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Vanadium salts stimulate mitogen-activated protein (MAP) kinases and ribosomal S6 kinases

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Abstract

Effect of several vanadium salts, sodium orthovanadate, vanadyl sulfate and sodium metavanadate on protein tyrosine phosphorylation and serine/threonine kinases in chinese hamster ovary (CHO) cells overexpressing a normal human insulin receptor was examined. All the compounds stimulated protein tyrosine phosphorylation of two major proteins with molecular masses of 42 kDa (p42) and 44 kDa (p44). The phosphorylation of p42 and p44 was associated with an activation of mitogen activated protein (MAP) kinase as well as increased protein tyrosine phosphorylation of $p42^{mapk}$ and $p44^{mapk}$. Vanadium salts also activated the 90 kDa ribosomal s6 kinase ($p90^{rsk}$) and 70 kDa ribosomal s6 kinase ($p70^{s6k}$). Among the three vanadium salts tested, vanadyl sulfate appeared to be slightly more potent than others in stimulating MAP kinases and $p70^{s6k}$ activity. It is suggested that vanadium-induced activation of MAP kinases and ribosomal s6 kinases may be one of the mechanisms by which insulin like effects of this trace element are mediated. (Mol Cell Biochem **153:** 69–78, 1995)

Key words: vanadium salts, MAP kinase, ribosomal s6 kinases ($p90^{rsk}$ and $p70^{s6k}$), insulinomimesis, protein tyrosine phosphatase

Abbreviations: eIF-4 – eukaryotic protein synthesis initiation factor-4; GRB-2 – growth factor receptor bound protein-2; GSK-3 – Glycogen Synthase Kinase-3; IRS-1 – insulin receptor substrate-1; ISPK – insulin stimulated protein kinase; MAPK – mitogen activated protein kinase, also known as: ERK – extracellular signal regulated kinase; MAPKK – mitogen activated protein kinase kinase, also known as – MEK, MAPK or ERK kinase; PHAS-1 – phosphorylated heat and acid stable protein regulated by insulin; PI3K – phosphatidyl inositol 3-kinase; PP1-G – protein phosphatase-glycogen bound form; PTK – protein tyrosine kinase; PTPase – protein tyrosine phosphatase; rsk – ribosomal s6 kinases; shc – src homology domain containing protein; SOS – son of sevenless

Introduction

Vanadium is a group V transition element and is an endogenous constituent of all or most mammalian tissues [1–3]. Vanadium salts such as sodium orthovanadate (Na_3VO_4), sodium metavanadate ($NaVO_3$) and vanadyl sulfate ($VOSO_4$) mimic several of the metabolic and growth promoting effects of insulin (4–24]; for a review see reference [25]). However, the most remarkable insulinomimetic effect of vanadium salts is their ability to normalise blood glucose in type I (insulin-dependent diabetes mellitus – IDDM) [26, 27] and type II (non insulin-dependent diabetes mellitus – NIDDM) [28–30] animal models of diabetes mellitus. Vanadium salts also improved the metabolic abnormalities associated with both type I as well as type II models of diabetes mellitus ([27, 31–43; for a review see reference [44]).

It is well established that the biological actions of insulin are initiated by binding of insulin to a specific receptor located on the membrane of target cells [45–46]. The insulin receptor is a heterotetrameric glycoprotein composed of two extracellular α-subunits and two transmembrane β-subunits. The α-subunit possesses the insulin binding activity and the β-subunit has an intrinsic protein tyrosine kinase (PTK) activity [44–48]. The binding of insulin to the α-

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subunit of its receptor activates the PTK activity of the β-subunit and results in autophosphorylation of the β-subunit in tyrosine residues as well as the tyrosyl phosphorylation of endogenous substrate [45–48]. Insulin receptor β-subunit autophosphorylation and activation of its PTK activity is believed to be a major pathway mediating the insulin action since cells with PTK deficient insulin receptors are unable to elicit several of the biological effects of insulin [49–50]. However, the mechanism by which vanadium compounds exert their insulin like effects remain to be clarified.

Vanadium salts are potent inhibitors of protein tyrosine phosphatases (PTPases) [51] and thus were initially thought to activate the insulin receptor PTK activity by preventing the dephosphorylation of insulin receptor β-subunit [11, 52–55]. However, recent work has suggested that the site of action of vanadium might not involve insulin receptor PTK [56–61].

A critical step in insulin signalling cascade appears to be activation of a group of protein serine/threonine kinases which include mitogen activated protein (MAP) kinases, 90 kDa ribosomal S6 kinase ($p90^{rsk}$) and 70 kDa ribosomal s6 kinase, ($p70^{s6k}$) [48, 62–66]. We have shown earlier that sodium orthovanadate stimulates the tyrosyl phosphorylation and activation of MAP kinases in an insulin receptor protein tyrosine kinase independent manner [60, 61]. Therefore, we were interested to examine if $p90^{rsk}$, which is immediate downstream of MAPK in insulin signalling cascade [48], could also be activated by vanadium salts. In addition, we also questioned whether $p70^{s6k}$ which is also activated by insulin by as yet undefined pathway [48], will be similarly activated by these salts. Furthermore, in the light of the suggestion that vanadyl sulfate may be more potent than sodium orthovanadate in eliciting hypoglycemic effects [67], we have compared the effects of various vanadium salts on MAPK, $p90^{rsk}$ and $p70^{s6k}$ activities in chinese hamster ovary cells overexpressing a normal human insulin receptor (CHO-HIRc).

Materials and methods

CHO cells overexpressing a normal human insulin receptor (CHO-HIRc) were a kind gift of Dr. Morris F. White (Joslin Diabetes Centre, Boston, MA, USA). Insulin was from Eli Lilly Co. Indianapolis, IN, USA). Myelin basic protein (MBP), sodium orthovanadate and sodium metavanadate were purchased from Sigma Chemical Company (St. Louis, MO, USA). Vanadyl sulfate was from Aldrich Chemical Co. (Milwaukee, WI, USA). Antiphosphotyrosine antibody, anti-MAP kinase antibody (raised against a peptide based upon residue 333–367 of the C-terminus of the rat 43 kDa ERK1) that detects several isoforms of MAP kinases including $p44^{mapk}$ and $p42^{mapk}$, antiphosphotyrosine agarose beads and S6 peptide RRRLSSLRA were from Upstate Biotechnology (Lake Placid, NY, USA). Goat anti-mouse or anti-rabbit IgG conjugated to alkaline phosphatase was from Bio-Rad (Mississauga, Ontario, Canada). The $p70^{s6k}$ antiserum (raised against a peptide corresponding to amino acids 2–30 of rat $p70^{s6k}$ [68] and $p90^{rsk}$ antiserum raised against bacterially produced chicken S6 kinase II [69] were generously provided by Drs. Frederic Hall of USC, School of Medicine, Los Angeles and John Blenis of Harvard Medical School, Boston respectively.

Cell culture

CHO cells were maintained on HAM's F-12 medium containing 10% fetal bovine serum. Cells were grown to confluence in 100 mm plates and incubated in serum-free F-12 medium for 20 h prior to the experiment [61].

Detection of phosphotyrosine-containing proteins

Tyrosine phosphorylation of cellular proteins stimulated in the absence or presence of insulin or vanadium compounds was assessed by immunoblotting using antiphosphotyrosine antibodies. Cells were stimulated with insulin or different vanadium compounds. The cells were lysed on ice in 400 µl of buffer A (25 mM Tris-Hcl pH 7.5, 25 mM NaCl, 1 mM Na orthovanadate, 10 mM Na fluoride, 10 mM Na pyrophosphate, 20 nM Okadaic acid, 0.5 mM EGTA, 1 mM phenylmethylsulphonyl fluoride (PMSF), 10 µg/ml aprotinin, 1% Triton X-100 and 0.1% SDS). The lysates were clarified by centrifugation for 12 min at 10,000 × g. Equal amounts of protein samples were electrophoresed on 10% SDS-polyacrylamide gels and transferred to polyvinilidine difluoride (PVDF) membranes and blotted with antiphosphotyrosine antibody (1:1000) and detected using goat-anti-mouse IgG conjugated to alkaline phophatase (1:3000).

Immunoaffinity purification of phosphotyrosyl proteins and MAP kinase immunoblotting

The cell lysates were affinity purified on antiphosphotyrosine-agarose column and immunoblotted using a MAP kinase antibody [61]. For affinity purification of phosphotyrosyl proteins, briefly, the clarified lysates from control or stimulated cells were incubated for 1 hour at 4°C with antiphosphotyrosine agarose beads equilibrated in buffer B (20 mM Tris-HCl pH 7.4, 1% Nonidet p-40, 10 mM EDTA, 0.2 mM Na vanadate, 0.01% Na azide and 100 mM NaCl). The beads were washed 3 times with buffer B and phospho-

tyrosine containing proteins were eluted with 1 mM phosphotyrosine in buffer B. The eluates were boiled in 3× Laemmli's sample buffer, electrophoresed on 10% SDS-polyacrylamide gels, transferred to PVDF membranes and blotted with anti-MAP kinase antibody (1:500) and detected using goat-anti-rabbit IgG conjugated to alkaline phosphatase.

MAP kinase assay

For MAP kinase assay cell lysates were prepared in buffer A devoid of Triton X-100 or SDS. To 5 µl of cell lysate (approximately 3–4 µg protein), 40 µl of kinase buffer (25 mM TrisHCl pH 7.4, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM dithiothreitol, 40 µM ATP, 1 µM staurosporine, 15 µg MBP, 0.5 mM EGTA and 0.5 µCi (γ^{32}P)ATP) was added. After 12 min at 30°C, the reaction was stopped by spotting 20 µl of the reaction mixture onto P-81 filter papers (2 × 2 cm), washed 4 times in 0.5% phosphoric acid and counted for radioactivity [70].

Assay of $p70^{s6k}$ and $p90^{rsk}$

The clarified lysates prepared in buffer A were normalized to contain equal amounts of protein (100 µg) and incubated for 4 h at 4°C with 3 µl of either $p70^{s6k}$ or $p90^{rsk}$ antibody preadsorbed to protein A sepharose beads (Pharmacia Biotech Inc, Missisauga, Ontario, Canada). The immune complex was collected by centrifugation followed by washing 3 times with buffer A and once with buffer B (20 mM HEPES pH 7.4, 10 mM $MgCl_2$, 1 mM DTT, 10 mM β-glycerophosphate). The beads were resuspended in 20 µl of buffer B containing S6 peptide, RRLSSLRA and phosphotransferase reaction was initiated by adding 5 µl of 100 µM ATP containing 2 µCi (γ^{32}P)ATP, (Amersham, Canada). After 15 min at 30°C, the reaction was stopped by spotting on P-81 filter paper, washing in 0.5% phosphoric acid and counted for radioactivity.

Results

Effect of various vanadium salts on protein tyrosine phosphorylation

We have shown earlier that sodium orthovanadate (OV) causes the tyrosyl phosphorylation and activation of $p42^{mapk}$ and $p44^{mapk}$ in a concentration and time dependent manner [60, 61]. Therefore, to examine if a similar response is observed with other vanadium salts such as sodium metavanadate (MV) and vanadyl sulfate (VS) (Fig. 1), the CHO-HIRc cells were treated with 100 µM of OV, MV or VS and lysates were immunoblotted with antiphosphotyrosine antibodies. As shown in Fig. 2, all the three vanadium salts stimulated the tyrosyl phosphorylation of two major proteins having molecular masses of 44 kDa (p44) and 42 kDa (p42) respectively. However VS appeared to be about 40% more potent than either OV or MV. Furthermore, in contrast to insulin, none of the vanadium salts stimulated the tyrosyl phosphorylation of either the insulin receptor or any other proteins (Fig. 2).

Na_3VO_4

(Sodium Orthovanadate)

$VOSO_4.3H_2O$

(Vanadyl sulfate)

$NaVO_3$

(Sodium Metavanadate)

Fig. 1. Structural formula of vanadium salts used.

Effect of vanadium salts on MAP kinase activity

Since the molecular masses of p44 and p42 corresponded to that of MAP kinases $p44^{mapk}$ and $p42^{mapk}$ which are activated in response to insulin [71] it was of interest to examine if increased tyrosyl phosphorylation of these proteins by MV and VS correlated with an increase in MAP kinase activity. As shown in Fig. 3, stimulation of CHO-HIR cells with all the 3 vanadium compounds resulted in the activation of MAP kinase activity as judged by increased phosphorylation of myelin basic protein (MBP) as an exogenous substrate. The stimulatory effect of VS on MAP kinase activity was slightly more than that of insulin (5.8 fold for VS versus 4.5. fold for insulin). However, VS was about 2 times more potent than either OV or MV in stimulating the MAP kinase activity (Fig. 3). Vanadium salt-mediated activation of MAP kinase was accompanied by an increase in the phosphotyrosyl content of $p44^{mapk}$ (ERK 1) and $p42^{mapk}$ (ERK 2) as determined by immunoprecipitation using antiphosphotyrosine antibody followed by immunoblotting with a specific antibody to MAP kinase which recognises both ERK 1 and ERK 2 (data not shown, [61]).

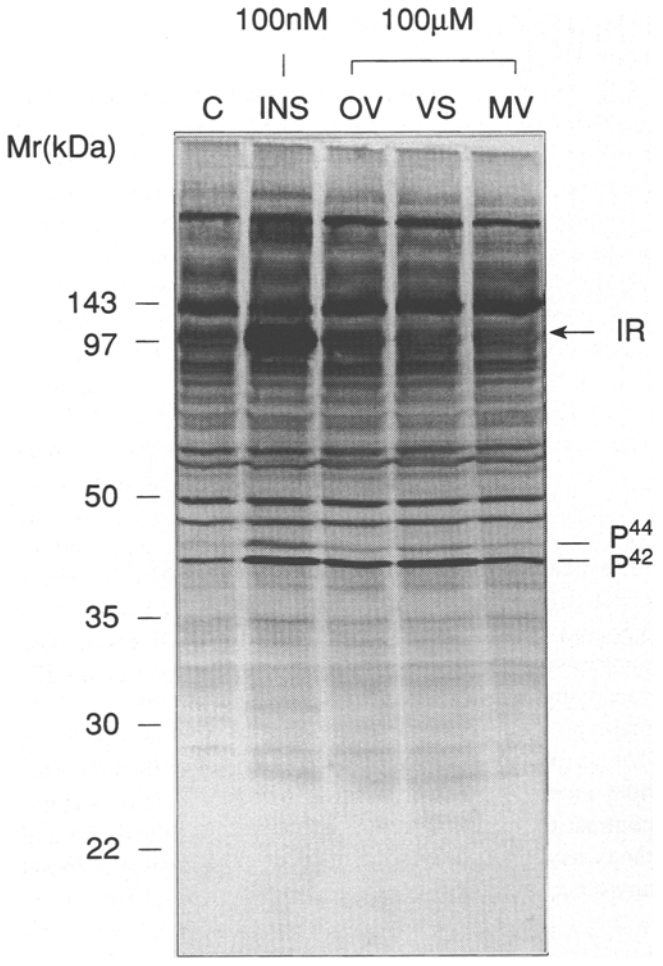

Fig. 2. Effect of various vanadium salts or insulin on protein tyrosine phosphorylation. CHO-HIRc cells were treated in the absence (C) or presence of insulin (INS, 100 nM) or 100 μM each of sodium orthovanadate (OV), vanadyl sulfate (VS) or sodium metavanadate (MV) for 5 min. Cell lysates were prepared and immunoblotted with antiphosphotyrosine antibodies as described in Materials and methods. The numbers in the left indicate the position of molecular weight standards. IR denotes the position of the insulin receptor β-subunit. This is a representative result from one of three experiments.

Effect of vanadium salts on 90 kDa ribosomal S6 kinase ($p90^{rsk}$)

In insulin stimulated cells MAP kinase activation is associated with the activation of an immediate downstream serine/threonine kinase-a 90 kDa ribosomal S6 kinase ($p90^{rsk}$) [48, 63, 66]. Therefore, to evaluate if vanadium salts exerted a similar effect, the $p90^{rsk}$ activity was assessed in CHO-HIR cells stimulated with various vanadium compounds. The $p90^{rsk}$ activity was determined by using an immune complex kinase assay in which $p90^{rsk}$ protein from cell lysates was immunoprecipitated using a specific antiserum. This antiserum was prepared against a recombinant chicken rsk S6

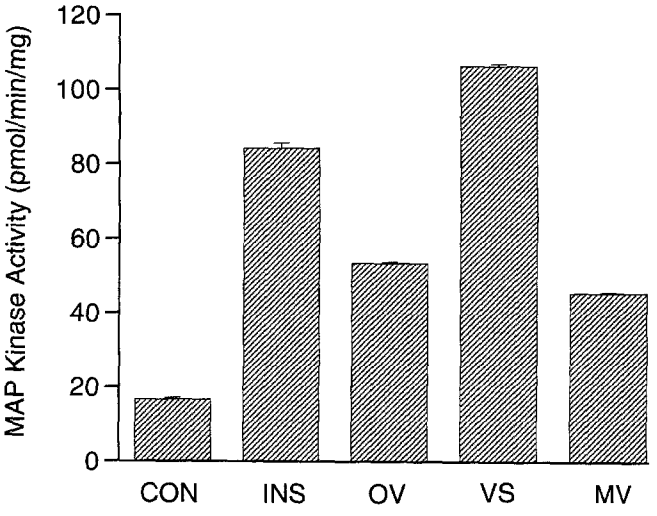

Fig. 3. Effect of insulin or vanadium salts on MAP kinase activity from CHO-HIRc cells. CHO-HIRc cells were treated in absence (CON) or presence of insulin or different vanadium compounds as mentioned in legend to Fig. 2, and MAP kinase activity was assayed in cell lysate using MBP (myelin basic protein) as exogenous substrate as described in Materials and methods. These results represent one of three independent experiments done in triplicate.

protein kinase which specifically immunoprecipitated $p90^{rsk}$ in various cell types [69]. The phosphotransferase reaction in the immune complex was performed by using a synthetic peptide substrate corresponding to amino acid 231–239 in human 40 S ribosomal protein S6. As shown in Fig. 4, incubation of CHO-HIR cells with all the vanadium salts (100 μM) activated the phosphotransferase activity up to 3-fold

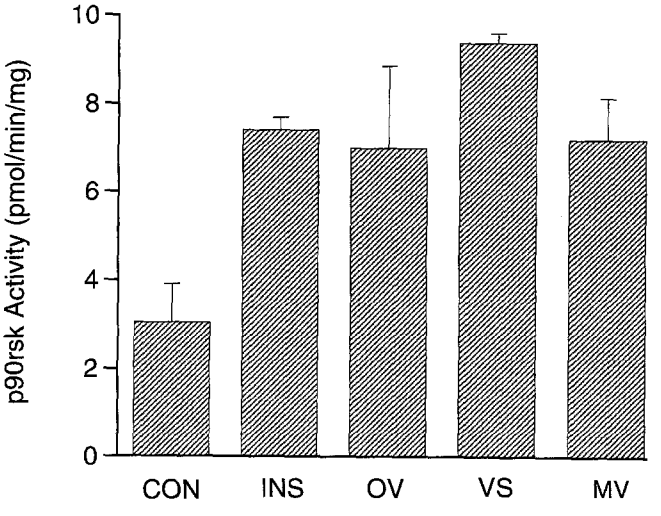

Fig. 4. Effect of insulin or various vanadium salts on 90 kDa ribosomal protein ($p90^{rsk}$) activity in CHO-HIRc cells. CHO-HIRc cells were treated as described in Fig. 2 and $p90^{rsk}$ activity was determined in immune complex using S6 peptide RRRLSSLRA as substrate as described in Materials and methods. Results are representative of one of three experiments performed in triplicate.

as compared to untreated control cells. All the salts tested were almost equipotent in activating the $p90^{rsk}$ activity and the response was similar to that observed with insulin.

Effect of vanadium salts on 70 kDa ribosomal S6 kinase ($p70^{s6k}$)

In addition to $p90^{rsk}$, insulin also stimulates a 70 kDa ribosomal S6 kinase termed as $p70^{s6k}$ [48, 64, 65]. However, in contrast to $p90^{rsk}$, MAPK is not the upstream kinase mediating $p70^{s6k}$ phosphorylation and activation, and the mechanism by which $p70^{s6k}$ is activated remains to be clarified [48]. We therefore asked whether similar to insulin, vanadium salts could also activate $p70^{s6k}$ in CHO-HIR cells. For these experiments also an immune complex kinase assay using an antiserum raised against a synthetic peptide corresponding to amino acid 2–30 of rat $p70^{s6k}$ which specifically recognizes $p70^{s6k}$ was performed. The results shown in Fig. 5 indicate that OV, VS and MV all caused a potent stimulation in the phosphotransferase activity of $p70^{s6k}$. The fold stimulation ranged between 2.5–4 fold as compared to untreated control. Among the 3 salts tested, the stimulatory response of VS was higher than that of either OV or MV. VS caused about 4 fold stimulation whereas only 2.5 fold stimulation was observed with OV or MV in comparison to control cells (Fig. 5). Furthermore the stimulatory response of VS was almost equal to that of insulin (Fig. 5).

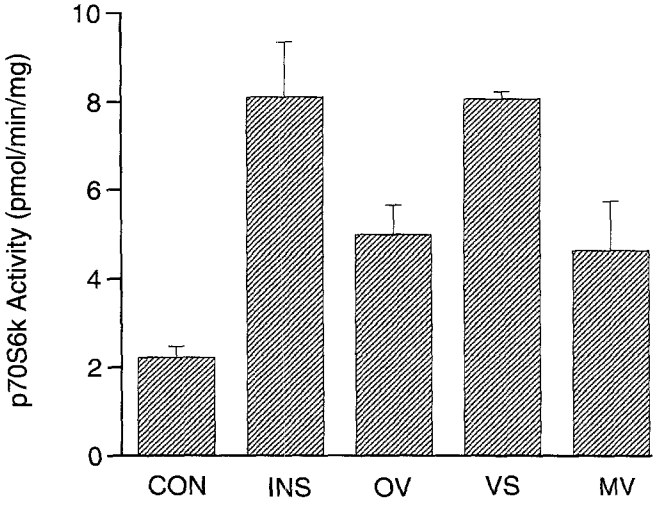

Fig. 5. Effect of insulin or vanadium compounds on 70 kDa ribosomal protein s6 kinase activity ($p70^{s6k}$) CHO-HIR cells were treated with vanadium compounds as described in Fig. 2 and $p70^{s6k}$ activity was determined in immune complex using S6 peptide, RRRLSSLRA as substrate as described in Materials and methods. The results are one of three independent experiments performed in triplicate.

Discussion

In this study we have shown that various vanadium salts activate serine/threonine kinases – MAP kinase, $p90^{rsk}$ and $p70^{s6k}$ in CHO cells overexpressing a normal human insulin receptor. The activation observed by vanadium salts was comparable to that observed with insulin and occurred in the absence of detectable tyrosyl phosphorylation of any proteins other than $p44^{mapk}$ and $p42^{mapk}$. Most notably, the tyrosyl phosphorylation of neither insulin receptor β-subunit nor the insulin receptor substrate I (IRS-I) could be detected even after 30 min incubation of cells with any of the vanadium salts. Vanadate-mediated activation of MAPK and $p90^{rsk}$ has previously been shown in other cell types but in these studies no attempts were made to correlate it with tyrosyl phosphorylation of insulin receptor β-subunit [72, 73]. Our earlier observation that tyrosyl phosphorylation and activation of MAP kinases can occur in the absence of IRS-1 phosphorylation [60, 61] has recently been confirmed by Tamemoto *et al.* who demonstrated that in the livers of mice deficient in IRS-1, MAP kinase activation was not significantly altered as compared to normal mice [74].

Recent studies have implicated MAP kinase signalling pathways in the regulation of glycogen metabolism by insulin [75–78]. Dent *et al.* demonstrated that an insulin-stimulated protein kinase (ISPK) is able to phosphorylate and thereby activate the regulatory subunit of the glycogen bound form of protein phosphatases-1 (PP1-G) [75]. Activated PP1-G dephosphorylates glycogen synthase and phosphorylase kinase and thus stimulates glycogen synthesis [75]. ISPK has subsequently been identified as an isoform of $p90^{rsk}$ or rsk II [79, 80]. Moreover, both $p90^{rsk}$ and $p70^{s6k}$ catalyze *in vitro* phosphorylation of glycogen synthase kinase-3 (GSK-3) [81–83]. GSK-3, which is able to phosphorylate and inhibit the activity of glycogen synthase is phosphorylated and inactivated in response to insulin, is believed to play an important role in the glycogen metabolism [83]. Recent studies with rapamycin, an immunosuppressant and specific inhibitor of $p70^{s6k}$, revealed that $p70^{s6k}$ may not be involved in the phosphorylation and inactivation of GSK-3 [76, 77] However, based on experiments using wortmannin, an inhibitor of phosphatidyl-inositol kinase (PI3K), a possible role of MAP kinase/$p90^{rsk}$ signalling pathway in insulin-mediated inactivation of GSK-3 has been suggested [76, 77]. Furthermore, insulin-stimulated glycogen synthesis and glycogen synthase activation was also blocked by wortmannin and rapamycin in 3T3-L1 adipocytes indicating the involvement of PI3K and $p70^{s6k}$ signalling pathway in this process [78]. These results are however in contrast to the studies of Lin and Lawrence who demonstrated that rapamycin did not attenuate the stimulatory effect of insulin on glycogen synthase and suggested that activation of MAP kinase and ribosomal s6

kinases may not be sufficient for the activation of glycogen synthesis in adipocytes [84].

An involvement of PI3K in insulin-mediated glucose transport and antilipolytic effects has also been suggested [85, 86]. Inhibition of PI3K activity by wortmannin and LY294002, another specific inhibitor of PI3K, resulted in complete inhibition of insulin-stimulated $p70^{s6k}$ as well as glucose uptake in 3T3-L1 adipocytes [85]. LY294002 treatment also inhibited the translocation of GLUT 4 glucose transporters to the plasma membrane [85]. Rapamycin, however, failed to exert any effect on insulin-stimulated glucose transport in 3T3-L1 adipocytes [87] suggesting the existence of alternate mechanisms.

Thus, the ability of vanadium salts to stimulate MAP kinase, $p90^{rsk}$ and $p70^{s6k}$ might be one of the mechanisms by which these compounds exert insulinomimetic effects in

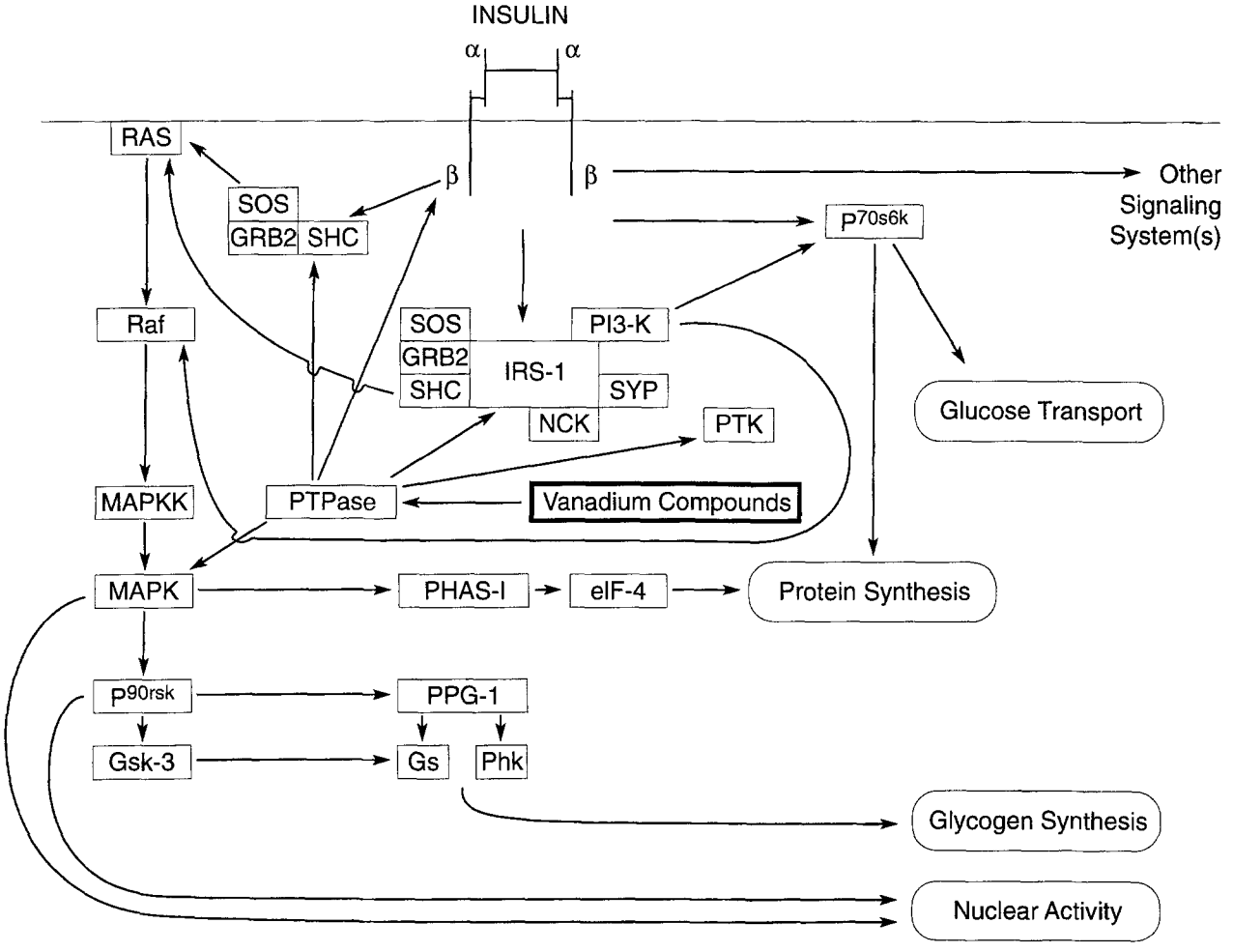

Fig. 6. Schematic model showing possible target sites of vanadium actions in relation to insulin signalling cascade: Insulin initiates its action by binding to α-subunit of its receptor, which is a heterotetrameric protein, and activates the intrinsic protein tyrosine kinase activity of β-subunit of the receptor by autophosphorylation. The activated β-subunit receptor in turn phosphorylates and thereby activates several proteins among which insulin receptor substrate-1 (IRS-l) is most widely characterized. The activated IRS-1 serves as docking protein and forms a complex with several SH2 domains containing proteins e.g. sos (son of sevenless), GRB2, Shc, Nck, Syp (PTPase) and phosphatidyl inositol 3-kinase (PI3K). This complex, in turn, stimulates Ras, MAPKK, MAPK and $p90^{rsk}$ cascade. Activated MAPK phosphorylates PHAS-1 (a heat stable protein) which is involved in protein synthesis via activation of eIF-4 (elongation initiation factor-4). The stimulated ribosomal protein $p90^{rsk}$ phosphorylates and activates glycogen bound protein phosphatase-1 (PP1-G). The activated PP1-G dephosphorylates glycogen synthase (GS) and phosphorylase kinase (Phk) and stimulates glycogen synthesis. P90rsk mediated phosphorylation of glycogen synthase kinase-3 (Gsk-3). MAPK and $p90^{rsk}$ may regulate nuclear activity by phosphorylating transcription factors. The other form of ribosomal protein s6 kinase, the 70 kDa protein, $p70^{s6k}$ lies on the other side of insulin signalling cascade and among other events is also implicated in modulating glucose transport and protein synthesis. Vanadium compounds are inhibitors of PTPases and by preventing dephosphorylation are able to increase tyrosine phosphorylation of key proteins involved in insulin signalling cascade. The potential target of this PTPase(s) could be IR-subunit, IRS-1, Shc, MAPK or a cytosolic PTK. Based on the results presented here and elsewhere the IR-β subunit/IRS-1 may not be the sites of vanadium action, however, MAPK-specific PTPase and/or Shc could be possible targets of vanadium compounds. With regard to $p70^{s6k}$ activation by vanadium compounds a role of PI3-K may be suggested. The possibility that vanadium may act at other sites can not be excluded.

various systems. This notion is further supported by a recent study in which OV-stimulated glucose transport was partially inhibited by wortmannin treatment [86]. In this regard, it is noteworthy that, in circulating mononuclear leucocytes from diabetic subjects, the insulin-mediated activation of ribosomal S-6-kinases (s6k) and MAP kinase was completely diminished, as compared to non-diabetics. $NaVO_3$ therapy of diabetic subjects not only improved the glucose homeostasis but also stimulated the basal s6k and MAPK activity between 1.7 and 3.9-fold [88] further suggesting a role of these serine/threonine kinases in the insulinomimetic effects of vanadium. Involvement of additional mechanisms in insulinomimesis by vanadium compounds is also possible. For example, studies of Fantus *et al.* have demonstrated a possible role of vanadium (OV) in enhancing insulin sensitivity and prolonging insulin action at the level of insulin receptor PTK via PTPase [89, 90]. An involvement of OV-stimulated, staurosporine-inhibited cytosolic PTK in vanadium action has been proposed in rat adipocytes [91]. It has been suggested that cytosolic PTK activation is secondary to the inhibition of a PTPase [91].

The mechanism by which vanadium salts activate MAP kinase and $p90^{rsk}$ and $p70^{s6k}$ is not clearly understood. A tentative scheme with potential sites where vanadium compounds might act in insulin signalling cascade is shown in Fig. 6. Vanadium compounds are inhibitors of PTPases [51] and by preventing the dephosphorylation are able to increase the phosphotyrosyl content of key protein molecules in insulin signalling cascade [48] (Fig. 6) and thus could result in the activation of the MAP kinases as well as $p70^{s6k}$ activating pathway. The potential target could be insulin receptor β-subunit and/or IRS-I (Fig. 6); however results presented here and elsewhere [58–61] do not support this notion. Shc protein which is tyrosyl phosphorylated and activated in response to insulin [92] may serve as another target. Shc is able to interact with GRB2 in an IRS-1 dependent and independent manner and may activate the $p21^{ras}$ signalling pathway leading to the activation of MAPK and $p90^{rsk}$ [48] (Fig. 6). Alternatively, vanadium compounds might activate MAP kinase pathway by inhibiting a constitutively active MAP kinase-specific PTPase. A vanadium-inhibitable MAPK-specific protein tyrosine phosphatase (PTP) has recently been described in Xenopus-oocytes [93] and a similar PTPase might also be expressed in CHO cells. Since several lines of evidence link PI3K with the activation of $p70^{s6k}$ [85, 94] and recent studies have shown a modulation of PI3K activity by vanadate [86], it may be suggested that vanadium compounds utilize a similar pathway in activating $p70^{s6k}$ activity.

In conclusion, these results demonstrate that similar to insulin, vanadium compounds activate serine/threonine kinases, MAP kinases, $p90^{rsk}$ and $p70^{s6k}$. However, in contrast to insulin vanadium mediated activation is independent of the tyrosine phosphorylation of either the insulin receptor or IRS-I. It is suggested that vanadium-mediated activation of MAP kinases, $p90^{rsk}$ and $p70^{s6k}$ plays an important role in mimicking insulin-like effects.

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