The effect of prostaglandins E₁, E₂ and F_{2α} and indomethacin on the sensitivity of glycolysis and glycogen synthesis to insulin in stripped soleus muscles of the rat

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Prostaglandins E₁ and E₂ increased the sensitivity of glycolysis to insulin in the isolated stripped soleus muscle of the rat, but prostaglandin F_{2α} had no effect. Indomethacin, which inhibits prostaglandin formation, markedly decreased the sensitivity of glycolysis to insulin. These findings suggest that prostaglandins of the E series increase the sensitivity of muscle glycolysis to insulin *in vivo*.

Insulin influences the rates of a large number of processes in both adipose tissue and muscle (see Newsholme & Leech, 1983, for review). There are several reports that prostaglandins either mimic or are involved in the effects of insulin on some of these processes (see, e.g., Willebrandt & Tasserone, 1968; Wieser & Fain, 1975; Dietze, 1982; Reeds & Palmer, 1983). The fact that administration of indomethacin, which is an inhibitor of prostaglandin synthesis, results in insulin resistance in man suggests that many of the reported effects of prostaglandins could be explained if prostaglandins increased the sensitivity of muscle to the effects of insulin (see Dietze *et al.*, 1978; Dietze, 1982). We have shown previously that adenosine or adenosine-receptor agonists can dramatically decrease the sensitivity of glycolysis to insulin in isolated soleus muscle, but that they have no effect on the sensitivity of glycogen synthesis to insulin; adenosine antagonists or the presence of adenosine deaminase increase the sensitivity of glycolysis to insulin, but do not affect the sensitivity of glycogen synthesis (Espinal *et al.*, 1983a; Budohoski *et al.*, 1984). Hence we decided to carry out similar studies, but with various prostaglandins and indomethacin. The results are presented and discussed below.

Materials and methods

Male Wistar rats were purchased from Bantin and Kingman, Hull, U.K. Indomethacin and prostaglandins were obtained from Sigma Chemi-

cal Co., Poole, Dorset, U.K. All other chemicals and enzymes were obtained from the sources given previously (Challiss *et al.*, 1983; Budohoski *et al.*, 1984).

Food was withheld from the rats for about 14 h before experiments. Soleus muscles were isolated and dissected longitudinally to produce two halves of similar weight as described by Crettaz *et al.* (1980). The muscle strips were attached to steel clips and incubated in 3.5 ml of modified Krebs–Ringer bicarbonate buffer containing 1.5% (w/v) defatted bovine serum albumin as described by Challiss *et al.* (1983). The muscles were preincubated for 30 min, transferred to Erlenmeyer flasks containing fresh incubation medium plus 5.5 mM-glucose (containing 0.25 μ Ci of [U-¹⁴C] glucose/ml) and insulin at various concentrations (see Table 1). The flasks were gassed continuously with O₂/CO₂ (19:1) during the preincubation and for the first 15 min of the second incubation (Espinal *et al.*, 1983a).

At the end of the incubation, the muscles were removed from the flasks and freeze-clamped. The flasks were rapidly re-sealed and, after acidification of the medium with 4% (w/v) HClO₄ (final concn.), they were incubated for a further 60 min, during which time ¹⁴CO₂ was absorbed in 0.2 ml of 2-phenethylamine/methanol (1:1, v/v). The radioactivity in the latter was measured in a Beckman LS7500 liquid-scintillation counter, and from this the rate of oxidation of glucose was calculated. [U-¹⁴C]Glucose incorporation into glycogen was assessed by the method outlined by Espinal *et al.* (1983b). The acid-deproteinized medium was neutralized with KOH, and precipitated KClO₄ was removed by centrifugation. The

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supernatant was used for the assay of lactate as described by Engel & Jones (1978). As found previously (Espinal *et al.*, 1983b; Challiss *et al.*, 1983), the stimulation of glycolysis by insulin was similar whether lactate was measured radiochemically or spectrophotometrically.

Results

The quantitative effects of insulin on rates of glycogen synthesis, glycolysis (Table 1) and glucose oxidation (results not shown) were similar to those previously reported (Espinal *et al.*, 1983a; Budohoski *et al.*, 1984). The rate of glucose oxidation was always less than 10% of the rate of glycolysis, and an increase in insulin concentration from 1 to 10000 μ units/ml increased the rate of glucose oxidation by 2-fold, which is also similar to previous findings (Budohoski *et al.*, 1984). Addition of prostaglandins to the incubation medium had no effect on the stimulation of the rate of glucose oxidation by insulin at any concentrations of the hormone from 1 to 10000 μ units/ml. It is not known why the rate of glycolysis was considerably higher than that of glucose oxidation, but this observation has been reported previously (Crettaz *et al.*, 1980). It is unlikely to be due to poor oxygenation, since preliminary experiments established that the ATP/ADP and ATP/AMP concentration ratios were maintained throughout the incubation period at values similar to those found in the muscle freeze-clamped *in situ* or immediately after dissection (results not shown); the ATP/AMP concentration ratio is a very sensitive index of the state of oxygenation of muscle, and the maintenance of a high value indicates that oxygenation is satisfactory (see Newsholme & Start, 1973). This is supported by the findings that rates of both glycolysis and glycogen synthesis were linear with time (results not shown).

The effects of insulin concentrations on the rates of glycolysis in the presence of prostaglandins E_1 , E_2 and $F_{2\alpha}$ are shown in Table 1. In the presence of prostaglandin E_1 , 10 μ units of insulin/ml resulted in a marked increase in rate of glycolysis, which was not observed in the absence of the prostaglandin. The concentration of insulin required to stimulate glycolysis by 50% was observed at about 70 and 10 μ units/ml in the absence and presence of prostaglandin E_1 respectively (Fig. 1). The effect of prostaglandin E_2 in improving insulin-sensitivity was not as large as that of E_1 , so that a statistically significant effect was not observed until 100 μ units of insulin/ml (Table 1). The concentration of insulin required to stimulate glycolysis by 50% was decreased from about 70 to about 30 μ units/ml in the presence of prostaglandin E_2 (Fig. 1). However, prostaglandin $F_{2\alpha}$ had no

Fig. 1. Effects of prostaglandins E_1 (○), E_2 (●), $F_{2\alpha}$ (▲) and indomethacin (□) on insulin-stimulated rates of lactate formation by isolated stripped soleus muscles. Control incubations are indicated by (△). Results are presented as means of increments of lactate formation from basal values.

effect on the response of glycolysis to insulin (Table 1).

In contrast with the effects on glycolysis, prostaglandins E_1 , E_2 and $F_{2\alpha}$ had no effect on the sensitivity of glycogen formation to insulin (Table 1).

Indomethacin, which is a cyclo-oxygenase inhibitor (see Flower, 1974), and would be expected to decrease the rate of prostaglandin synthesis, and hence the concentration of prostaglandins, decrease the sensitivity of glycolysis to insulin (Table 1); the concentration of insulin that stimulated glycolysis by 50% was increased from about 70 to about 1200 μ units/ml by indomethacin (Fig. 1). This finding is consistent with endogenous prostaglandins increasing the sensitivity of glycolysis in muscle to insulin. It is possible that indomethacin decreases the rate of glycogen synthesis, but it does not appear to affect the sensitivity of this process to insulin (Table 1).

Discussion

The results demonstrate that prostaglandins E_1 and E_2 , but not prostaglandin $F_{2\alpha}$, increase the sensitivity of glycolysis to insulin (Table 1). The fact that prostaglandin E_1 is more effective than E_2 and that prostaglandin $F_{2\alpha}$ has no effect indicates that the effect of E_1 is specific, perhaps influencing the sensitivity via a specific receptor.

Indomethacin markedly decreases the sensitivity of glycolysis to insulin (Table 1; Fig. 1).

Table 1. *Effects of prostaglandins E₁, E₂ and F_{2α} and indomethacin on the rates of lactate formation and glycogen synthesis at different concentrations of insulin in stripped soleus muscle preparations from rats*

The methods for measuring rates of lactate formation and glycogen synthesis are given in the Materials and methods section. Results are presented as means $\pm$ S.E.M., and statistically significant differences (Student's *t* test) between control and experimental value are indicated by **P* < 0.05 and ***P* < 0.01.

Insulin concn. (μ units/ml)	Prostaglandin E ₁ (1 μ g/ml)		Prostaglandin E ₂ (1 μ g/ml)		Prostaglandin F _{2α} (1 μ g/ml)		Indomethacin (1 μ M)	
	Control	Prostaglandin E ₁ (1 μ g/ml)	Control	Prostaglandin E ₂ (1 μ g/ml)	Control	Prostaglandin F _{2α} (1 μ g/ml)	Control	Indomethacin (1 μ M)
Rate of lactate formation (μ mol of lactate/h per g)								
1	8.12 $\pm$ 0.56 (5)	9.23 $\pm$ 0.21 (9)*	11.88 $\pm$ 0.47 (18)	12.48 $\pm$ 0.51 (19)	10.76 $\pm$ 0.66 (14)	10.89 $\pm$ 0.50 (12)	7.82 $\pm$ 0.32 (8)	8.54 $\pm$ 0.37 (5)
10	8.31 $\pm$ 0.72 (5)	12.93 $\pm$ 0.80 (9)**	12.73 $\pm$ 0.57 (20)	14.08 $\pm$ 0.52 (18)	11.20 $\pm$ 0.62 (16)	11.91 $\pm$ 0.55 (16)	7.86 $\pm$ 0.38 (8)	9.29 $\pm$ 1.09 (5)
100	11.05 $\pm$ 0.67 (5)	14.76 $\pm$ 0.99 (9)**	15.40 $\pm$ 0.66 (16)	18.54 $\pm$ 0.60 (20)**	16.88 $\pm$ 0.78 (14)	15.44 $\pm$ 0.76 (14)	11.22 $\pm$ 0.64 (7)	9.30 $\pm$ 0.50 (5)**
1000	13.46 $\pm$ 0.72 (5)	16.02 $\pm$ 0.86 (9)*	19.95 $\pm$ 0.76 (21)	19.80 $\pm$ 0.74 (20)	16.98 $\pm$ 0.93 (15)	17.12 $\pm$ 0.78 (15)	14.65 $\pm$ 0.71 (7)	11.32 $\pm$ 1.04 (5)**
10000	13.74 $\pm$ 0.98 (5)	16.53 $\pm$ 0.97 (9)*	18.28 $\pm$ 0.55 (14)	19.50 $\pm$ 0.65 (16)	17.59 $\pm$ 0.75 (15)	17.22 $\pm$ 0.75 (15)	16.19 $\pm$ 1.27 (7)	14.65 $\pm$ 1.28 (5)
Rate of glycogen formation (μ mol of glucosyl equiv./h per g)								
1	1.67 $\pm$ 0.15 (6)	1.75 $\pm$ 0.18 (6)	2.51 $\pm$ 0.27 (8)	2.38 $\pm$ 0.33 (5)	2.88 $\pm$ 0.25 (8)	2.38 $\pm$ 0.22 (8)	1.79 $\pm$ 0.18 (6)	1.52 $\pm$ 0.22 (5)
10	1.83 $\pm$ 0.23 (6)	1.98 $\pm$ 0.21 (10)	3.03 $\pm$ 0.33 (9)	2.52 $\pm$ 0.33 (9)	2.80 $\pm$ 0.17 (8)	3.01 $\pm$ 0.32 (8)	1.98 $\pm$ 0.13 (6)	1.46 $\pm$ 0.21 (5)
100	2.89 $\pm$ 0.41 (6)	2.80 $\pm$ 0.25 (10)	5.48 $\pm$ 0.32 (9)	4.51 $\pm$ 0.86 (9)	5.68 $\pm$ 0.36 (8)	5.38 $\pm$ 0.31 (8)	3.73 $\pm$ 0.17 (6)	3.33 $\pm$ 0.34 (5)
1000	3.64 $\pm$ 0.41 (6)	3.75 $\pm$ 0.34 (7)	7.24 $\pm$ 0.73 (9)	7.29 $\pm$ 0.41 (9)	7.43 $\pm$ 0.58 (8)	6.70 $\pm$ 0.57 (8)	5.36 $\pm$ 0.21 (6)	4.41 $\pm$ 0.17 (5)*
10000	4.25 $\pm$ 0.47 (5)	4.10 $\pm$ 0.45 (8)	6.95 $\pm$ 0.53 (5)	8.03 $\pm$ 0.84 (5)	6.99 $\pm$ 0.61 (8)	7.58 $\pm$ 0.56 (8)	5.62 $\pm$ 0.47 (6)	4.86 $\pm$ 0.60 (5)

Previous work has shown that muscle is similar to other tissues in that prostaglandins neither are stored nor accumulate intracellularly during an incubation, but are released into the extracellular space (Reeds & Palmer, 1984). Indomethacin completely inhibits the release of prostaglandin E_2 from rat diaphragm muscle *in vitro* (Rodemann & Goldberg, 1982), so if a similar effect occurs in soleus muscle the effect of indomethacin on the sensitivity of glycolysis to insulin supports the view that endogenously produced prostaglandins are effective in modifying the sensitivity of glycolysis to insulin.

Dietze and co-workers have suggested that locally produced factors such as kinins or prostaglandins are involved in insulin action and may increase the sensitivity of some processes to insulin (see Dietze, 1982). These workers have suggested that increased glucose utilization during exercise and after hypoxia (reactive hyperaemia) is due, in part, to the release from kininogen of kinins, which in turn cause an increase in the rate of production of prostaglandins that increase the sensitivity of some process in muscle to insulin. They have also shown that the administration of indomethacin to man results in resistance of glucose utilization to insulin (Dietze *et al.*, 1978). However, the precise means by which prostaglandins could influence the effects of insulin were not considered. The present findings suggest that at least some of the observations by Dietze and co-workers could be explained by a specific effect of prostaglandins of the E series that results in an increase in the sensitivity of glycolysis in muscle to insulin. The effect of indomethacin in markedly decreasing the sensitivity of glycolysis to insulin in the isolated soleus muscle of the rat (Table 1) might be the means by which it causes insulin resistance in man (Dietze *et al.*, 1978).

Despite the marked effects of prostaglandin of the E series and indomethacin on the sensitivity of glycolysis to insulin, no effects were observed on the sensitivity of glycogen synthesis to insulin (Table 1). This is characteristic of the effects of adenosine, adenosine agonists and adenosine an-

tagonists on insulin-sensitivity in muscle (Espinal *et al.*, 1983a; Budohoski *et al.*, 1984). Consequently, it is possible that adenosine and prostaglandins influence insulin-sensitivity of muscle through a common mechanism, and hence both could play a role in changes in insulin-sensitivity *in vivo*.

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