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Possible mode of action for insulinomimetic activity of vanadyl(IV) compounds in adipocytes

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Abstract

Vanadyl(IV) ions (+4 oxidation state of vanadium) and their complexes have been shown to have in vitro insulinomimetic activity and to be effective in treating animals with diabetes mellitus. Although, researchers have proposed many vanadyl compounds for the treatment of diabetes patients, the mode of action of vanadyl compounds remains controversial. In order to evaluate the mode of action of these compounds, we examined the insulinomimetic activity of $VOSO_4$, bis(picolinato)oxovanadyl(IV), and bis(maltolato)oxovanadyl(IV) in the presence of several inhibitors relevant to the glucose metabolism. After confirming that these vanadyl compounds were incorporated in the adipocytes as estimated by ESR method, we evaluated the mode of action by examining free fatty acids (FFA) release in the adipocytes. Inhibition of FFA release by these vanadyl compounds was found to be reversed by the addition of inhibitors, typically by cytochalasin B (glucose transporter 4 (GLUT4) inhibitor), cilostamide (phosphodiesterase inhibitor), HNMPA-$(AM)_3$ (tyrosine kinase inhibitor), and wortmannin (PI3-k inhibitor), indicating that these compounds affect primarily GLUT4 and phosphodiesterase, as named "ensemble mechanism". Based on these results, we suggest that vanadyl compounds act on at least four sites relevant to the glucose metabolism, and on GLUT4 and phosphodiesterase in particular in rat adipocytes, which in turn normalizes the blood glucose levels of diabetic animals. The obtained results provide evidence for the role of vanadyl ion and its complexes in stimulation of the uptake and degeneration of glucose.

Keywords: Diabetes mellitus; Vanadyl complex; Action mechanism; Adipocyte

Introduction

In recent years, the prevalence of diabetes mellitus (DM) in adults in the world has been reported to be 5.4%, and the number of adults with DM is forecasted to increase to approximately 300 million worldwide by the year 2025 (King et al., 1998; Wild et al., 2003). Insulin injections to treat type 1 DM and administration of several synthetic pharmaceuticals to treat type 2 DM have often many defects involving physical and mental pain, and some side effects, respectively. Many researchers in this field have therefore tried to find orally active therapeutic compounds in place of insulin injections and other synthetic pharmaceuticals. Previous studies have revealed that chromium, manganese, selenium, zinc, and vanadium ions show both in vitro insulinomimetic action and in vivo antidiabetic activity in animal experiments (Schwarz and Mertz, 1959; Rubenstein et al., 1962; Shechter and Karlish, 1980; Ezaki, 1989; Ghosh et al., 1994). Among them, antidiabetic activity of vanadium compounds has been intensively studied in recent years (Sakurai et al., 1990a,b; Brichard and Henquin, 1995; Sekar et al., 1996; Badmaev et al., 1999; Brichard et al., 1999; Thompson et al., 1999; Thompson and Orvig, 2000; Sakurai et al., 2002). Together with such fundamental research on vanadium compounds in animals, clinical trials of vanadium compounds have also been reported (Goldfine et al., 1995; Cohen et al., 1995; Halberstam et al., 1996; Goldfine et al., 2000; Cusi et al., 2001), in which vanadium salts such as $VOSO_4$ and $NaVO_3$ were administered in diabetic patients. However, such vanadium compounds have high solubility in aqueous solution, and thus have low bioavailability. In order to enhance both lipophilicity and bioavailability of vanadium compound, we prepared several types of less toxic vanadyl (+4 oxidation state of vanadium) complexes with different coordination structures, and evaluated

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their insulinomimetic activity by using in vitro cell systems and in vivo experimental diabetic model animals since 1990 (Sakurai et al., 1990a,b, 2003, 2004; Kawabe et al., 1998; Sakurai, 2002; Sasagawa et al., 2002; Sakurai and Yasui, 2003).

In order to reveal why vanadium compounds exhibit insulinomimetic activity, the mode of action of vanadium compounds has been examined by many researchers, and important data have been reported in regard to the inhibition of protein tyrosine phosphatase activity, which is involved in the activation of insulin receptor as well as the cytosolic non-receptor tyrosine kinase, direct phosphorylation of insulin receptor substrate 1, and the activation of phosphatidylinositol 3 kinase, leading to glucose transporter 4 translocation (Swarup et al., 1982; Posner et al., 1994; Fantus et al., 1995; Tsiani et al., 1998; Shafrir et al., 2001; Seale et al., 2005). However, the obtained results were sometimes controversial, and furthermore, there have been few reports dealing with the comprehensive mode of action of the vanadyl compounds (Sakurai and Tsuji, 1998; Shafrir et al., 2001; Marzban and McNeill, 2003).

We previously reported that glucose, which was taken up in rat adipocytes in the presence of metal ions or their complexes, suppressed free fatty acids (FFA) release similarly to the action of insulin (Nakai et al., 1995; Sakurai et al., 2002). Following the finding, we found that glucose uptake correlated well with inhibition of the FFA release in rat adipocytes (Adachi and Sakurai, 2004). Thus we were able to elucidate the action mechanism of vanadyl complexes by simultaneous measuring the FFA level released from the rat adipocytes.

In the present study, we aimed at an analysis of the comprehensive mode of action of insulinomimetic vanadyl compounds involving ionic vanadyl sulfate ($VOSO_4$) and chelated forms of vanadyl compounds such as vanadyl-picolinate ($VO(pic)_2$) and vanadyl-maltolate ($VO(mal)_2$) complexes with a $VO(N_2O_2)$ and $VO(O_4)$ coordination structure, respectively, in rat adipocytes, by using several inhibitors relevant to glucose metabolism. The obtained results will provide evidence for the role of vanadyl ion and the complexes in stimulation of the uptake and degradation of glucose.

Methods

Materials

All reagents and solvent used were of the highest grade commercially available and were used as obtained. $VOSO_4 \cdot nH_2O$ (VS) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was standardized complexometrically with ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA) and identified as the $2.3H_2O$ adduct. Picolinic acid and maltol were obtained from Tokyo Kasei Inc. (Tokyo, Japan), and (±)-epinephrine hydrochloride, collagenase, and bovine serum albumin (BSA, fraction V) from Sigma Chemical Co. (St. Louis, MO, USA). Hydroxy-2-naphthalenylmethyl phosphonic acid tris acetoxy methyl ester (HNMPA-$(AM)_3$), wortmannin (Wort), cytochalasin B (Cyt-B), cilostamide, and forskolin were purchased from Biomol Inc. (Philadelphia, PA, USA).

Instruments

Elemental analyses of the complexes were carried out on a 240C Elemental Analyzer (Perkin-Elmer, Inc., Wellesley, MA, USA). Fourier-transform infrared (FT-IR) spectra were recorded with KBr pellets on a FT/IR-420 spectrophotometer (Jasco, Tokyo, Japan). The uptake of vanadyl compounds was measured by a JEOL RE1X spectrometer (X-band) (Shimazu, Tokyo, Japan).

Preparations of vanadyl complexes

Bis(picolinato)oxovanadyl(IV) ($VO(pic)_2$) with $VO(N_2O_2)$ coordination mode, and bis(maltolato)oxovanadyl(IV) ($VO(mal)_2$) with $VO(O_4)$ coordination mode were prepared following the methods previously described (Sakurai et al., 1995; McNeill et al., 1992).

Preparation of isolated rat adipocytes

Isolated rat adipocytes were prepared according to the method previously described by Rodbell (1964). In brief, male 7-week-old male Wistar rats (Shimizu Experimental Materials Co., Kyoto, Japan) were sacrificed by decapitation under anesthesia with ether, and adipocytes were isolated from epididymal fat pads. The fat pads were chopped up with scissors and incubated for 1 h in Krebs Ringer bicarbonate (KRB) buffer (120 mM NaCl, 1.27 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.75 mM KCl, 1.2 mM KH_2PO_4, 24 mM $NaHCO_3$, 2% BSA, and 0.5 mg collagenase/mL) at 37 °C. Adipocytes were separated from the undigested tissues by filtration through nylon gauze, were washed three times with KRB buffer without collagenase, and were prepared for 2.5×10^6 cell/mL. This animal study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University (KPU) and was performed according to the Guideline for Animal Experimentation of KPU.

Vanadyl determination in rat adipocytes treated with vanadyl complexes by ESR spectroscopy

The isolated adipocytes in KRB buffer containing 5 mM glucose were incubated in the presence of 1000 μM vanadyl sulfate or 250 μM vanadyl complexes at a total volume of 300 μL for 0, 1, and 2 h. After incubation, the adipocytes were separated by centrifugation (3000 rpm, 5 min), and the outer solution was collected. We added 5 M HCl in the outer solution, because $VOSO_4$ forms ESR silent oligomeric or polymeric spin-paired species at physiological pH. Electron spin resonance (ESR) spectra were measured on the outer solution at room temperature with a JEOL RE1X spectrometer (X-band) operated at a modulation frequency of 100 kHz, a modulation amplitude of 2 mT, a magnetic field of 340 ± 50 mT, and microwave power of 5 mW using ESR quartz cells. The linear equation for a working curve was obtained as $y = 3697.4x + 100.48$ (y, signal intensity; x, concentration of vanadyl compound), in which the correlation coefficient for the linear regression was $r = 0.9992$ for a total of 3 vanadyl concentrations.