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Selectivity of the insulin-like actions of vanadate on glucose and protein metabolism in skeletal muscle

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To determine if vanadate has insulin-like actions in skeletal muscle, we measured its effects on glucose and protein metabolism in epitrochlearis muscles of rats. Compared with insulin, vanadate increased glucose uptake, glycogen synthesis and glycolysis to a lesser degree, but caused a greater stimulation of lactate and glucose oxidation. Unlike insulin, vanadate did not change either protein synthesis or degradation. These different metabolic responses could be related to the different pattern of insulin-receptor phosphorylation caused by insulin and vanadate.

INTRODUCTION

In muscle, insulin initiates the transport of glucose, ions and amino acids, and also regulates the synthesis and degradation of macromolecules. However, the initial steps in the regulation of muscle protein synthesis and degradation by insulin are unknown, and it is unclear whether insulin-induced changes in protein synthesis and degradation are related to other functions of insulin. In other tissues, certain metabolic effects of insulin require phosphorylation reactions, suggesting that regulation by insulin occurs by related processes (Kasuga *et al.*, 1983; Tamura *et al.*, 1983). For example, when insulin binds to adipose cells, it causes phosphorylation of threonine, tyrosine and serine residues in the insulin receptor, and stimulates glucose transport, glycogen synthesis and glucose oxidation (Larner *et al.*, 1982). The mechanism(s) by which insulin regulates intracellular events is unknown, but does involve phosphorylation of enzymes, e.g. glycogen synthase and pyruvate dehydrogenase.

A novel method for studying how insulin exerts its biochemical effects has been to use compounds that mimic the effects of the hormone (Tolman *et al.*, 1979). One such compound, vanadate, causes phosphorylation of the insulin receptor of adipose cells and stimulates glucose transport, activates glycogen synthase and increases glycogen synthesis in these cells (Tamura *et al.*, 1984). This raises the possibility that phosphorylation of the insulin receptor might initiate all of the metabolic effects of insulin (Cheng *et al.*, 1985). Vanadate, however, differs from insulin in that it does not cause phosphorylation of serine residues in the insulin receptor (Tamura *et al.*, 1984). Consequently, vanadate might not exert a full range of insulin-like effects. To pursue this, we compared the effects of insulin and vanadate on glucose metabolism and on protein synthesis and degradation in muscle. We reasoned that a difference might suggest an initial step in the mechanism of insulin-mediated changes in protein turnover; it also might help to explain the correlation we found previously between insulin-induced changes in the ratio of glycolysis (or lactate release) to glucose uptake and net protein degradation in muscle (Clark & Mitch, 1983*b*; Clark *et al.*, 1984; May *et al.*, 1985).

EXPERIMENTAL

Animals

Male Sprague–Dawley rats weighing 180–200 g (Charles River Breeding Laboratory) were maintained on Purina RMH-1000 chow (Agway Country Foods) and water *ad libitum* for 3 or more days before they were placed in individual cages and deprived of food and water for 44–48 h. Krebs & Henseleit (1932) bicarbonate buffer, pH 7.4, containing 10 mM-glucose, 2 mM-lactate, 0.5 mM-phenylalanine, fatty acid and globulin-free bovine albumin (0.2 g/dl) and other additions as indicated was prepared and gassed with O_2/CO_2 (19:1). Epitrochlearis muscles of anaesthetized rats were removed, placed in 3 ml of medium and incubated at 37 °C for a 30 min equilibration period. Subsequently, the muscles were transferred to fresh medium containing radiolabelled compounds with or without either 1 mM-sodium vanadate or 10 munits of insulin/ml. Medium containing vanadate was adjusted with HCl to pH 7.4. The flasks were regassed and incubated for a 2 h experimental period (Clark & Mitch, 1983*a*).

Glucose and lactate metabolism

Glucose and lactate metabolism were assessed simultaneously by incubating muscles for 2 h in medium containing 10 mM-D-glucose, D-[5-^{3}H]glucose (1 mCi/mmol), 2 mM-L-lactate and L-[1-^{14}C]lactate (10 μCi/mmol) (Clark *et al.*, 1984). Glucose uptake was calculated as the sum of D-[5-^{3}H]glucose incorporated into glycogen and ^3H_2O formed during glycolysis. To measure glycogen synthesis during the incubation, muscles were immediately dissolved in 0.5 ml of 30% (w/v) KOH at 100 °C. Glycogen was precipitated in cold 85% (v/v) ethanol and washed with 2% (w/v) LiBr and then with 85% ethanol, and the concentration and radioactivity of glycogen were determined as described previously (Clark *et al.*, 1984). Recovery of glycogen by using a higher concentration of ethanol than suggested by Walaas & Walaas (1950) was $91 \pm 1\%$ (May *et al.*, 1985). Glycogen synthesis from glucose and lactate respectively were determined by measuring the amounts of ^{3}H and ^{14}C incorporated into glycogen and dividing

by the specific radioactivities of D-[5-^{3}H]glucose and L-[1-^{14}C]lactate in the medium. Lactate oxidation was calculated by measuring the amount of $^{14}CO_2$ evolved and dividing by the specific radioactivity of L-[1-^{14}C]lactate in the medium (Schadewalt *et al.*, 1983). In separate experiments D-[U-^{14}C]glucose (5 μCi/mmol) was substituted for L-[1-^{14}C]lactate, and $^{14}CO_2$ was collected to measure rates of glucose oxidation.

Protein metabolism

During the experiments in which glucose and lactate metabolism were assessed, tyrosine release into the media was measured fluorimetrically (Waalkes & Udenfriend, 1957) to estimate the net rate of protein degradation. Because tyrosine is neither synthesized nor catabolized by muscle and because the tissue pool of tyrosine remains unchanged during incubation with insulin or vanadate, its production reflects net protein breakdown. In separate experiments 0.5 mM-cycloheximide was added to block protein synthesis so that total protein degradation could be measured.

Protein synthesis was calculated in paired muscles as the difference in L-[U-^{14}C]phenylalanine incorporated into muscle protein between 30 and 150 min of incubation. After the experimental incubation, muscles were removed, blotted, homogenized in 3 ml of 10% (w/v) trichloroacetic acid and washed successively with 10% trichloroacetic acid and ethanol/diethyl ether (1:1, v/v). The homogenate was dissolved in Soluene (Packard) and the radioactivity was determined by scintillation spectrometry (Mitch & Clark, 1984). Incorporation of phenylalanine into muscle protein was corrected for the extracellular phenylalanine specific radioactivity. The intra- and extra-cellular specific radioactivities of phenylalanine remain equal after 30 min of incubation (Clark & Mitch, 1983*a*).

Materials and analyses

Reagent-grade chemicals and enzymes were obtained from Sigma Chemical Co. Sodium vanadate was obtained from Fisher Scientific Co. and radiolabelled compounds were purchased from New England Nuclear. NADH was purchased from P-L Biochemicals. Incubated muscles were homogenized in 0.2 M-$HClO_4$ and analysed spectrophotometrically for phosphocreatine (Lamprecht *et al.*, 1974) and ATP (Lamprecht & Trautschold, 1974) and fluorimetrically for ADP and AMP (Jaworek *et al.*, 1974). ATP was also determined by a chemiluminescence assay (Deluca & McElroy, 1978) with firefly luciferase (Analytical Luminescence Laboratory). Both measurements of ATP content gave similar results. Results are expressed as means $\pm$ S.E.M. and the significance of differences was tested by Student's *t* test.

RESULTS AND DISCUSSION

Vanadate and insulin caused qualitatively similar changes in muscle glucose metabolism, but the magnitude of the changes differed substantially (Table 1). Incubation with a maximally effective concentration of insulin, 10 munits/ml (Clark *et al.*, 1984), stimulated glucose uptake 20-fold, glycolysis 6-fold and glycogen synthesis 30-fold. Vanadate at 1 mM, a maximally effective concentration in adipose cells (Tamura *et al.*, 1984), increased glucose uptake only 2-fold, glycolysis 2-fold and glycogen synthesis 3-fold. Although vanadate and insulin increased the incorporation of D-[5-^{3}H]glucose into glycogen, only insulin increased the glycogen content of muscle. This suggests that vanadate increased glycogen turnover, but not net glycogen synthesis in muscle. The stimulation of glycolysis by vanadate could not be attributed to altered tissue concentrations of ADP or AMP (Table 2). Although incubation with vanadate caused a small decrease in muscle ATP content and no change in phosphocreatine, it seems unlikely that this could account for the observed changes in glucose metabolism. Thus in muscle, as in adipose tissue, vanadate exerts insulin-like effects on glucose metabolism *in vitro*. Vanadate has also been reported to have beneficial effects *in vivo*. Oral administration of vanadate to diabetic rats improved cardiac muscle performance and normalized blood glucose concentrations (Heyliger *et al.*, 1985). This suggests that vanadate might exert positive effects in insulin-resistant hypercatabolic conditions.

The differences in magnitude of the stimulation by vanadate of glucose uptake, glycogen synthesis and glycolysis (Table 1) compared to insulin may be related to the different patterns of insulin-receptor phosphorylation. In the 95000 Da β-subunit of the insulin receptor, insulin causes phosphorylation of serine, threonine and tyrosine residues. In contrast, vanadate does not cause phosphorylation of serine residues in the receptor, though it stimulates a similar phosphorylation of tyrosine residues and only a slight phosphorylation of threonine residues (Tamura *et al.*, 1984). It has also been proposed that phosphorylation of specific amino acids in the insulin receptor leads to the formation of mediators that regulate specific intracellular metabolic pathways; to date, four mediators have been identified (Larner *et al.*, 1982; Cheng *et al.*, 1985). This hypothesis could be tested by selectively phosphorylating serine residues in the absence of insulin or selectively inhibiting insulin-mediated formation of phosphoserine in the β-subunit of the insulin receptor.

Vanadate, in contrast with insulin, had a 2-fold greater effect on lactate (Table 1) and glucose oxidation. Glucose oxidation increased from 0.12 ± 0.01 to 0.76 ± 0.06 μmol of glucose/h per g with vanadate and from 0.10 ± 0.01 to 0.33 ± 0.02 μmol of glucose/h per g with insulin. Differences in receptor phosphorylation do not offer an obvious explanation for our finding that vanadate stimulated lactate oxidation to a greater extent than did insulin (Table 1). It is possible that vanadate, like dichloroacetate (Whitehouse *et al.*, 1974), directly stimulates pyruvate dehydrogenase activity.

Previously, we have found that the lower rate of net protein degradation in muscle incubated or perfused with different concentrations of insulin is correlated with a lower ratio of glycolysis (or lactate release) to glucose uptake (Clark & Mitch, 1983*b*; Clark *et al.*, 1984; May *et al.*, 1985). As shown in Tables 1 and 3, insulin decreased this ratio from 0.82 to 0.51, and increased muscle protein synthesis by 60% and inhibited total and net protein degradation by approx. 24%. Since vanadate did not change the ratio of glycolysis to glucose uptake in incubated muscle (Table 1), we did not expect that it would change net protein degradation. Indeed, vanadate, in contrast with muscle, had no effect on either muscle protein synthesis or total protein degradation (Table 3). These results differ from those reported to occur in hepatocytes and reticulocytes. In hepatocytes 10 mM-vanadate inhibited lysosomal proteolysis (Seglen &

Table 1. Effects of vanadate and insulin on glucose, lactate and protein metabolism in incubated epitrochlearis muscles

Values are the means ± S.E.M. of measurements in paired muscles from six to eight starved rats. Muscles were incubated for 2 h in buffer containing 10 mM-glucose, [5-^{3}H]glucose (1 mCi/mmol), 2 mM-lactate and [1-^{14}C]lactate (10 μCi/mmol) with or without 1 mM-vanadate or with or without 10 munits of insulin/ml. *$P < 0.01$ by paired t test compared with muscles without vanadate or insulin; †$P < 0.01$ by unpaired t test compared with muscles incubated with insulin.

	Control	Vanadate	Control	Insulin
Glucose uptake (μmol of glucose/h per g)	1.69 ± 0.17	3.42 ± 0.30*†	1.83 ± 0.20	19.38 ± 0.45*
Glycogen synthesis from glucose (μmol of glucose/h per g)	0.29 ± 0.02	0.89 ± 0.10*†	0.36 ± 0.05	10.87 ± 0.69*
Glycogen synthesis from lactate (μmol of lactate/h per g)	0.28 ± 0.03	0.31 ± 0.04	0.19 ± 0.03	0.26 ± 0.06
Glycolysis (μmol/h per g)	1.34 ± 0.13	2.54 ± 0.21*†	1.50 ± 0.21	9.46 ± 0.89*
Lactate oxidation (μmol of lactate/h per g)	0.12 ± 0.01	1.05 ± 0.09*†	0.11 ± 0.02	0.49 ± 0.04*
Glycogen content (μmol/g)	24.0 ± 1.1	23.2 ± 1.3†	20.4 ± 1.2	39.7 ± 2.7*
Glycolysis/glucose uptake (ratio)	0.80 ± 0.03	0.77 ± 0.03	0.82 ± 0.04	0.51 ± 0.03*
Net protein degradation (nmol of tyrosine/h per g)	113.6 ± 6.7	107.6 ± 8.5	126.4 ± 6.5	84.7 ± 3.9*

Table 2. Effects of insulin and vanadate on tissue adenine nucleotides and phosphocreatine

Values are means ± S.E.M. of tissue concentrations measured in paired muscles incubated in buffer containing 10 mM-glucose with or without 10 munits of insulin/ml and with or without 1 mM-vanadate. *$P < 0.01$ by paired comparsion with muscle incubated without insulin or vanadate.

	Tissue concentration (nmol/mg)			
	ATP	ADP	AMP	Phosphocreatine
Basal ($n = 5$)	5.27 ± 0.34	0.90 ± 0.04	0.22 ± 0.04	16.22 ± 1.90
Insulin (10 munits/ml)	6.81 ± 0.21*	1.05 ± 0.08	0.23 ± 0.02	17.86 ± 1.79
Basal ($n = 6$)	6.10 ± 0.46	0.77 ± 0.05	0.20 ± 0.03	16.89 ± 1.29
Vanadate (1 mM)	5.13 ± 0.43*	0.83 ± 0.04	0.22 ± 0.04	17.96 ± 0.80

Table 3. Effects of vanadate and insulin on protein synthesis and degradation in incubated epitrochlearis muscles of starved rats

Values are the means ± S.E.M. of measurements in paired muscles from six starved rats incubated with or without 1 mM-vanadate or with or without 10 munits of insulin/ml. Protein degradation was measured during incubation in media containing 0.5 mM-cycloheximide. *$P < 0.01$ by paired t test compared with control values.

	Control	Vanadate	Control	Insulin
Protein synthesis (μmol of phenylalanine/h per g)	41.6 ± 2.3	40.4 ± 1.8	36.5 ± 2.1	58.3 ± 2.7*
Protein degradation (nmol of tyrosine/h per g)	151.4 ± 8.3	153.8 ± 6.6	148.3 ± 7.4	112.6 ± 4.5*

Gordon, 1981), whereas in reticulocytes vanadate inhibited ATP-dependent proteolysis (Tanaka *et al.*, 1984). Unfortunately, our results suggest that vanadate would not limit the accelerated muscle protein breakdown associated with the response to catabolic conditions such as acute renal failure or thermal injury (Clark & Mitch, 1983*b*; Clark *et al.*, 1984; May *et al.*, 1985).

With regard to the relevance of these results to the action of insulin, they do not elucidate how vanadate exerts its insulin-like effects on muscle glucose metabolism

or why the compound does not affect protein metabolism. It is tempting to speculate that the lack of any effect of vanadate on muscle protein synthesis or degradation (Table 3) and the difference in the magnitude of changes in glucose metabolism compared with insulin (Table 1) may be linked to the difference in insulin-receptor phosphorylation.

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