Effect of corticosterone treatment on muscle protein turnover in adrenalectomized rats and diabetic rats maintained on insulin

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The effect of corticosterone on protein turnover in skeletal muscle was investigated in growing rats. Protein synthesis was measured *in vivo* by the constant infusion of [^{14}C]tyrosine. The extent to which any effect of corticosterone is modulated by the hyperinsulinaemia induced by steroid treatment was examined by giving the hormone not only to adrenalectomized rats but also to streptozotocin-induced diabetic rats maintained throughout the treatment period on two dosages of insulin by an implanted osmotic minipump. Approximate rates of protein degradation were also estimated in some cases as the difference between synthesis and net change in muscle protein mass. Measurements were also made of free 3-methylhistidine concentration in muscle and plasma. At 10 mg of corticosterone/100 g body wt. per day, growth stopped and muscle wasting occurred, whereas at 5 mg of corticosterone/100 g body wt. per day no net loss of protein occurred. However, this low dose did induce muscle wasting when insulin concentration was regulated by a dose of 1.2 units/day. Protein synthesis was markedly depressed in all treated groups, the depression in the insulin-maintained rats being marginally more than in the hyperinsulinaemic adrenalectomized rats. The oxidative soleus muscle appeared to be less susceptible to the effect of the corticosterone than was the more glycolytic plantaris or gastrocnemius muscle. Any effect of the corticosterone on protein degradation was much less than its effects on protein synthesis. Where increases in the degradation rates appeared to occur in the rats treated with 10 mg of corticosterone/100 g body wt. per day, the increases were less than 20%. The free intracellular 3-methylhistidine concentrations were doubled in all groups treated with 5 mg of corticosterone/100 g body wt. per day and increased 5-fold in the adrenalectomized rats treated with 10 mg of corticosterone/100 g body wt. per day, with no change in plasma concentration in any of the groups. It is therefore concluded that: (a) the suppression of protein synthesis is the main effect of glucocorticoids in muscle; (b) marked increases in insulin afford only minor protection against this effect; (c) stimulation of protein degradation may occur, but to a much lesser extent.

Corticosterone, the major glucocorticoid in the rat, is thought to exert a net catabolic effect on skeletal-muscle protein. An undisputed part of this action is the hormone's powerful suppression of protein synthesis (Young, 1970; Millward *et al.*, 1976a,b; Shoji & Pennington, 1977; Rannels *et al.*, 1978). We are concerned with two aspects of this particular action of the hormone: firstly, whether the suppression of protein synthesis is sufficient to account for its observed actions, or whether an increase in degradation is also part of the response. This is a long-standing controversy, but the strongest evidence for an effect on degradation is in the

reports of increased 3-methylhistidine excretion in response to large doses of the hormone in rats (Tomas *et al.*, 1979; Santidrian *et al.*, 1981). This contrasts with the inability of Millward *et al.* (1976a) or Shoji & Pennington (1977) to observe substantial increases in degradation after glucocorticoid administration. These differences have been explained in a report showing that intraperitoneal injection of glucocorticoids [as performed by Millward *et al.* (1976a,b) and Shoji & Pennington (1977)] is a much less effective route of administration than a subcutaneous route (Santidrian *et al.*, 1981). However, the validity of measure-

ment of 3-methylhistidine excretion as an index of skeletal-muscle degradation has been questioned (Millward *et al.*, 1980; Bates & Millward, 1981b; Millward *et al.*, 1982). We have therefore repeated the experiments of Tomas *et al.* (1979), measuring rates of protein synthesis *in vivo* and calculating where possible rates of protein degradation as the difference between protein synthesis and net change in muscle protein mass.

Our second objective is to examine whether the marked hyperinsulinaemia in rats treated with glucocorticoids (Tomas *et al.*, 1979) minimizes glucocorticoid actions because of the known anabolic effects of insulin (Jefferson *et al.*, 1977). We have therefore measured the effect of corticosterone on protein synthesis in muscle of diabetic rats maintained on physiological dosages of insulin administered by osmotic minipump. A preliminary report of some of these measurements has been presented (Odedra *et al.*, 1980).

Experimental

Materials

L-[U-¹⁴C]Tyrosine was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and dissolved in 0.9% NaCl to yield 2 (Expt. 1) or 3 (Expt. 2) μ Ci of tyrosine/ml. Streptozotocin and corticosterone were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. A test kit (GOD/PEROXIDASE) for glucose determination was obtained from Boehringer Corp. (London), Lewes, Sussex, U.K. Uranyl acetate and all other chemicals, unless otherwise stated, were obtained from BDH (Poole, Dorset, U.K.). The protamine zinc insulin (40 units/ml; Wellcome Foundation, Beckenham, Kent, BR3 3BS, U.K.) and Actrapid monocomponent insulin (80 units/ml; Novo Industries, distributed by Farillon Ltd., Romford, Essex, U.K.) were obtained from a pharmacy. ALZET (R) osmotic minipumps (model 2001; 1 μ l/h) were purchased from Scientific Marketing Associates, London N1 4RH, U.K.

Experimental design

In each of these experiments an attempt was made to compare the effect of hormone treatment on muscle protein turnover with two control groups, one initial group representative of the rats at the start of the treatment and one final group which was sham treated (i.e. given vehicle alone). This is necessary because of the possible developmental changes in muscle protein turnover that accompany growth (Millward *et al.*, 1975).

Expt. 1. The plan in this case was to repeat the experiments of Tomas *et al.* (1979), at least as far as the doses of corticosterone and the weights of the adrenalectomized rats were concerned. However, in

their experiments treated rats were not fed *ad libitum*, but were given fixed amounts of food. In order to avoid the possibility of restricting food intake and enhancing any catabolic effects of the hormone treatment, we decided to feed all animals *ad libitum*. However, actual food intakes were not measured. Male rats of weight range 110–130 g were adrenalectomized, housed individually in cages and given 0.9% NaCl to drink. All rats were fed *ad libitum* on a purified diet based on 20% (w/w) casein, which was free of 3-methylhistidine (Millward *et al.*, 1974). At least 5 days after the operation, the rats were assigned to four groups of six rats each. One group was used as an initial control group for measurements of protein turnover. The other groups were injected with either 5 or 10 mg of corticosterone (Sigma)/day per 100 g body wt., administered subcutaneously in a vehicle containing 0.8% NaCl, 0.4% Polysorbate 80, 0.5% sodium CM-cellulose and 0.9% benzyl alcohol as previously reported (Tomas *et al.*, 1979), or with vehicle only. The corticosterone treatment continued for 6 days, and on day 7 protein synthesis was measured.

Expt. 2. In this experiment the plan was to make rats diabetic with streptozotocin, then maintain them on insulin, delivered by implanted osmotic minipumps, at two dosages giving low and normal plasma insulin concentrations. Half the rats in each of these two groups were then given corticosterone at 5 mg/day per 100 g body wt. for 5 days, after which time protein synthesis was measured. Preliminary trials were carried out with the minipumps to establish the dose-response relationship between insulin-infusion rates and plasma glucose and insulin concentrations.

Induction of diabetes and initial treatments

Male rats weighing approx. 90–100 g were caged individually, fed *ad libitum* on Oxoid cubed diet and maintained on a 12h-light/12h-dark cycle. The daily weighing of rats took place between 10:00h and 11:00h. The rats were made diabetic by intravenous injection of streptozotocin (13 mg/100 g body wt., dissolved in 0.4 ml of 0.9% NaCl adjusted to pH 4.5 with 50 mM-citric acid) under light diethyl ether anaesthesia (day 0). Plasma glucose concentrations were measured 48 h later by the glucose oxidase method. Rats with plasma glucose concentrations of 300–500 mg/100 ml were selected for the experiment.

The rats were divided into three groups of 6, 12 and 12 rats and given the purified diet as in Expt. 1. The first group served as the initial controls, the second group was to be implanted with minipumps delivering insulin at the rate of 0.6 unit/day and the third group was to be similarly implanted with minipumps that delivered insulin at 1.2 units/day.

All the groups were injected subcutaneously with insulin (protamine zinc insulin, Wellcome) at 2 units/day per 100 g body wt. at 16:00 h for 2 days (days 4 and 5). On day 6, measurements of muscle protein turnover were made on the first group of six rats.

Implantation of minipumps

Implantation of the minipump into the peritoneal cavity involved making a small (approx. 1 cm) incision on the lower abdomen, carefully separating the musculoperitoneal layer and incising this. A minipump filled with appropriately diluted insulin was then inserted, delivery portal first. The wound was sutured with monofilament nylon and the skin incision then closed with wound clips.

Group 2, comprising 12 rats, were implanted on day 6, under anaesthesia, with minipumps filled with approx. 230 μ l of appropriately diluted insulin (Actrapid monocomponent insulin, 80 units/ml stock solution, Novo Industries) to deliver at the rate of 0.6 unit/day per rat. Group 3 (12 rats) were injected subcutaneously with 2 units of insulin/day per 100 g body wt. (protamine zinc insulin; Wellcome) on day 6, and implanted with minipumps on day 7 to deliver 1.2 units of insulin (Actrapid monocomponent insulin; Novo Industries)/day. The 24 h difference in minipump implantation between the two groups was solely to facilitate their infusion on two consecutive days.

Six rats each from group 2 and group 3 were injected subcutaneously with 5 mg of corticosterone/100 g body wt. in a vehicle (as stated above) at 16:00 h each day for 5 days, beginning the day after implantation of minipump. The remaining six rats in each group served as controls, and were injected with the vehicle only. Group 2 (0.6 unit of insulin/day) was infused on day 12, and group 3 (1.2 units of insulin/day) on day 13, 1 day before the minipumps expired.

Constant infusion and measurement of muscle protein synthesis

Protein synthesis in rat skeletal muscle was measured by the constant-intravenous-infusion method (Waterlow & Stephen, 1968; Garlick *et al.*, 1973). L-[U- 14 C]Tyrosine was infused via a lateral tail vein at the rate of 0.5 ml/h for 6 h, as described previously (Millward *et al.*, 1975; Odedra *et al.*, 1982). At the end of the infusion rats were killed by decapitation, and the blood was collected into heparinized tubes for analysis of plasma. Gastrocnemius muscle was removed in Expt. 1 and gastrocnemius, plantaris and soleus were removed in Expt. 2. These were quickly frozen in liquid nitrogen, until further analysis. Subsequently, protein and RNA as well as tyrosine specific radioactivity were measured as previously described (Garlick & Marshall, 1972; Millward *et al.*, 1974). The frac-

tional rate of muscle protein synthesis was calculated from the specific-radioactivity ratio of protein-bound to free intracellular tyrosine (Garlick *et al.*, 1973).

Estimations of degradation rates from comparison of growth data and protein-synthesis measurement

When the rate of change of body weight is reasonably constant for 3 days before the infusion, the rate of degradation can be estimated if measurements are also made in the change (if any) in the muscle protein as a proportion of body weight. This involves assuming that the growth rate of muscle protein calculated from these two measurements is maintained during the infusion so that degradation can be estimated as the difference between synthesis and growth of the muscle protein. Although the precision of these estimates is not as great as the precision of the measurement of protein synthesis, they do enable the range of possible changes in degradation to be estimated (see Millward *et al.*, 1975; Laurent *et al.*, 1978; Maruyama *et al.*, 1978; MacDonald & Swick, 1981).

However, in the present experiments it was decided to calculate degradation rates only in the initial control and two hormone-treated groups in Expt. 1. This was because either the growth rates were too variable before measurement (groups 2, 5, 6 and 7 as described in Table 1) or because of uncertainty about the time-course of changes in the muscle-protein/body-weight ratio (groups 8 and 9).

The 3-methylhistidine concentrations were measured in ether-extracted trichloroacetic acid supernatants of muscle homogenates and plasma on a Rank-Hilger Chromaspek amino acid analyser. The amino acid was eluted with 0.2 M-lithium citrate, pH 2.2, followed by 0.3 M-lithium citrate, pH 3.36. Plasma insulin was measured by using a charcoal-separation radioimmunoassay (Herbert *et al.*, 1965), with human insulin standards (Wellcome).

Results and discussion

Effect of corticosterone on muscle protein synthesis in adrenalectomized rats

The first experiment was designed to examine the extent to which protein synthesis was suppressed by doses of corticosterone similar to those that increased 3-methylhistidine excretion in the experiments of Tomas *et al.* (1979). However, we fed our rats *ad libitum*, unlike the study of Tomas *et al.* (1979), in which a fixed amount of food was given. This may account for the fact that, although the overall response to the steroid in terms of body weight was the same in our experiment, i.e. growth suppression at the lower dose and an actual fall in body weight at the higher dose (Fig. 1), we did not observe selective wasting of muscle. As shown in

Fig. 1. Changes in body weight in response to corticosterone treatment

Adrenalectomized rats were either untreated (▲), or treated with vehicle (●), with 5mg of corticosterone/100g body wt. per day (○) or 10mg of corticosterone/100g body wt. per day (△). Values are means for six rats. Variability (s.d.) is shown in Table 1.

Table 1, gastrocnemius protein as a proportion of body weight was if anything increased at both doses. Furthermore, these effects were delayed by about 2 days, unlike the immediate response to the hormone reported by Tomas *et al.* (1979). However, since we did not measure food intake, we do not know whether our rats consumed more than the 10g/day given by Tomas *et al.* (1979).

The rates of protein synthesis were estimated by assuming the measured intracellular tyrosine labelling to be similar to that of the precursor. The rationale for this has been discussed previously (Garlick *et al.*, 1973; Laurent *et al.*, 1978; Waterlow *et al.*, 1978). Protein synthesis is expressed as the fractional rate and as the rate per unit of RNA (RNA activity). As shown in Table 2, the rate of protein synthesis was markedly suppressed at each dose of corticosterone. This fall in protein synthesis involved a loss of RNA and a decreased RNA activity (i.e. the rate of protein synthesis per unit of RNA). The fact that the decrease was greater than that previously observed by us (Millward *et al.*, 1976a) may reflect the longer period of treatment in the present study. Rannels & Jefferson (1980) also found a progressive effect on muscle RNA concentration in their studies of cortisone treatment. The subcutaneous injection used here is also likely to be more effective than our previous intraperitoneal injection, according to the results of Santidrian *et al.* (1981). The combined effects on muscle weight and RNA concentration were a complete suppression of RNA accumulation at the lower dose and 25% loss at the higher dose. The decrease in RNA activity,

Table 1. Effect of corticosterone treatment on body weight, gastrocnemius protein and insulin concentrations

Values shown are means $\pm$ s.d. for six rats in each group. The first group in Expts. 1 and 2 represents the initial controls, which were infused with L-[U-¹⁴C]tyrosine for 6h. The other groups of adrenalectomized rats (Expt. 1) and diabetic rats treated with insulin via minipump (Expt. 2), were injected with vehicle alone (0), 5mg of corticosterone/100g body wt. (5) or 10mg of corticosterone/100g body wt. each day for 6 days in Expt. 1 and 5 days in Expt. 2.

Treatment (mg of corticosterone/day per 100 g body wt.)	Body wt. on day 1 of treatment (g)	Final body wt. (at death) (g)	Gastrocnemius protein (mg/ 100 g body wt.)	Plasma insulin concentrations (μ units/ml)
Expt. 1				
1. Adrenalectomized	137 $\pm$ 13	—	81.8 $\pm$ 5.0	7.8 $\pm$ 3
2. Adrenalectomized (0)	136 $\pm$ 10	195 $\pm$ 19	81.5 $\pm$ 2.1	28.0 $\pm$ 17
3. Adrenalectomized (5)	150 $\pm$ 13	159 $\pm$ 13	84.7 $\pm$ 6.9	144.0 $\pm$ 21
4. Adrenalectomized (10)	140 $\pm$ 10	136 $\pm$ 10	84.6 $\pm$ 4.8	113.0 $\pm$ 21
Expt. 2				
5. Diabetic + insulin (2 units/day) (injected)	105 $\pm$ 3.7	—	57.5 $\pm$ 2.6	31.7 $\pm$ 17
6. Diabetic + insulin (0.6 unit/day) (0)	128 $\pm$ 6.0	159 $\pm$ 9	63.0 $\pm$ 3.1	23.3 $\pm$ 11
7. Diabetic + insulin (0.6 unit/day) (5)	130 $\pm$ 6.0	142 $\pm$ 9	47.0 $\pm$ 4.3	13.6 $\pm$ 6
8. Diabetic + insulin (1.2 units/day) (0)	128 $\pm$ 10.0	158 $\pm$ 14	65.0 $\pm$ 4.5	47.0 $\pm$ 12
9. Diabetic + insulin (1.2 units/day) (5)	121 $\pm$ 4.0	108 $\pm$ 8	51.5 $\pm$ 4.2	41.2 $\pm$ 15

Table 2. *Effect of corticosterone treatment on protein synthesis and RNA content in gastrocnemius muscle*

Experimental procedure is as described in the text. Measurements were made of the specific radioactivity of tyrosine in plasma (S_p), the intracellular pool (S_i) and protein-bound tyrosine in gastrocnemius (S_b). The fractional synthesis rate was calculated from the ratio S_b/S_i as described in the text. The RNA activity was calculated from the fractional synthesis rate and the RNA/protein ratio. Values are means $\pm$ S.D. for six rats.

Treatment (mg of corticosterone/day per 100 g body wt.)	S_i/S_p ratio	S_b/S_i ratio	Fractional synthesis rates (%/day)	RNA/ protein (μ g/mg)	RNA activity (g of protein synthesis/day per g of RNA)
Expt. 1					
1. Adrenalectomized	0.79 $\pm$ 0.09	0.0242 $\pm$ 0.002	11.50 $\pm$ 2.01	7.33 $\pm$ 0.35	15.8 $\pm$ 3.20
2. Adrenalectomized (0)	0.83 $\pm$ 0.08	0.0264 $\pm$ 0.003	11.80 $\pm$ 1.40	8.57 $\pm$ 1.26	14.0 $\pm$ 1.85
3. Adrenalectomized (5)	0.81 $\pm$ 0.07	0.0116 $\pm$ 0.002	5.70 $\pm$ 0.66	6.88 $\pm$ 1.20	8.4 $\pm$ 1.42
4. Adrenalectomized (10)	0.85 $\pm$ 0.12	0.0109 $\pm$ 0.004	5.30 $\pm$ 0.86	5.39 $\pm$ 0.40	9.8 $\pm$ 2.04
Expt. 2					
5. Diabetic + insulin (2 units/day) (injected)	0.69 $\pm$ 0.13	0.0225 $\pm$ 0.006	9.26 $\pm$ 2.40	6.86 $\pm$ 0.88	13.8 $\pm$ 2.90
6. Diabetic + insulin (0.6 unit/day) (0)	0.75 $\pm$ 0.09	0.0252 $\pm$ 0.004	10.73 $\pm$ 1.22	8.87 $\pm$ 0.65	12.1 $\pm$ 1.36
7. Diabetic + insulin (0.6 unit/day) (5)	0.77 $\pm$ 0.08	0.0087 $\pm$ 0.002	4.16 $\pm$ 0.84	4.42 $\pm$ 0.47	9.4 $\pm$ 1.52
8. Diabetic + insulin (1.2 units/day) (0)	0.72 $\pm$ 0.07	0.0297 $\pm$ 0.003	12.17 $\pm$ 1.14	8.80 $\pm$ 0.50	13.7 $\pm$ 0.80
9. Diabetic + insulin (1.2 units/day) (5)	0.79 $\pm$ 0.13	0.0088 $\pm$ 0.003	4.64 $\pm$ 0.75	4.80 $\pm$ 0.84	9.8 $\pm$ 1.50

however, was not dose-dependent (in this range), with a 40–50% fall over the period of treatment. This fall in RNA activity is consistent with the impaired peptide-chain initiation suggested by the studies of Rannels *et al.* (1978).

When these changes in protein synthesis are compared with the estimated net change in muscle protein, the rate of degradation can be calculated. As discussed below, this was only possible in Expt. 1 (groups 1, 3 and 4). These rates of protein degradation at the end of the corticosterone treatment are shown in Table 3. In the group treated with 10 mg of corticosterone/100 g body wt. per day, the rate was 16% higher than in the initial control group, whereas the group treated with 5 mg/100 g body wt. per day exhibited an 18% lower rate of protein degradation. The significance of these findings must be assessed with due consideration given to the problems associated with the methods used in this study.

There is no entirely satisfactory method for the measurement of the rate of protein degradation in muscle *in vivo*. The method that we have used in this study has proved to be useful in evaluating changes in protein degradation in several studies in our laboratory (e.g. Millward *et al.*, 1975, 1976a; Laurent *et al.*, 1978; Brown *et al.*, 1981) and in the hands of other workers (e.g. Maruyama *et al.*, 1978; MacDonald & Swick, 1981). However, it is not without its limitations. The most difficult problem lies with the measurement of the rate of net change in muscle protein mass at the time of the measurement of the protein synthesis. Since changes over a 6 h period cannot be measured, the assumption must always be made that growth rates observed in the previous days and changes in body composition

occurring over the entire period are maintained on the day of the infusion. This can never be proved, although growth-rate measurements made before and after the time of protein-synthesis measurement provide the most satisfactory experimental design (e.g. Laurent *et al.*, 1978). The assumption is particularly questionable when growth rate is not constant from day to day, as we observed in some of the groups in these experiments. Thus in Expt. 1 the vehicle-injected group grew erratically in the days before measurement, for no good reason (Fig. 1); in this case no assumption about the growth rate, and hence no calculation of the degradation rate, can be reasonably made. Similarly, in Expt. 2 uncertainty about the time course of the change in the muscle-protein/body-weight ratio after insulin injection and minipump treatment precludes calculation of degradation rates in this experiment.

A second problem in deciding whether the rate of degradation has changed is the problem of choice of control for comparison. Since rates of degradation fall with development in growing rats (Millward *et al.*, 1975; Bates & Millward, 1981a), experiments that slow or stop growth necessitate both age and weight controls, and rates of degradation may on some occasions differ between these groups (e.g. Maruyama *et al.*, 1978). In Expt. 1 we could only sensibly calculate the degradation rate for the initial control group. Thus the results indicate the effect of the hormone compared with the initial group only and do not indicate the extent of any changes in comparison with an age control group.

A final problem concerns the error associated with the measurement of degradation. Since it is the combined error associated with two other measure-

Table 3. *Estimates of effect of corticosterone treatment on muscle protein degradation (where possible to calculate)* Values shown are means $\pm$ s.d. for six rats. The fractional degradation rate was calculated from the fractional synthesis rate and the fractional growth rate of the gastrocnemius protein. This latter value was calculated from the average whole-body rate over the three preceding measurements and from the change in the gastrocnemius protein as a proportion of body weight over the treatment period. As discussed in the text, these calculations could only be made in groups 1, 3 and 4 in Expt. 1.

Treatment (mg of corticosterone/day per 100g body wt.)	Fractional synthesis rate (%/day)	Growth of gastrocnemius protein (%/day)	Fractional degradation rate (%/day)
Expt. 1			
1. Adrenalectomized	11.5 $\pm$ 2.01	5.30 $\pm$ 0.40	6.20
3. Adrenalectomized (5)	5.7 $\pm$ 0.66	0.58 $\pm$ 0.40	5.12
4. Adrenalectomized (10)	5.3 $\pm$ 0.86	-1.91 $\pm$ 0.76	7.12

ments (synthesis and growth), it is large, and therefore the sensitivity of this method to detect small changes in degradation is limited. Thus the rates of protein degradation shown in Table 3 in the corticosterone-treated rats are not statistically different from controls. What can be concluded from these measurements is that, whereas rates of protein synthesis were more than halved by the treatment, if rates of protein degradation were changed after 6 days of treatment the changes were much less than this.

This was the conclusion of Shoji & Pennington (1977) and Rannels & Jefferson (1980). However, 6 days after treatment with 10mg of corticosterone/100g body wt. per day, Tomas *et al.* (1979) reported that 3-methylhistidine excretion was markedly elevated (by about 80%). Although we did not measure 3-methylhistidine excretion in our experiments, we did measure the concentration of free 3-methylhistidine in muscle and plasma. The concentrations in the plasma did not change at either dose, but in the muscle corticosterone treatment increased the concentration, particularly at the highest dose (Table 4). We fully recognize that these changes could reflect either increased actin degradation rates in muscle or decreased efflux from muscle, and we cannot differentiate between these two possibilities in the study. Adrenal steroids do interfere with amino acid transport (Kostyo & Redmond, 1966). We can calculate the trans-membrane flux of tyrosine infused in our experiments, however, from the values of S_i/S_p shown in Table 2, the rate of protein synthesis and the pool size of free tyrosine according to the equations described elsewhere (Waterlow *et al.*, 1978). In the rats treated with 10mg of corticosterone/100g body wt. per day showing the largest increase in free 3-methylhistidine, the free tyrosine concentration increased to 75% (results not shown), but the transmembrane flux did not significantly change. Furthermore, Lewis & Goldspink (1982) report that cortisol increases the efflux of α -aminoisobutyrate out of

muscle, in accord with the increased efflux of most amino acids from the perfused hemicorpus of cortisone-treated rats (Rannels & Jefferson, 1980). Whether 3-methylhistidine transport was affected by the hormone cannot be ascertained in the current experiments, and could only be determined by direct measurement of arterio-venous differences. Since the overall rate of degradation is not markedly elevated by the hormone, we might conclude that the changes in 3-methylhistidine concentration did result from decreased transport out of the muscle. However, the fact that increased muscle concentration occurred when, according to Tomas *et al.* (1979), increased 3-methylhistidine excretion occurred does suggest increased actin degradation in muscle. The only explanation for this would be that actin degradation was increased to a disproportionately greater extent than the overall rate. If this is the case, and our arguments supporting it are presented elsewhere (Millward *et al.*, 1982), then 3-methylhistidine excretion would be a sensitive qualitative indication of any change in muscle protein degradation.

Effect of corticosterone treatment on insulin-maintained diabetic rats

As previously shown by us (Millward *et al.*, 1976b) and by Tomas *et al.* (1979), treatment with high doses of glucocorticoids induced a marked hyperinsulinaemia (Table 1). We were concerned with establishing whether this response was protective against the catabolic effects of the hormone. The circulating insulin concentrations in the diabetic rats treated with insulin via a minipump were, in our experience, in the upper and lower regions of the normal range observed in well-fed rats, and these rats were growing at normal rates. Because of possible complicating effects of streptozotocin treatment, an ideal control group for comparison would have been diabetic rats maintained on large doses of insulin giving plasma concentrations similar to those observed in the steroid-treated adrenalectomized rats. However, such rats become hypoglycaemic and

Table 4. Concentration of free 3-methylhistidine in skeletal muscle and plasma

Values shown are means $\pm$ s.d. for six rats. 3-Methylhistidine was measured as described in the Experimental section.

Treatment (mg of corticosterone/day per 100g body wt.)	3-Methylhistidine (nmol/g)	
	Muscle	Plasma
Expt. 1		
2. Adrenalectomized (0)	5.2 $\pm$ 2.1	6.5 $\pm$ 2.1
3. Adrenalectomized (5.0)	12.2 $\pm$ 3.0	5.3 $\pm$ 2.0
4. Adrenalectomized (10.0)	25.8 $\pm$ 4.0	4.7 $\pm$ 1.0
Expt. 2		
6. Diabetic + insulin (0.6 unit/day) (0)	4.92 $\pm$ 1.80	5.3 $\pm$ 1.8
7. Diabetic + insulin (0.6 unit/day) (5)	8.92 $\pm$ 0.45	5.4 $\pm$ 1.7
8. Diabetic + insulin (1.2 units/day) (0)	5.28 $\pm$ 1.34	6.2 $\pm$ 1.9
9. Diabetic + insulin (1.2 units/day) (5)	9.25 $\pm$ 0.02	4.9 $\pm$ 1.8

do not survive, so could not be used as controls. Thus we accept the possibility that any differences in the response of the adrenalectomized or insulin-maintained diabetic rats to corticosterone do not necessarily reflect the difference in circulating insulin concentrations.

When insulin concentrations were maintained (in Expt. 2) at the lower values, the effect of the corticosterone treatment was somewhat surprising. In the group treated with insulin at 1.2 units/day, 5 mg of corticosterone/day per 100g body wt. was more catabolic than with the adrenalectomized rats, suggesting that hyperinsulinaemia can be protective against glucocorticoid-induced muscle wasting. We would have expected therefore even more muscle wasting in the rats maintained on 0.6 unit of insulin/day, with consequent lower plasma insulin concentration. This was not observed, however. These rats were exhibiting slight growth rather than wasting before infusion. Their relative muscle weight was falling, the overall muscle protein content did not change markedly and the net effect of the corticosterone treatment on the muscle protein content in this group (0.6 unit of insulin/day) was not markedly different from that observed when the same dose was given to the adrenalectomized rats (Table 1). We cannot offer any explanation for the difference between the two groups in Expt. 2. These rats were not adrenalectomized, so that we cannot be certain that the actual corticosterone concentrations were the same in each treatment group. However, since exogenous glucocorticoids suppress endogenous production and since a dose rate of 5 mg/day results in plasma concentrations that are only observed in severe stress (Tomas *et al.*, 1979), it is unlikely that plasma corticosterone concentrations were different in the two treated groups. Both doses of insulin infusion appeared to result in normal muscle growth rates for rats of this weight (i.e. about 6%/day), so that, at least according to

Fig. 2. Changes in body weight of insulin-maintained diabetic rats in response to corticosterone treatment. Rats were made diabetic on day 0 and treated with insulin (2 units/day per 100g body wt.) on days 4–5 (■, ●), or 4, 5 and 6 (▲, △). Minipumps were implanted on day 6 delivering 0.6 unit/day (●, ○) or on day 7 delivering 1.2 units/day (▲, △). Corticosterone (5 mg/100g body wt. per day) was given on days 7–11 (○) or 8–12 (△).

this criterion, each insulin infusion rate was adequate. One difference between the two experiments was the generally low muscle protein mass (mg/100g body wt.) in the insulin-maintained-diabetic

Table 5. *Effect of corticosterone treatment on protein content, protein synthesis and RNA activity in plantaris and soleus muscle*
Experimental details are as given in the text and in the legend to Table 3. Values shown are means $\pm$ s.d. for six rats.

Treatment (mg of corticosterone/day per 100 g body wt.)	Protein in the muscle/protein in gastrocnemius (mg/mg)		Fractional synthesis rate (%/day)		RNA activity (g of protein synthesis/day per g of RNA)	
	Plantaris	Soleus	Plantaris	Soleus	Plantaris	Soleus
Expt. 2						
5. Diabetic + insulin (2 units/day) (injected)	0.150 $\pm$ 0.016	0.100 $\pm$ 0.006	10.20 $\pm$ 2.50	15.22 $\pm$ 0.80	11.60 $\pm$ 2.10	20.40 $\pm$ 2.30
6. Diabetic + insulin (0.6 unit/day) (0)	0.148 $\pm$ 0.020	0.098 $\pm$ 0.010	12.53 $\pm$ 0.50	14.96 $\pm$ 1.40	11.75 $\pm$ 0.63	14.63 $\pm$ 1.40
7. Diabetic + insulin (0.6 unit/day) (5)	0.155 $\pm$ 0.009	0.127 $\pm$ 0.014	4.77 $\pm$ 0.65	8.73 $\pm$ 1.06	8.87 $\pm$ 0.97	12.60 $\pm$ 0.77
8. Diabetic + insulin (1.2 units/day) (0)	0.155 $\pm$ 0.020	0.093 $\pm$ 0.013	14.67 $\pm$ 0.67	14.42 $\pm$ 0.64	14.84 $\pm$ 2.80	14.31 $\pm$ 0.43
9. Diabetic + insulin (1.2 units/day) (5)	0.176 $\pm$ 0.020	0.141 $\pm$ 0.022	4.53 $\pm$ 0.95	8.44 $\pm$ 0.55	8.07 $\pm$ 1.25	11.30 $\pm$ 1.90

rats. This may have reflected changes in body composition in the period before minipump implantation, since muscle protein content increased relative to body weight after the minipump was implanted, particularly in the 1.2 units/day treatment group (Table 1). However, corticosterone treatment prevented this increase in relative muscle protein content, inducing an 18% fall in the rats treated with the low dose of insulin (Table 1).

As shown in Table 2, corticosterone treatment markedly suppressed muscle protein synthesis in both experimental groups treated with hormone, and this reflected lower RNA concentration and lower RNA activities. Once again it is not obvious whether the hyperinsulinaemia was protective in the steroid-treated rats in Expt. 1, since, although the fractional synthesis rates were lower in the steroid-treated rats when insulin concentrations were lower in Expt. 2, the differences were small. Certainly, since insulin is thought to regulate the initiation step in the translational phase of protein synthesis in muscle (Jefferson, 1980), any protective effect of hyperinsulinaemia might be observed by comparing RNA activities in the steroid-treated groups with the different insulin concentrations. There was no significant difference between RNA activities in any of the four groups of steroid-treated rats. Thus the slightly lower rates of protein synthesis in the steroid-treated rats in Expt. 2 (Table 2) reflected the slightly lower RNA concentrations.

The present results indicate that at the dosages of steroids in these experiments (5–10 mg/100 g body wt. per day) the effectiveness of the hormone is not markedly changed by the presence of insulin at concentrations between 14 μ units/ml (which might be regarded as in the lower part of the physiological normal range; Millward *et al.*, 1974) and markedly elevated concentrations (113–144 μ units/ml).

There is little indication therefore of any competition between the two hormones with respect to the translational phase of protein synthesis. Only at the level of the control of RNA concentration can we demonstrate a protective effect of insulin on corticosterone-induced loss, and even this difference is small.

Rates of protein degradation could not be calculated in this experiment, but, as shown in Table 4, the concentrations of free 3-methylhistidine were increased by 75–81% (with no changes in plasma concentrations), values that were not markedly different from those observed in adrenalectomized rats treated with the same dose of corticosterone. In this respect, then, the hyperinsulinaemia had no protective effect on actin degradation, assuming, as argued above, that these changes in free 3-methylhistidine concentration gradients do reflect changes in actin degradation rather than decreased transport.

Relative susceptibility of different muscles to corticosterone

In addition to the gastrocnemius muscle, measurements were also made in Expt. 2 on the plantaris and soleus muscles. This was done in order to verify previous reports that the oxidative slow-twitch soleus muscle is less susceptible to nutritional and hormonal influences in terms of protein balance and turnover (Waterlow *et al.*, 1978; Tomas *et al.*, 1979; Rannels & Jefferson, 1980). The effect of the treatment on the growth of these two muscles is shown in Table 5, in terms of the relative protein contents of the muscles compared with that of the gastrocnemius muscle. This shows whether there are selective effects on these muscles. As far as the plantaris is concerned, although there were small increases in the plantaris/gastrocnemius ratios in the steroid-treated rats, indicating less wasting of the plantaris than of the gastrocnemius, these differences were not significant. In contrast, the increased ratios of soleus to gastrocnemius protein show the soleus to be invariably less affected by corticosterone treatment than the gastrocnemius.

The RNA/protein ratio was decreased by corticosterone treatment in both plantaris and soleus, but, as expected, the effect was greater in the plantaris (50–55% decrease) than in the soleus (a 25–30% decrease). This was again reflected in the lower rates of protein synthesis and RNA activity in plantaris than in soleus from corticosterone-treated rats (Table 5). Although the decrease in protein synthesis in gastrocnemius from perfused hemi-corpus preparation of rats previously treated with cortisone acetate (Rannels & Jefferson, 1980) was quite similar to the effect observed by us (Table 2), no apparent effect of corticosterone on soleus was observed by these authors in their experiment. Whereas we observed a 25–33% loss of RNA in soleus on corticosterone treatment, there was a complete lack of change in RNA content or in ribosomal subunits in soleus from perfused hemi-corpus preparation (Rannels & Jefferson, 1980). This would suggest that soleus does become susceptible to long-term administration of corticosterone, as reflected in the 42% decrease in protein synthesis, especially when the concentrations of insulin are kept low. To summarize, the degree of response in fast-twitch glycolytic muscles such as gastrocnemius and plantaris to corticosterone treatment appears to be rapid and of greater intensity than that elicited in soleus, a slow-twitch muscle.

Relationship between insulin and corticosterone

As discussed above, insulin is known to stimulate RNA activity in muscle through increasing the rate of peptide-chain initiation (Jefferson *et al.*, 1977). Glucocorticoids, on the other hand, appear to

suppress rates of peptide-chain initiation in muscle (Rannels *et al.*, 1978). Insulin is also reported to suppress protein degradation in muscle (Jefferson *et al.*, 1977; Garlick *et al.*, 1981), although, like any stimulatory effect of glucocorticoids on protein degradation, effects of insulin on protein degradation have not been shown to be as marked as its effect on protein synthesis. Physiologically, insulin and glucocorticoids usually change in opposite directions (e.g. in energy deficiency; Coward *et al.*, 1977), and this would have the obvious advantage of imparting great sensitivity to the regulation of protein balance in muscle if both hormones act at similar sites. If there was direct competition at similar regulatory sites, the effect of glucocorticoids on muscle protein synthesis and degradation in Expt. 1 might have been markedly modified in the presence of persistent hyperinsulinaemia. However, when the plasma insulin concentrations were controlled to yield more physiological values, the response of protein synthesis to corticosterone treatment was not significantly different from that observed in Expt. 1. In the diabetic rat, acute administration of insulin is not effective in stimulating protein synthesis unless the animal has been adrenalectomized, probably because of high concentrations of corticosterone (Oedra *et al.*, 1982). This together with the present observation would suggest that increased glucocorticoids exert an anti-anabolic action on protein balance in muscle through a mechanism that is separate from and insensitive to any anabolic action of insulin, and that, in agreement with Rannels & Jefferson (1980), protein synthesis is the main target process for the hormone.

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