Effects of the β -adrenoceptor agonist isoprenaline on insulin-sensitivity in soleus muscle of the rat

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1. The interactions between a β -adrenoceptor agonist (isoprenaline) and insulin on rates of hexose transport, glucose phosphorylation, glycogen synthesis and glycogenolysis were investigated in the incubated stripped soleus-muscle preparation of the rat. 2. In the presence of 1 μ M-isoprenaline, insulin was less effective in stimulating glucose phosphorylation and glycogen synthesis. The stimulation of glycogenolysis by isoprenaline was only slightly decreased even at high (10000 μ units/ml) concentrations of insulin. 3. Insulin-stimulated phosphorylation of 2-deoxyglucose was decreased by isoprenaline. It is suggested that this decrease in the rate of glucose phosphorylation is caused by the observed elevated concentration of glucose 6-phosphate, which inhibits hexokinase activity. 4. This conclusion is supported by the fact that isoprenaline had no effect on the stimulation of 3-O-methylglucose transport by insulin.

INTRODUCTION

Mature obese (*fa/fa*) Zucker rats exhibit marked resistance to insulin *in vivo* (Jeanrenaud, 1979), and this can be shown with isolated soleus muscle *in vitro* (Crettaz *et al.*, 1980; Challiss *et al.*, 1984c). Chronic treatment of obese Zucker rats with the β -adrenoceptor agonist BRL 26830 improves glucose tolerance *in vivo* (Cawthorne *et al.*, 1984; Smith *et al.*, 1985), and it has been shown that this treatment dramatically increases insulin-sensitivity in skeletal muscle that was isolated from these animals and incubated *in vitro* (Challiss *et al.*, 1985).

In view of these effects, it is surprising that adrenaline infusion into an intact animal acutely decreases rates of glucose utilization and induces apparent resistance to insulin (Shikama & Ui, 1975; Deibert & DeFronzo, 1980). However, this effect of adrenaline may be indirect (e.g. by increasing the plasma concentration of fatty acids). It seemed possible, therefore, that adrenaline might acutely increase the sensitivity of glucose utilization in muscle tissue, but this effect is masked *in vivo* by the indirect effect(s). Since skeletal muscle is considered to be a major site of insulin-stimulated glucose utilization *in vivo*, such an effect of adrenaline (or other β -adrenoceptor agonists) would be of considerable physiological importance. To investigate this possibility, studies have to be carried out on isolated muscle incubated *in vitro*. It is known that at high physiological and supra-physiological concentrations β -adrenoceptor agonists increase the rate of glycogenolysis, which can indirectly decrease the rate of glucose utilization by muscle (Dietz *et al.*, 1980): an increased rate of glycogenolysis raises the concentration of glucose 6-phosphate, which results in inhibition of hexokinase activity, and hence decreases the rate of glucose utilization (Newsholme & Leech, 1983). This could interfere in the interpretation of the interactive effects of a β -adrenoceptor agonist and insulin on rates of glucose utilization: in an attempt to clarify this, the effects of insulin at several different concentrations of isoprenaline on the rates of glycogenolysis, glycogen synthesis and glycolysis were investigated.

To investigate specifically the effect of isoprenaline on

the sensitivity of glucose transport to insulin, which is normally the rate-limiting step in glucose utilization in muscle (see Newsholme & Leech, 1983), the rate of intracellular accumulation of 3-O-methylglucose by isolated incubated muscle was measured. Previous studies of this process provide conflicting conclusions: Chiasson *et al.* (1981) have shown that adrenaline does not affect hexose transport into skeletal muscle of the perfused hindquarter of the rat; in contrast, Bihler and co-workers have demonstrated an inhibitory effect of 10 nM–10 μ M-adrenaline on hexose transport in isolated muscle preparations of diaphragm and soleus (Bihler *et al.*, 1978; Sloan *et al.*, 1978).

MATERIALS AND METHODS

Male Wistar rats (160–170 g; purchased from Bantin & Kingman, Hull, U.K.) were maintained in the Department of Biochemistry animal house for 4 days before use. All enzymes, biochemicals and radiochemicals were obtained from sources given previously (Challiss *et al.*, 1983; Budohoski *et al.*, 1984a), except that (–)-isoprenaline (isoproterenol) bitartrate was obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K., and 2-deoxy[2,6- 3 H]glucose, 3-O-methyl[U- 14 C]glucose and [3 H]inulin were obtained from Amersham International, Amersham, Bucks., U.K.

Stripped soleus muscles were prepared and incubated as described by Crettaz *et al.* (1980), with the modifications given by Challiss *et al.* (1983). Additions of isoprenaline (10 nM–1 μ M) and/or insulin (1–10000 μ units/ml) were made at the start of the incubation period. Rates of total lactate production (*J*), [14 C]lactate production (λ) and 14 C incorporation into glycogen (γ) were measured as described previously (Espinal *et al.*, 1983b; Challiss *et al.*, 1984a). The rate of hexose oxidation to CO₂ was measured as described by Leighton *et al.* (1985); measured 14 CO₂ production rates were corrected for the decrease in specific radioactivity in pyruvate owing to glycogenolysis.

The rates of glucose oxidation were never greater than 15% of the rate of lactate formation under any of the incubation conditions investigated; it was therefore

Table 1. Effects of insulin on rates of formation of lactate, [¹⁴C]lactate and [¹⁴C]glycogen by the stripped soleus-muscle preparation of the rat in the presence and absence of isoprenaline

Results are presented as means $\pm$ S.E.M. for the numbers of separate incubations in parentheses.

	Insulin concn. (μ units/ml)	Rates (μ mol/h per g of tissue)			
		0	0.01	0.1	1
(a) Lactate formation	1	8.67 $\pm$ 0.50 (9)	12.96 $\pm$ 0.78 (9)	15.54 $\pm$ 0.86 (9)	18.18 $\pm$ 0.63 (9)
	100	11.95 $\pm$ 0.37 (12)	16.06 $\pm$ 0.40 (12)	18.82 $\pm$ 0.43 (12)	19.09 $\pm$ 0.65 (12)
	10000	13.93 $\pm$ 0.88 (9)	15.60 $\pm$ 0.90 (9)	18.27 $\pm$ 0.52 (8)	18.81 $\pm$ 0.56 (8)
(b) [¹⁴ C]Lactate formation	1	7.73 $\pm$ 0.65 (8)	7.71 $\pm$ 0.99 (9)	7.45 $\pm$ 0.99 (9)	7.63 $\pm$ 0.80 (9)
	100	11.13 $\pm$ 0.91 (11)	11.93 $\pm$ 0.76 (10)	10.68 $\pm$ 0.89 (10)	11.52 $\pm$ 0.98 (11)
	10000	13.95 $\pm$ 0.81 (8)	14.20 $\pm$ 0.61 (9)	13.59 $\pm$ 0.85 (9)	12.42 $\pm$ 0.66 (8)
(c) [¹⁴ C]Glycogen formation	1	1.45 $\pm$ 0.13 (9)	1.19 $\pm$ 0.13 (9)	0.57 $\pm$ 0.06 (9)	0.55 $\pm$ 0.06 (9)
	100	3.35 $\pm$ 0.29 (12)	1.35 $\pm$ 0.11 (12)	0.81 $\pm$ 0.09 (12)	0.70 $\pm$ 0.05 (12)
	10000	5.91 $\pm$ 0.52 (9)	4.18 $\pm$ 0.36 (9)	2.23 $\pm$ 0.19 (9)	1.54 $\pm$ 0.06 (8)

considered satisfactory to use the rate of lactate production as an index of glycolytic flux. If it is also assumed that, under the conditions investigated, glycogen acts as a 'sink' (i.e. the flux of ¹⁴C from glycogen to the hexose monophosphate pool is negligible), then it is possible to calculate the hexose monophosphate flux to glycogen (*B*), the glycogen flux to hexose monophosphate (*A*) and the flux from glucose to hexose monophosphate (*G*) (see Challiss *et al.*, 1984b) as follows:

$$B = J \cdot \frac{\gamma}{\lambda}$$

$$G = \frac{J+B}{J} \cdot \lambda$$

and

$$A = B + (J - G)$$

Glucose 6-phosphate concentration was determined in neutralized HClO₄ extracts of incubated soleus muscle strips by the method of Lamprecht & Trautshold (1974).

For the investigation of glucose transport and glucose phosphorylation by using 3-*O*-methyl[U-¹⁴C]glucose (0.15 μ Ci/ml) and 2-deoxy[2,6-³H]glucose (0.4 μ Ci/ml) respectively, it was considered important to carry out experiments under conditions identical with those used previously. Thus the radiolabelled compounds were added to 5.5 mM-glucose in the incubation medium so that the actual concentrations of 2-deoxyglucose and 3-*O*-methylglucose were negligible. In the former experiments, [³H]inulin (0.1 μ Ci/ml) was also added to enable the extracellular space in muscle to be measured. In the latter experiments, the frozen muscle was homogenized in 4% (w/v) HClO₄, neutralized after deproteinization, and deoxy[³H]glucose 6-phosphate was separated from deoxy[³H]glucose by Dowex-2 (formate form) ion-exchange chromatography (Challiss *et al.*, 1984a). Preliminary experiments established that rates of lactate release, 3-*O*-methyl[U-¹⁴C]glucose transport and 2-deoxy[2,6-³H]glucose 6-phosphate accumulation increased linearly over the 60 min time course of the experiment (results not shown).

RESULTS

The effects of insulin concentration (1, 100, 10000 μ units/ml) on rates of total lactate formation (i.e.

that from both glucose and glycogen) and the radioisotopic fluxes from glucose to lactate and to glycogen have been measured in the isolated stripped soleus-muscle preparation in the presence of different concentrations of the β -adrenoceptor agonist isoprenaline (0, 0.01, 0.1 and 1.0 μ M) (Table 1). With the assumptions given in the Materials and methods section, glucose phosphorylation (*G*), glycogenic (*B*) and glycogenolytic (*A*) fluxes have been calculated at the three insulin concentrations investigated in the absence and presence of 1 μ M-isoprenaline, and are presented in Table 2.

In the absence of the β -adrenoceptor agonist, increasing the concentration of insulin stimulates the rate of glucose phosphorylation more than 2-fold, and that of glycogenesis by 3-fold, but decreases that of glycogenolysis almost to zero (Table 2). For each process, the half-maximal effect of insulin was found to be at about 100 μ units/ml, which is consistent with previous findings for glycolysis and glycogenesis (Espinal *et al.*, 1983b).

In the presence of 1 μ M-isoprenaline, insulin was less effective in stimulating the rate of glucose phosphorylation, and this was particularly evident at the higher insulin concentrations (Table 2a), the inhibitory effect of isoprenaline on insulin-stimulated glucose phosphorylation being statistically significant at 100 and 10000 μ units of insulin/ml (Student's *t* test; *P* < 0.05). Isoprenaline (1 μ M) had no effect on the rate of glycogenesis in the presence of 1 μ unit of insulin/ml; however, isoprenaline markedly decreased the effect of increasing concentrations of insulin (Table 2b), such that the effect of 10000 μ units of insulin/ml on the glycogenic flux was decreased by 58%. The rate of glycogenolysis was stimulated 7-fold (to 5.74 $\pm$ 0.96 μ mol of glucosyl/h per g) by 1 μ M-isoprenaline at the basal insulin concentration, and this stimulation was only slightly less at high concentrations of insulin (Table 2c). In the absence of isoprenaline, 10000 μ units of insulin/ml decreased glycogenolytic flux to zero (0.08 $\pm$ 0.14 μ mol of glucosyl/h per g), whereas in the presence of 1 μ M-isoprenaline and 10000 μ units of insulin/ml the glycogenolytic rate was 3.91 $\pm$ 0.85 μ mol h per g.

The mechanism by which isoprenaline decreases the stimulation by insulin of glucose phosphorylation was investigated by using the analogues 2-deoxyglucose and 3-*O*-methylglucose. The accumulation of 2-deoxyglucose

Table 2. Effects of insulin on the calculated rates of (a) glucose phosphorylation, (b) glycogen synthesis and (c) glycogen breakdown by isolated stripped soleus muscles in the absence or presence of 1 μ M-isoprenaline

Fluxes are calculated from the data presented in Table 1 by using the equations given in the Materials and methods section. Values are means $\pm$ S.E.M., with the numbers of separate incubations given in parentheses. Statistically significant differences (Student's *t* test) between incubations in the absence and presence of 1 μ M-isoprenaline at a given concentration of insulin are indicated by **P* < 0.05, ***P* < 0.01.

	Insulin concn. (μ units/ml) Concn. of isoprenaline (μ M) . . .	Flux (μ mol of glucosyl/h per g)	
		0	1.0
(a) Glucose phosphorylation	1	5.32 $\pm$ 0.51 (6)	4.35 $\pm$ 0.71 (6)
	100	8.92 $\pm$ 0.84 (9)	6.88 $\pm$ 0.80 (9)*
	10000	12.19 $\pm$ 1.07 (6)	8.76 $\pm$ 0.60 (6)*
(b) Hexose monophosphate to glycogen	1	1.63 $\pm$ 0.23 (6)	1.29 $\pm$ 0.17 (6)
	100	3.60 $\pm$ 0.25 (9)	1.22 $\pm$ 0.09 (9)**
	10000	5.85 $\pm$ 0.48 (6)	2.43 $\pm$ 0.31 (6)**
(c) Glycogen to hexose monophosphate	1	0.85 $\pm$ 0.19 (6)	5.74 $\pm$ 0.96 (6)**
	100	0.60 $\pm$ 0.23 (9)	3.93 $\pm$ 0.51 (9)**
	10000	0.08 $\pm$ 0.14 (6)	3.91 $\pm$ 0.85 (6)**

Fig. 1. Effects of insulin on rates of 2-deoxy-D-[3 H]glucose phosphorylation by the stripped soleus-muscle preparation of the rat in the absence (■) and presence (▲) of 1 μ M-isoprenaline

All points represent means $\pm$ S.E.M. for at least six separate incubations. Statistically significant differences (Student's *t* test) between incubations in the absence or presence of 1 μ M-isoprenaline at a given concentration of insulin are indicated by **P* < 0.05.

6-phosphate is a direct indication of the rate of hexose phosphorylation, and the accumulation of 3-*O*-methylglucose is a direct indication of the activity of the hexose transporter process (Kipnis & Cori, 1960; Post *et al.*, 1961). It was considered important to use the glucose

analogues under experimental conditions identical with those used to obtain the results presented in Tables 1 and 2; thus, as stated in the Materials and methods section, tracer amounts of the radiolabelled analogues were used to follow the metabolism of 5.5 mM-glucose in the medium. Preliminary time-course experiments confirmed the validity of this method for 1 h muscle incubations.

The effects of insulin on rates of 2-deoxyglucose phosphorylation in the absence and presence of 1 μ M-isoprenaline are shown in Fig. 1. Isoprenaline decreases the rate of phosphorylation of 2-deoxyglucose at the higher concentrations of insulin, this effect being statistically significant at 1000 and 10000 μ units of insulin/ml, findings that are in excellent agreement with those for glucose phosphorylation given in Table 2(a). However, the stimulation of 3-*O*-methylglucose accumulation by insulin was not modified by the presence of 1 μ M-isoprenaline (Fig. 2). In addition, there was no effect of isoprenaline on the sensitivity of 3-*O*-methylglucose transport in insulin (Fig. 2). The effect of increasing insulin concentration on glucose 6-phosphate concentration in the presence and absence of isoprenaline is shown in Table 3. The presence of isoprenaline increases the concentration of glucose 6-phosphate in incubated soleus muscle, the effect being statistically significant (*P* < 0.05) in the presence of 10000 μ units of insulin/ml.

DISCUSSION

In the present series of experiments we have assessed the interaction between insulin and a β -adrenoceptor agonist on carbohydrate metabolism in the stripped soleus-muscle preparation. Isotopically labelled glucose, 2-deoxyglucose and 3-*O*-methylglucose have been used to measure the rates of glycogenolysis, glycogen synthesis, glucose phosphorylation and glucose transport. It has been shown that isoprenaline inhibits insulin-stimulated glucose phosphorylation (as measured by the calculation of glucose-phosphorylation rates from the data presented

Fig. 2. Effects of insulin on rates of 3-O-methyl-D-[14 C]glucose uptake by the stripped soleus-muscle preparation of the rat in the absence (■) and presence (▲) of 1 μ M-isoprenaline

All points represent means $\pm$ S.E.M. for at least six separate incubations.

Table 3. Effect of insulin on the concentration of glucose 6-phosphate in stripped soleus muscle of the rat in the absence and presence of 1 μ M-isoprenaline

Values shown are means $\pm$ S.E.M., with the numbers of separate incubations shown in parentheses. Glucose 6-phosphate concentration is expressed as μ mol/g of tissue. Statistically significant differences (Student's *t* test) between incubations in the absence and presence of 1 μ M-isoprenaline at a given concentration of insulin are indicated by **P* < 0.05.

Insulin concn. (μ units/ml)	[Glucose 6-phosphate] (μ mol/g)	
	Concn. of isoprenaline (μ M) . . . 0	1.0
1	0.212 $\pm$ 0.21 (5)	0.327 $\pm$ 0.065 (5)
100	0.271 $\pm$ 0.037 (5)	0.401 $\pm$ 0.083 (5)
10000	0.296 $\pm$ 0.033 (6)	0.463 $\pm$ 0.052 (6)*

in Table 1 or from the rate of accumulation of 2-deoxyglucose 6-phosphate). However, isoprenaline had no effect on insulin-stimulated hexose transport (as measured by 3-O-methylglucose accumulation).

These results suggest that β -adrenoceptor agonists decrease the effect of insulin on glucose utilization, not by a direct effect on insulin-stimulated recruitment of glucose transporters to the sarcolemma (Wardzala & Jeanrenaud, 1981) or by changing the sensitivity of the transporter to insulin, but indirectly by elevation of the

concentration of glucose 6-phosphate (Table 3), causing inhibition of hexokinase activity. To inhibit glucose utilization, the inhibition of hexokinase must result in a marked increase in the concentration of intracellular glucose such that the transport process approaches near-equilibrium and no longer limits the rate of glycolysis [as has been shown in the glucose-, acetoacetate- and insulin-perfused heart by Randle *et al.* (1964)]. Consequently, stimulation of transport by insulin has no effect on the rate of glucose utilization, a conclusion in agreement with that of Chiasson *et al.* (1981), but not that of Bihler and co-workers (Bihler *et al.*, 1978; Sloan *et al.*, 1978).

The lack of an acute effect on glucose transport in muscle indicates that β -adrenoceptor agonists do not directly or acutely influence the sensitivity of glucose utilization in muscle to insulin. The well-established decrease in sensitivity to insulin caused by such agonists *in vivo* is therefore likely to be indirect, either by increasing the rate of muscle glycogenolysis or by raising the plasma fatty acid concentration.

It has been shown that adenosine-receptor agonists can decrease (and adenosine-receptor antagonists increase) insulin-sensitivity in isolated incubated soleus muscle (Espinal *et al.*, 1983b; Budohoski *et al.*, 1984a). The present results rule out the possibility that these effects might be achieved by changing the sensitivity of muscle to locally produced noradrenaline or that β -adrenoceptor agonists might influence the effectiveness of adenosine on insulin sensitivity.

It is, however, tempting to speculate that the effect of chronic administration of β -adrenoceptor agonists on insulin-sensitivity in muscle (Challiss *et al.*, 1985) might be caused by a decrease in the adenosine concentration or a decrease in the number or affinity of adenosine receptors. In this way, catecholamines could be responsible for the improvement in insulin-sensitivity in muscle observed *in vitro* from cold-exposed (Budohoski *et al.*, 1984b) or exercise-trained (Espinal *et al.*, 1983a) rats; in these two conditions plasma catecholamine concentrations have been reported to be raised for prolonged periods (Leduc, 1961; Maron *et al.*, 1977; Picotti *et al.*, 1981).

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