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Changes in prostaglandin release associated with inhibition of muscle protein synthesis by dexamethasone

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Forelimb digit extensor muscles from fed rabbits were incubated in the absence or presence of dexamethasone (100nM). The presence of dexamethasone decreased the rates of protein synthesis, prostaglandin $F_{2\alpha}$ and prostaglandin E_2 release after a time lag of 2.5–3h. Although intermittent stretching stimulated both protein synthesis and prostaglandin release in the presence of dexamethasone, the absolute activities of both processes were lower in the presence of the steroid than in its absence. It is suggested that the inhibitory action of dexamethasone on muscle protein synthesis *in vitro* results from its effect on the activity of plasma-membrane phospholipase A_2.

High circulating concentrations of both synthetic and natural glucocorticoid hormones are associated with decreased weight gain, and specifically a decrease in the deposition of protein in skeletal muscle (Bullock *et al.*, 1968, 1972; Goldberg & Goodman, 1969; Millward *et al.*, 1976; Tomas *et al.*, 1979; Odedra *et al.*, 1983). It is generally agreed that a decrease in the rate of synthesis of total and of contractile proteins is induced by glucocorticoid injections (Millward *et al.*, 1976; Rannels & Jefferson, 1980; Odedra & Millward, 1982; Odedra *et al.*, 1983). Chronic treatment of animals with glucocorticoid hormones leads to a decrease in muscle RNA content, but in the short term the administration of corticosterone decreases the rate of formation of the initiation complex (Bullock *et al.*, 1972; Rannels & Jefferson, 1980), and incubation of isolated muscles with natural or synthetic glucocorticoids inhibits protein synthesis (Kostyo & Redmond, 1966; McGrath & Goldspink, 1982). It appears therefore that these hormones directly and acutely influence ribosomal activity.

Previous results have suggested that an increase in the synthesis of $PGF_{2\alpha}$ is closely involved in the stimulation of muscle protein synthesis brought about by stretch (Smith *et al.*, 1983; Palmer *et al.*, 1983), by leucocyte pyrogen (Baracos *et al.*, 1983) and by insulin (Reeds & Palmer, 1983). It is also possible that an inhibition of $PGF_{2\alpha}$ synthesis could result in a decrease in the rate of protein synthesis, and it is noteworthy therefore that in other cells the glucocorticoids inhibit synthesis of prostaglandins and thromboxanes by inhibiting plasma-membrane phospholipase A_2 (Kantowitz *et al.*, 1975; Tashjian *et al.*, 1975; Nijkamp *et al.*, 1976; Hong & Levine, 1976; Hirata *et al.*, 1980; Blackwell *et al.*, 1980). The present work was undertaken to investigate whether changes in $PGF_{2\alpha}$ synthesis were involved in the inhibition of protein synthesis in isolated muscles brought about by the synthetic steroid dexamethasone.

Abbreviation used: PG, prostaglandin.

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Materials and methods

L-[2,6-^{3}H]Phenylalanine was purchased from Amersham International, materials for the assay of $PGF_{2\alpha}$ were from Travenol Laboratories (Thetford, Norfolk, U.K.) and for PGE_2 from New England Nuclear (Dreieich, Germany). Dexamethasone was purchased from Sigma Chemical Co. (Poole, Dorset, U.K.).

Forelimb digit extensor muscles (Palmer *et al.*, 1981) were removed from male rabbits (850–1100g) which had been allowed free access to food until they were killed. The dissection and incubation (Palmer *et al.*, 1981) and the basic incubation medium (Reeds *et al.*, 1980) have been described in full. Protein synthesis was measured for the last 0.5h of each incubation by adding labelled phenylalanine together with unlabelled phenylalanine to give a specific radioactivity of 1500–2500d.p.m./nmol and a concentration of

2.5mM. At the end of the labelling period the muscles were removed from the incubation vessel, washed briefly in ice-cold NaCl (9g/l), blotted, weighed and frozen. After homogenization in ice-cold 0.5M-$HClO_4$, the precipitated proteins were purified (Palmer *et al.*, 1981), hydrolysed and analysed for phenylalanine and radioactivity. Phenylalanine was analysed by the method given in Palmer *et al.* (1983), and radioactivity was measured by liquid-scintillation counting with NE 265 scintillator (Nuclear Enterprises, Edinburgh, Scotland, U.K.) and a Packard 460CD counter.

The concentration of the two prostaglandins $F_{2\alpha}$ and E_2 was measured in the incubation medium by radioimmunoassay. In the former assay the label in the antibody precipitate was measured by liquid-scintillation counting after dissolving the precipitate in 1ml of 0.1M-NaOH, together with a washing of 1ml of 0.1M-HCl. In the latter the label was measured by gamma-counting in a Tracer Lab (Spectromatic) NaI well counter. The anti-$PGF_{2\alpha}$ serum was stated to cross-react 28% with $PGF_{1\alpha}$, but the anti-PGE serum had a high (>98%) specificity for PGE_2. Because labelled phenylalanine in the medium interferes with the $PGF_{2\alpha}$ assay, the release of the two prostaglandins was measured during the 30min period immediately before the addition of labelled phenylalanine. Dexamethasone was added in 10μl of ethanol, and all control incubations contained 10μl of ethanol/3ml of medium.

The rate of protein synthesis was calculated as described in Smith *et al.* (1983). The significance of differences between means was assessed by Student's *t* test for paired data. A value of *P* (two-tailed test) <0.05 was taken as statistically significant.

Results

Expt. 1

Muscles, held at a constant tension of 10g, were preincubated for 15min in normal medium. This was then replaced with medium either with no additions or containing dexamethasone (100nM). The incubation was then continued for periods of up to 4.5h. In the experiments lasting 1h, PG release was measured during the first 30min of incubation. The results (Table 1) demonstrate that during the 1h incubations dexamethasone (100nM) stimulated protein synthesis by approx. 30% and then, after a time lag of at least 2.5h, protein synthesis was inhibited. The time course of changes in protein synthesis was closely followed by changes in $PGF_{2\alpha}$ release. There was a close correlation between $PGF_{2\alpha}$ and PGE_2 release under all conditions of incubation (Fig. 1).

Expt. 2

Muscles were incubated for 4.5h either under a constant tension of 10g or with intermittent mechanical stretching, as described previously (Palmer *et al.*, 1981). Incubations were carried out in the absence or presence of dexamethasone (100nM). Release of $PGF_{2\alpha}$ and PGE_2 was measured between 3 and 4h of incubation, and protein synthesis between 4 and 4.5h. The results (Table 2) show that intermittent stretching increased both protein synthesis and $PGF_{2\alpha}$ release, and that the presence of dexamethasone, like the cyclo-oxygenase inhibitors indomethacin and meclofenamic acid, inhibited but did not abolish the effect of mechanical stimulation on both these processes.

Discussion

The presence of dexamethasone was associated with time-dependent changes in both protein synthesis and prostaglandin release. The two were positively correlated, confirming previous observations (Palmer *et al.*, 1983; Reeds & Palmer, 1983). The delayed decrease in protein synthesis was similar both in time course and in magnitude (−30%) to that observed by McGrath & Goldspink (1982) in rat extensor digitorum longus muscle incubated in the presence of dexamethasone. On a potency basis, the dexamethasone concentrations that brought about this effect were similar to the concentrations of natural adrenal glucocorticoids encountered in starved and stressed rats (Barrett & Stockham, 1963; Bellamy *et al.*, 1968; Bellinger *et al.*, 1975), and we believe that this effect is of physiological relevance. Indeed, Millward *et al.* (1983) have proposed that, in addition to changes in insulin (Garlick *et al.*, 1983), the changes in corticosterone concentrations that occur during starvation and on re-feeding of starved animals are an important factor in the control of muscle protein biosynthesis.

The delay in the inhibition of protein synthesis by dexamethasone may be due simply to the slow penetration of the steroid into the cell, since the action of insulin on protein synthesis in isolated muscles can be delayed for at least 1h (Stirewalt & Low, 1983), yet *in vivo* (Garlick *et al.*, 1983) and in the perfused hemicorpus (Flaim *et al.*, 1980) insulin stimulates protein synthesis more rapidly. However, dexamethasone stimulated protein synthesis and $PGF_{2\alpha}$ release in the first 1h, and this suggests that the steroid does rapidly penetrate the muscle cell.

Other quite dissimilar effects of the glucocorticoids, e.g. the enhancement of lipolysis in isolated adipocytes (Fain *et al.*, 1965) and the inhibition of chemotaxis in neurophils (Hong & Levine, 1976),

Table 1. *Changes in the rate of protein synthesis (k_s, %/day) and $PGF_{2\alpha}$ release (pg/h per mg of muscle) with time: effect of addition of dexamethasone*

Muscles were incubated as described in the Materials and methods section for the various periods of time indicated below in the absence or presence of dexamethasone (100nM). Results are means ± S.E.M. for *n* experiments. Differences between means with and without dexamethasone (paired *t* test) are indicated by: $*P<0.05$, $**P<0.01$, $***P<0.001$.

Time period (h)	*n*	Protein synthesis: Control	Protein synthesis: +Dexamethasone	Time period (h)	$PGF_{2\alpha}$ release: Control	$PGF_{2\alpha}$ release: +Dexamethasone
0.5–1	14	3.67±1.06	4.82±1.76*	0–0.5	7.6±0.5	9.5±0.8**
1.5–2	6	2.38±0.27	2.81±0.18	1–1.5	4.8±1.1	5.0±1.2
2.5–3	6	2.44±0.27	1.96±0.16	2–2.5	8.0±1.9	7.1±0.3
3.5–4	8	2.87±0.27	1.95±0.26***	3–3.5	7.2±0.8	4.6±0.5**
4–4.5	4	2.50±0.11	1.81±0.11***	3.5–4	7.0±0.20	5.7±0.6**

Table 2. *Effect of dexamethasone (100nM) on protein synthesis (%/day) and $PGF_{2\alpha}$ release (pg/h per mg) in muscles incubated under a constant tension or with intermittent stretching*

Muscles were incubated either under a constant tension of 10g or with intermittent stretching (100ms every 3s) as described previously (Palmer *et al.*, 1981). Protein synthesis was measured between 4 and 4.5h and $PGF_{2\alpha}$ release between 3 and 4h of incubation. Results are means ± S.E.M. for eight observations. Differences between means (paired *t* test) are indicated by $**P<0.01$ and $***P<0.001$ with respect to constant tension without dexamethasone and by $\dagger P<0.05$ and $\dagger\dagger P<0.01$ with respect to intermittent stretching without dexamethasone.

	Constant tension: Control	Constant tension: +Dexamethasone	Intermittent stretching: Control	Intermittent stretching: +Dexamethasone
Protein synthesis	2.78±0.24	1.84±0.22***	3.88±0.39**	2.65±0.39†
$PGF_{2\alpha}$ release	9.00±1.50	6.70±1.50**	15.40±2.90*	10.30±0.80††

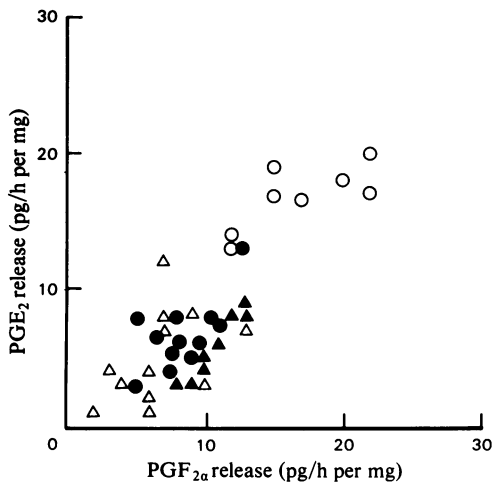

Fig. 1. *Relationship between $PGF_{2\alpha}$ and PGE_2 release by isolated muscles incubated under constant tension (●, △) or subjected to intermittent stretching (○, ▲) in the presence (△, ▲) or absence (○, ●) of dexamethasone*

Values are taken from the 3.5–4 and 4–4.5h time points in Expts. 1 and 2 respectively. The best line is:

$$PGE_2 = 0.92\ (\pm 0.15)\ PGF_{2\alpha} - 1.4\ (\pm 1.6)$$
$$n = 40,\ r = 0.714.$$

occur after a time lag of at least 2h. This is consistent with the proposal that the cytoplasmic steroid receptor interacts with nuclear DNA and leads to the induction of new enzyme synthesis (Thompson & Lippman, 1974), and glucocorticoid-induced activation of lipolysis (Fain *et al.*, 1965), inhibition of release of prostaglandins (Danon & Assouline, 1978) and thromboxanes (Flower & Blackwell, 1979) can all be inhibited by actinomycin D. In several cell types it has now been shown that the glucocorticoids affect prostaglandin synthesis, not by inhibiting cyclo-oxygenase (indeed, they may activate this enzyme; Chandrabose *et al.*, 1978), but by stimulating the synthesis of a protein or proteins that inhibit phospholipase A_2 (Hirata *et al.*, 1980; Blackwell *et al.*, 1980; Hassid, 1981; Rothut *et al.*, 1983).

Previous evidence strongly suggests that a variety of stimuli of muscle protein turnover (Rodemann *et al.*, 1982; Smith *et al.*, 1983; Palmer *et al.*, 1983; Baracos *et al.*, 1983; Reeds & Palmer, 1983) also stimulate arachidonic acid metabolism and lead to an increase in the synthesis of $PGF_{2\alpha}$ and PGE_2. Furthermore, protein synthesis is stimulated by the addition of arachidonic acid or $PGF_{2\alpha}$ itself to skeletal muscle (Rodemann &

Goldberg, 1982; Smith *et al.*, 1983) and to 3T3 fibroblasts (De Asua *et al.*, 1977), and the stimulation of protein synthesis by intermittent stretching and by insulin can be blocked by inhibitors of cyclo-oxygenase. As muscles taken from starved animals have low rates of protein synthesis and $PGF_{2\alpha}$ release (Reeds & Palmer, 1983) and the presence of dexamethasone also decreased the rates of both processes, it appears that $PGF_{2\alpha}$ synthesis is involved both in the stimulation and in the inhibition of protein synthesis. However, although we would propose that a decrease in the activity of phospholipase A_2 underlies the effect of dexamethasone on protein synthesis, its effect is different from and independent of the actions of other effectors. Thus *in vivo* Goldberg & Goodman (1969) found that muscles still gave a growth response to increased work even in animals that received catabolic doses of corticosterone, and in the present experiments intermittent mechanical stretching still stimulated protein synthesis and $PGF_{2\alpha}$ release in the presence of dexamethasone. The absolute increases were smaller in the presence of the steroid than in its absence, but stretching produced a similar proportional increase, and these results suggest that, although the glucocorticoid may have decreased the absolute activity of phospholipase A_2, the enzyme retained its ability to respond to other short-term stimuli.

The presence of dexamethasone was also associated with a decrease in PGE_2 release, and this effect may help to resolve the continuing controversy over glucocorticoid effects on protein degradation. In other experiments Rodemann *et al.* (1982) and Baracos *et al.* (1983) have shown that *in vitro* enhanced PGE_2 release accompanies increased protein degradation, that PGE_2 itself stimulates this process and that pharmacological inhibition of cyclo-oxygenase decreases the rate of muscle protein degradation. By analogy to the relationship between $PGF_{2\alpha}$ release and protein synthesis, it seems reasonable to propose that, in circumstances in which PGE_2 release is decreased, protein degradation will also be decreased.

One of the most interesting features of the control of muscle protein accretion is that, in the large majority of reports of altered muscle growth, protein synthesis and degradation rose and fell in concert (Turner & Garlick, 1974; Millward *et al.*, 1975, 1976; Vernon & Buttery, 1976; Laurent *et al.*, 1978; Reeds *et al.*, 1982). These observations suggest that a single stimulus or inhibition or simultaneous stimuli are exerted on both processes. On the basis of previous observations (Rodemann & Goldberg, 1982; Palmer *et al.*, 1983) a simultaneous change in $PGF_{2\alpha}$ and PGE_2 concentrations could explain the dual response of protein synthesis and degradation. This would be the likely consequence of an activation of phosphopholipase A_2, as this would lead to a change in their common precursor, arachidonic acid, the rate-limiting factor for the synthesis of the prostaglandins (Irvine, 1982). Alterations in the activity of this enzyme of the plasma membrane may therefore be a common link between a number of different changes in the extracellular environment and the ability of muscles to synthesize and degrade protein.

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References

Baracos, V., Rodemann, H. P., Dinarello, C. A. & Goldberg, A. L. (1983) *N. Engl. J. Med.* **308**, 553–558

Barrett, A. M. & Stockham, M. A. (1963) *J. Endocrinol.* **26**, 97–105

Bellamy, D., Leonard, R. A., Dulieu, K. & Stevenson, A. (1968) *Gen. Comp. Endocrinol.* **10**, 119–125

Bellinger, L. L., Mendel, V. E. & Moberg, G. P. (1975) *Horm. Metab. Res.* **7**, 132–135

Blackwell, G. J., Carnuccio, R., Di Rossa, M., Flower, R. J., Parente, L. & Persico, P. (1980) *Nature (London)* **287**, 147–149

Bullock, G. R., White, A. M. & Worthington, J. (1968) *Biochem. J.* **108**, 417–425

Bullock, G. R., Carter, E. E., Elliott, P., Peters, R. F., Simpson, P. & White, A. M. (1972) *Biochem. J.* **127**, 881–892

Chandrabose, K. A., Lapetina, E. G., Schmitges, C. J., Siegel, M. I. & Cuatrecasas, P. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 214–217

Danon, A. & Assouline, G. (1978) *Nature (London)* **273**, 552–554

De Asua, L. J., O'Farrell, M., Bennett, D., Clingan, D. & Rudland, P. (1977) *Nature (London)* **265**, 151–153

Fain, J. N., Kovacev, V. P. & Scow, R. O. (1965) *J. Biol. Chem.* **240**, 3522–3529

Flaim, K. E., Copenhaver, M. E. & Jefferson, L. S. (1980) *Am. J. Physiol.* **239**, E88–E95

Flower, R. J. & Blackwell, G. J. (1979) *Nature (London)* **278**, 456–459

Garlick, P. J., Fern, M. & Preedy, V. R. (1983) *Biochem. J.* **210**, 669–676

Goldberg, A. L. & Goodman, H. M. (1969) *J. Physiol. (London)* **20**, 667–675

Hassid, A. (1981) *Prostaglandins* **21**, 985–1001

Hirata, F., Schiffman, E., Venkatasubramanian, K., Saloman, D. & Axelrod, J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2533–2536

Hong, S.-C. & Levine, L. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1730–1734

Irvine, R. F. (1982) *Biochem. J.* **204**, 3–16

Kantowitz, F., Robinson, D. R. & McGuire, M. B. & Levine, L. (1975) *Nature (London)* **258**, 737–738

Kostyo, J. L. & Redmond, A. F. (1966) *Endocrinology* **79**, 531–540

Laurent, G. J., Sparrow, M. P. & Millward, D. J. (1978) *Biochem. J.* **176**, 407–417

McGrath, J. A. & Goldspink, D. F. (1982) *Biochem. J.* **206**, 641–645

Millward, D. J., Garlick, P. J., Stewart, R. J. C., Nnanyelugo, D. O. & Waterlow, J. C. (1975) *Biochem. J.* **150**, 235–243

Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976) *Biochem. J.* **156**, 185–188

Millward, D. J., Odedra, B. & Bates, P. C. (1983) *Biochem. J.* **216**, 583–587

Nijkamp, F. R., Flower, R. J., Moncada, S. & Vane, J. R. (1976) *Nature (London)* **263**, 479–482

Odedra, B. R. & Millward, D. J. (1982) *Biochem. J.* **204**, 663–672

Odedra, B. R., Bates, P. C. & Millward, D. J. (1983) *Biochem. J.* **214**, 616–627

Palmer, R. M., Reeds, P. J., Lobley, G. E. & Smith, R. H. (1981) *Biochem. J.* **198**, 491–498

Palmer, R. M., Reeds, P. J., Atkinson, T. & Smith, R. H. (1983) *Biochem. J.* **214**, 1011–1014

Rannels, S. R. & Jefferson, L. S. (1980) *Am. J. Physiol.* **238**, E564–E572

Reeds, P. J. & Palmer, R. M. (1983) *Biochem. Biophys. Res. Commun.* **116**, 1084–1090

Reeds, P. J., Palmer, R. M. & Smith, R. H. (1980) *Int. J. Biochem.* **11**, 7–14

Reeds, P. J., Haggarty, P., Wahle, K. W. J. & Fletcher, J. M. (1982) *Biochem. J.* **204**, 393–398

Rodemann, H. P. & Goldberg, A. L. (1982) *J. Biol. Chem.* **257**, 1632–1638

Rodemann, H. P., Waxman, L. & Goldberg, A. L. (1982) *J. Biol. Chem.* **257**, 8716–8723

Rothut, B., Cloix, J. F. & Russo-Marie, F. (1983) *Adv. Prostaglandin Thromboxane Leukotriene Res.* **9**, 51–56

Smith, R. H., Palmer, R. M. & Reeds, P. J. (1983) *Biochem. J.* **214**, 153–161

Stirewalt, W. S. & Low, R. B. (1983) *Biochem. J.* **210**, 323–330

Tashjian, A. H., Voelkel, E. F., McDonough, J. & Levine, L. (1975) *Nature (London)* **258**, 739–741

Thompson, E. B. & Lippman, M. E. (1974) *Metab. Clin. Exp.* **23**, 159–202

Tomas, F. M., Munro, H. N. & Young, V. R. (1979) *Biochem. J.* **178**, 139–146

Turner, L. V. & Garlick, P. J. (1974) *Biochim. Biophys. Acta* **349**, 109–113

Vernon, B. G. & Buttery, P. J. (1976) *Br. J. Nutr.* **36**, 575–579