Effects of dexamethasone treatment on insulin-stimulated rates of glycolysis and glycogen synthesis in isolated incubated skeletal muscles of the rat

Brendan LEIGHTON,* R. A. John CHALLISS,† Fred J. LOZEMAN‡ and Eric A. NEWSHOLME

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

1. Rats were treated with dexamethasone for 4 days before measurement of the rates of lactate formation [which is an index of hexose transport; see Challiss, Lozeman, Leighton & Newsholme (1986) *Biochem. J.* 233, 377–381] and glycogen synthesis in response to various concentrations of insulin in isolated incubated soleus and extensor digitorum longus muscle preparations. 2. The concentration of insulin required to stimulate these processes half-maximally in soleus and extensor digitorum longus muscles isolated from control rats was about 100 μ units/ml. 3. Dexamethasone increases the concentration of insulin required to stimulate glycolysis half-maximally in soleus and extensor digitorum longus preparations to 250 and 300 μ units/ml respectively. The respective insulin concentrations necessary to stimulate glycogen synthesis half-maximally were about 430 and 370 μ units/ml for soleus and extensor digitorum longus muscle preparations isolated from steroid-treated rats. 5. Dexamethasone treatment did not change the amount of insulin bound to soleus muscle.

INTRODUCTION

Chronic elevation of the blood glucocorticoid concentration results in prolonged increases in the plasma concentrations of both glucose and insulin in the rat and man (Conn & Fajans, 1956; Bates & Garrison, 1971). Early work demonstrated that the rates of glucose utilization were decreased in isolated rat heart and diaphragm removed from animals treated with glucocorticoids (Manchester *et al.*, 1959; Morgan *et al.*, 1961; Park *et al.*, 1961; Czech & Fain, 1972). More recently, it has been shown that corticosterone decreased the insulin-stimulated rates of hexose transport in skeletal-muscle preparations of the mouse *in vitro* (Tan & Bonen, 1985). Apart from this latter work, there have been no systematic studies on the effects of the administration of glucocorticoids on the sensitivity of glucose metabolism in skeletal muscle to insulin. Nonetheless, it is generally accepted that glucocorticoid-induced insulin resistance involves, at least in part, a decrease in rates of glucose disposal in peripheral tissues (Chap *et al.*, 1986).

The present study was undertaken to investigate the effect of chronic glucocorticoid administration *in vivo* on the sensitivity of the rates of both glycolysis (i.e. glucose transport; see Challiss *et al.*, 1986) and glycogen synthesis to insulin in isolated incubated soleus and extensor digitorum longus muscles of the rat. The synthetic glucocorticoid dexamethasone was used, since it has low mineralocorticoid activity, it has prolonged action and it is easy to administer (French *et al.*, 1985).

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 140–150 g and 80–90 g were used for soleus and extensor digitorum longus

muscle preparations respectively. Rats were purchased from OLAC 1976 Ltd., Bicester, Oxon., U.K., and were kept in the department's animal quarters for at least 7 days before experimentation. Animals were randomly divided into control and dexamethasone-treated groups (treated daily at 16:00 h for 4 days with intraperitoneal injections of dexamethasone acetate suspended in sterile 0.9% NaCl; 2.5 mg/kg body wt.). Control rats received injections of saline. The rats were housed in groups of ten, under controlled 12 h-light/12 h-dark cycle at a temperature of 23 ± 1 °C. Food and water were provided *ad libitum*, except 16 h before experimentation, when food was withdrawn.

Chemicals and enzymes

All enzymes, biochemicals and radiochemicals were obtained from sources given previously (Espinal *et al.*, 1983; Challiss *et al.*, 1986), except for dexamethasone acetate (Sigma Chemical Co., Poole, Dorset, U.K.) and mono-[¹²⁵I]iodinated (pig) insulin (The Radiochemical Centre, Amersham, Bucks., U.K.).

Incubation procedures

Soleus muscles were isolated and stripped as previously described (Challiss *et al.*, 1983). Extensor digitorum longus muscles were isolated as described by Maizels *et al.* (1977), and both muscles were placed under resting tension on stainless-steel clips. Incubations were carried out in Krebs–Ringer bicarbonate buffer containing 5.5 mM-glucose (0.5 μ Ci of [¹⁴C]glucose/ml), 1.5% (w/v) defatted bovine serum albumin (Chen, 1967) and insulin, at concentrations given in the Results section [the details of the protocol have been given previously (see Challiss *et al.*, 1983; Leighton *et al.*, 1985)]. After 60 min incubation at 37 °C, muscles were removed from the

* To whom correspondence should be addressed.

† Present address: Department of Pharmacology, University Road, University of Leicester, Leicester LE1 7RH, U.K.

‡ Present address: Howard Hughes Medical Institute, Research Laboratories, University of Washington, Seattle, WA 98195, U.S.A.

incubation flasks and frozen in liquid N₂; the concentration of lactate in the medium and the rates of incorporation of [U-¹⁴C]glucose incorporated into glycogen (assumed to measure the rate of glycogen synthesis) or lactate (assumed to measure the rate of glycolysis from glucose) were measured as described previously (Challiss *et al.*, 1983). Rates of hexose transport plus phosphorylation were measured by monitoring the rate of conversion of 2-deoxy[2,6-³H]glucose into 2-deoxy[2,6-³H]glucose 6-phosphate in the isolated muscle as described by Challiss *et al.* (1986). The rate of oxidation of ¹⁴C-labelled glucose to ¹⁴CO₂ was measured by the method of Leighton *et al.* (1985). The rates of glucose oxidation were never greater than 10% of the rate of lactate formation under any of the incubation conditions investigated; it was therefore considered satisfactory to use the rate of lactate production as an index of glycolytic flux. Also, chronic dexamethasone administration had no effect on basal or insulin-stimulated rates of oxidation of [¹⁴C]glucose to ¹⁴CO₂. Glycogen content in skeletal muscles was determined by the method of Keppler & Decker (1973).

Insulin-binding studies

Isolated stripped soleus muscles were incubated in medium containing mono-[¹²⁵I]iodinated insulin and unlabelled insulin in Erlenmeyer flasks. More than 95% of the radioactivity of ¹²⁵I-insulin was precipitated by 10% (w/v) trichloroacetic acid. Optimal binding conditions were achieved at 20 °C during 4 h of incubation. The muscles were removed from the flasks and washed in 0.9% NaCl containing 1% (w/v) bovine serum albumin. The washing procedure was repeated four times. All results were corrected for non-specific binding (percentage of total binding), i.e. the radioactivity associated with muscles in the presence of 10⁶ μunits of unlabelled insulin/ml (Table 2).

RESULTS AND DISCUSSION

Rats treated with dexamethasone lost approx. 20 g of body weight during the 4-day treatment period, whereas control rats gained 8 g, but dexamethasone did not cause a decrease in food intake. Similar observations have been reported previously (Kelly & Goldspink, 1982; Osegawa *et al.*, 1986), and it has been suggested that dexamethasone induces atrophy of intestinal smooth muscle, resulting in malabsorption of nutrients from the gut (Kelly & Goldspink, 1982). Dexamethasone treatment caused an increase in the plasma glucose concentration

[from 5.3 ± 0.3 (5) to 7.7 ± 0.3 (5)] in 14 h-starved animals. In soleus and extensor digitorum longus muscles, dexamethasone caused marked increase in the glycogen content (130% and 55% respectively) and significant decreases in the weight of both muscles (about 10%) (see Table 1).

Dexamethasone, added to the incubation medium at a concentration of 0.1 μM, had no effect on the insulin-stimulated rates of glycolysis or glycogen synthesis in the isolated soleus muscle (results not shown). This lack of effect is consistent with previous reports that only unphysiological concentrations of glucocorticoids influence insulin-responsive processes *in vitro* (Conn & Fajans, 1956; Tan & Bonen, 1985).

The effects of 4 days' dexamethasone treatment on the rates of glycogen synthesis in incubated soleus muscle at various insulin concentrations are given in Fig. 1. The concentration of insulin required to stimulate the rate of glycogen synthesis half-maximally is increased by dexamethasone from about 80 to 430 μunits/ml (see Fig. 1). The rate of glycogen synthesis at supraphysiological concentrations of insulin was decreased (by about 25%). A similar decrease in the sensitivity of the rate of glycogen synthesis to insulin was observed for the extensor digitorum longus muscle (the concentration of insulin required to stimulate glycogen synthesis was increased from about 140 to 370 μunits/ml). However, there was no decrease in the rate of glycogen synthesis at the maximal concentrations of insulin (Fig. 1).

The effects of dexamethasone treatment on the incremental rates of glycolysis in the incubated soleus muscles at various insulin concentrations are shown in Fig. 2; the concentration of insulin required to stimulate the rate of glycolysis half-maximally was increased from about 100 to 250 μunits/ml. The effects of dexamethasone treatment were similar whether rates of glycolysis were measured by monitoring the increase in lactate concentration or the incorporation of ¹⁴C from [U-¹⁴C]glucose into lactate or whether transport plus phosphorylation was studied by monitoring the accumulation of 2-deoxy[2,6-³H]glucose 6-phosphate in the muscle (results not shown). It is concluded, therefore, that dexamethasone treatment of rats for 4 days increases the sensitivity of glucose transport to insulin in isolated soleus muscle. Similar but more dramatic effects of dexamethasone were observed in the experiments with extensor digitorum longus muscle (Fig. 2): the concentration of insulin required to stimulate glycolysis half-maximally was increased from about 80 to 300 μunits/ml.

Table 1. Effects of 4-day dexamethasone treatment on soleus and extensor digitorum longus muscle weights and glycogen content

Glycogen contents were measured by the methods described in the Materials and methods section. Results are presented as means ± S.E.M. for the numbers of observations given in parentheses. Statistically significant differences (non-paired Student's *t* test) between control and dexamethasone values are indicated by * (*P* < 0.05) and *** (*P* < 0.001). Results are from 70 g rats; however, similar changes were found in muscles from control and dexamethasone-treated 150 g rats.

	Soleus		Extensor digitorum longus	
	Control	Dexamethasone treatment	Control	Dexamethasone treatment
Wet wt. (mg)	31.4 ± 0.7 (14)	28.6 ± 0.6 (14)*	39.2 ± 0.6 (14)	35.3 ± 0.6 (14)*
Glycogen content (μmol/g)	17.6 ± 1.1 (7)	40.4 ± 1.3 (7)***	31.1 ± 2.2 (12)	48.6 ± 2.2 (10)***

Fig. 1. Effects of dexamethasone treatment on the rates of glycogen synthesis at various concentrations of insulin in incubated soleus (○ and ●) and extensor digitorum longus (△ and ▲) muscle preparations isolated from control (○ and △) and steroid-treated (● and ▲) rats

The methods for measuring rates of glycogen synthesis are given in the Materials and methods section. Results are presented as means $\pm$ S.E.M., and statistically significant differences for control versus dexamethasone-treated rats were determined by using the unpaired Student's *t* test (**P* < 0.05; ****P* < 0.001).

There was no effect of dexamethasone treatment on the amount of insulin bound to the stripped soleus muscle (Table 2); this suggests that the effects of dexamethasone on the sensitivity of glycolysis or glycogen synthesis to insulin are post-receptor effects. This is supported by the observations that, although the concentrations of insulin required to stimulate either glycolysis or glycogen synthesis half-maximally in muscles from control animals are about the same, they are considerably different in muscles from the dexamethasone-treated animals (i.e. 250–300 and 370–430 μunits/ml respectively).

Since steroid hormones increase the maximal concentration of cyclic AMP that can be produced in response to adrenoceptor agonists (by decreasing the agonist's capacity to uncouple the receptor-adenylate cyclase complex; Davies & Lefkowitz, 1984), it seemed possible that dexamethasone could cause increased sensitivity of soleus muscles to adrenergic agonists. Hence any endogenous catecholamines in the isolated incubated muscles could have resulted in decreased rates of glycogen synthesis in response to insulin, as described by Challiss *et al.* (1986). Hence the effect of various concentrations of isoprenaline on the rates of glycogen synthesis (at 100 μunits of insulin/ml) were studied in

Fig. 2. Effects of dexamethasone treatment on the incremental rates of glycolysis at various concentrations of insulin in incubated soleus (○ and ●) and extensor digitorum longus (△ and ▲) muscle preparations isolated from control (○ and △) and treated (● and ▲) rats

The methods for measuring rates of lactate formation are given in the Materials and methods section. Basal rates of lactate formation for control and dexamethasone-treated muscles were, respectively, 8.6 ± 0.33 (20) and 9.17 ± 0.47 (17) μmol/h per g for soleus and 8.72 ± 0.59 (13) and 9.88 ± 0.92 (9) μmol/h per g for extensor digitorum longus muscle. Results are presented as means $\pm$ S.E.M., and statistically significant differences for control versus dexamethasone-treated rats were determined by using the unpaired Student's *t* test (**P* < 0.05).

isolated soleus muscle from control and dexamethasone-treated animals. No differences were observed (results not shown).

At present there is no information as to how dexamethasone treatment is causing a decrease in sensitivity of either glycolysis or glycogen synthesis to insulin. It is known that an increase in the concentration of adenosine or a decrease in that of prostaglandins of the E series can decrease the sensitivity of the rates of glycolysis to insulin in isolated muscles (Budohoski *et al.*, 1984; Leighton *et al.*, 1985); such changes may be brought about by dexamethasone treatment. Indeed, it is known that glucocorticoids inhibit phospholipase A_2 activity and hence can decrease the availability of arachidonic acid for prostaglandin formation (Russo-Marie *et al.*, 1979). However, neither of these local hormones influences the sensitivity of glycogen synthesis

Table 2. Effect of dexamethasone treatment on amount of insulin bound by stripped soleus muscles of the rat

Results are presented as means $\pm$ S.E.M. for at least four separate experiments.

Concn. of insulin (μ units/ml)	Insulin binding (c.p.m. of 125 I-insulin/mg of protein)		Specific insulin binding (fmol/mg of protein)	
	Control	Dexamethasone treatment	Control	Dexamethasone treatment
4	96 $\pm$ 5	108 $\pm$ 8	0.14 $\pm$ 0.01	0.16 $\pm$ 0.01
14	91 $\pm$ 3	106 $\pm$ 4	0.38 $\pm$ 0.02	0.45 $\pm$ 0.02
50	89 $\pm$ 4	103 $\pm$ 7	1.44 $\pm$ 0.14	1.68 $\pm$ 0.17
250	76 $\pm$ 4	86 $\pm$ 4	5.27 $\pm$ 0.42	5.87 $\pm$ 0.53
2500	64 $\pm$ 4	68 $\pm$ 0	37.42 $\pm$ 4.51	44.46 $\pm$ 11.76
1000000	32 $\pm$ 1	37 $\pm$ 3	—	—

to insulin in the isolated muscle (Budohoski *et al.*, 1984; Leighton *et al.*, 1985), so that one or both local hormones may be responsible for the glucocorticoid-induced decrease in insulin-sensitivity of glycolysis. This cannot be the case for glycogen synthesis. The change in sensitivity of the latter process may be explained by the change in glycogen content. Thus dexamethasone results in increased glycogen contents in skeletal muscle, and, since increased glycogen contents *per se* can decrease rates of glycogen synthesis (Danforth, 1965; Ivy & Holloszy, 1981; Fell *et al.*, 1982), it is possible that the change in insulin-sensitivity is due to the dexamethasone-induced increment in muscle glycogen content.

We thank the British Diabetic Association for financial support and acknowledge the superb experimental help from Dr. George Dimitradis and Mark Parry-Billings and the excellent technical assistance of Mrs. Bronwyn McManus and Mr. Simon Owen. F.J.L. was a Commonwealth Commission Research Scholar and B.L. is supported by the British Medical Research Council.

REFERENCES

- Bates, R. W. & Garrison, M. M. (1971) *Endocrinology* (Baltimore) **88**, 1429–1436
- Budohoski, L., Challiss, R. A. J., McManus, B. & Newsholme, E. A. (1984) *FEBS Lett.* **167**, 1–4
- Challiss, R. A. J., Espinal, J. & Newsholme, E. A. (1983) *Biosci. Rep.* **3**, 675–679
- Challiss, R. A. J., Lozeman, F. J., Leighton, B. & Newsholme, E. A. (1986) *Biochem. J.* **233**, 377–381
- Chap, Z., Joines, R. H., Chow, J., Hartley, C. J., Entman, M. L. & Field, J. B. (1986) *J. Clin. Invest.* **78**, 1355–1361
- Chen, R. J. (1967) *J. Biol. Chem.* **242**, 173–181
- Conn, J. W. & Fajans, S. S. (1956) *Metab. Clin. Exp.* **5**, 114–127
- Czech, M. P. & Fain, J. N. (1972) *Endocrinology* (Baltimore) **91**, 518–522
- Danforth, W. H. (1965) *J. Biol. Chem.* **240**, 588–593
- Davies, A. O. & Lefkowitz, R. J. (1984) *Annu. Rev. Physiol.* **46**, 119–130
- Espinal, J., Challiss, R. A. J. & Newsholme, E. A. (1983) *FEBS Lett.* **158**, 103–106
- Fell, R. D., Terblanche, S. E., Ivy, J. L., Young, J. C. & Holloszy, J. D. (1982) *J. Appl. Physiol.* **52**, 434–437
- French, T. J., Good, A. W., Palmer, T. N. & Sugden, M. C. (1985) *Biosci. Rep.* **5**, 729–734
- Ivy, J. L. & Holloszy, J. D. (1981) *Am. J. Physiol.* **241**, C200–C203
- Kelly, F. J. & Goldspink, D. F. (1982) *Biochem. J.* **208**, 147–151
- Keppler, D. & Decker, K. (1973) in *Methods of Enzymic Analysis* (Bergmeyer, H.-U., ed.), pp. 1127–1131, Academic Press, New York and London
- Leighton, B., Budohoski, L., Lozeman, F. J., Challiss, R. A. J. & Newsholme, E. A. (1985) *Biochem. J.* **227**, 337–340
- Maizels, E. Z., Ruderman, N. B., Goodman, M. N. & Law, D. (1977) *Biochem. J.* **162**, 557–568
- Manchester, K. L., Randle, P. J. & Young, F. G. (1959) *J. Endocrinol.* **18**, 395–408
- Morgan, H. E., Regen, D. M., Hendersen, M. J., Sawyer, T. K. & Park, C. R. (1961) *J. Biol. Chem.* **236**, 2162–2168
- Osegawa, M., Makino, H., Kanatsuka, A., Suzuki, T. & Yashida, S. (1986) *Metab. Clin. Exp.* **33**, 754–759
- Park, C. R., Morgan, H. E., Hendersen, M. J., Regen, D. M., Cadenase, D. & Post, R. L. (1961) *Recent Prog. Horm. Res.* **17**, 493–538
- Russo-Marie, F., Paing, M. & Duval, D. (1979) *J. Biol. Chem.* **254**, 8498–8504
- Tan, M. H. & Bonen, A. (1985) *Can. J. Physiol. Pharmacol.* **63**, 1133–1138

Received 28 January 1987/15 June 1987; accepted 2 July 1987