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Vanadate potentiates the glycogenic action of insulin-like growth factors on isolated diaphragm

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Na_3VO_4 (6.5 μmol/100 g rat weight), co-injected with a trace amount of [^{14}C]glucose, increased within 15 min the incorporation of radiolabel in diaphragmal glycogen. After 2 h the vanadate-induced increases were 12-fold in the diaphragm and 7–8-fold in heart and liver. In contrast, when added to isolated diaphragms for up to 1 h, vanadate (0.1–5 mM) had no effect on the synthesis of glycogen from 5 mM glucose. In search of a putative mediator of vanadate's action in vivo, insulin and the insulin-like growth factors (IGFs) were considered. Their plasma concentration was not affected by vanadate treatment. In isolated diaphragms, 1 mM vanadate did not potentiate insulin-induced glycogen synthesis, but it caused a several-fold increase in glycogen synthesis in the presence of concentrations of IGF-I which, alone, had no effect. A similar synergism occurred between vanadate and IGF-II. We propose that the glycogenic action of vanadate in vivo, at least in some tissues, involves a potentiation of the action of IGF-I.

Vanadate; Insulin-like growth factor I; Insulin-like growth factor II; Insulin; Glycogen; Diaphragm

1. INTRODUCTION

Insulin and the insulin-like growth factors, I and II (IGF-I and IGF-II) are structurally related hormones with a similar spectrum of biological activities [1,2]. In general, though, insulin is more potent as a stimulator of anabolic pathways, whereas the IGFs are far more efficient in stimulating cell proliferation and differentiation. Nearly all the IGFs in the bloodstream are derived from the liver and are associated with inhibitory binding proteins, however, the IGFs are also synthesized in nearly all other tissues, where they serve an autocrine/paracrine function. There are distinct receptors for insulin, IGF-I and IGF-II on the plasma membrane, but their specificity is not absolute, except for the lack of binding of insulin to the IGF-II receptor [2]. Signal transduction by the IGF-I and insulin receptors probably involves activation of the tyrosine kinase activity of their β-subunit. The molecular function of the IGF-II receptor is still unknown.

Orthovanadate (VO_4^{3-}), metavanadate (VO_3^-), and vanadyl compounds (VO^{2+}) mimic several effects of insulin [3] when administered in vivo [4] or added to isolated adipocytes [5–9]. Many alterations in insulin-dependent and non-insulin-dependent diabetes, including hyperglycaemia and deficient glucose transport and glycogen synthesis, can also be corrected by chronic oral administration of vanadate or vanadyl salts [3,10–15]. The exact mechanism of these insulin-like effects still remains unclear [15]. Earlier data indicated that vanadate stimulated the autophosphorylation and the tyrosine kinase activity of the insulin receptor [6]. However, it has become clear that vanadate can bypass the insulin receptor. Thus, insulin-like effects have been obtained at concentrations of vanadate that do not affect the tyrosine kinase activity or the phosphorylation state of the insulin receptor [4,7,14]. Also, the efficiency of vanadate in stimulating glucose transport appears to be independent of the number of insulin receptors in the plasma membrane [8]. Further, vanadate does not exactly mimic all the effects of insulin in vitro [9,16–18]. In isolated hepatocytes, it can even produce effects opposite to insulin [19]. An exception should probably be made for peroxovanadates (formed by reaction of orthovanadate with H_2O_2) which have full insulin-like effects, perhaps via activation of the insulin-receptor tyrosine kinase [18,20].

We report here the results of a comparative study of the effects of vanadate and insulin on glycogen synthesis in vivo and in vitro. It appears that the stimulatory effect of vanadate, in diaphragm and probably also in liver, is indirect but not mediated by the insulin signaling pathway. Our results suggest a mediatory role of IGFs.

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2. EXPERIMENTAL

2.1. *Materials*

Human recombinant insulin (humulin regular) was obtained from Eli Lilly. Human recombinant IGF-I and IGF-II were gifts from Ciba-Geigy (Basel, Switzerland) and Eli Lilly (Indianapolis, IN, USA), respectively. Na_3VO_4 was purchased from Fisher Scientific and D-[U-^{14}C]glucose from Amersham.

2.2. *In vivo studies*

Overnight-fasted female Wistar rats of about 100 g were injected intraperitoneally with 0.5 ml saline containing 2 μCi of [^{14}C]glucose only, or plus either 6.5 μmol of Na_3VO_4 or 0.1 nmol of insulin. After 5, 20, 50 or 110 min, 8 mg of sodium pentobarbital was injected intraperitoneally. 10 min later the abdomen was opened and blood was collected from the portal vein in an heparinized tube. Then the liver, the diaphragm, and the heart were successively isolated and at once frozen in liquid N_2. A fragment was later weighed and dropped in 2 vol. of a solution containing 1 M NaOH and 1% (w/v) shellfish glycogen, which was heated for 30 min at 90°C.

2.3. *Isolated (hemi)diaphragms*

These were prepared with part of the rib cage attached from normally fed female Wistar rats of 60–80 g [21]. They were first preincubated for 2 × 15 min at 37°C in 12 ml (diaphragms) or 6 ml (hemidiaphragms) of Kreb's–Henseleit bicarbonate buffer plus 5 mM glucose. Subsequently, the (hemi)diaphragms were incubated in half the previous volume of the same medium, with or without vanadate (1 mM unless stated otherwise), and [^{14}C]glucose and hormones were added as indicated. After 10–60 min the (hemi)diaphragms were isolated from the attached ribs, weighed and digested as described above.

2.4. *Assays and statistics*

For the determination of [^{14}C]glycogen, 150 μl of the NaOH digest was spotted onto filter paper (Whatman ET31), which was washed 4 × 20 min in 66% (v/v) ethanol. The papers were then washed once in acetone, dried and counted. Plasma glucose was assayed by the glucose oxidase method [22]. Insulin, IGF-I and IGF-II were measured by radioimmunoassays. The antibodies used were a polyclonal guinea pig antiserum against recombinant human IGF-I [23] and a monoclonal mouse anti-IGF-II (Sera-Lab, Sussex, UK). Plasma binding proteins were removed by treatment with acid ethanol and acid acetone, respectively, for the IGF-I and IGF-II assays. It was checked with 20 samples that near-identical results were obtained when the IGFs were separated from their binding proteins by gel filtration on Superdex in an acid medium as described in [24].

The results are given as means ± S.E.M. for the indicated number (n) of observations. Statistical treatment was by Student's t-test for unpaired data.

3. RESULTS

Thirty minutes after an intraperitoneal co-injection of insulin and [^{14}C]glucose into rats, the amount of label incorporated into diaphragmal glycogen was 22-times higher than in the control condition (Table I). Ninety minutes later the amount of labeled glycogen had again increased fourfold in the diaphragm of the insulin-treated animals, in spite of the decreasing specific radioactivity of the plasma glucose. However, insulin failed to stimulate glycogen synthesis in liver and heart within the 2-h observation period. The incorporation of glucose into glycogen in the diaphragm was also stimulated by an injection of vanadate. This effect was already significant after 15 min and the label continued to be rapidly incorporated into glycogen for up to 2 h, when the amount of radioactive glycogen reached 40% of that deposited after insulin administration. Vanadate also stimulated by about 10-fold the incorporation of label from [^{14}C]glucose into glycogen in heart and liver, as measured after 1 h and 2 h, respectively.

The administration of vanadate caused a significant hypoglycaemia (−20%) after 15 min, which had turned into a mild hyperglycaemia (+20%) by 60 min (Table II). However, vanadate did not affect the circulating concentrations of insulin or of IGF-I. Adult rats have very little circulating IGF-II [2], and the detection level was not reached 15 min and 60 min after the administration of vanadate (not illustrated).

In view of the marked in vivo effects of vanadate and

Table I

Effect of an intraperitoneal injection of insulin or vanadate on the incorporation of label from [^{14}C]glucose into glycogen in various rat tissues

Tissue	Period of treatment (min)	Glucose incorporated into glycogen (cpm/mg of tissue)		
		Saline	Insulin	Vanadate
Diaphragm	15	1.2±0.2 (5)	–	4.6±0.8 (5)*
	30	1.7±0.4 (5)	37.2±12.9 (5)*	7.5±3.1 (5)*
	60	2.9±0.9 (14)	–	20.2±3.4 (16)*
	120	5.5±1.1 (5)	161.0±9.6 (4)*	64.5±8.0 (5)*
Heart	30	1.5±0.2 (5)	1.7±0.1 (5)	1.7±0.1 (5)
	60	1.0±0.3 (14)	–	10.7±1.5 (16)*
	120	1.7±0.3 (5)	1.6±0.2 (5)	11.4±1.5 (5)*
Liver	30	3.8±0.9 (5)	4.0±0.8 (5)	5.3±1.4 (5)
	60	1.8±0.3 (14)	–	3.0±0.4 (16)*
	120	3.8±0.5 (5)	2.2±0.4 (5)	31.3±4.0 (5)*

The details of the experiment are described in section 2. *$P < 0.01$ vs. saline-treated.

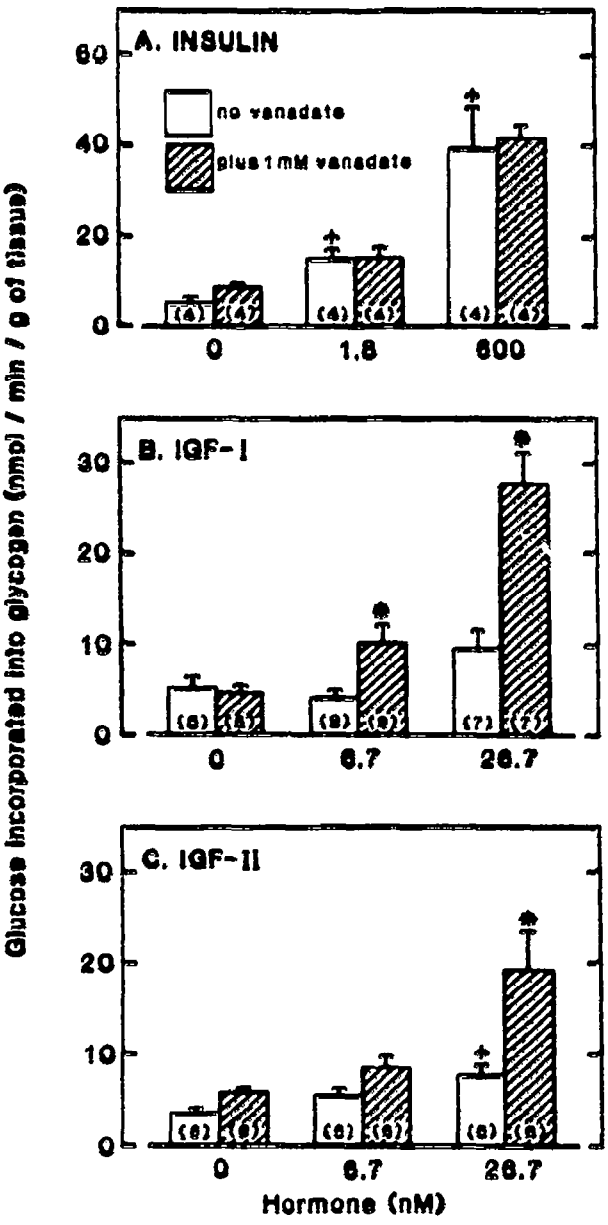

Fig. 1. The effects of insulin, IGF-I or IGF-II, without or with vanadate, on the rate of glycogen synthesis in isolated (hemi)diaphragms. (A) Hemidiaphragms were incubated for 1 h in 3 ml of medium with 5 mM glucose plus or minus 1 mM Na_3VO_4. After 50 min, 2 μCi of [^{14}C]glucose were added plus the indicated concentrations of insulin, and the hemidiaphragms were removed 10 min later. (B and C) Entire diaphragms were incubated for 1 h in 6 ml of medium containing 5 mM (4 μCi) [^{14}C]glucose, with or without 1 mM Na_3VO_4, and the indicated concentrations of IGF-I or IGF-II. Vertical bars represent +S.E.M. The number of observations is indicated between parentheses at the bottom of each column. $^*P \leq 0.04$ vs. absence of vanadate; $^*P \leq 0.012$ vs. absence of hormone.

insulin on the diaphragm, we have also compared their capacity to stimulate glycogen synthesis in isolated hemidiaphragms. While an exposure in vitro to 1.8 nM and 600 nM insulin for 10 min increased the incorporation of labeled glucose into glycogen 3-fold and 7-fold, respectively (Fig. 1A), the presence of 1 mM vanadate for 1 h did not affect the labeling of glycogen (Fig. 1A,B,C: $P = 0.06$; $n = 15$). Lower (0.1 mM) or higher (5 mM) concentrations of vanadate for 10–60 min were also ineffective (not illustrated). Neither did the presence of 1 mM vanadate modify the effect of either insulin concentration (Fig. 1A).

Fig. 1 illustrates also the results of experiments with two concentrations of IGF-I (panel B) and IGF-II (panel C). Neither concentration of IGF-I caused a significant increase in glycogen synthesis, but in the presence of 1 mM vanadate the rate was increased 2.3-fold and 6-fold by 6.7 nM and 26.7 nM IGF-I, respectively. A qualitatively similar pattern was noted for IGF-II, but here a significant synergism with vanadate required 26.7 nM IGF-II, which by itself doubled the rate of glycogen synthesis.

4. DISCUSSION

Sodium orthovanadate only stimulated the incorporation of [^{14}C]glucose into diaphragmal glycogen when it was administered in vivo (Fig. 1; Table I). This agrees with reports that orthovanadate is a very weak stimulator of glycogen synthesis when added to skeletal muscle preparations in vitro [16,18]. Moreover, we have found that vanadate increases hepatic glycogen synthesis in vivo (Table I), whereas it does not mimic the stimulatory effect of insulin on glycogen synthesis in cultured Hep-G2 cells (unpublished data). Rodriguez-Gil et al. [19] observed that vanadate even causes glycogenolytic changes in isolated hepatocytes. Taken together, these data indicate that the glycogenic effect of vanadate requires, at least in some tissues, a factor that is absent during incubation in vitro.

Our results eliminated insulin as a candidate for such a factor. Indeed, vanadate did not increase the plasma insulin level (Table II) and it did not enhance the action of insulin on the isolated diaphragm (Fig. 1A). In fact, our data are in keeping with the emerging conclusion that many metabolic effects of vanadate are not mediated by the insulin receptor (see Introduction).

It is noteworthy that in our studies in vivo the action of insulin was limited to the diaphragm, whereas vana-

Table II

Concentrations of glucose, insulin and IGF-I in the portal plasma at the indicated times after an intraperitoneal injection of vanadate

Vanadate treatment	Glucose (mM)	Insulin (pM)	IGF-I (nM)
0 min	5.0±0.1 (10)	148±17 (15)	82±4 (23)
15 min	4.0±0.2 (5)*	108±24 (5)	99±8 (10)
60 min	6.0±0.4 (5)*	178±17 (14)	88±5 (21)

$^*P < 0.001$, * 0.05 vs. 0 min.

date affected liver, diaphragm and heart (Table I). There is a striking parallel between these observations and the results of Jacob et al. [25] who studied the incorporation of labeled glucose into tissue glycogen in rats during a 3-h euglycaemic clamp while either insulin or IGF-I was infused; they found that IGF-I stimulated glycogen synthesis not only in skeletal muscle, but also in tissues where glycogen synthesis was not (liver) affected or was negatively (heart) affected by insulin. These striking similarities prompted us to consider whether IGF-I could be the factor required for the action of vanadate in vivo. The marked synergism between vanadate and IGF-I in vitro (Fig. 1B) is in agreement with this hypothesis. Vanadate also potentiated the action of IGF-II (Fig. 1C). This may seem irrelevant in view of the very low circulating levels of IGF-II in adult rats, but it has been pointed out that the rat is rather the exception in this regard [2].

Further experiments will be required to see whether the putative mediatory role of IGF-I also applies to other metabolic effects of vanadate. It also remains to be established how vanadate amplifies the action of IGF-I on glycogen synthesis in isolated diaphragms. Among the possibilities are an increased binding efficiency or capacity for IGF-I, and an increased tyrosine kinase activity of the β-subunit of the IGF-I receptor. Vanadate increases the number of IGF-II receptors, at least on adipocytes [26]. Finally, it is tempting to speculate on a role of IGF-I in the long-term effects of vanadate in diabetes [3,10–15].

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