in both muscles was 2 times the control values 24 h after dexamethasone administration and increased to about 6-fold greater than control after 7 daily injections of the steroid hormone.

Enhancement of glutamine synthetase activity by dexamethasone probably is mediated via interaction of the steroid hormone with intracellular glucocorticoid receptors, which are present in rat muscles (4, 31). This conclusion is based upon the data of Fig. 2, in which it is seen that RU38486, a potent and selective glucocorticoid antagonist in a number of tissues (32) including muscle (4), significantly reduced the dexamethasone-mediated increase in enzyme activity. Lack of complete inhibition of the dexamethasone effect by this large dose of RU38486, compared with complete blockade *in vitro* (19), may be due to a higher level of non-specific binding of RU38486 to plasma proteins (34). Administration of RU38486 by itself was without effect on glutamine synthetase activity (33). That the induction of glutamine synthetase is selective for glucocorticoids is shown further by the data of Fig. 4. Dexamethasone and triamcinolone

acetonide caused major increases in glutamine synthetase. Progesterone caused a 50% increase in enzyme activity. Muscle is considered to be devoid of progestin receptors (35,36). The effect of this steroid hormone on glutamine synthetase, which we also observed *in vitro* (19), may be due to direct agonist action on glucocorticoid receptors in muscle. Such agonist action has been demonstrated in certain hepatoma cells (37). Estradiol was effective, albeit less so, than progesterone. Possibly, estrogen acted directly via estrogen receptors, which are known to exist in muscle (38). Testosterone was without effect.

Heart muscle glutamine synthetase increased by only 15% ($p < 0.05$) after 7 days of daily s.c. injections of dexamethasone at 5 mg/kg, in striking contrast to the large increases (+500%) in enzyme activity in plantaris and soleus muscles (Fig. 4).

The dexamethasone-mediated increase in glutamine synthetase activity is at least partly a result of an increased level of glutamine synthetase mRNA. Northern blot analysis (Fig. 5) demonstrates that glutamine synthetase mRNA (a major band of 3 kb) was increased dramatically in plantaris muscles from dexamethasone-treated rats compared with control animals. By contrast, there was a small, if any, change in glutamine synthetase mRNA level in heart. Thus, there is good correlation between enhanced glutamine synthetase activity and increased levels of glutamine synthetase mRNA (Figs. 5 and 6).

Exposure of autoradiograms for prolonged periods revealed a second smaller (ca. 1.5 Kb) glutamine synthetase mRNA species, in agreement with Kumar *et al*. (39) and Bhandari *et al*. (40).

Further work is needed to evaluate whether this smaller species also is regulated by dexamethasone and to confirm that it codes for glutamine synthetase.

Induction of glutamine synthetase precedes muscle atrophy and appears to be a common denominator in atrophy of a number of causes (18, 33, 42). Therefore, we have hypothesized that glutamine synthetase may be involved in a fundamental manner in the process of atrophy.

It has been demonstrated that glucocorticoid hormones are anabolic in heart muscle, actually causing an increase in protein synthesis (41). The lack of induction of glutamine synthetase by dexamethasone in this tissue supports our hypothesis of a role for glutamine synthetase in glucocorticoid-mediated muscle atrophy (19). However, our hypothesis is not supported by the observation that glutamine synthetase activity is enhanced in soleus muscles from dexamethasone-treated rats (Figs. 2, 3). Soleus muscle is notoriously resistant to glucocorticoid-mediated muscle atrophy (e.g., 4). Paradoxically, soleus muscle contains a high level of cytosolic glucocorticoid receptors (43). The difference in response to glucocorticoids may reflect the activity patterns of these muscles. Plantaris is a fast-twitch, fatiguable muscle that fires phasically; soleus is a slow-twitch, fatigue-resistant muscle that fires tonically (44). Perhaps the continuous activity of the soleus muscle protects it from wasting. Indeed, glucocorticoid-mediated muscle atrophy is reversed by physical activity (45). It is also possible that differences in rates of protein turnover between these muscles (46-50) may account for the

disparity in hormonal responsiveness. Further experimentation will be necessary to evaluate these possibilities.

Glutaminase activity in rat muscles was not induced by dexamethasone treatment (Table I), indicating that the dexamethasone effect on glutamine synthetase is selective. In fact, dexamethasone caused a significant 30% decrease in glutaminase activity in soleus muscle (Table I). Whether this decrease is biologically significant is not known.

These data demonstrate that glutamine synthetase is induced by glucocorticoids in rat muscles; that this induction involves the activity of intracellular glucocorticoid receptors; and that it occurs, at least in part, at the transcriptional level. These observations agree with our data on L6 muscle cells in vitro (19). To our knowledge, glutamine synthetase appears to be the first glucocorticoid-induced protein to be identified in skeletal muscle. It should provide a valuable biochemical marker for further studies of glucocorticoid hormone-regulated gene expression in muscle.

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Figure Legends

Figure 1: Time-course of the effect of dexamethasone (5 mg/kg) on glutamine synthetase activity in rat soleus and plantaris muscles. Data are means ± SEM of 6 determinations. Experimental procedures are described in the text. *Significantly different from control, $p < 0.02$, plantaris, $p < 0.005$, soleus. **Significantly different from day 1 control, $p < 0.005$, plantaris, $p < 0.01$, soleus. ***Significantly different from day 3 control, $p < 0.005$, soleus and plantaris.

Figure 2: Effect of RU38486 on the dexamethasone-mediated increase in glutamine synthetase activity in rat plantaris muscle. Dexamethasone was injected s.c. at 5 mg/kg. RU38486 was administered p.o. at 50 mg/kg. Glutamine synthetase was assayed after 3 days of administration of dexamethasone (5 mg/kg) and RU38486 (50 mg/kg). Data are means ± SEM of six determinations. Experimental procedures are described in the text. *Significantly different from control, $p < 0.005$. **Significantly different from the dexamethasone group, $p < 0.01$. CTL, control, DEX, dexamethasone.

Figure 3: Steroid hormone specificity of glutamine synthetase induction in rat plantaris muscles. Steroid hormones were injected at 5 mg/kg (s.c.). TA, triamcinolone acetonide; E_2 estradiol-17β ; P, progesterone; T, testosterone. Glutamine synthetase was assayed 24 h after a single injection of steroid hormone. Data are means ± SEM of six determinations (except control, n = 16). Experimental procedures are described in the text. *Significantly different from control, $p < 0.001$.

Figure 4: Effect of dexamethasone (5 mg/kg, s.c.) on glutamine synthetase activity in heart, plantaris, and soleus muscles. Glutamine synthetase was assayed after 7 days of daily dexamethasone injections. *Significantly different from control, $p < 0.05$; **Significantly different from control, $p < 0.001$.

Figure 5: Northern blot analysis of glutamine synthetase induction by dexamethasone (5 mg/kg, s.c.) in plantaris muscle and heart after 5 daily injections. Ctl, control; dex, dexamethasone. Equal (2.5 μg) amounts of total cellular RNA were applied to the gel in each instance. Experimental procedures are described in the text. A section of an autoradiogram containing cellular RNA hybridized to a radiolabeled glutamine synthetase RNA probe (3 Kb) is shown.

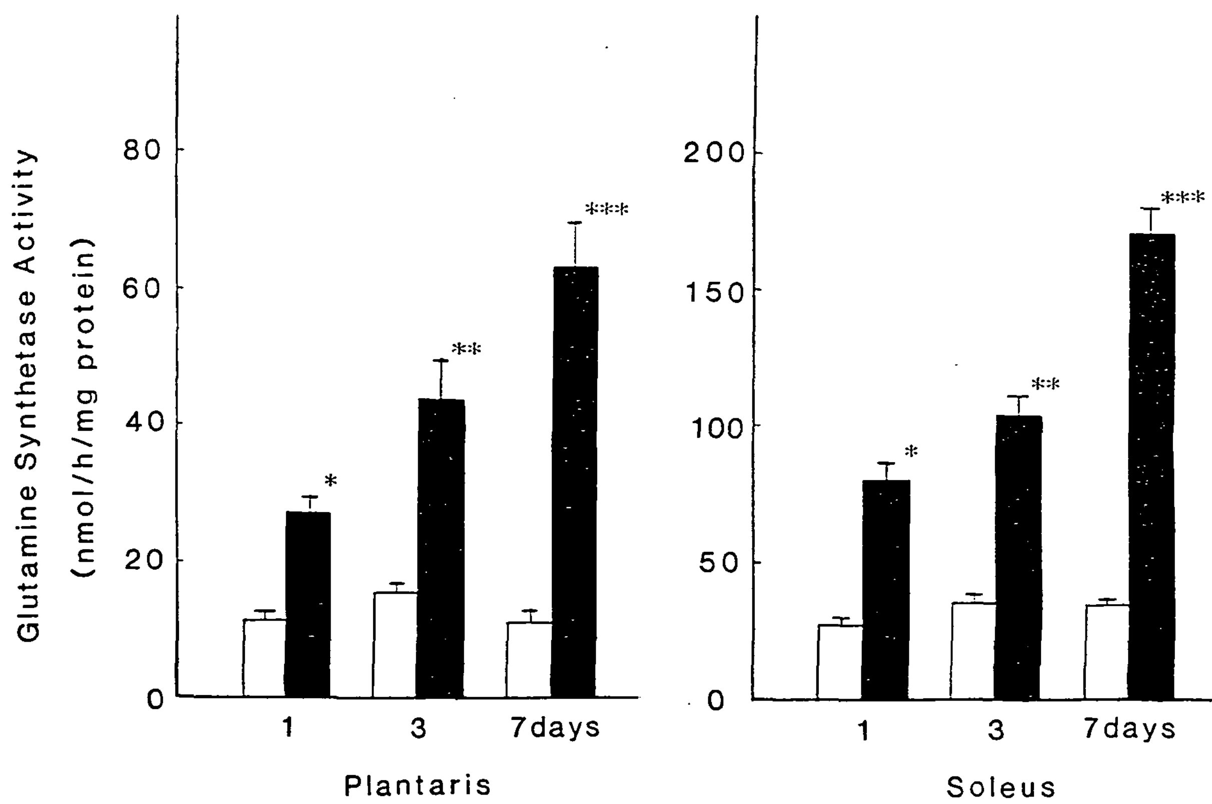
Table 1

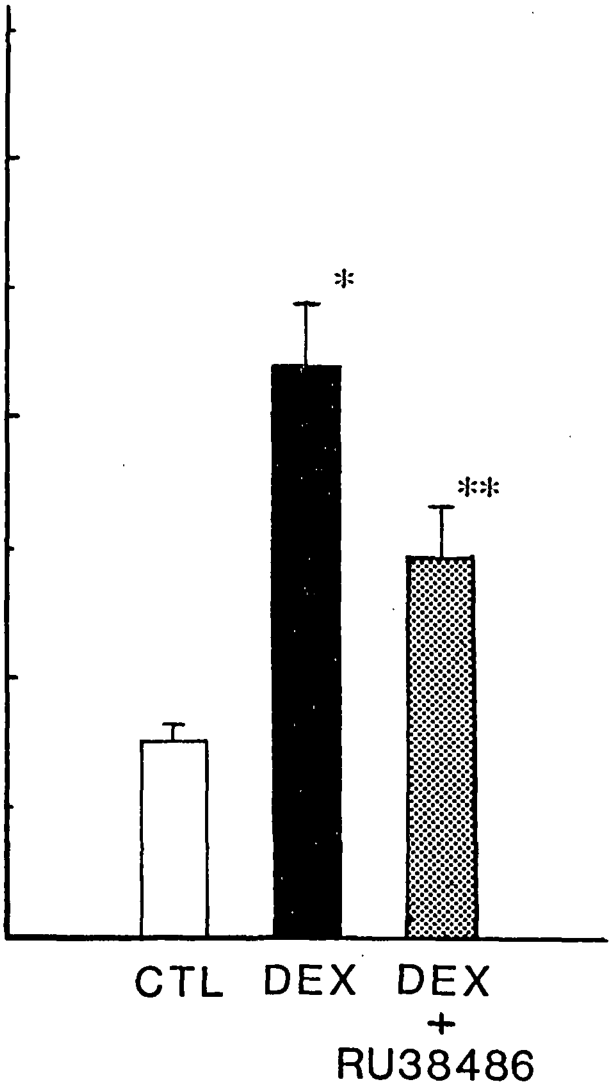
Glutaminase Activity in Soleus and Plantaris

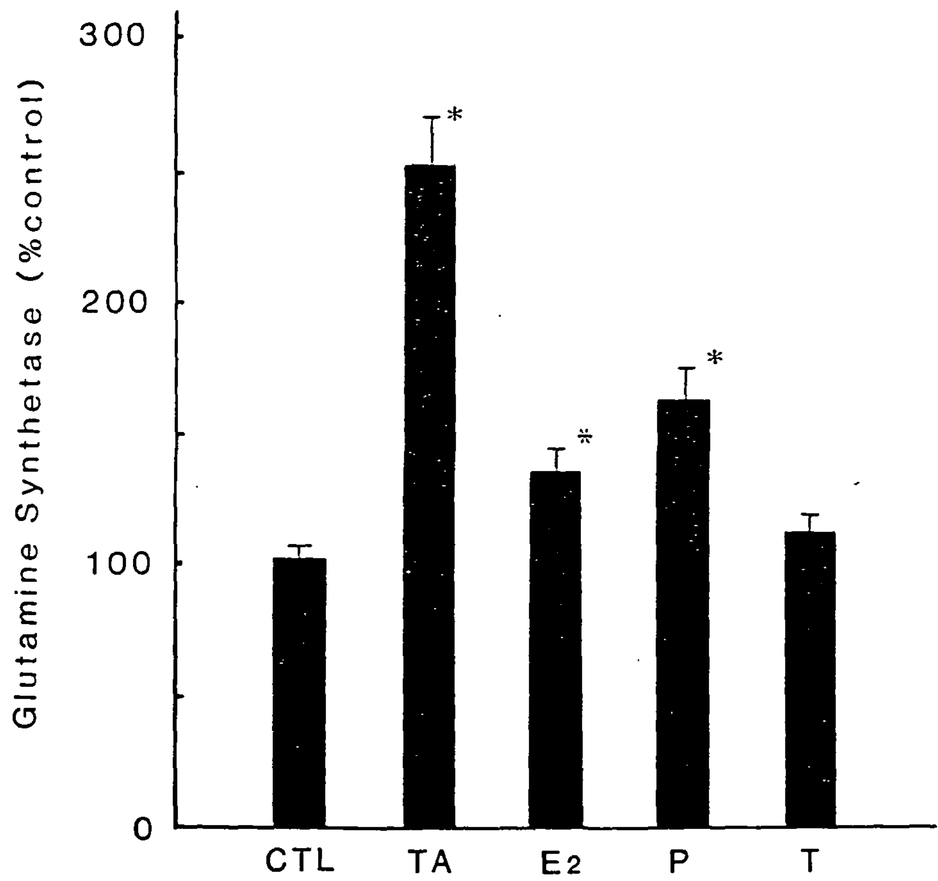
Muscles of Dexamethasone-Treated Rats

Treatment	Soleus	Plantaris
	(nmols/mg protein/h)	
vehicle	$76.40 \pm 13.39^{+}$	83.54 ± 16.11
dexamethasone	$53.83 \pm 7.36^{*}$	76.67 ± 16.9

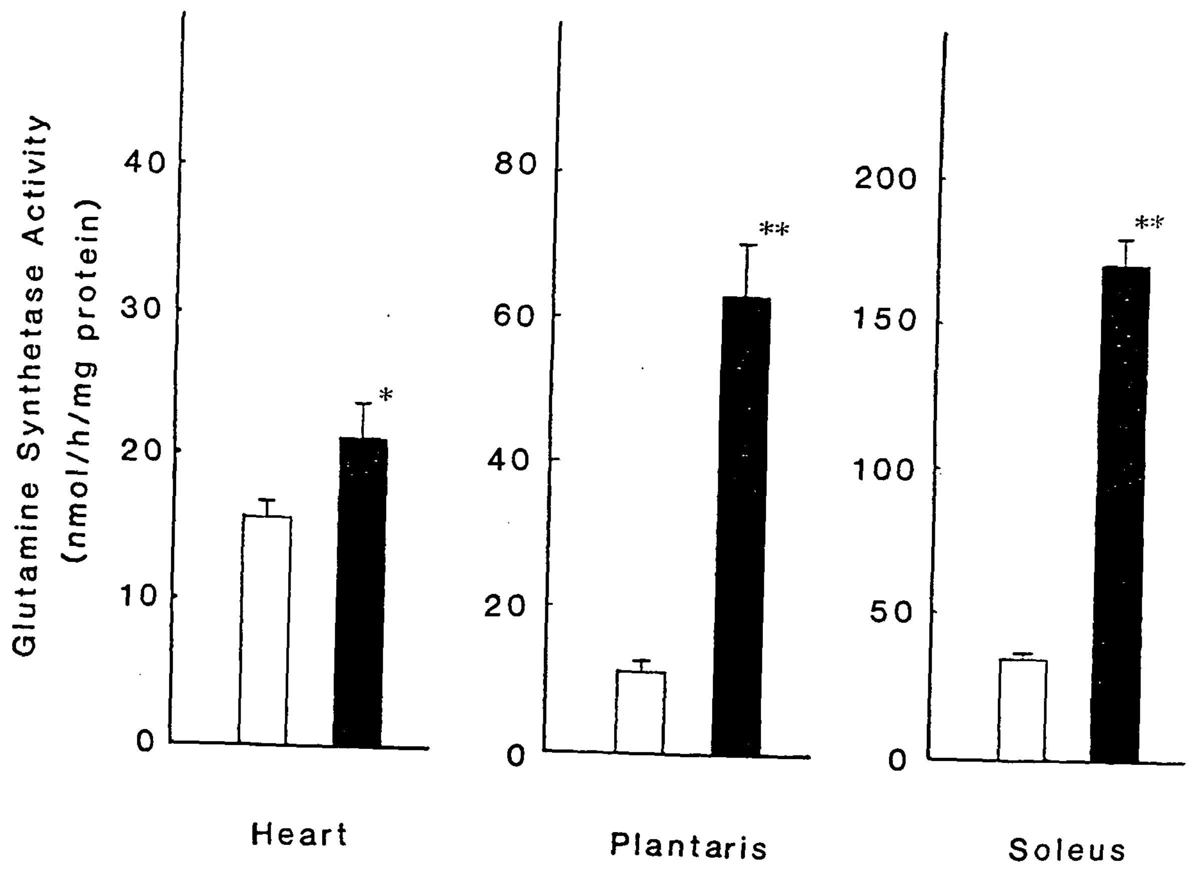
Rats were injected with dexamethasone daily for 7 days, at a dose of 5 mg/kg, s.c. $^{+}$Data are means ± SEM, n = 6. *Significantly different from control, $p < 0.005$.


Glutamine Synthetase Activity
(nmol/h/mg protein)
80
60
40
20
0
*
**
***
1
3
7days
Plantaris
200
150
100
50
0
*
**
***
1
3
7days
Soleus


Glutamine Synthetase Activity
(nmol/h/mg protein)
60
40
20
0
*
**
CTL
DEX
DEX
+
RU38486



Glutamine Synthetase (%control)
300
200
100
0
CTL
TA
E2
P
T
*
*
*


Glutamine Synthetase Activity
(nmol/h/mg protein)
40
30
20
10
0
*
80
60
40
20
0
**
200
150
100
50
0
**
Heart
Plantaris
Soleus