Short-term effects of corticosterone treatment on muscle protein synthesis in relation to the response to feeding

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1. Rates of protein synthesis in liver and muscle of 100 g male rats were measured *in vivo* at 1 h or 4 h after injection of 2.5 mg of corticosterone and compared with those from animals given carrier medium alone. 2. In post-absorptive rats, corticosterone for 1 h had no effect on either muscle or liver protein synthesis. After 4 h there was a decrease in both tissues, but this was only statistically significant in muscle. 3. In fed rats, rates of protein synthesis were higher than those in post-absorptive animals, but the effects of corticosterone injection were similar. 4. Re-feeding of post-absorptive rats led to an increase in muscle protein synthesis after 1 h and 4 h. At 1 h this increase was not inhibited when plasma corticosterone concentrations were maintained high by injection of the hormone immediately before feeding commenced, but at 4 h there was a small inhibition. 5. It is concluded that the action of corticosterone in depressing muscle protein synthesis is time-dependent and requires longer than 1 h to develop. The failure of the hormone to alter the response to re-feeding for 1 h in post-absorptive rats suggest that corticosteroids are not important mediators of the acute stimulation of muscle protein synthesis by food intake.

INTRODUCTION

High levels of corticosteroid hormones result in muscle wasting, as in Cushing's syndrome, and have been shown to inhibit muscle protein synthesis both *in vivo* (Oedra & Millward, 1982) and *in vitro* (McGrath & Goldspink, 1982; Reeds & Palmer, 1984). It has been suggested that a decrease in the concentration of corticosterone when starved rats are re-fed might be, in part, responsible for the observed increase in the rate of muscle protein synthesis (Millward *et al.*, 1983). However, this effect on protein synthesis was observed within 1 h, whereas the available evidence suggests that corticosterone requires some hours for its effects to become apparent. In studies in incubated muscles *in vitro*, addition of corticosteroids resulted in a decrease in protein synthesis only after 3–4 h (McGrath & Goldspink, 1982; Reeds & Palmer, 1984). Indeed, during the first 1 h of exposure to the substance, an increase in protein synthesis was observed (Reeds & Palmer, 1984). No equivalent data for the time course of corticosteroid action in muscle *in vivo* appear to be available. We have therefore studied the effect of corticosterone on muscle protein synthesis *in vivo* during the first few hours after injection. Our interest is in the mediators of the acute response of muscle protein synthesis to feeding in post-absorptive animals (Garlick *et al.*, 1983; Preedy & Garlick, 1986), and studies were planned to reveal any involvement of corticosterone in this process.

MATERIALS AND METHODS

Animals

Male hooded Lister rats of the Rowett strain were individually caged from 80 g body wt. (29 days of age) in a temperature-controlled room (21 °C) with a 12 h-light/12 h-dark cycle (lights on at 06:00 h) until the day of the experiment, when they weighed about 100 g. In each experiment animals were allocated to groups of six, with

equal mean body weight. Food was removed from 'post-absorptive' animals at 23:00 h on the night before the experiment. Starting at 08:00 h, animals were injected subcutaneously with corticosterone or injection medium only (controls) and returned to their cages for 1 h or 4 h before death. The corticosterone (Sigma, Poole, Dorset, U.K.) was dissolved in ethanol (50 mg/ml) at 65 °C before being mixed with water (100 µl to 300 µl of water) and injected (200 µl, containing 2.5 mg of corticosterone) rapidly through a 21-gauge needle. Exactly 10 min before death, each rat was injected with 30 µCi of [2,6-³H]phenylalanine (300 µCi/mmol; Amersham International, Amersham, Bucks., U.K.) into a tail vein for measurement of protein synthesis. The injection schedule was such that rats treated for 4 h were killed on average about 2 h later than those treated for 1 h. The procedures for injection, killing and sampling of tissues and blood have been described previously (Garlick *et al.*, 1980, 1983).

Analytical procedures

Preparation of the tissues for analysis and measurement of specific radioactivity of free and protein-bound phenylalanine have been described previously (Garlick *et al.*, 1980; McNurlan *et al.*, 1982). Plasma glucose was measured as described by Trinder (1969), insulin by radioimmunoassay (Basset & Thorburn, 1971) and corticosterone by radioimmunoassay (Fang & Strobel, 1982). The rate of protein synthesis in liver and muscle was calculated from the specific radioactivity of free and protein-bound phenylalanine as described by McNurlan *et al.* (1982). Results were evaluated statistically by analysis of variance, means being compared by two-tailed *t* tests by using the pooled estimate of variance.

RESULTS

The concentration of corticosterone in plasma during the first 0.5 h after the subcutaneous injection of 2.5 mg

of the hormone is shown in Fig. 1. A maximum concentration of about 11 times the control value was observed at 15 min, and at 30 min the concentration was still elevated 6-fold. Rats given the injection medium without hormone had similar concentrations to those of uninjected controls.

The effect of corticosterone injection on protein synthesis in tissues of post-absorptive rats is shown in Table 1. At 1 h after injection there was no effect on protein synthesis in either muscle or liver, although the concentration of corticosterone in plasma was still elevated ($P < 0.001$) at this time. Insulin was also increased ($0.05 < P < 0.1$). At 4 h after injection, the rate of muscle protein synthesis in control animals was lower than at 1 h ($0.05 < P < 0.1$), probably because of the additional period of food deprivation. Muscle protein synthesis was decreased by 25% ($P < 0.01$) by corticosterone injection, and that in liver by 11% (not

Fig. 1. Changes in plasma corticosterone concentration after subcutaneous injection of 2.5 mg of corticosterone into fed rats

Samples of blood were taken by decapitation from untreated rats (□) or at various times after injection of corticosterone (●) or medium alone (○). Results are means $\pm$ S.E.M.

significant). Both corticosterone and insulin concentrations were higher in the hormone-injected group, but the difference was only significant for insulin ($P < 0.02$).

In fed rats (Table 2), rates of protein synthesis in muscle and liver were higher than in post-absorptive animals, but statistical comparison would be inappropriate, as the rats were from different batches. At 1 h after injection there was no difference between groups in any of the parameters measured. At 4 h there was a 17% lower rate of protein synthesis in muscle of the hormone-treated animals ($P < 0.05$) and a 9% lower rate in liver (not significant). Plasma insulin concentration was also raised, but not significantly.

Table 3 shows the effect of corticosterone injected immediately before re-feeding of post-absorptive rats. At 1 h after re-feeding commenced, muscle protein synthesis was increased, but there was no difference between corticosterone-injected and control groups. After 4 h, muscle protein synthesis remained elevated relative to the post-absorptive group. In the hormone-injected group the rate was 10% lower than in controls, but this difference was not quite significant ($0.05 < P < 0.1$). Corticosterone and glucose concentrations were significantly increased in the hormone-treated groups at both 1 h and 4 h.

DISCUSSION

The present experiments show clearly that muscle protein synthesis does not respond to corticosterone within the first 1 h after injection. This is in agreement with data from rat muscle *in vitro*, when no decrease in protein synthesis was observed during the first 2 h of incubation with cortisol (McGrath & Goldspink, 1982). By contrast, in rabbit muscle Reeds & Palmer (1984) showed an increase during the first 1 h of incubation with dexamethasone, but an increase at 1 h was not apparent in the rat *in vivo* (Tables 1 and 2). At 4 h after hormone injection, there was a significant fall in protein synthesis *in vivo* in both fed and fasted rats. This is in complete agreement with data obtained from muscle incubation *in vitro* from both rats and rabbits, when a decline in protein synthesis became apparent after 3–4 h of exposure to the hormone (McGrath & Goldspink, 1982; Reeds & Palmer, 1984). This concurrence between the time course of glucocorticoid action *in vivo* and *in vitro* does not parallel the effects of insulin, which stimulates muscle protein synthesis within 10 min *in vivo* (Garlick *et al.*,

Table 1. Effects of corticosterone in post-absorptive rats at 1 h and 4 h after injection

Rats were fasted overnight before injection of corticosterone or medium alone. After 1 h or 4 h animals were killed, rates of protein synthesis (%/day) being measured during the last 10 min. Glucose (mM), insulin (μ units/ml) and corticosterone (μ g/l) were measured in samples of plasma taken at the end. Significance of differences between corticosterone and control values: * $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$.

Treatment	Protein synthesis		Glucose	Insulin	Corticosterone
	Muscle	Liver			
Control (1 h)	11.3 $\pm$ 0.8	60 $\pm$ 8	6.0 $\pm$ 0.2	6.4 $\pm$ 1.1	350 $\pm$ 50
Corticosterone (1 h)	10.9 $\pm$ 0.6	58 $\pm$ 6	7.1 $\pm$ 0.3**	8.5 $\pm$ 0.5	1030 $\pm$ 100***
Control (4 h)	9.8 $\pm$ 0.3	58 $\pm$ 7	6.2 $\pm$ 0.3	6.4 $\pm$ 0.9	360 $\pm$ 30
Corticosterone (4 h)	7.3 $\pm$ 0.5**	51 $\pm$ 6	6.8 $\pm$ 0.2	9.4 $\pm$ 0.7*	490 $\pm$ 70

1983), but not until 30–60 min *in vitro* (Stirewalt & Low, 1983). Previous studies of the time course of the response to glucocorticoids *in vivo* do not appear to have investigated periods shorter than 1 day (e.g. Odedra *et al.*, 1983).

The effects of corticosterone on liver protein synthesis were not statistically significant in either fed or fasted rats, but in both experiments the trend was the same. The data seem to indicate a small decrease at 4 h after injection, but no effect at 1 h. This is consistent with the observation by Jeejeebhoy *et al.* (1972) that albumin synthesis by liver *in vivo* was decreased at 3 h after cortisol injection. However, Kim & Kim (1975) showed a biphasic response to prednisolone injection, whereby protein synthesis was decreased at 1–2 h and increased at about 8 h, with little change at 4 h. This rapidly varying response was not noted in the present study, but the method used for measuring liver protein synthesis *in vivo* by Kim & Kim (1975) did not make adequate allowance for precursor specific radioactivity. Odedra *et al.* (1983) used the same technique as that in the present study, and showed an increase in liver synthesis at 1 day after corticosterone administration, but did not report measurements at shorter time intervals.

The dose of corticosterone was chosen to maintain higher than normal concentrations in fasting over the

chosen study period. The time course of concentration shown in Fig. 1 was obtained in animals that were not given the large dose of [³H]phenylalanine used to measure protein synthesis, because the injection of amino acid and the associated stress have previously been shown to stimulate corticosterone secretion (Preedy & Garlick, 1986). In the experiment shown in Fig. 1, no special precautions were adopted to avoid stressing the animals, with the result that basal concentrations were very high. In addition, the concentrations shown in Tables 1 and 3 were obtained from [³H]phenylalanine-injected rats. Thus the data can be used as a guide to the increase in concentration after hormone injection, but the changes in concentration with feeding are not considered reliable. Very high concentrations (6–11 times control values) were seen in corticosterone-injected rats over the period 0–30 min (Fig. 1), and elevated values persisted up to 4 h (Tables 1 and 3). By comparison, when the effects of feeding on corticosterone concentration were measured in uninjected and relatively unstressed post-absorptive rats, there was a fall of about 3-fold (Preedy & Garlick, 1986). With re-feeding for 1 h after 4 days of starvation, Millward *et al.* (1983) reported decreases ranging from 4 to 22-fold, but these were in animals that had been injected with phenylalanine.

The main aim of the present work was to elucidate the role of corticosterone in the stimulation of muscle protein synthesis in post-absorptive animals by feeding (see the Introduction). Rates of synthesis were higher in fed animals than in fasted ones, but this was apparently not due to higher corticosterone concentrations in the latter, as injection of the hormone had an inhibitory effect at 4 h in both states (Tables 1 and 2). The lack of effect of corticosterone for 1 h in either fasted or fed rats suggests that this hormone might not be involved in mediating the response to re-feeding, but it is not proof. Re-feeding would involve a fall in concentration of the hormone, and hence a removal of any inhibition (Millward *et al.*, 1983), instead of the acute rise in concentration studied in Tables 1 and 2. The experiment shown in Table 3 was therefore performed, where the effect of re-feeding was studied while preventing any feeding-induced fall in corticosterone concentration by injection of exogenous hormone. The lack of a detectable decrease in protein synthesis in response to the hormone is strong evidence that corticosterone is not involved in

Table 2. Effects of corticosterone in fed rats at 1 h and 4 h after injection

The experimental procedures were the same as in Table 1, except that animals were fed. Significance of differences between corticosterone and control values: **P* < 0.05.

Treatment	Protein synthesis (%/day)		Insulin (μ units/ml)
	Muscle	Liver	
Control (1 h)	14.3 $\pm$ 0.3	68 $\pm$ 4	17.4 $\pm$ 1.6
Corticosterone (1 h)	14.4 $\pm$ 0.7	66 $\pm$ 4	18.5 $\pm$ 1.8
Control (4 h)	13.9 $\pm$ 1.1	62 $\pm$ 3	16.4 $\pm$ 2.4
Corticosterone (4 h)	11.6 $\pm$ 0.8*	57 $\pm$ 3	22.2 $\pm$ 2.6

Table 3. Influence of corticosterone at 1 h and 4 h after feeding of post-absorptive rats

Rats were fasted overnight before injection of corticosterone or medium alone, followed by the re-introduction of food. After 1 h or 4 h animals were killed, and samples of plasma were collected for measurement of glucose, insulin and corticosterone. The rate of muscle protein synthesis was measured during the last 10 min before death. Those rats that were not re-fed were killed at the same time as those that were re-fed for 1 h. Significance of differences between corticosterone and control values: **P* < 0.1, ***P* < 0.05, ****P* < 0.001.

Treatment	Muscle k_s (%/day)	Glucose (mM)	Insulin (μ units/ml)	Cortico- sterone (μ g/l)
Fasted	8.3 $\pm$ 0.6	5.5 $\pm$ 0.2	4 $\pm$ 1	203 $\pm$ 33
Re-fed (1 h), control	13.0 $\pm$ 0.5	9.2 $\pm$ 0.2	53 $\pm$ 3	254 $\pm$ 30
Re-fed (1 h), +corticosterone	12.4 $\pm$ 0.4	10.6 $\pm$ 0.3***	41 $\pm$ 8	1100 $\pm$ 170***
Re-fed (4 h), control	12.9 $\pm$ 0.5	9.0 $\pm$ 0.2	22 $\pm$ 9	340 $\pm$ 45
Re-fed (4 h), +corticosterone	11.6 $\pm$ 0.6*	9.9 $\pm$ 0.3**	13 $\pm$ 2	556 $\pm$ 89**

this acute response. The hypothesis that a decrease in corticosterone contributed to an increase in muscle protein synthesis within 1 h was advanced from studies of re-feeding of 4-day-starved rats (Millward *et al.*, 1983). Injection of corticosterone before re-feeding resulted in a diminished response at 60 min, although this was not statistically significant. However, the hormone was given 2 h before the food, allowing 3 h to elapse before protein synthesis was measured. On the basis of the present results, this extended period of hormone treatment might be expected to inhibit protein synthesis, but it would not properly represent the effect of a change in corticosterone concentration that occurred within the last 1 h in response to food. Our own interest is not in the recovery from 4 days of starvation, but in defining the mediators of the acute response to meals in post-absorptive animals (Garlick *et al.*, 1983; Preedy & Garlick, 1986). However, it seems unlikely that corticosterone is involved in the re-feeding response, even after longer-term starvation, because none of the data from experiments either *in vivo* or *in vitro* suggest that inhibitory effects of this hormone can be detected within 1 h.

The metabolic responses to feeding are complex, and undoubtedly involve a number of regulatory factors. Previous data have suggested that the acute effect of food intake on muscle protein synthesis depends on the presence of insulin and amino acids (Garlick *et al.*, 1983; Preedy & Garlick, 1986). Both of these factors have also been shown to stimulate protein synthesis *in vitro* (e.g. Fulks *et al.*, 1975; Jefferson *et al.*, 1977). Glucagon has been shown to inhibit muscle protein synthesis both *in vivo* and *in vitro* (Preedy & Garlick, 1985; Preedy *et al.*, 1980), and its concentration does rise on fasting (Unger *et al.*, 1963). Despite this, it is unlikely that glucagon is important in mediating the effect of feeding, since the effective concentration was above the physiological range (Preedy & Garlick, 1985). Furthermore, although responses were detected after 6 h of infusion, there was no effect at 1 h (Preedy & Garlick, 1985). The present results show that corticosterone, like glucagon, does not appear to mediate the acute response to feeding within 1 h. The ability of corticosteroids to inhibit muscle protein synthesis after 4 h suggests that they may

have a more important role in chronic wasting conditions such as malnutrition or injury.

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