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EFFECTS OF CHRONIC ADMINISTRATION OF VANADATE TO THE RAT ON THE SENSITIVITY OF GLYCOLYSIS AND GLYCOGEN SYNTHESIS IN SKELETAL MUSCLE TO INSULIN

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Abstract—Male Wistar rats were given sodium orthovanadate in their drinking water for at least 14 days. This treatment increased the hypoglycaemic effect of intravenously administered insulin and increased the sensitivity of isolated soleus muscle strips to insulin with respect to both glycolytic and glycogen synthetic rates. This effect of chronic vanadate administration was shown not to be a consequence of a change in the insulin binding characteristics of soleus muscle. It is suggested that these changes may be brought about by the interaction of vanadate with insulin-mediated alterations in tyrosine kinase/phosphotyrosyl phosphatase activities.

Vanadium is now recognised as an essential nutritional element in higher animals and although its precise physiological functions are unclear [1], it has been shown to influence a variety of enzyme activities and cellular functions [2–4].

One such effect of vanadate is on carbohydrate metabolism: Tolman *et al* have reported that, *in vitro*, vanadate stimulates glucose transport and oxidation in adipocytes and enhances glycogen synthesis in liver and diaphragm muscle [5]; this insulin-mimetic effect of vanadate has been confirmed by other investigators [6–9]. Tamura *et al.* [8, 10] have demonstrated that vanadate increases the extent of phosphorylation of tyrosine residues in the β-subunits of the insulin receptor, an effect which may be brought about by activation of a specific tyrosyl-protein kinase or inhibition of a phosphotyrosyl-protein phosphatase [8, 10–12].

Recently, it has been shown that chronic administration of vanadate to diabetic rats prevents the decline in cardiac performance observed in untreated animals [13]; it was also shown in this study that chronic exposure to vanadate reduced hyperglycaemia in diabetic animals. Such an effect may be brought about either by vanadate mimicking insulin action or by altering the sensitivity of target tissues to insulin.

In the present study the effects of chronic administration of vanadate to normal rats on the sensitivity of the rates of glycolysis and glycogen synthesis in isolated preparations of skeletal muscle to insulin have been investigated. In addition, the sensitivity to insulin *in vivo* has been assessed and the effect of vanadate on plasma glucose and insulin concentrations are reported.

MATERIALS AND METHODS

Male Wistar rats (120–150 g) were obtained from OLAC1976 Ltd. (Bicester, Oxon, U.K.) and were maintained at 23 ± 1° under a 12 hr light–12 hr dark cycle. Unless stated, animals were given free access to Rat & Mouse No. 1 maintenance diet (S.D.S., Witham, Essex, U.K.) and water. For vanadate treatment, the drinking water was supplemented with 1% sucrose and 0.06% sodium orthovanadate for at least 14 days prior to study.

After 14 days, rats were starved for 12 hr during which time both control and vanadate-treated animals had free access to water only. Anaesthesia was induced by administration of 60 mg/kg sodium pentabarbital intraperitoneally and animals were given 0.75 i.u./kg of bovine insulin (Sigma Chemical Co. Ltd., Poole, Dorset, U.K.) via the left femoral vein. Blood samples were obtained immediately before insulin administration and at regular intervals for 60 min to monitor the hypoglycaemic response. After deproteinisation with $HClO_4$, blood glucose was determined [14].

After the hypoglycaemic response test, animals were given free access to food and water (± vanadate) for at least 4 days before subsequent study. For determination of insulin sensitivity of skeletal muscle *in vitro*, rats were starved for 12 hr prior to sacrifice; soleus muscle strips were prepared and incubated as previously described [15, 16] and rates of lactate formation and glycogen synthesis measured at several concentrations of insulin [15, 16].

Insulin binding was also determined in isolated stripped soleus muscles prepared from control or 14 day vanadate-treated animals. Muscles were first

pre-incubated at 37° for 30 min as previously described [16]. Muscles were then transferred to flasks containing 1–100000 μunit/ml insulin and 0.05 μCi/ml [^{125}I]insulin (porcine insulin, monoiodinated (receptor grade), 2200 Ci/mmol; Du Pont de Nemours, GMBH NEN products, Dreieich, F.R.G.) in the incubation medium previously described [16]. Muscles were incubated for 4 hr at 20°. At the end of this time muscles were washed with 6 × 10 ml ice-cold isotonic saline, pH 7.4 containing 1% defatted bovine serum albumin. Muscles were solubilized in 0.5 ml 1 M KOH and counted for ^{125}I radioactivity in a gamma spectrometer.

Plasma insulin and glucose concentrations were determined for post-prandial and overnight fasted animals which had received vanadate in their drinking water for at least 14 days. Blood samples were obtained by insertion of a heparinized cannula into the tail artery. Freshly-drawn blood was centrifuged and insulin [17] and glucose [14] determined.

Results are given as mean ± SEM. Statistically significant differences were determined using Student's *t*-test.

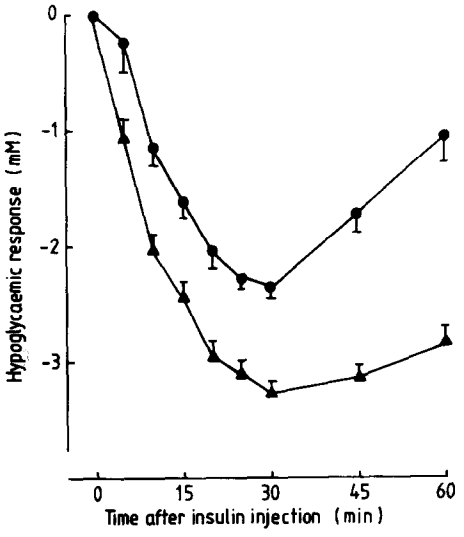

Fig. 1. The effect of chronic vanadate treatment on the hypoglycaemic action of insulin *in vivo*. Insulin (0.75 i.u./kg) was given intravenously. The hypoglycaemic response was followed in control (●) and vanadate-treated (▲) rats. Results are given as mean ± SEM of five rats.

RESULTS

During the 14-day treatment period, control animals gained weight at the rate of 5.1 ± 0.2 g per day, whereas those receiving vanadate in their drinking water gained weight at a statistically significantly slower rate (3.8 ± 0.2 g per day; $P < 0.01$). Despite reducing their fluid intake compared to controls, the vanadate-treated animals consumed 5–10 mg of sodium orthovanadate daily, a dose which, if given orally in a single dose, is similar to the LD_{50} [18]. However, despite the 25% growth retardation, which has been reported by others [19], no other symptoms of vanadium toxicity [20] were observed.

The arterial blood concentration of glucose and insulin for post-prandial and overnight-fasted rats are presented in Table 1: no differences were observed in the post-prandial rats, but overnight fasting resulted in maintenance of higher plasma glucose and insulin concentrations. This occurred despite an increase in whole-body sensitivity to insulin (see below).

The effect of intravenously administered insulin on the blood glucose concentration in control and vanadate-treated animals is shown in Fig. 1. The hypoglycaemic effect of insulin was more marked in the vanadate-treated rats, suggesting that the sensitivity to insulin *in vivo* was increased: from the areas representing deviation from euglycaemia for 1 hr following insulin administration it was calculated that the hypoglycaemic effect of insulin (expressed as mM.hr) was 1.53 ± 0.21 (5) for control and 2.62 ± 0.16 (5) for vanadate-treated rats, which is a statistically significant improvement in insulin sensitivity ($P < 0.01$).

The effect of insulin concentrations on the rates of glycolysis and glycogen synthesis in isolated soleus muscle is shown in Table 2. Comparison of the effect of a given concentration of insulin on glycolytic rate in muscles isolated from control and vanadate-treated animals reveals only a lower rate of lactate formation at a 1 μunit of insulin/ml in the vanadate-treated group (Table 2). However, it can be seen that increasing the concentration of insulin in the incubation from 1 to 10 μunits/ml has a significant effect on glycolytic rate ($P < 0.05$) in muscles from vanadate-treated rats, whereas no increase is observed in muscle strips from control rats. This observation is illustrated in Fig. 2 where the increase in glycolytic rate for each increment in insulin con-

Table 1. Plasma glucose and insulin concentrations of control and vanadate-treated rats

	Post-prandial		15 hr Starved	
	Glucose*	Insulin†	Glucose*	Insulin†
Control	9.7 ± 0.3 (6)	32.3 ± 6.6 (6)	5.5 ± 0.2 (5)	8.6 ± 2.0 (5)
Vanadate-treated	10.5 ± 0.7 (8)	26.5 ± 2.6 (8)	7.1 ± 0.2 (5)‡	13.4 ± 2.1 (5)

* Glucose concentration expressed as μmol/ml of plasma; † insulin concentration expressed as μunits/ml of plasma. Arterial blood samples were drawn as described in the Materials and Methods section. Results are presented as means ± SEM with the number of observations in parentheses. Statistically significant differences for control vs vanadate-treated rats were determined using Student's *t*-test (‡ $P < 0.01$).

Table 2. Effects of chronic vanadate treatment on the rates of lactate formation and glycogen synthesis at different concentrations of insulin in stripped soleus muscle preparations

Conc. of Insulin μunit/ml	Lactate formation (μmol/hr per g of muscle)		Glycogen synthesis (μmol glucosyl equiv./hr/g)	
	Control	Vanadate-treated	Control	Vanadate-treated
1	9.18 ± 0.45 (6)	7.49 ± 0.48 (9)*	1.93 ± 0.18 (7)	2.23 ± 0.79 (8)
10	9.00 ± 0.53 (9)	9.25 ± 0.64 (9)	2.19 ± 0.20 (8)	3.00 ± 0.26 (9)*
100	12.02 ± 0.72 (8)	11.38 ± 0.76 (7)	3.87 ± 0.23 (8)	4.70 ± 0.32 (7)*
1000	14.26 ± 0.69 (6)	11.92 ± 0.84 (5)	5.24 ± 0.36 (6)	5.17 ± 0.37 (5)
10000	13.51 ± 1.06 (6)	12.64 ± 0.49 (6)	5.64 ± 0.24 (8)	5.05 ± 0.48 (5)

The methods for measuring rates of lactate formation and glycogen synthesis are referenced in the Materials and Methods section. Results are presented as means ± SEM with number of observations in parentheses. Statistically significant differences for control vs vanadate-treated rats were determined using Student's *t*-test (* $P < 0.05$)

centration above 1 μunit/ml is shown graphically; from this plot it can be seen that the concentrations of insulin required to cause a half-maximal increase in the rates of glycolysis are about 85 and 25 μ unit of insulin/ml for soleus muscle strips from control and vanadate-treated rats respectively. Similar results were obtained for the effect of insulin concentrations on rates of glycogen synthesis (Table 2, Fig. 3); in this case the concentrations of insulin giving half-maximal effects were 90 and 25 μunits/ml for muscle from control and vanadate-treated rats respectively.

The fact that the responses to insulin of the rates of glycolysis and glycogen synthesis are similarly affected by chronic vanadate administration may indicate that a change in insulin binding properties has occurred. Insulin binding characteristics of soleus muscles from control and chronically vanadate-treated rats are shown in Table 3. No change in the number or affinity of insulin receptors is suggested by these data.

DISCUSSION

Many studies have demonstrated metabolic effects of vanadate *in vitro*: addition of millimolar concentrations of vanadate to incubations of isolated cells or muscle preparations produce similar effects on carbohydrate metabolism to addition of insulin [5–8]. However, few studies have investigated the effect of maintaining sub-toxic plasma levels of vanadate on carbohydrate metabolism *in vivo*. In the present study, chronic exposure to vanadate increased markedly the hypoglycaemic effect of a given dose of intravenously administered insulin (Fig. 1). In addition, the sensitivities of glucose transport (as measured by the rate of lactate formation [15, 16]) and glycogen synthase (indicated by the rate of glycogen synthesis) to insulin in isolated soleus muscle were increased (Figs. 2 and 3). Basal and

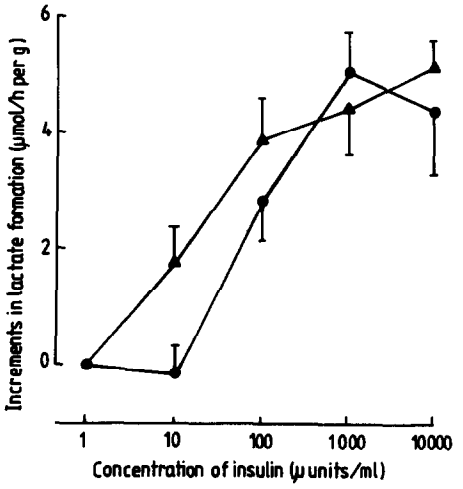

Fig. 2. Effect of insulin on the rate of lactate formation by incubated stripped soleus muscles. Muscle strips were prepared from control (●) and vanadate-treated (▲) rats. Each point is the mean of at least five separate incubations (see Table 1).

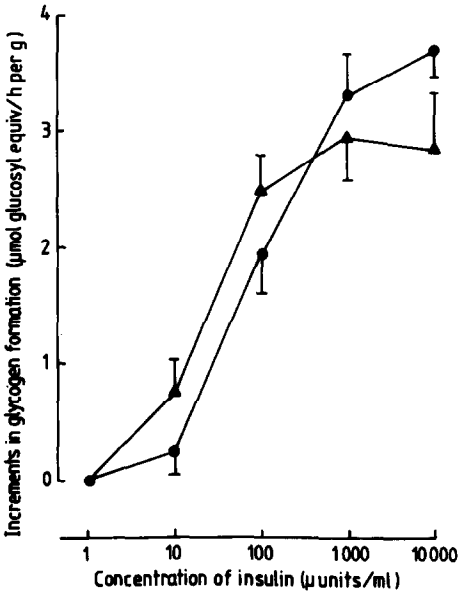

Fig. 3. Effect of insulin on the rate of glycogen synthesis by incubated stripped soleus muscles. Muscle strips were prepared from control (●) and vanadate-treated (▲) rats. Each point is the mean of at least five separate incubations (see Table 1).

Table 3. Effect of chronic vanadate treatment on insulin binding in stripped soleus muscle preparations

Insulin added to incubation medium (μunit/ml)	Insulin binding (c.p.m. ^{125}I bound/mg)		Specific insulin binding (fmol/mg protein)	
	Control	Vanadate-treated	Control	Vanadate-treated
4	110 ± 5	121 ± 6	0.23 ± 0.01	0.25 ± 0.01
13	97 ± 9	113 ± 8	0.61 ± 0.06	0.71 ± 0.07
50	88 ± 7	94 ± 9	2.17 ± 0.17	2.20 ± 0.21
250	78 ± 4	83 ± 6	8.62 ± 0.45	8.63 ± 0.62
2500	54 ± 2	53 ± 3	44.2 ± 1.67	35.9 ± 2.11
100000	27 ± 3	32 ± 3	—	—

Results are given as means ± SEM of at least three separate experiments. [^{125}I] insulin was added to each experiment to give approximately 100000 d.p.m./ml. It was therefore calculated that 3 μunit of insulin/ml was added to each incubation, this has been taken into account in the left-hand column of the table.

maximally insulin-stimulated rates of lactate formation and glycogen synthesis *in vitro* were similar in muscles from control and vanadate-treated animals indicating that insulin responsiveness was not changed (Figs. 2 and 3). Previously, it has been shown that manipulation of the skeletal muscle adenosine concentration *in vitro* has dramatic effects on the sensitivity (but not the responsiveness) of glucose transport to insulin [16, 23, 24], thus, by decreasing the adenosine concentration by addition of adenosine deaminase it is possible to increase sensitivity to insulin, whereas addition of adenosine-receptor agonists cause insulin resistance [16, 23]. However, the sensitivity of glycogen synthesis to insulin appears to be unaffected by changes in adenosine concentration [23, 24], thus, a decrease in the concentration of adenosine in the muscles of vanadate-treated rats would not fully account for the observed increase in sensitivity of glucose transport and glycogen synthesis to insulin demonstrated in this work.

Tamura *et al.* [8, 10] have reported that vanadate, like insulin [25], increases tyrosine phosphorylation of the β-subunits of the insulin receptor in adipocytes. It has been proposed [25, 26] that activation of the tyrosine kinase activity of the β-subunits of the insulin receptor, autophosphorylation [27], and tyrosine phosphorylation of a number of, as yet unidentified, cellular proteins [28, 29] are important initial events in the action of insulin. As would be predicted for a key regulatory process, a dynamic balance exists between tyrosine phosphorylation and dephosphorylation reactions [30]. It is possible that vanadate may exert its insulin-mimetic action by activation of insulin receptor β-subunit tyrosine kinase or by inhibition of a cellular phosphotyrosyl phosphatase activity. In this communication it has been shown in experiments using an *in vitro* soleus muscle preparation that prior chronic vanadate treatment of donor animals had no effect on rates of glucose uptake or glycogen synthesis *per se*, but markedly affected the ability of insulin to stimulate these processes; in addition, no change in insulin receptor number or affinity was detected suggesting that the effect of vanadate on insulin-stimulation cellular processes occurs distally to the insulin–receptor interaction. It is therefore tempting to speculate that the observed metabolic actions of chronic vanadate treatment are brought about by the ability of vanadate to inhibit phosphotyrosyl phosphatase activity. Consequently, vanadate may provide an important biochemical tool to probe the coupling of insulin receptor tyrosine kinase activation and subsequent modification of the activities of key regulatory enzymes, particularly as it has recently been reported [21, 22] that it is the insulin receptor tyrosine kinase which may be defective in a number of pathophysiological states.

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