Mechanism on Insulin-Like Action of Vanadyl Sulfate: Studies on Interaction between Rat Adipocytes and Vanadium Compounds

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When rats with streptozotocin (STZ)-induced diabetes were given a daily intraperitoneal (i.p.) injection of VO\(\text{SO}_4\)\(_2\) (+4 oxidation state of vanadium), their serum glucose dropped from hyperglycemic level to normal level within 2 d and serum free fatty acid (FFA) level also dropped to normal level. Vanadium was incorporated in most organs as well as in the adipose tissues, as detected by neutron activation analysis (NAA). The mechanism for the insulin-like action of vanadium in terms of FFA release from isolated rat adipocytes was investigated: (1) Vanadyl (IV) and vanadic (III) ions normalize the FFA release in the adipocytes treated with epinephrine; (2) vanadate (V) ion treated with ascorbic acid, cysteine or glucose is effective in normalizing the FFA release but vanadate ion alone has no effect on FFA release; (3) vanadyl ion is incorporated into the adipocytes, while vanadate ion is not, as indicated by ESR spectroscopy; and (4) vanadyl ion can act on the glucose transporter, as indicated by experiments using cytochalasin B which is an inhibitor of this transporter. From these results, the normalization of both serum glucose and FFA levels by vanadyl ion was concluded to be due to the incorporation of vanadyl ion into the adipocytes, in which the metal ion acts on the glucose transporter and induces both the promotion of glucose uptake and the decrease of FFA release from peripheral adipocytes. The vanadyl state was suggested to be a possible pharmacologically active form of vanadium allowing the insulin-like action. We further propose that the monitoring of serum FFA level is another sensitive index in addition to serum glucose level by which to know the degree of the diabetes, and the FFA release from adipocytes is a good in vitro evaluation system to find a compound which shows an insulin-like action.

Key words vanadyl sulfate; insulin-mimetic action; diabetes; rat adipocyte; free fatty acid; glucose transporter

The biological and physiological roles of essential trace elements in living organisms have recently become apparent and their importance recognized in relation to our health and disease. Vanadium is known to be an essential trace element for rats and chicks and since it has a broad of redox potentials which depend on oxidation states and differ from the potentials of iron or copper ions, it is thought to have various physiological roles. In fact, vanadate ion (+5 oxidation state of vanadium) was found to be a potent inhibitor of Na\(^+\), K\(^+\)-ATPase in 1977 and interests in the biological functions of vanadium have focused on its structure and function in living systems. Among them, the relationship between vanadium and diabetes mellitus has been studied and numerous reports on the insulin-like effect of vanadium have been published. The insulin-like action of vanadate ion has been found to stimulate various systems such as glucose uptake into adipocytes and hepatocytes in rats, glycolysis and the binding of insulin-like growth factor-I. Vanadate ion given orally was shown to normalize blood glucose level of experimental diabetic rats. However, vanadate is more than 10 times as toxic as vanadyl (+4 oxidation state of vanadium) to rats. Most of the vanadium in living organisms is incorporated in the form of vanadyl ion VO\(^{2+}\) which has a square pyramidal structure and in which VO\(^{2+}\) is coordinated with four oxygen ligands from either water or oxamino acid residues in proteins. We found that both vanadyl ion and vanadyl complexes given orally normalize the blood glucose levels of diabetic rats induced by STZ. Furthermore, we recognized that vanadyl ion promotes the incorporation of glucose into adipocytes of rats, similar to the function of insulin. Such insulin-like effect of vanadium has been restricted to in vitro and in vivo observations on blood glucose regulation. To obtain further insights on the detailed mechanism of this insulin-like effect, the vanadium effect on lipid metabolism was studied to determine whether this element normalizes the level of serum FFA in diabetic rats and decreases FFA release from adipocytes. We also looked at the interactions of vanadium and adipocytes using ESR spectroscopy. We report here a new mechanism of insulin-like action of vanadium in terms of release of FFA, in which vanadyl ion is proposed to be an active form promoting the insulin-like effect by acting on the glucose transporter.

MATERIALS AND METHODS

Materials BSA (essentially fatty acid free, prepared from fraction V), insulin (bovine pancreatic, crystalline), Epi, Cyt B and STZ were purchased from Sigma Chemical Co. VC\(_1\), VO\(_2\), NaVO\(_3\), glucose, ascorbic acid, cysteine, nitric acid and collagenase were obtained from Wako Chemicals (Tokyo, Japan). All other chemicals used were of special analytical grade.

Preparation of STZ-Induced Diabetic Rats (STZ-Rats)

Diabetes was induced in male Wistar rats (about 200 g body weight) by a single intravenous injection of a freshly prepared solution of STZ (50 mg/kg body weight) in 0.1 M
citrate buffer (pH 5.0) via the tail vein under light anaesthesia with ether. The serum glucose levels were not significantly changed by the anaesthesia. STZ-rats with serum glucose levels of 300–350 mg/dl one week after STZ administration were used for experiments.

Analyses of Blood Samples Blood samples for analyses of serum glucose, FFA and insulin were obtained from the heart of the rats under light anaesthesia with ether. The serum concentrations of glucose, FFA and insulin were determined by Glucose CII-test Wako, NEFA C-test Wako and Insulin Kit Wako (Wako Pure Chemicals), respectively.

Vanadium Treatment for STZ-Rats STZ-rats were given a daily intraperitoneal (i.p.) injection of VOSO₄ at a dose of 10 mg (200 μm) vanadium/kg body weight in 0.9% (w/v) NaCl solution for the first 2 d, of 5 mg (100 μm) vanadium/kg for the next 6 d, and then 2.5 mg (50 μm) vanadium/kg for 1 month thereafter. Blood samples for analyses were taken at 10–11 a.m. before injection of VOSO₄.

Determination of Vanadium Concentrations in VOSO₄ Treated Normal and STZ-Rats Normal and STZ-rats were treated with VOSO₄ by i.p. injection at doses of 10 mg (200 μm) vanadium/kg for the first 2 d, and 5 mg (100 μm) vanadium/kg for the next 2 d. STZ-rats whose serum glucose levels had been normalized by administrations of VOSO₄ were used for experiments. The rats were sacrificed under ether anaesthesia and their organs were removed. Subcellular fractions of the liver and kidney were prepared by the method of Hogeboom. The organs and subcellular fractions were lyophilized. Vanadium was determined by NAA at the Research Reactor Institute of Kyoto University using the peak area of 1434.4 keV based on 51V(n, γ)52V reaction (half-life of 52V: 3.75 min). Protein in subcellular fractions was determined by the Lowry method.

Preparation of Isolated Rat Adipocytes Male Wistar rats, weighing 200 g, were killed by decapitation under anaesthesia with ether, and the adipocytes were isolated from the epididymal fat pads by the method of Rodbell. Fat tissues were chopped up with scissors and digested for 1 h at 37 °C in KRB buffer (120 mM NaCl, 1.27 mM CaCl₂, 1.2 mM MgSO₄, 4.75 mM KCl, 1.2 mM KH₂PO₄ and 24 mM NaHCO₃, pH 7.4) containing 20 mg BSA and 2 mg collagenase/ml. Adipocytes were then separated from the undigested tissues by filtration through nylon mesh (250 μm), washed three times with the above buffer without collagenase, and prepared for 2.5 × 10⁶ cells/ml.

Effects of Three Vanadium Compounds on FFA Release in Isolated Rat Adipocytes Isolated adipocytes (2.5 × 10⁶ cells/ml) in siliconized vials were preincubated at 37 °C for 0.5 h with various concentrations (10⁻⁴–10⁻⁶ M) of three vanadium compounds in 1 ml KRB buffer containing 20 mg BSA/ml in the absence or presence of 1 mg/ml glucose. A 10⁻⁵ M Epi was then added to the reaction mixtures and the resulting solutions were incubated at 37 °C for 3.0 h. The reactions were stopped by soaking in ice water and the mixtures were centrifuged at 1200 rpm for 10 min. For the outer solution of the cells, FFA levels were determined with an NEFA kit. The three vanadium compounds used were, VCl₃, VOSO₄, and NaVO₃. In the systems containing 10⁻³ M NaVO₃, the effects of reducing agents such as ascorbic acid or cysteine (10⁻² M) were tested.

Relationship between Glucose and FFA in Rat Adipocytes Three types of the isolated adipocyte solution were prepared as follows: (1) adipocytes (2.5 × 10⁶ cells/ml) alone in 1 ml KRB buffer containing 1 mg/ml glucose, (2) adipocytes with glucose (10⁻⁴–10⁻⁶ M) in 1 ml KRB buffer and (3) adipocytes with 10⁻² M glucose and Cyt B (10⁻²–10⁻³ M) in 1 ml KRB buffer. These three solutions were incubated at 37 °C for 3.0 h. FFA concentrations in the outer solution released from the adipocytes were determined as described above.

Inhibitory Effect of Cyt B on the Suppressed FFA Release by VOSO₄ in Adipocytes Adipocytes (2.5 × 10⁶ cells/ml) were treated with 10⁻⁵ M Cyt B, preincubated with 10⁻⁴ M VOSO₄ in KRB buffer containing 1 mg/ml glucose at 37 °C for 0.5 h and incubated with 10⁻⁵ M Epi for 3.0 h. FFA levels in the outer solution of the cells were determined as described above.

ESR Measurements on Rat Adipocytes Treated with Vanadium Compounds Two types of the isolated adipocyte were prepared with or without glucose in KRB buffer. The adipocytes (2.5 × 10⁶ cells/ml) prepared without glucose were incubated in the presence of 10⁻⁴ M VOSO₄ or 10⁻³ M NaVO₃ at a total volume of 300 μl. After incubation for 3.0 h, the adipocytes were separated by centrifugation (1200 rpm, 10 min), and washed three times with KRB buffer. ESR spectra were measured for both adipocytes and the outer solution at liquid nitrogen temperature (77 K) to detect the vanadyl state, and were compared with the spectrum of 10⁻³ M VOSO₄ in KRB buffer. ESR spectra were also measured both for undigested adipocyte solutions and for washed adipocytes digested with 61% nitric acid, followed by the addition of ascorbic acid. The outer solutions and 10⁻⁷ M NaVO₃ in KRB buffer were also treated with the same procedures. The adipocytes containing 1 mg/ml or 1 M glucose were incubated in the presence of 10⁻² M NaVO₃ and ESR spectra were recorded after 3.0 h. ESR spectra at 77 K were recorded with a JEOL RE1X spectrometer (X band) operated at modulation frequency of 100 kHz, modulation amplitude of 0.63 mT and microwave power of 5 mW using ESR quartz cells. The microwave frequency applied to the sample was monitored by an Advantest R5372 digital frequency counter. The magnetic field was calibrated by Mn (II) doped in MgO powder. The g-values were estimated with TCNQ-Li (tetracyanoquinodimethane-Li salt, g = 2.00252) as a standard. Other instrument settings were: magnetic field 330 ± 100 mT, amplitude 7.9–1000, response 0.03 s and scan speed 4 min.

RESULTS

In Vivo Observations on Normalization of Serum Glucose and FFA Levels by Vanadyl Treatment in STZ-Rats Normalization of Both Serum Glucose and FFA Levels by Vanadyl Sulfate in STZ-Rats: The effects of VOSO₄ (VO²⁺ form as +4 oxidation state) on the serum glucose, FFA and insulin levels of STZ-rats were examined. When STZ-rats were given a daily i.p. injection of VOSO₄
(10 mg V/kg for the first 2 d, 5 mg V/kg for the next 6 d and then 2.5 mg V/kg for 1 month), their serum glucose decreased from hyperglycemic level to hypoglycemic level within 2 d, and this normal level was maintained for 1 month (Fig. 1) supporting our previous observations. Their serum FFA decreased concomitantly from an abnormal by high level to a normal level; however, their serum insulin levels remained low. During the experiments no significant loss of body weight in these rats was observed. The serum parameters and body weight for the control non-diabetic rats, STZ-rats and STZ-rats treated with VOSO₄ after one month are summarized in Table 1.

Distributions of Vanadium in Normal- and STZ-Rats Treated with VOSO₄: Vanadyl treatment was confirmed to normalize the serum glucose in STZ-rats, so the total vanadium distribution in these rats was studied by NAA method. When normal- and STZ-rats were treated with VOSO₄ (10 mg V/kg for the first 2 d, and 5 mg V/kg for the next 2 d), vanadium was found to be incorporated in all organs examined. In terms of μg V/g wet weight organ, large amounts of the metal were incorporated in the following order in both normal- and STZ-rats: kidney > liver > bone > pancreas (Fig. 2). It is interesting to note that vanadium was uptaken in adipose tissues as well as the blood. Furthermore, the vanadium distribution in subcellular fractions of the liver and kidney was examined and found to be well incorporated in mitochondria of the liver and in supernatant of the kidney in both rat groups (Fig. 3). No significant difference in vanadium-uptake of normal- and STZ-rats was not observed (Figs. 2, 3).

Correlation of Glucose and FFA in Normal- and STZ-Rats: Since both serum glucose and FFA levels in STZ-rats were normalized by vanadyl treatment, the relationship between these two parameters was examined. The correlation coefficient of the linear regression (\( y = 177.5x + 97.3 \)) was 0.844 for a total of 64 (data not shown) for normal- and STZ-rats. When glucose levels in sera increase, FFA levels increase, indicating that the
serum FFA level is a good index as is the serum glucose level to know the degree of diabetes mellitus.

The Normalization of Serum Glucose by Vanadyl Treatment in STZ-Rats (in Vitro Studies) Since vanadyl treatment was confirmed to normalize both serum glucose and FFA levels in STZ-rats and the administered vanadium was found in most organs involving the adipose tissues, we examined the mechanism by in vitro experiments. We earlier demonstrated that [3H]glucose was uptaken into the adipocytes in the presence of vanadyl ion as well as in the presence of insulin. In the present study, we found that the glucose levels correlate with the levels of FFA as described above. Therefore, the relationship between the FFA and glucose in the presence of vanadyl ion using rat adipocytes was of interest.

Effects of Vanadium Compounds on FFA Release from Rat Adipocytes: Effect of vanadium compounds in various oxidation states on FFA release from rat adipocytes was examined. When isolated rat adipocytes (2.5 x 10^6 cells/ml) in 1 ml KRB buffer without glucose were preincubated with various concentrations of three vanadium compounds at 37°C for 0.5 h and then incubated with Epi for 3.0 h, both vanadic (III) and vanadyl (IV) ions were found to suppress FFA release from adipocytes in a dose-dependent manner, while vanadate (V) ion did not (Fig. 4). Furthermore, when adipocytes in the absence of glucose were treated with 10^-3 M vanadate plus a reducing agent like 10^-2 M ascorbic acid or cysteine, the suppressed release of FFA was observed (about 1.1 m eq/l FFA level) (data not shown). However, when adipocytes in the presence of 1 mg/ml glucose were incubated with three vanadium compounds under the same conditions, the three compounds inhibited FFA release from adipocytes. Strong dose-dependent inhibition was particularly observed in the systems containing vanadic and vanadyl ions, while the effect of vanadate was less effective (Fig. 5). From these observations, the normalization of serum FFA levels by vanadium is suggested to be due to inhibition of FFA release induced by vanadium ions in low oxidation states such as vanadic and vanadyl forms.

Effect of Glucose on FFA Release in Rat Adipocytes: Effect of glucose on FFA release in rat adipocytes was investigated. Both incubation time (0-3 h; data not shown) and glucose concentration (10^-4-10^-2 M; Fig. 6)-dependent suppressed FFA releases from the adipocytes were observed. When adipocytes with 10^-2 M glucose were treated with Cyt B, which is an inhibitor of glucose transporter, in KRB buffer at 37°C for 3.0 h, the...

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Fig. 4. Inhibitory Effects of VCl3, VO3 and NaVO3 on FFA Release from Rat Adipocytes Treated with Epi in the Absence of Glucose

Isolated adipocytes (2.5 x 10^6 cells/ml) plus vanadium compound (10^-4-10^-2 M) in KRB buffer containing 2% BSA were preincubated at 37°C for 0.5 h. A 10^-3 M Epi was added to the mixture (total volume, 0.3 ml) and the resulting solution was incubated at 37°C for 3.0 h. The reaction was terminated by adding the solution to an ice-water and then centrifuging at 1200 rpm for 10 min. FFA concentration in medium was determined by NEFA c-test Wako. Values are the means ± S.D.s for three experiments. * p < 0.05 vs. Epi 10^-3 M; ** p < 0.01 vs. Epi 10^-3 M.

Fig. 5. Inhibitory Effects of VCl3, VO3 and NaVO3 (10^-4-10^-2 M) on FFA Release from Rat Adipocytes (2.5 x 10^6 Cells/ml) Treated with 10^-3 M Epi in the Presence of 1 mg/ml Glucose

Experimental procedures were the same as for Fig. 4. Values are the means ± S.D.s for three experiments. * p < 0.01 vs. Epi 10^-3 M.

Fig. 6. Inhibitory Effect of Cyt B on the Suppressed FFA Release from Rat Adipocytes (2.5 x 10^6 Cells/ml) Treated with Glucose (Glu) in Glucose-Free Medium

Adipocytes (2.5 x 10^6 cells/ml) treated with (10^-4-10^-3 M) Glu were mixed with 10^-4-10^-3 M Cyt B and incubated at 37°C for 3.0 h. The other experimental procedures were the same as for Fig. 4. Values are the means ± S.D.s for three experiments. * p < 0.01 vs. saline; ** p < 0.05 vs. Glu 10^-3 M; *** p < 0.01 vs. Glu 10^-3 M.
suppressed FFA release (about 0.5 m eq/l FFA) by glucose was inhibited or restored at the inhibitor concentrations of 10^{-6}–10^{-5} m (about 1.0 m eq/l FFA at 10^{-5} m Cyt B) (Fig. 6).

Effect of Cyt B on the Suppressed FFA Release by VOSO_4 in Adipocytes: When 10^{-5} m Cyt B was added to adipocytes treated with 10^{-4} m VOSO_4 and 10^{-5} m Epi in KRB buffer containing 1 mg/ml glucose at 37°C for 3.5 h, the suppressed FFA release (about 1.4 m eq/l FFA) by VOSO_4 was inhibited or restored by the inhibitor (about 1.8 m eq/l FFA) (Fig. 7).

ESR Studies on Adipocytes Treated with Vanadium Compounds: As the suppressed FFA release from adipocytes depended on the oxidation state of vanadium, the interaction of adipocytes with vanadium compounds and adipocytes was studied by ESR spectrometry. When rat adipocytes were incubated with 10^{-3} m VOSO_4 in glucose-free medium at 37°C for 3.0 h, the ESR signals (77 K) due to vanadyl ion with an 8-line hyperfine structure ($g_\perp = 1.950$, $g_\parallel = 1.986$, $A_\perp = 164 \times 10^{-4} \text{ cm}^{-1}$ and $A_\parallel = 77 \times 10^{-4} \text{ cm}^{-1}$) were detected in adipocytes after washing three times with KRB buffer (Fig. 8a), and in the outer solution, a small signal was observed (b vs. c). In contrast, after the incubation with 10^{-3} m NaVO_3 under the same conditions, no ESR signals due to vanadyl state were found in adipocytes (d; e), in the outer solution treated with nitric acid and ascorbic acid, intense ESR signals due to vanadyl ion were detectable (f, g). These results strongly indicate that vanadyl ions are uptaken in adipocytes or bind with membrane of adipocytes, while vanadate ions do not interact with the isolated rat adipocytes. When adipocytes in KRB buffer containing 1 mg/ml or 1 m glucose were incubated with 10^{-2} m NaVO_3 at 37°C for 3.0 h, the signals due to the vanadyl state developed both incubation time (0–3 h; data not shown) and glucose concentration (1 mg/ml–1 m; h, i) dependently, indicating that vanadate is reduced by glucose in the outer solution and then incorporated in adipocytes.

![Fig. 7. Inhibitory Effects of Cyt B on the Suppressed FFA Release from Rat Adipocytes Treated with VOSO_4 and Epi in 1 mg/ml Glucose-Containing Medium](image)

Values are the means ± S.D.s for three experiments. * p < 0.01 vs. Epi + VOSO_4 10^{-4} m. Adipocytes (2 x 10^6 cells/ml) were mixed with 10^{-6} m Cyt B and incubated with 10^{-6} m VOSO_4 in KRB buffer containing 1 mg/ml glucose at 37°C for 3.0 h. Then, 10^{-6} m Epi was added to the solution and the resulting solution was incubated at 37°C for 3.0 h. The other experimental procedures were the same as for Fig. 4.

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![Fig. 8. ESR Spectra (77 K) of Vanadyl States Recorded after the Incubation of Adipocytes (2.5 x 10^6 Cells/ml) with 1 or 10 mM Vanadium Compounds in the Absence or Presence of 1 mg/ml or 1 mM Glucose for 3.0 h at 37°C](image)

Volume of the sample was adjusted to 300 μl. (a) adipocytes + VOSO_4 10^{-4} m; washed cells; (b) adipocytes + VOSO_4 10^{-4} m; outer solution; (c) KRB buffer + VOSO_4 10^{-3} m; whole solution; (d) adipocytes + NaVO_3 10^{-3} m; whole solution; (e) adipocytes + NaVO_3 10^{-3} m + 61% nitric acid + ascorbic acid; washed cells; (f) adipocytes + NaVO_3 10^{-3} m + 61% nitric acid + ascorbic acid; outer solution; (g) KRB buffer + NaVO_3 10^{-3} m + 61% nitric acid + ascorbic acid; whole solution; (h) adipocytes + NaVO_3 10^{-3} m in the presence of 1 mg/ml glucose; whole solution; (i) adipocytes + NaVO_3 10^{-3} m in the presence of 1 mM glucose; whole solution. Amplitudes for recording were 200, 50, 50, 100, 100, 7.9, 7.9, 1.000 and 250 for (a), (b), (c), (d), (e), (f), (g), (h) and (i), respectively.
DISCUSSION

The chemical form, oxidation state and distribution of vanadium in rats has been studied to understand the various physiological functions of vanadium. Vanadyl species in a living organism are found to be predominantly in oxo-vanadium form $\text{VO}^{2+}$ with a square pyramidal structure.\textsuperscript{14,15} Since this state of the metal ion was highly incorporated in most organs such as kidney, liver, pancreas and bone as well as in supernatant of the kidney and mitochondria of the liver (Figs. 2, 3), vanadium was suggested to act on the islet of the pancreas,\textsuperscript{20} mineralization of the bone,\textsuperscript{21} electron transport systems or induction of metallothionein in the kidney or liver. In fact, a number of biochemical and pharmacological function of vanadium ions are known,\textsuperscript{32–34} among which the anti-diabetic activity of vanadate and vanadyl ion has been investigated by many researchers.\textsuperscript{11,12,15,16,20–37}

Vanadate ion is known to be reduced in vivo to vanadyl ion, in which vanadate is transported into erythrocytes via an anion channel\textsuperscript{19} and is reduced chemically to the vanadyl form by endogenous reducing agents like cysteine and glutathione.\textsuperscript{39} Vanadyl ion is less toxic to rats than vanadate.\textsuperscript{13} Therefore, we get the reason to use the vanadyl ion to know the mechanism to improve a diabetic condition in experimental animals. We confirmed that daily i.p. injection of vanadyl sulfate to STZ-rats results in normalization of both serum glucose and FFA levels without causing significant loss of body weight of the animals (Fig. 1 and Table 1). This observation together with our previous results\textsuperscript{15} is analogous to the findings that NaVO$_3$ given orally in the drinking water normalized the blood glucose levels in STZ-rats.\textsuperscript{11,12} However, in these observations the serum insulin levels were not restored (Table 1). From the results of vanadium distribution study, vanadium was found to be incorporated in most organs as well as in adipose tissue and no remarkable difference in total vanadium distribution between normal- and STZ-rats was observed (Fig. 2). We thus consider that vanadium is sufficiently accumulated in each organ of STZ-rats to normalize blood glucose level after the treatment of vanadyl sulfate.

Vanadyl ion\textsuperscript{35} as well as vanadate ion\textsuperscript{5,6} has been reported to enhance glucose uptake in peripheral adipocytes. Therefore, the use of adipocytes may give valuable informations on the mechanism of the vanadium-dependent normalization of both serum glucose and FFA levels in STZ-rats.

We obtained the following results on the interaction of adipocytes and vanadium ions; (1) vanadyl ion enhances glucose-uptake in adipocytes\textsuperscript{15}; (2) vanadyl ion inhibits FFA release in the absence of glucose (Fig. 4) and then strongly suppresses FFA release in the presence of glucose (Fig. 5); (3) glucose inhibits FFA release and the effect is suppressed by Cyt B (Fig. 6); (4) the suppressed FFA release by vanadyl ion is restored by Cyt B (Fig. 7); (5) vanadyl ion is uptake into adipocytes (Fig. 8a); and (6) vanadate ion are not incorporated into the adipocytes (Fig. 8e) and hence it is not active in suppressing FFA release from adipocytes (Fig. 4). Although both vanadyl\textsuperscript{15} and vanadate\textsuperscript{5,6} have been found to enhance glucose-uptake in adipocytes and vanadate has been proposed to enter the cells through non-specific anion channels followed by intracellular reduction to vanadyl,\textsuperscript{38,40} our present ESR study clearly indicated that vanadate is reduced extracellularly in the presence of glucose and the reduced vanadyl is then incorporated into the cells (Figs. 8h and i). On the basis of these results, the vanadyl state is proposed to be a possible active form of vanadium for the insulin mimetic action and to act on the glucose transporter. These findings support the observations that vanadate, which is in turn reduced to vanadyl, restores expression of the insulin-sensitive glucose transporter of skeletal muscle in STZ-diabetic rats\textsuperscript{41} and induces the recruitment of glut-4 glucose transporter to the plasma membrane of adipocytes.\textsuperscript{42} Even after obtaining these results, the detailed mechanism by which vanadium may affect glucose regulation cannot be directly deduced. However, the basic mechanism for the vanadium-dependent glucose regulatory effect may involve the enhanced auto-phosphorylation of the insulin receptor.\textsuperscript{43} Although it is still unclear whether this effect lead to a stimulation of tyrosine kinase in the receptor,\textsuperscript{8,43,44} esterification of tyrosine residues of the receptor\textsuperscript{45} or inhibition of phosphotyrosine phosphatase,\textsuperscript{46,47} an insulin-like effect due to vanadium\textsuperscript{48–50} may be associated with increase of protein-tyrosine phosphates in adipocytes, followed by increase of glucose transporter. Quite recently, an interesting observation was reported that vanadyl but not vanadate inhibits receptor tyrosine kinases such as insulin receptor and insulin-like growth factor-I receptor,\textsuperscript{51} supporting our present result on the biochemically active oxidation state of vanadium.

Vanadium effect on lipid metabolism was also examined. The adenosine 3',5'-cyclic monophosphate (C-AMP)-mediated protein phosphorylation cascade in adipocytes is activated during diabetes (in vivo) or in the presence of Epi (in vitro), and glucose and vanadyl ion which are uptake in adipocytes by vanadyl treatment leads to a restored regulation of this cascade in peripheral cells.\textsuperscript{52} Thus, it is proposed that FFA release from adipocytes is inhibited by vanadyl ion. The suppressed FFA release by vanadate ion depended on the enhancement of glucose-uptake by the metal ion, which was reduced to vanadyl by the added glucose (Figs. 4 and 5). Therefore, vanadate does not inhibit FFA release in adipocytes, and probably this metal ion is not reduced without glucose. In contrast, insulin inhibited FFA release in adipocytes in a dose-dependent manner (0.256–25.6 mU/ml) and the effect was completely reversed by 10$^{-7}$ M Cyt B in the presence of 1 mg/ml glucose. These effects were not observed in the absence of glucose (data not shown). Therefore, glucose which is uptake in adipocytes by either insulin or vanadyl ion is suggested to suppress FFA release. An investigation to learn the mechanism of glucose effect in suppressing FFA release is now under way (manuscript in preparation).

In conclusion, vanadyl ion was found to be incorporated in adipose tissue of rats and to act on the glucose transporter of the peripheral cells to normalize both glucose and FFA levels in STZ-diabetic rats. The vanadyl effect in terms of FFA release was shown to depend on
glucose, being different from the effect of insulin. We further propose that (1) serum FFA levels is another sensitive index to know the degree of the diabetes and (2) FFA release from adipocytes is a good evaluation system by which to find a compound which has an insulin-like action in vitro.

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REFERENCES AND NOTES

1) Abbreviations: STZ, streptozotocin; FFA, free fatty acid; BSA, bovine serum albumin; VC13, vanadic chloride; VOSO4, vanadyl sulfate; NaVO3, sodium vanadate; Cyt B, cytochalasin B; KRB, Kreb's-Ringer bicarbonate; Glu, glucose; Epi, epinephrine; NAA, neuron activation analysis.


