

# Oral administration of vanadate to streptozotocin-diabetic rats restores the glucose-induced activation of liver glycogen synthase

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Isolated hepatocytes from streptozotocin-diabetic rats failed to respond to a glucose load with an activation of glycogen synthase. This lesion was associated with severely decreased activities of glycogen-synthase phosphatase and of glucokinase. All these defects were abolished after consumption for 13–18 days of drinking water containing  $\text{Na}_3\text{VO}_4$  (0.7 mg/ml), and they were partially restored after 3.5 days, when the blood glucose concentration was already normalized. In all conditions the maximal extent of activation of glycogen synthase in cells closely paralleled the activity of glycogen-synthase phosphatase.

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## INTRODUCTION

Vanadate is a potent effector of many enzymes, including ion-transport ATPases, phosphotyrosyl-protein phosphatases and adenylate cyclase (see [1,2]). Addition of vanadate to isolated cells or tissues mimics many [2–9], but not all [10,11], metabolic effects of insulin. Although vanadate can stimulate the tyrosine kinase activity of the insulin receptor [4], recent data rather indicate that the insulin-like effects of vanadate are elicited at a post-receptor level [5,7,8].

Heyliger *et al.* [12] for the first time showed that the blood glucose concentration in insulin-dependent diabetic rats was almost normalized after oral administration of vanadate for several weeks. The normalization of the glycaemia is associated with a restoration of the hepatic 6-phosphofructo-2-kinase and glucokinase activities [13], as well as the fructose 2,6-bisphosphate and glycogen contents [13,14]. It is not clear whether or not the deposition of glycogen in skeletal muscle is also restored [14,15].

A few days after the onset of insulin-dependent diabetes, isolated hepatocytes or perfused livers cease to respond to an increased glucose concentration with an activation of glycogen synthase [16–18]. This lesion has been explained by a loss of the glycogen-bound synthase phosphatase activity, and both defects can be slowly reversed by the administration of insulin [16,19]. We report here that vanadate mimics the effects of insulin on the activity of synthase phosphatase and on the response of glycogen synthase to a glucose load in livers from streptozotocin-diabetic rats.

## EXPERIMENTAL

### Materials

$\text{Na}_3\text{VO}_4$  and streptozotocin were purchased from Fisher Scientific and Sigma Chemical Co. respectively. Ketamine hydrochloride was obtained from Parke-Davis. Purified glycogen synthase from dog liver was a pool of the  $b_2$  and  $b_3$  forms [20].

### Handling of animals and livers

Male Sprague-Dawley rats of about 250 g were injected intravenously with either streptozotocin (60 mg/kg) dissolved in 50 mM-sodium citrate (pH 4.5) plus 150 mM-NaCl, or with sol-

vent only. One week later, blood was collected from the tail vein of the streptozotocin-injected rats and deproteinized with  $\text{ZnSO}_4$  and  $\text{Ba(OH)}_2$  for the assay of glucose. Only diabetic rats with a blood glucose concentration exceeding 3.5 mg/ml were retained for the experiments. From this time until death (either 3.5 or 13–18 days later), both the control and the diabetic animals received drinking water with NaCl (5 mg/ml) with or without  $\text{Na}_3\text{VO}_4$  (0.7 mg/ml). Glycosuria was checked daily in all diabetic rats.

On the morning of the final day, blood was collected from a sublingual incision and deproteinized. The animals were then anaesthetized with ketamine. At the beginning of each liver perfusion, the large median lobe was ligated and removed. Part of this lobe was immediately homogenized in 4 vol. of an ice-cold buffer containing 50 mM-imidazole (pH 7.4), 0.5 mM-dithiothreitol and 0.25 M-sucrose, for the assay of synthase phosphatase; the remainder was freeze-clamped and stored at  $-80^\circ\text{C}$  for the assay of glucokinase. Hepatocytes were isolated from the remaining liver and incubated as described in ref. [17]. The cells were preincubated for 10 min with 2 mM-glucose before addition of 60 mM-glucose. At the indicated times a sample of the cell suspension was added to a mixture of inhibitors of protein kinases and phosphatases, and frozen in liquid  $\text{N}_2$  [17] for the assay of glycogen synthase.

### Assays

Glycogen synthase ( $a$  and  $a+b$ ) was assayed as in [17]. The activity of synthase phosphatase was determined in fresh liver homogenates at a final concentration of 2% (w/v), from the rate of activation of purified liver synthase  $b$  [20]. The hepatic synthase phosphatase activity in treated rats is expressed as a percentage of that of a control animal that was included each day. Glucose and glucokinase were measured as described in ref. [13]. One unit of enzyme converts 1  $\mu\text{mol}$  of substrate into product/min under the appropriate assay conditions.

## RESULTS AND DISCUSSION

Addition of 60 mM-glucose to isolated hepatocytes from normal rats resulted in a rapid activation of glycogen synthase, which was close to 80% conversion within 10 min (Fig. 1). The

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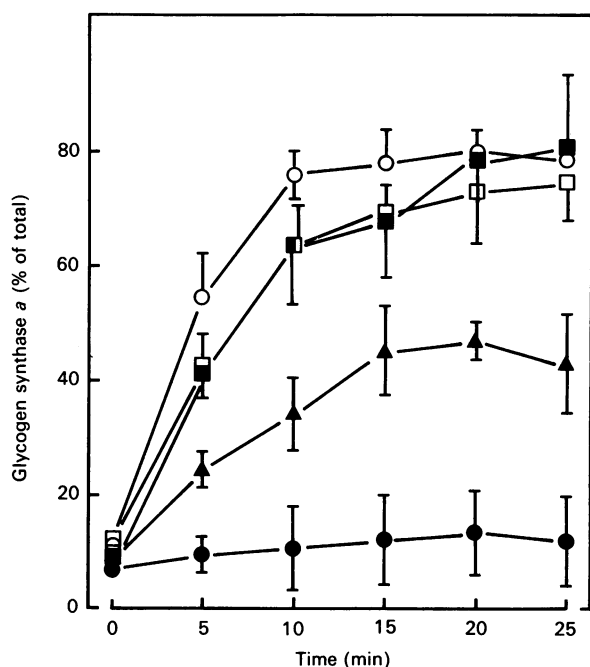


Fig. 1. Effect of oral vanadate administration on the response of glycogen synthase to glucose in isolated hepatocytes from normal and diabetic rats

Liver cells were isolated from control rats (○), normal rats treated with vanadate during 13–18 days (□), diabetic rats (●), and diabetic rats treated with vanadate for 3.5 (▲) or 13–18 days (■). Each group comprised 4–6 animals. Hepatocytes were incubated at 37 °C. At 0 min the glucose concentration was raised from 2 to 62 mM, and at the indicated time intervals samples were taken for the assay of glycogen synthase. Vertical bars represent  $\pm$  S.E.M. Total glycogen synthase, measured in control liver homogenates, was  $1.29 \pm 0.17$  units/g of liver; the values in the other experimental groups were not significantly different ( $P > 0.1$ ).

response was somewhat slower in the group of rats that had consumed vanadate during 13–18 days. In liver cells from streptozotocin-diabetic rats glycogen synthase was hardly activated at all in the presence of 60 mM-glucose. This is in keeping with earlier results in alloxan-diabetic Wistar rats [17] and in spontaneously diabetic BB rats [18]. However, the response to glucose improved after oral administration of vanadate to the diabetic rats (Fig. 1). The restoration was partial after 3.5 days and virtually complete after 13–18 days.

The glycogen-synthase phosphatase activity in dilute liver homogenates from the diabetic rats amounted to only 15% of the activity in control rats (Table 1). Treatment of the diabetic rats with vanadate increased the synthase phosphatase activity, to

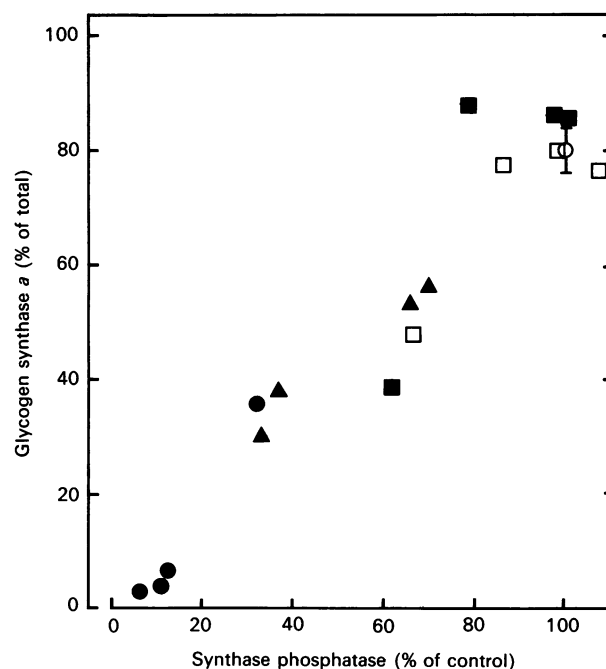


Fig. 2. Correlation between the activity of glycogen-synthase phosphatase and the maximal activation of glycogen synthase in isolated hepatocytes

The activity of synthase phosphatase was plotted against the mean activity of glycogen synthase, recorded during the 15–25 min interval after addition of 60 mM-glucose to hepatocytes isolated from the same liver. For the control group synthase is expressed as the mean  $\pm$  S.E.M. Symbols are as in Fig. 1. Correlation coefficient  $r = 0.95$  ( $P = 0.0001$ ).

52% and 85% of the control activity after 3.5 and 13–18 days respectively. The glucokinase activity was restored in a similar way, but with a higher degree of variability after 3.5 days. In contrast, the blood glucose concentration was already normalized 3.5 days after the initiation of the treatment.

Administration of vanadate to non-diabetic rats (Table 1) did not significantly affect the synthase phosphatase activity or the glycaemia, but it resulted in a 25% lower glucokinase activity ( $P = 0.006$ ). This may be related to a lesser intake of food by the vanadate-treated animals (specifically, 35% less during the 2 days before the experiment;  $P = 0.022$ ), and to significantly lower plasma insulin levels [12,21,22].

It has been proposed that the impaired glycogenic response of the diabetic liver to glucose results from the decreased activity of glycogen-synthase phosphatase [16,18,19]. This hypothesis is further strengthened by the finding of an excellent linear cor-

Table 1. Effect of a treatment with vanadate on the blood glucose concentration and on the hepatic activities of glycogen-synthase phosphatase and glucokinase

The results are presented as means  $\pm$  S.E.M. for the numbers of animals in parentheses. The synthase phosphatase activity in the control group was  $150 \pm 43$  m-units/g of liver.

Experimental group (n)	Synthase phosphatase (% of control)	Blood glucose (mg/ml)	Glucokinase (units/g of liver)
Control (6)	100	$0.98 \pm 0.03$	$2.13 \pm 0.13$
Control + 13–18 days vanadate (4)	$90.0 \pm 8.5$	$0.91 \pm 0.06$	$1.61 \pm 0.05$
Diabetic (4)	$15.5 \pm 5.7$	$4.52 \pm 0.43$	$0.05 \pm 0.05$
Diabetic + 3.5 days vanadate (4)	$51.5 \pm 9.6$	$0.83 \pm 0.15$	$1.55 \pm 0.63$
Diabetic + 13–18 days vanadate (4)	$84.5 \pm 8.8$	$1.11 \pm 0.11$	$1.61 \pm 0.11$

relation between the activity of synthase phosphatase and the maximal extent of the synthase activation in isolated hepatocytes (Fig. 2). A less good correlation ( $r = 0.68$ ;  $P = 0.004$ ) existed also between the latter parameter and the activity of glucokinase (results not shown).

The above results clearly show that vanadate, like insulin [19], can restore the glycogenic response of isolated hepatocytes from diabetic rats to glucose. Like insulin [23], vanadate also increases acutely (by about 25%) the synthase phosphatase and phosphorylase phosphatase activities in the isolated perfused liver from normal rats [24]. Further exploration of the mechanism of these vanadate effects may shed light on the link(s) between the activation of the insulin receptor and the ultimate metabolic effect.

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