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Vanadyl sulphate differently influences insulin response to glucose in isolated pancreas of normal rats after in vivo or in vitro exposure

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Abstract

The effect of the antidiabetic agent vanadyl sulphate ($VOSO_4$) on the endocrine pancreas function of normal rats was studied using the isolated pancreas preparation. A short-term (8 days) i.p. treatment (15 mg/kg per day) resulted in attenuation of high glucose-stimulated insulin release, at day 9 but also at days 19, i.e., after full recovery of appetite and weight, while blood and pancreas vanadium concentrations were still elevated. Six months of oral $VOSO_4$ treatment (0.75 mg/ml in drinking water) resulted in elevated vanadium concentrations while glucose-stimulated insulin release was attenuated as compared to pair-fed animals. Conversely, when directly perfused in pancreas, $VOSO_4$ potentiated glucose-stimulated insulin release. These apparently opposite effects may be related to the ability of $VOSO_4$ to exert both peripheral insulinomimetic effects – leading to chronic reduction in insulin demand –, and a direct pancreatic insulinotropic activity.

Keywords: Vanadyl sulphate; Endocrine pancreas; Insulin secretion; (Rat)

1. Introduction

Vanadium derivatives have been shown to possess insulin-like properties and antidiabetic activities (Brichard and Henquin, 1995). In vitro effects include activation of glucose transport in adipocytes (Shechter and Karlish, 1980), stimulation of glycolysis or inhibition of lipolysis (Rodriguez-Gil et al., 1991) and stimulation of lipid synthesis (Aguis and Vaartjes, 1982; Castro et al., 1984). In vivo studies have shown that the administration of vanadium under the forms of vanadyl, vanadate or pervanadate, corrects hyperglycaemia in both streptozotocin-diabetic rat (Heyliger et al., 1985; Ramanadham et al., 1989b; Shisheva et al., 1994) and genetically diabetic *db/db* mice (Pugazhenthi et al., 1991), reduces the requirement of insulin in a model of type I diabetes, the BB rat (Ramanadham et al., 1990) and improves glucose tolerance in senescent animals (De Tata et al., 1993).

Vanadyl sulphate ($VOSO_4$) administered in drinking water was also reported to exert antidiabetic effects for 13–20 weeks after cessation of treatment (Ramanadham et al., 1989a; Dai et al., 1994; Cam et al., 1995). Furthermore, we have recently observed that an 8-day i.p. $VOSO_4$ treatment of streptozotocin-diabetic rats induced a long-term correction of diabetes associated with a partial preservation of the glucose-induced insulin response from the isolated perfused pancreas (Poucheret et al., 1995). This protective effect of $VOSO_4$ could be due to a direct influence of the metal on the endocrine pancreatic function. This has not been investigated so far, although it is known that in non-diabetic animals, vanadium treatment lowers plasma insulin concentrations without affecting blood glucose levels.

The aim of the present study was to investigate a possible pancreatic effect of $VOSO_4$ in normal rats. Using the isolated perfused rat pancreas preparation, we compared the effects of $VOSO_4$, administered either in vivo under short- or long-term treatment conditions, or in vitro upon direct infusion in the preparation, on glucose-induced insulin secretion.

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2. Materials and methods

2.1. Isolated perfused pancreas preparation

Pancreases were isolated from male Wistar rats of 250–300 g body weight under pentobarbital anaesthesia (60 mg/kg i.p.). Blood samples were collected from anaesthetized animals for measurement of plasma glucose and insulin levels.

The technique of Loubatières et al. (1969) was used to isolate the pancreas from the neighbouring tissues. The organ was then transferred into a plastic chamber maintained at 37.5°C. Perfusion medium was Krebs-Ringer bicarbonate buffer containing 2 g/l bovine serum albumin and 5 mM glucose, and continuously bubbled with a mixture of 95% O_2/5% CO_2. Infusion pressure was selected to provide a pancreatic outflow of 2.5 ml/min.

The first sample was taken 30 min after initiation of perfusion. Two more samples were collected 10 and 15 min later; glucose concentration was increased from 5 to 11 mM to test the insulin response. Samples were then collected every min for 5 min, then every 5 min for 15 min. Glucose concentration was then reduced to 5 mM and two more samples were taken. All samples were collected for 1 min allowing determinations of pancreatic effluent output, and were immediately frozen for insulin assay. Insulin output rate was calculated by multiplying the hormone concentration (ng/ml) in the effluent by the corresponding flow rate (ml/min).

2.2. Experimental protocols

Glucose insulin responses of isolated pancreas were evaluated (a) after $VOSO_4$ in vivo administration and (b) upon in vitro $VOSO_4$ infusion.

2.2.1. In vivo treatments

2.2.1.1. Experiment 1. Male rats were treated with an i.p. $VOSO_4 \cdot 5H_2O$ daily dose of 15 mg/kg for 8 days ($VOSO_4$-treated). At this dose, $VOSO_4$ was previously shown to promote in diabetic animals long-term normoglycaemia after cessation of treatment (Poucheret et al., 1995), and to lower plasma insulin levels in normal rats without hypoglycaemia or mortality. Insulin secretory capability was tested at the end of treatment (day 9) or 10 days after treatment withdrawal (day 19). One group of food-restricted animals for which the daily amount of food was adjusted to that eaten by $VOSO_4$-treated rats and one group of control animals were included in this study.

2.2.1.2. Experiment 2. Male rats were given $VOSO_4 \cdot 5H_2O$ in drinking water for 180 days at a concentration (0.75 mg/ml) previously shown to be administrable for long periods of time and able to correct diabetes in streptozotocin animals (Dai et al., 1994), decrease insulin requirement in BioBred (BB) insulin-dependent diabetic rats (Battell et al., 1992), or lower blood pressure in spontaneously hypertensive (SHR) animals (Bhanot and McNeill, 1994). A group of food-restricted animals (amount of food adjusted to that eaten by $VOSO_4$-treated rats on a weekly basis) was followed in parallel.

The effects of treatments on food and fluid intakes, body growth and plasma glucose and insulin levels as well as blood concentration of vanadium were evaluated for each set of experiments.

2.2.2. In vitro experiments

$VOSO_4$ at the concentration of 1 μM was added to the perfusion buffer of isolated pancreas preparation from 45 min after initiation until the end of perfusion, i.e., in the presence of 5 mM glucose for 15 min, 11 mM glucose for 20 min and again 5 mM glucose for 10 min. The concentration of $VOSO_4$ chosen was compatible with the blood vanadium levels measured after in vivo $VOSO_4$ treatment and proved to be ineffective on the pancreatic vascular flow rate (not shown).

2.3. Assays

2.3.1. Determination of insulin levels in plasma and isolated pancreas effluent

Insulin concentrations were measured by the method of Herbert et al. (1965) using an antibody supplied by Miles Laboratories (Paris, France). [^{125}I]Insulin was obtained from International CIS (Gif-sur-Yvette, France) and standard rat insulin from Novo (Copenhagen, Denmark). The intra- and inter-assay coefficients of variation were respectively 9% and 13.5%. The analytical sensitivity was 0.1 ng/ml.

2.3.2. Determination of vanadium levels

Vanadium levels in blood and pancreas were measured by atomic absorption spectrophotometry, according to a previously described method (Mongold et al., 1990), with the difference that argon instead of nitrogen was used as purge gas.

2.3.3. Determination of glycaemia

Plasma glucose concentrations were determined by the glucose-oxidase method (Trinder, 1969).

2.4. Expression and statistical analysis of results

Data are expressed as mean ± S.E.M. All the results were submitted to analysis of variance followed by the multiple comparison test (Zar, 1974). $P \leq 0.05$ was viewed as statistically significant. The areas under the curves (AUC) of insulin secretory response to 11 mM glucose were calculated by the trapezoidal rule.

2.5. Materials

Krebs-Henseleit buffer components and $VOSO_4 \cdot 5H_2O$ were from Prolabo (France).

3. Results

3.1. In vivo treatment with $VOSO_4$

3.1.1. General feature of the animals

3.1.1.1. Experiment 1. Table 1 indicates body weight changes as well as food and fluid intakes of control, food restricted and $VOSO_4$-treated rats during the experimental period. $VOSO_4$ treatment was associated with impairment of growth and decrease in food intake. A similar body weight loss was found in food-restricted animals. After treatment, $VOSO_4$-treated and food-restricted rats resumed normal feeding and their weight gain was higher than that of control rats, so that 19 days later, body weights were not significantly different between control (362.2 ± 6.9 g), $VOSO_4$-treated (357.0 ± 11.8 g) or food-restricted (360.2 ± 5.9 g) groups.

Blood glucose was unchanged by treatment and averaged 8.2 mM throughout the experimental period. Plasma insulin levels measured at sacrifice were significantly lower ($P \leq 0.05$) in $VOSO_4$-treated or food-restricted than in control animals at day 9 (4.0 ± 0.5, 1.4 ± 0.4, and 0.8 ± 0.3 ng/ml in control, $VOSO_4$-treated and food-restricted rats, respectively). At day 19, plasma insulin was significantly lower in $VOSO_4$-treated (2.0 ± 0.3 ng/ml) than in control

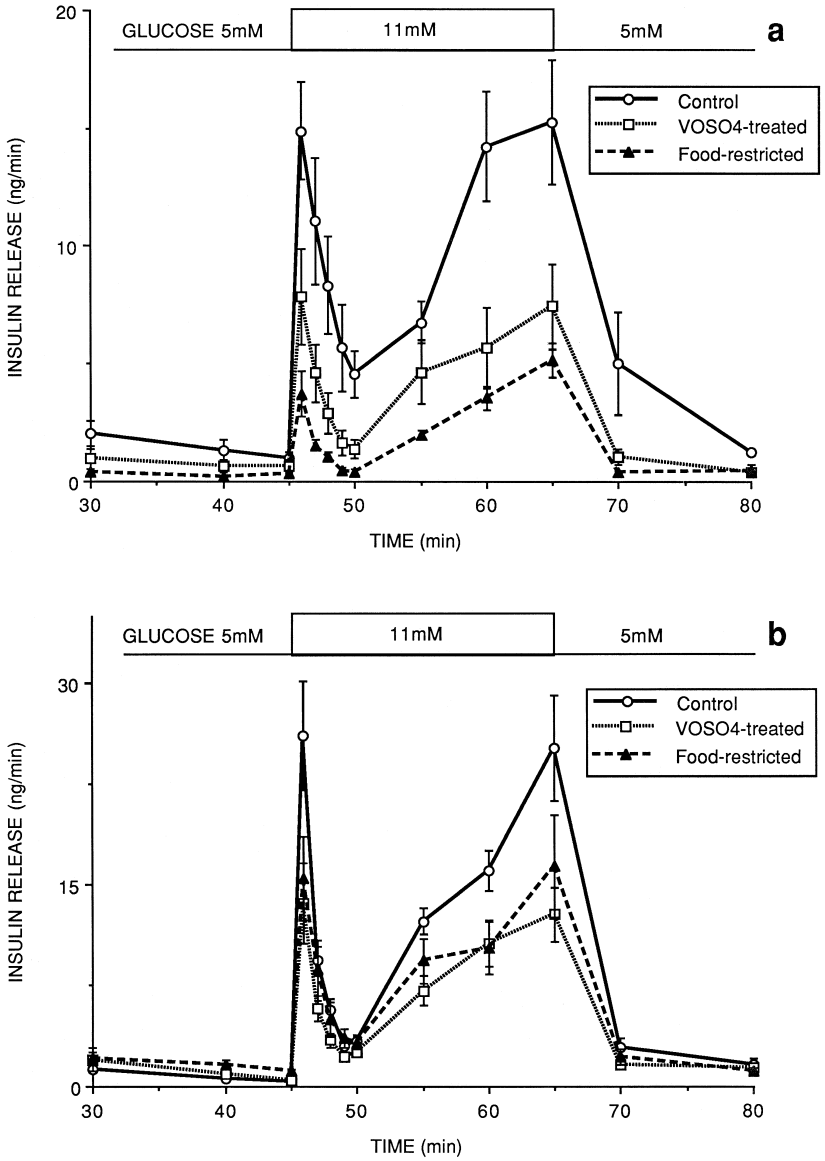

Fig. 1. Effect of 8-day i.p. $VOSO_4 \cdot 5H_2O$ (15 mg/kg per day) treatment on insulin response to glucose from isolated perfused pancreas of normal rats, evaluated (a) at termination or (b) 10 days after the end of the treatment. Insulin responses of food-restricted and control animals are shown. Values are means ± S.E.M. of six experiments.

Table 1
General feature of rats treated with i.p. $VOSO_4$ for 8 days and of the corresponding food-restricted or control animals (Experiment 1)

Days	1–8			9–19		
	Weight gain (g/rat per day)	Food intake (g/day per rat)	Fluid intake (ml/day per rat)	Weight gain (g/rat per day)	Food intake (ml/day per rat)	Fluid intake (g/day per rat)
Control	+5.5	25.9	27.1	+3.8	25.8	25.9
(6)	±0.4	±0.6	±1.3	±0.4	±0.4	±2.1
$VOSO_4$-treated	−2.5 [a]	15.3 [a]	27.7	+7.6 [a]	26.1	30.3
(6)	±1.4	±1.7	±1.1	±1.2	±1.1	±0.8
Food-restricted	−3.0 [a]	(1)	28.8	+9.2 [a]	(1)	29.3
(6)	±0.6		±1.6	±1.0		±1.9

Number of animals are indicated in parentheses. Values are mean ± S.E.M. [a] Significantly different from control group. Day 1 corresponds to the first day of treatment. (1) Amount of food adjusted to $VOSO_4$-treated group.

animals (5.7 ± 1.0 ng/ml), and was not significantly different from control or $VOSO_4$-treated in food-restricted (3.7 ± 0.6 ng/ml) rats.

3.1.1.2. Experiment 2. Table 2 indicates body weight changes as well as food and fluid intakes of food-restricted and $VOSO_4$-treated rats during the experimental period. While food intake and growth were similar between the two groups, fluid intake was reduced in $VOSO_4$-treated as compared to food-restricted animals. Daily intake of vanadium averaged 3.5 mg/day in $VOSO_4$-treated animals. Glycaemia (12.1 ± 1.3 mM for food-restricted vs. 9.6 ± 0.8 mM for $VOSO_4$-treated rats, respectively) and insulinaemia (2.9 ± 0.6 for food-restricted vs. 2.9 ± 0.8 ng/ml for $VOSO_4$-treated rats, respectively) measured at sacrifice were not significantly different between groups.

3.1.2. Effect of in vivo $VOSO_4$ treatments on glucose-stimulated insulin release in the isolated perfused pancreas

Increment in glucose concentration from 5 to 11 mM resulted in a clear biphasic response of insulin secretion which peaked after 1 min of high glucose, achieved the minimum after 5 min and then rose again until the end of high glucose infusion (Figs. 1, 2 and 4).

3.1.2.1. Experiment 1 (Fig. 1). In $VOSO_4$-treated rats, glucose-stimulated insulin release was clearly attenuated with respect to controls with corresponding AUCs being significantly lower than in control animals both at day 9 (105 ± 19 and 197 ± 16 ng × 20 min in $VOSO_4$-treated and controls, respectively) and day 19 (154 ± 23 and 259 ± 15 ng × 20 min in $VOSO_4$-treated and controls, respectively). In food-restricted animals, an attenuation of response was also observed at day 9, with the corresponding AUC (49 ± 5 ng × 20 min) being significantly lower than in control or $VOSO_4$-treated rats. At day 19, AUC in food-restricted rats (195 ± 29 ng insulin × 20 min) was still lower than in controls but not significantly different from $VOSO_4$-treated rats.

3.1.2.2. Experiment 2 (Fig. 2). After 6 months of treatment, the insulin response was lower in $VOSO_4$-treated than in food-restricted animals. Indeed, corresponding 11

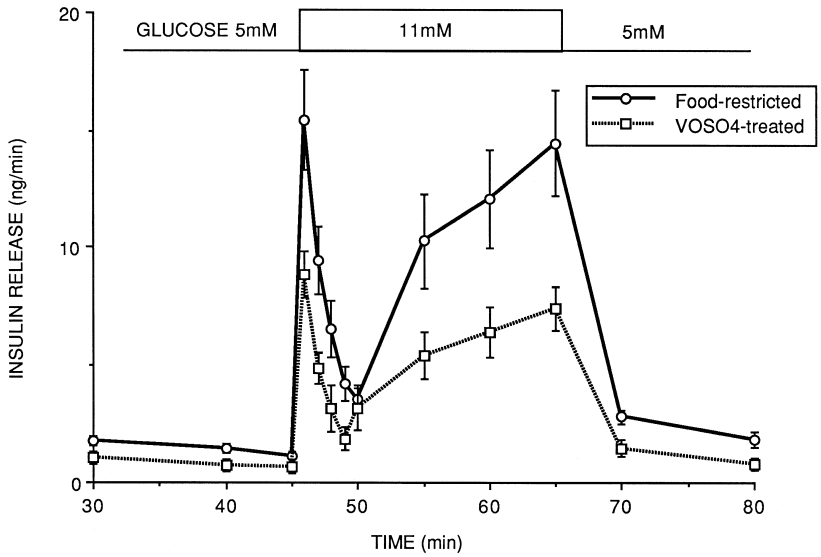

Fig. 2. Effect of 180-day oral $VOSO_4 \cdot 5H_2O$ (0.75 mg/ml in drinking water) treatment on insulin response to glucose from isolated perfused pancreas of normal rats. Results are compared to food-restricted rats. Values are means ± S.E.M. of six experiments.

Table 2
General feature of rats treated with oral $VOSO_4$ for 180 days ($VOSO_4$-treated) and of the corresponding food-restricted controls

	Weight gain (g/rat per day)	Food intake (g/day per rat)	Fluid intake (ml/day per rat)
Food-restricted (6)	+1.3 ±0.7	25.5 ±0.1	32.1 ±0.7
$VOSO_4$-treated (6)	+1.2 ±0.1	25.9 ±0.4	23.4 [a] ±0.7

Number of animals are indicated in parentheses. Values are mean ± S.E.M.
[a] Significantly different from food-restricted group.

mM glucose AUCs were 195 ± 31 and 106 ± 17 ng × 20 min for food-restricted and $VOSO_4$-treated rats, respectively ($P \leq 0.05$).

3.1.3. Effect of in vivo $VOSO_4$ treatments on vanadium blood or pancreas levels (Fig. 3)

In untreated animals, vanadium blood or pancreas levels were slightly higher than the detection limit and similar between untreated controls, 8-day or 180-day food-restricted groups.

After an 8-day i.p. $VOSO_4$ treatment, vanadium levels were dramatically elevated as compared to non-treated animals. At day 19, blood and pancreas levels were still 10- and 3.5-fold more elevated than in non-treated animals. Similar elevated levels were found after 6 months of oral $VOSO_4$ treatment.

3.2. Effect of in vitro $VOSO_4$ infusion on the perfused pancreas (Fig. 4)

Direct $VOSO_4$ infusion at the concentration of 1 μM did not modify insulin secretion in the presence of 5 mM glucose but induced a marked enhancement of the response to 11 mM glucose. Thus corresponding 11 mM glucose AUCs were 187 ± 49 ng × 20 min in control vs. 472 ± 84 ng × 20 min in $VOSO_4$-perfused pancreas ($P \leq 0.01$).

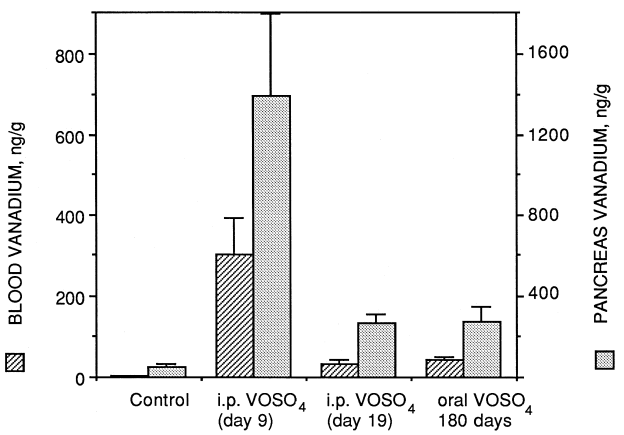

Fig. 3. Effect of $VOSO_4$ treatments on blood and pancreas vanadium levels. Vanadium was measured at termination of an 8-day i.p. $VOSO_4 \cdot 5H_2O$ (15 mg/kg per day) treatment (i.p. $VOSO_4$, day 9) or 10 days after (i.p. $VOSO_4$, day 19), and at termination of an oral 180-day $VOSO_4 \cdot 5H_2O$ (0.75 mg/ml in drinking water) treatment (oral $VOSO_4$, 180 days). Controls correspond to non-$VOSO_4$-treated animals.

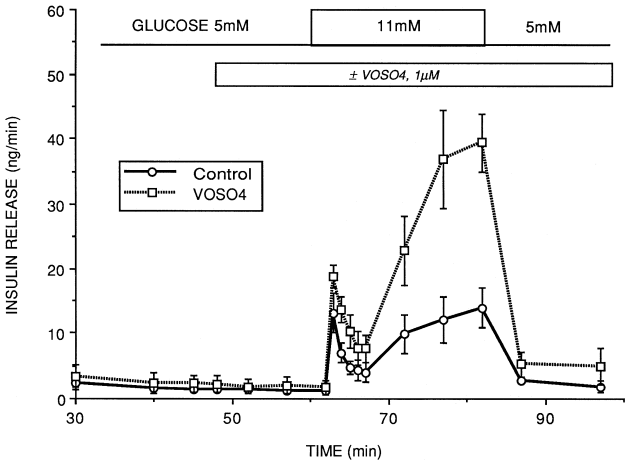

Fig. 4. Effect of $VOSO_4$ (1 μM) perfused in isolated pancreas of normal rats on insulin response to glucose. Control experiments are shown. Values are means ± S.E.M. of six experiments.

4. Discussion

Previous studies have shown that, in addition to their in vitro insulinomimetic properties, vanadium derivatives are able to correct hyperglycemia in various experimental models of diabetes. Our own data, obtained on 8-day streptozotocin-diabetic rats, indicated that short-term (8-day) i.p. treatment with $VOSO_4$ alone or in association with insulin was able to induce a long-term period of euglycaemia (Poucheret et al., 1995). In addition, isolated pancreas from long-term corrected animals retained some insulin-secreting capacity, indicating that $VOSO_4$ treatment could positively influence endocrine pancreatic function in diabetic animals.

In order to assess the influence of $VOSO_4$ treatment on endocrine pancreas, we therefore studied the insulin response to glucose in the isolated perfused pancreas from non-diabetic rats under short- and long-term $VOSO_4$ treatment conditions, and compared the responses obtained to that following direct $VOSO_4$ perfusion of pancreas from untreated animals. An original finding of our work is that after chronic $VOSO_4$ administration a decreased insulin response to glucose was obtained, whereas an increase in β-cell response to glucose occurred when $VOSO_4$ was directly perfused into the pancreas.

An 8-day i.p. treatment of non-diabetic rats with a dose of $VOSO_4$ previously shown to promote long-term correction of diabetes (Poucheret et al., 1995) induced a decrease in the amplitude of the pancreatic insulin response to glucose, as well as a decrease in plasma insulin levels. These effects were still apparent 10 days after the end of

treatment, i.e., at a time when treated animals had recovered normal feeding and body weight. Malabu et al. (1994) recently suggested that the antidiabetic activity of vanadate was entirely attributable to the anorectic effect of the metal. In order to determine the role of anorexia in the reduction of insulin secretion induced by the $VOSO_4$ treatment, a food-restricted animal group was included in the present study. Surprisingly, we observed that an 8-day food restriction period induced an even more important reduction in insulin secretion than that observed with $VOSO_4$ treatment, indicating that drastic food reduction has an immediate effect on insulin secretory responsiveness. However, the effect of food reduction was less long-lasting than that of $VOSO_4$. Indeed, 10 days later, the insulin response of food-restricted animals was not significantly different whereas that of $VOSO_4$-treated rats was still significantly lower than that of controls. The persistence of elevated levels of vanadium in blood and pancreas at distance of treatment extends to normal rats our previous results obtained in diabetic animals (Poucheret et al., 1995) and may explain the prolonged activity of $VOSO_4$.

In order to further explore the role of food reduction in the effect of $VOSO_4$, we decided to initiate a chronic oral treatment (0.75 mg/ml in drinking water) which could be prolonged for extended periods of time and associated with slight food reduction (10–15%, Bhanot and McNeill, 1994). After 6 months, a reduction of insulin response to glucose could still be recorded in chronic $VOSO_4$-treated as compared to pair-fed animals.

Taken together, our results indicate that although drastic reduction in food intake has a significant impact on insulin secretion, the modifications of insulin secretion occurring upon $VOSO_4$ treatment are not entirely attributable to an anorectic effect. Other possible mechanisms of the reduced β-cell response may include (a) the long-term consequences of the chronic low insulin demand resulting from the peripheral insulinomimetic action of vanadium or (b) a proper inhibitory effect on insulin secretion of the elevated blood or pancreatic levels of the metal.

Surprisingly, a potentiation of glucose-stimulated insulin secretion was obtained when $VOSO_4$ was directly infused in the pancreas at a concentration compatible with vanadium blood levels measured under in vivo $VOSO_4$ treatment conditions. This result suggests that the decreased insulin response obtained in $VOSO_4$-treated animals may rather be the consequence of a chronic low insulin demand than that of a proper inhibitory effect of vanadium, although chronic (vs. single) pancreatic exposure to the metal may induce additional changes in β-cells. It should be emphasized, however, that in vitro potentiation occurred at high (11 mM) glucose concentration, but not under basal (5 mM) 'normoglycaemic' conditions, suggesting that vanadyl might somehow interfere with glucose metabolism and be of potential interest in the treatment of non insulin-dependent diabetes.

Other in vitro studies of the effect of vanadium salts on insulin secretion have been performed on isolated islets only and produced inconsistent data. Fagin et al. (1987) showed that sodium vanadate stimulates insulin release in a concentration-dependent manner (0.5–1 mM) and inhibits islet Na^+/K^+-ATPase activity. On the other hand, Voss et al. (1992) found an inhibition of insulin secretion and biosynthesis in islets exposed to sodium orthovanadate (1–50 μM). Zhang et al. (1991) observed that in isolated islets vanadate did not induce insulin release but markedly potentiated glucose-stimulated insulin secretion. Our results seem therefore in agreement with Zhang's data although further investigations are required to elucidate the mechanism of the potentiating effect of vanadium on glucose-induced insulin release.

In summary, our results showed that while in vivo chronic exposure to $VOSO_4$ results in a decreased responsiveness of β-cells, in vitro direct intrapancreatic administration provokes in contrast an enhanced insulin response to glucose. These apparently opposite effects are probably related to the ability of $VOSO_4$ to exert both peripheral insulinomimetic effects – leading to chronic reduction in insulin demand –, and direct pancreatic insulinotropic activity.

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