Biochem. J. (1982) **206**, 641–645
Printed in Great Britain

Glucocorticoid action on protein synthesis and protein breakdown in isolated skeletal muscles

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(*Received 14 May 1982/Accepted 2 June 1982*)

The direct actions of glucocorticoid hormones on protein turnover were studied in isolated soleus muscles. These steroids were found to decrease the rates of both protein synthesis and protein breakdown within 3h and 4h respectively. Synthetic steroids (e.g. dexamethasone) were found to be more potent than naturally secreted hormones (e.g. cortisol) in inducing these changes, but only at concentrations *in vitro* less than 10nM.

High circulating concentrations of glucocorticoid steroids are known to have a general catabolic effect on the body, inhibiting growth in children and young animals (Blodgett *et al.*, 1956; Loeb, 1976; Waterlow *et al.*, 1978; McGrath *et al.*, 1981) and causing a true atrophy (Peters *et al.*, 1970; Bullock *et al.*, 1972; Rannels & Jefferson, 1980; McGrath *et al.*, 1981) and increased excretion of urinary nitrogen (Long *et al.*, 1940) in older animals. In addition, muscle wasting and concomitant weakness are clinical features associated with the over-secretory activity of the adrenals in Cushing's syndrome and with prolonged therapeutic uses of these steroids. In the latter situations several synthetic hormones (e.g. dexamethasone, prednisone, triamcinolone acetonide etc.) have been widely used, since these steroids are more potent immuno-suppressants and anti-inflammatory agents than the naturally secreted hormones. However, these same synthetic steroids are also more powerful inducers of muscle wasting (Peters *et al.*, 1970; Bullock *et al.*, 1972; Kelly & Goldspink, 1981; McGrath *et al.*, 1981). Such differences in potency may represent genuine differences in the steroids' direct action on the musculature. Alternatively, the administration of these steroids *in vivo* may induce variable changes in the concentrations of other circulating hormones or metabolites, which may indirectly affect protein turnover and hence muscle growth.

Several studies have attempted to define the precise effects of these steroids on protein turnover in skeletal muscle. Generally, good agreement exists from a variety of experiments, both *in vivo* and *in vitro*, in ascribing to these steroids an inhibitory action on muscle protein synthesis (Wool & Weinshelbaum, 1959; Kostyo & Redmond, 1966; Goldberg, 1969; Shoji & Pennington, 1977*a*; McGrath & Goldspink, 1978; Waterlow *et al.*, 1978; Tomas *et al.*, 1979; Rannels & Jefferson, 1980; McGrath *et al.*, 1981). In contrast, their action on protein breakdown remains poorly defined. Of the few studies undertaken, the whole spectrum of possibilities has been described, i.e., ranging from an enhancement (Goldberg, 1969; Tomas *et al.*, 1979) to no effect (Waterlow *et al.*, 1978) or even an inhibition (Shoji & Pennington, 1977*a*; McGrath *et al.*, 1981) of the degradative rates.

In the present investigation we have examined the effects of both synthetic and naturally secreted glucocorticoids on protein turnover in isolated soleus muscles. Particular emphasis has been placed on defining the precise direct action(s) of these hormones on the rates of protein synthesis and breakdown, their latency of action and the existence of genuine differences in potency.

Methods

All experiments involved the use of male rats (CD strain; Charles River, Manston, Kent, U.K.), initially 45–50g body wt. After the animals had been killed, intact soleus muscles were rapidly dissected out and the average rates of protein synthesis and protein breakdown were measured in these isolated muscles by the method of Fulks *et al.* (1975). These methods involve the use of the amino acid tyrosine, since it is neither synthesized nor degraded by skeletal muscle. That is, protein synthesis was measured as the incorporation of tyrosine into muscle proteins after a 2h incubation in 3ml of oxygenated (O_2/CO_2, 19:1) Krebs–Ringer bicarbonate buffer, pH 7.4 (DeLuca & Cohen, 1964), containing 10mM-

0306-3283/82/090641-05$01.50/1

glucose, 5 times the normal concentration of all plasma amino acids, 0.05 μCi of L-[U-^{14}C]tyrosine (sp. radioactivity 483 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) and L-tyrosine hydrochloride (0.5 mM). The [^{14}C]tyrosine measured in protein was divided by the specific radioactivity of the intracellular tyrosine pool, to give the total number of moles of tyrosine incorporated into muscle proteins (Fulks *et al.*, 1975). Protein degradation was determined, independently of synthesis, by measuring the release of tyrosine (Waalkes & Udenfriend, 1957) into intracellular amino acid pools and the surrounding medium after a 2 h incubation. The medium used here was identical with that for synthesis (above) except that (i) tyrosine was omitted from the initial medium to increase the sensitivity for measuring the tyrosine released from the muscle, and (ii) cycloheximide (50 μM) was added to block protein synthesis and prevent any re-utilization of the released tyrosine.

Where appropriate, the steroid hormones were added to these incubation media. To prolong the periods of exposure to the steroids (i.e. in excess of 2 h), muscles were first preincubated in identical media (except for the exclusion of [^{14}C]tyrosine or cycloheximide) in the presence or in the absence (controls) of the appropriate hormones.

Results

Initially we studied the direct effects of both naturally secreted (i.e. corticosterone and cortisol) and synthetic (i.e. dexamethasone and triamcinolone acetonide) glucocorticoids on the rates of protein synthesis and protein breakdown in isolated soleus muscles. Since some of the hormones had to be dissolved in ethanol, an equivalent concentration of the alcohol (0.5% final concn.) was added to all systems, including the controls. The steroids were then added at 1 μM to both preincubation (2 h) and incubation (2 h) media. In all cases, after 4 h total exposure to the steroids the rates of both protein

Table 1. *Time-related changes in protein synthesis and protein breakdown in response to different glucocorticoid steroids*
Isolated intact soleus muscles were exposed *in vitro* to various naturally secreted or synthetic steroids, and the rates of protein synthesis and breakdown were measured by the method of Fulks *et al.* (1975). Each value represents the mean ± S.E.M. for at least six control or steroid-treated muscles, with the differences between these means analysed by Student's *t* test (*$P < 0.01$). Percentage differences are given in parentheses. (A) Muscles were exposed to 1 μM natural (i.e. cortisol or corticosterone) or synthetic (i.e. triamcinolone acetonide or dexamethasone) glucocorticoid hormones for a total of 4 h. This involved a 2 h preincubation before a 2 h incubation, during which time the average rates of protein synthesis or breakdown were measured. (B) In these experiments muscles were exposed to 1 μM-cortisol for a total of 2, 3, 4 or 5 h. In all instances the last 2 h involved the measurements for the protein synthetic or degradative rates.

	Protein synthesis (nmol of tyrosine incorporated/2 h per g of muscle)	Protein breakdown (nmol of tyrosine released/2 h per g of muscle)
(A) Steroid addition (1 μM)		
None (i.e. control)	225 ± 14	403 ± 13
Cortisol	181 ± 17 (−20*)	310 ± 22 (−23*)
Corticosterone	171 ± 10 (−24*)	209 ± 15 (−48*)
Triamcinolone acetonide	156 ± 10 (−31*)	252 ± 13 (−37*)
Dexamethasone	164 ± 11 (−27*)	239 ± 13 (−41*)
(B) Exposure time (h) ± 1 μM-cortisol		
2 h −	217 ± 11	455 ± 25
2 h +	199 ± 9 (−8)	472 ± 15 (+4)
3 h −	232 ± 21	415 ± 20
3 h +	162 ± 19 (−30*)	429 ± 23 (+3)
4 h −	225 ± 19	391 ± 14
4 h +	151 ± 11 (−32*)	325 ± 13 (−17*)
5 h −	271 ± 23	357 ± 20
5 h +	192 ± 9 (−29*)	268 ± 22 (−25*)

synthesis and protein breakdown were significantly ($P < 0.005$) decreased, compared with control values (Table 1A). However, at this concentration of the hormones there was little evidence to suggest that any particular glucocorticoid was more, or less, effective than the others in inducing these changes. To be certain that the muscles had been exposed to the steroids long enough for their full effects to have been exerted, the time course of action of cortisol on protein synthesis and breakdown was studied in more detail.

Isolated soleus muscles were exposed to cortisol (1 μM) for 0, 1, 2 or 3 h before a 2 h incubation (in the presence of the steroid) during which the synthetic or degradation rates were measured. After 3 h total exposure to cortisol, but not before, the incorporation of tyrosine into the muscle's proteins was significantly ($P < 0.01$) inhibited (Table 1B). Longer exposure times failed to produce any greater degree of inhibition. For the steroid's inhibitory influence on protein breakdown to be observed, a minimum of 4 h exposure to the hormone was required (Table 1B), i.e. 1 h longer than that necessary for the suppression of protein synthesis. These experiments therefore suggest that the 4 h used in comparing the natural and synthetic steroids (Table 1A) was adequate for the full expression of the steroids' action on protein turnover. These particular findings also suggested that there was no discernible difference in the potencies of these various glucocorticoid hormones (Table 1A). However, this concentration of the hormones (1 μM), as added to the media, is high, especially since in the absence of plasma proteins (*in vitro*) all of the hormones will exist in a free non-bound form, in contrast with the normal situation in the plasma. It is therefore probable that under these conditions *in vitro* the steroid receptors of the muscle would be fully saturated. Hence similar experiments to those of Table 1(A) were repeated, but this time with progressive lowering of the concentrations of cortisol and dexamethasone, these two steroids being chosen as representatives of the naturally secreted and synthetic hormones respectively. Both steroids continued to inhibit the protein synthetic and degradative rates down to concentrations of 10 nM (Table 2). Thereafter cortisol was without significant effect. In contrast, dexamethasone remained effective, significantly inhibiting synthesis down to 0.1 mM and breakdown at the even lower concentration of 1 pM (Table 2). Hence at steroid concentrations below those likely to saturate the steroid receptors (Mayer *et al.*, 1974) the differences between the natural and synthetic glucocorticoids become apparent. In this respect these direct actions of the steroids on muscle protein turnover (i.e. *in vitro*) are in agreement with the more powerful atrophic effects of the synthetic steroids on the musculature when administered to the intact animal (see above).

Discussion

In keeping with the existence of steroid receptors in the sarcoplasm (Mayer *et al.*, 1974; Shoji & Pennington, 1977*b*), our findings clearly emphasize the direct actions of the glucocorticoid hormones in regulating muscle protein turnover. Although the rates of both synthesis and breakdown were suppressed, a more rapid and/or marked effect on the former (as suggested by the times and percentage changes in Table 1B and McGrath, 1980) would be necessary to produce a net catabolic effect, which would ultimately cause either a slowing of muscle growth or muscle atrophy (McGrath *et al.*, 1981). However, caution has to be exercised in extrapolating these short-term direct actions (*in vitro*) to the situation in the intact animal. For example, under the precise conditions used in the present study the isolated soleus muscles (controls) were in a negative nitrogen balance, with the rates of breakdown exceeding those of synthesis (e.g. see Table 1). Clearly this is not a true reflection of the situation *in vivo* in rapidly growing animals. However, there are at least two plausible explanations for this. Firstly, the rates

Table 2. *Changes in protein turnover in response to various concentrations of natural and synthetic steroids*
Average rates of protein synthesis and protein breakdown were measured in soleus muscles after 4 h total exposure to various concentrations (1 μM to 1 pM) of either the naturally secreted (cortisol) or synthetic (dexamethasone) glucocorticoid. Each value was determined on six to eight control or steroid-treated muscles. Since the control rates differed slightly from day to day, the data are presented in percentage terms, with the controls assigned a value of 100%. Student's *t* test was used for assessing the statistical significance (*$P < 0.01$) of the differences between the means of steroid-treated and control muscles.

	Percentage changes in								
	Protein synthesis				Protein breakdown				
Concn. of steroid ...	1 μM	10 nM	0.1 nM	1 pM	1 μM	10 nM	1 nM	0.1 nM	1 pM
Cortisol	−20*	−33*	+17	+10	−23*	−33*	−	0	+6
Dexamethasone	−27*	−38*	−29*	−12	−41*	−39*	−33*	−31*	−20*

of synthesis and breakdown *in vitro* were not measured under strictly comparable conditions (see the Methods section), thus limiting the usefulness of direct comparisons between these rates. The main emphasis of the present study therefore must be found in comparing the steroid-induced changes within each individual system, i.e. for measuring either synthesis or breakdown. Secondly from other studies it is known that the absolute rates of synthesis and breakdown (i.e. in control muscles) *in vitro* differ from those measured *in vivo*, synthesis being approx. 50% lower and breakdown 70% higher *in vitro* than when measured *in vivo* (D. F. Goldspink, P. J. Garlick & M. A. McNurlan, unpublished work). Undoubtedly some of these differences can be explained by the absence (*in vitro*) of certain anabolic endocrine (e.g. insulin) and mechanical factors that would normally be present *in vivo* (Goldspink, 1981). Hormones other than the glucocorticoids were deliberately excluded from the media so as not to complicate interpretation of the results. Despite such differences in the absolute rates as measured *in vitro* and *in vivo* and the general difficulties associated with obtaining meaningful direct measurements of protein breakdown (Waterlow *et al.*, 1978), the use of isolated muscles as described in the present paper remains the only means of investigating direct hormonal action(s) on this tissue. Further, in spite of any deficiencies in these measurements of protein synthesis and breakdown, such isolated muscle preparations, like those *in situ*, respond similarly to hormones such as insulin and thyroxine (Fulks *et al.*, 1975; Goldberg *et al.*, 1976; Albertse *et al.*, 1980; Brown *et al.*, 1981; Goldspink, 1981) and to increased work demands (D. F. Goldspink, P. J. Garlick & M. A. McNurlan, unpublished work).

The more potent effects of the synthetic steroids on protein turnover *in vitro* (Table 2) correlate with both their higher affinities for steroid receptors (Mayer *et al.*, 1974; Shoji & Pennington, 1977*b*) and their more profound influences *in vivo* on muscle wasting. However, in the latter situations other explanations may also be involved. For example, the free circulating concentrations of these different steroids are likely to vary by virtue of their differing binding affinities for plasma proteins (Thompson & Lippman, 1974). In addition, the injected steroids may induce variable degrees of hyperglycaemia and hyperinsulinaemia (Perley & Kipnis, 1966; Tomas *et al.*, 1979). The ultimate explanation may therefore involve the combination of both direct and indirect responses. Of the naturally secreted hormones in the rat cortisol may be considered of smaller quantitative importance than corticosterone. However, most previous studies *in vivo* have involved the administration of suspensions of cortisone acetate with the aim of establishing a prolonged release of cortisol. For comparison with such studies, we too have placed a greater emphasis on investigating the direct actions of cortisol rather than corticosterone on protein turnover. Although limited, nothing in this (Table 1A) or a previous (Kostyo & Redmond, 1966) study suggests that differences exist in the direct actions of these two natural hormones.

The ability of these glucocorticoids to inhibit protein synthesis in either slow-twitch (i.e. the soleus, as above) or fast-twitch (extensor digitorum longus; results not presented) skeletal muscles verifies the findings of several previous workers (see above). Although fast-twitch muscles are usually considered to be more responsive to hormones, this was not the case here; the steroids *in vitro* (Table 1A) suppressed protein synthesis in a nearly identical manner in both the soleus and the extensor digitorum longus muscles. In most earlier experiments *in vitro* no allowance was made for possible changes in the size, or specific radioactivity, of the precursor amino acid pool(s) (Kostyo & Redmond, 1966). These investigators were perhaps fortunate that the glucocorticoids do not appear to change these parameters in isolated muscle preparations (Shoji & Pennington, 1977*a*; J. A. McGrath & D. F. Goldspink, unpublished work). The rapid suppression of protein synthesis (i.e. 3h; Table 1B) is in keeping with the suggestion that the acute response to these steroids is a decrease in the initiation of translation (Rannels & Jefferson, 1980) and is accompanied by a rapid (4h) decrease in the proportion of polyribosomes (Young *et al.*, 1968; Bullock *et al.*, 1972). Such initial responses are followed by an inhibition of transcription and consequent decreases in the muscle's RNA content.

Of particular note was the finding that protein breakdown was also inhibited by these steroids, whether administered *in vitro* (Tables 1 and 2) or *in vivo* (Shoji & Pennington, 1977*a*; McGrath & Goldspink, 1978; McGrath, 1980). These findings also correlate with decreased autolytic activities measured within glucocorticoid-treated muscles (McGrath, 1980). The rapidity (4h) with which breakdown was inhibited should enable further analysis of the mechanisms involved. Some inducible inhibitor/inactivation system or possibly a stabilization of lysosomal membranes (Berg & Bird, 1970) might be expected, since so little time was available for appreciable adjustment of the number of proteinase molecules. The apparent conflict between the above findings and those of other workers (Goldberg, 1969; Waterlow *et al.*, 1978; Tomas *et al.*, 1979; Santidrian *et al.*, 1981) cannot as yet be explained. Inevitably such an explanation(s) must lie within the various techniques used to measure protein breakdown, not all of which have, or are able to, define events within individual muscles (Tomas *et al.*, 1979): the use of different steroid doses (Odedra *et al.*, 1980) and routes of injection

(Santidrian *et al.*, 1981), and the general disregard for differing age-related responses (McGrath *et al.*, 1981) within the same or different species of animal and the marked variation in response within the different muscle types (Goldberg, 1969; Rannels & Jefferson, 1980; Kelly & Goldspink, 1981).

These and previous studies *in vitro* suggest a common and concerted mode of action of the glucocorticoids in arresting muscle growth. DNA synthesis (Goldberg & Goldspink, 1975), the net transfer of amino acids (Kostyo & Remond, 1966; Lewis & Goldspink, 1981) and the rates of protein synthesis (Tables 1 and 2; Young *et al.*, 1968) and protein breakdown (Tables 1 and 2) are all significantly inhibited in skeletal muscle within 4h of exposure to these steroid hormones.

The excellent technical assistance of Mr. Paul Anderson, B.Sc., and Mrs. Gwen Sanderson is gratefully acknowledged. We are also indebted to the British Heart Foundation for their financial support of this work.

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