



ROLE OF PKB/AKT SIGNALING IN THE INSULINOMIMETIC EFFECTS OF ORGANO-VANADIUM COMPOUNDS

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Review article

Abstract

Over the past several decades, multiple studies have shown that organo-vanadium compounds (OVC) have more potent anti-diabetic and insulin-mimetic effects than their inorganic vanadium salt counterparts, both in vitro and in vivo. Several of these OVCs, such as Bis(maltolato) Oxo Vanadium (IV) (BMOV) and Bis(alixinato) Oxo Vanadium (IV) (Valx), have been studied in depth, since they have demonstrated great potency in improving hyperglycemia and insulin-resistance in rodent models of both type 1 and type 2 diabetes, with fewer or no reported side effects. While the exact molecular mechanisms of OVCs are not yet fully known, studies have shown that these OVCs are potent activators of key elements of the insulin signaling pathway, such as protein kinase B (PKB). Since PKB and its downstream substrates, such as GSK-3 and FOXO are essential in regulating glucose transport, glycogen synthesis and gluconeogenesis, it may be suggested that the activation of the PKB pathway by OVCs plays an important role in mediating the insulin-like effects of these compounds.

Keywords: BMOV, PKB, Akt, GSK-3, FOXO, insulinomimesis, antidiabetic effect.

Resumen

En las últimas décadas numerosos estudios han mostrado que los derivados orgánicos del vanadio (OVC) tienen efectos antidiabéticos e insulinomiméticos más potentes que los de sus contrapartes, las sales inorgánicas de vanadio tanto *in vitro* como *in vivo*. Muchos de estos OVCs tales como el Bis(maltolato) Oxovanadio(IV) (BMOV) y el Bis(alixinato) Oxovanadio(IV) (Valx) han sido estudiados en profundidad, demostrando su gran potencia en mejorar la hiperglucemia y la insulino-resistencia en modelos de la diabetes tipo 1 y tipo 2, con ninguno o pocos efectos colaterales. Mientras que los mecanismos exactos de los OVCs no son todavía totalmente conocidos, los estudios han mostrado que los OVCs son potentes activadores de elementos clave de la vía de señalización de la insulina, tal como la proteína quinasa B (PKB). Ya que la PKB y sus sustratos, tales como el GSK-3 y FOXO son esenciales en el transporte de glucosa, síntesis de glucógeno y gluconeogénesis, se puede sugerir que la activación de la vía de la PKB por los OVCs desempeña un papel importante en mediar los efectos insulinomiméticos de estos compuestos.

Palabras clave: BMOV, PKB, Akt, GSK-3, FOXO, insulinomimesis, efecto antidiabético.

I. Introduction

Studies performed over the last 2 decades have established that vanadium compounds exert various insulin-mimetic and anti-diabetic effects *in vitro* and *in vivo* (reviewed in [1-3]), such as improved insulin sensitivity and glucose homeostasis in animal models of type 1 and 2 diabetes mellitus [4-6], as well as in a small number of diabetic human subjects [7-10]. Although the precise mechanism by which vanadium compounds elicit their insulin-like effects is not clear, their ability to enhance glucose transport [11-15], glycogen synthesis [11,16-18], lipogenesis [19,20] and to inhibit lipolysis [14,20], as well as gluconeogenesis [11], has been suggested to play an important role in this process [1].

Inorganic vanadium salts, such as sodium orthovanadate (SOV), sodium metavanadate (SMV) and vanadyl sulphate (VS) were utilized in earlier studies to test their insulin-like properties [1,3,4]. However, it was noted that treatment with vanadium salts resulted in gastro-intestinal side effects [4,5,21,22]. To overcome these side-effects, McNeill et al. introduced an organo-vanadium complex (OVC) bis(maltolato) oxovanadium (BMOV), in experimental studies aimed to test its ability to improve glucose homeostasis in rodent models [23]. Since then, a number of OVCs have been synthesised and tested as insulinomimetic agents. These include vanadium (IV) oxo bis(acetylacetonate) (VO(acac)₂/VAC) [24], vanadium (IV) oxo bis(3-ethylacetylacetonate) (VET), vanadium (IV) oxo bis(ethylmaltolato) (BEOV), vanadium (IV) oxo bis(6-methylpicolinato), and L-glutamic acid δ -monohydroxamate-NaOV complex [25-33]. These OVCs have been reported to be better absorbed and appear to be less toxic than inorganic salts, as well as exert more potent insulin-like effects than inorganic vanadium salts (reviewed in [1]). Due to the possibility that some of these OVCs may be eventually developed as therapeutic agents to treat diabetes, there is a great deal of interest to understand the molecular mechanism of their action. Our laboratory has been involved in examining the effects of these OVC on key elements of the insulin signaling pathways, such as protein kinase B (PKB), which is implicated in regulating glucose homeostasis. The aim of this review is to provide a summary of our studies, and those of others, highlighting the role of PKB and its downstream substrates in mediating the insulin-like effects of BMOV and other OVCs.

II. Protein Kinase B (PKB)

PKB, also known as Akt (a product of Akt proto-oncogene) is a serine/threonine kinase with 3 identified isoforms in mammalian systems: PKB α /Akt1, PKB β /Akt2 and PKB γ /Akt3 [34]. Various PKB isoforms share more than 80% sequence homology with each other, and are

composed of 3 functionally distinct regions: an amino-terminal pleckstrin homology (PH) domain, a central catalytic serine/threonine kinase domain, and a carboxy-terminal regulatory domain. The PH domain of PKB binds phosphatidylinositol 3,4,5 triphosphate (PIP₃). PIP₃ is generated by the phosphorylation of phosphatidylinositol 4,5 bisphosphate (PIP₂) at position 3 of the inositol ring, which is catalyzed by the activation of phosphatidylinositol 3-kinase (PI3-K) [35]. In the insulin signaling cascade, insulin-induced tyrosine phosphorylation and activation of the β -subunit of its receptor results in the tyrosine phosphorylation of insulin receptor (IR) substrates (IRS), promoting the binding and activation of the lipid kinase activity of PI3-K (Figure 1). Binding of PIP₃ to the PH domain of PKB allows its phosphorylation in threonine 308 and serine 473, catalyzed by the upstream phosphoinositide-dependent kinase (PDK) 1 and 2, respectively [35-37].

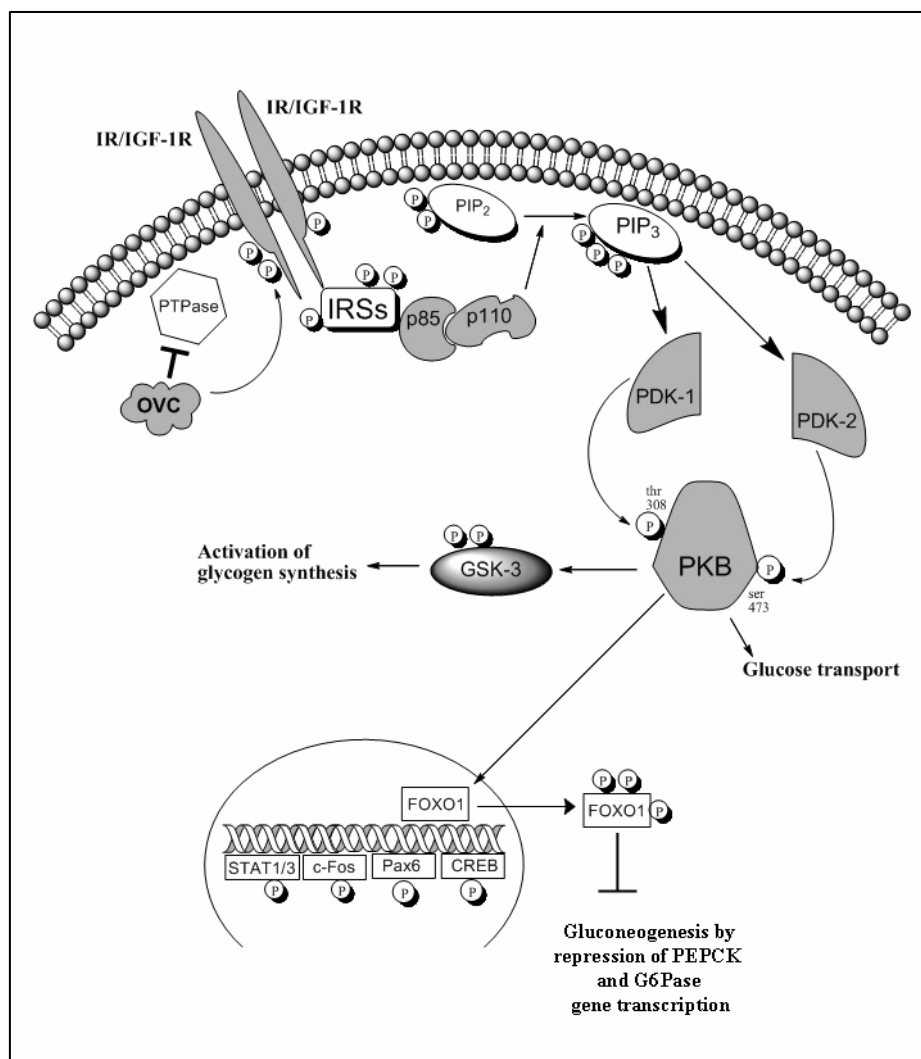


Figure 1: Activation of the IR signaling cascade by OVCs. OVCs are known to be potent inhibitors of protein tyrosine phosphatases (PTPases). PTPases dephosphorylate IR, causing a decrease in its downstream signaling. By inhibiting PTPase activity, OVC allow for an increased phosphorylation of IR- β subunits, which go on to phosphorylate several intracellular substrates, such as insulin receptor substrate-1 (IRS-1). Phosphorylated IRS-1 serves as a docking protein for binding several signaling molecules such as the p85 subunit of PI3-K, activating its p110 catalytic subunit, catalyzing the conversion of PIP₂ to PIP₃. PIP₃ recruits PKB, PDK-1 and PDK-2 to the plasma membrane, where PKB becomes phosphorylated and activated. PKB mediates a variety of cellular responses, such as glucose transport, glycogen synthesis, and gluconeogenesis, through several downstream substrates, such as GSK-3 and FOXO1.

In recent years, PKB and its downstream substrates have emerged as key contributors in modulating glucoregulatory responses. For example, PKB is involved in translocation of glucose transporter-4 (GLUT4) vesicles to the surface of fat and muscle cells, resulting in the activation of glucose uptake and transport [38-41]. Also, PKB, through phosphorylation and thereby inactivation of glycogen synthase kinase-3 (GSK-3), participates in the process of glycogen synthesis [42,43]. Furthermore, PKB-induced inactivation by phosphorylation of GSK-3 and forkhead box-containing protein (FOXO) transcription factor O1 (FOXO1) suppresses the transcription of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), downregulating gluconeogenesis [44,45].

III. Activation of PKB signaling by OVCs

Because of the importance of PKB in mediating glucoregulatory effects, several investigators examined the effect of OVCs on PKB phosphorylation. In 3T3-L1 adipocytes, human hepatoma (HepG2), Chinese hamster ovary (CHO), and human lymphocyte (IM9) cells, BMOV augmented the activation state of PKB through its phosphorylation on Ser 473 [24,46,47]. This phosphorylation occurred in a dose- and time dependent fashion, and was detectable within 10 minutes of treatment with BMOV [47]. Furthermore, BMOV-induced PKB phosphorylation required PI3-K, as pharmacological inhibition of PI3-K with wortmannin in CHO cells overexpressing human insulin receptor (IR) almost completely abolished the BMOV response [24]. In addition to BMOV, other OVCs, such as bis(acetylacetonato)-oxovanadium (IV) (Vac), bis(picolinato)-oxovanadium (IV), bis(3-methylpicolinato)-oxovanadium (IV), bis(6-methylpicolinato)-oxovanadium (IV), and bis(allixinato)-oxovanadium (IV) (Valx), were also shown to induce the Ser 473 phosphorylation of PKB in CHO-IR and 3T3-L1 adipocytes [24,48-50].

Valx was shown to potently enhance the phosphorylation of IR and IRS-1, as well as PKB in 3T3-L1 adipocytes [49]. This phosphorylation of PKB lead to an increase of GLUT4 translocation to the plasma membrane, which increased glucose transport into the cells [49]. The increased PKB activation by Valx also enhanced the phosphorylation of GSK-3 and FOXO1, which was associated with a decrease in the transcription of glucose-6-phosphatase (G6Pase) in HepG2 cells [49]. G6Pase, primarily found in the liver, catalyzes the hydrolysis of glucose-6-phosphate to glucose, the final step in both glycogenolysis and gluconeogenesis. Along with phosphoenolpyruvate carboxykinase (PEPCK), another important enzyme involved in gluconeogenesis, catalyzing the conversion of oxaloacetate to phosphoenolpyruvate, these enzymes have been shown to be increased in diabetic states [51-53].

While these results using isolated cells indicate that an enhanced PKB phosphorylation through PI3-K activation may be a link between BMOV and its glucose lowering effects, prior studies conducted in streptozotocin (STZ)-treated Wistar rats, as well as Zucker fatty (fa/fa) rats show otherwise [54,55]. In these studies, a 3-week oral administration of BMOV to these animal models of type 1 and type 2 diabetes, respectively, normalized their blood glucose levels, without showing any effect on PI3-K/PKB system [55]. Similar observations were made by Marzban et al., which showed that despite an increase in insulin sensitivity and a normalization of plasma glucose levels, a 3 week treatment of BMOV did not affect basal or insulin-stimulated PKB activity in skeletal muscle or liver of control, fa/fa diabetic and STZ-treated animals [54].

In contrast to these results, more recent studies have demonstrated a clear link between the insulin-mimetic actions of OVCs and PKB activation [50]. In STZ-diabetic mouse model, daily oral administration of Valx reduced hyperglycemia [50], which was associated with the restoration of blunted phosphorylation of PKB observed in the skeletal muscle of these mice [50]. These authors also reported that Valx-treated diabetic animals exhibited a reversal of the decreased

phosphorylation of GSK-3 β and displayed increased levels of GLUT4 in skeletal muscle, as well as a decrease in the levels of FOXO1 [50].

As stated earlier, GSK-3 is a downstream substrate of PKB which exists in two isozymic forms, GSK-3 α and GSK-3 β [43,56,57]. In the basal state, GSK-3 remains constitutively active; however, PKB catalyzed phosphorylation of GSK-3 renders it inactive [56,57]. In its basal state, GSK-3 suppresses glycogen synthase (GS) through inhibitory phosphorylation, thereby inhibiting glycogen synthesis. Upon phosphorylation by PKB, GSK-3 is inhibited, resulting in the activation of GS, leading to an increase in glycogen synthesis [58]. GSK-3 also plays a role in regulating the expression of PEPCK and G6Pase [39].

FOXO1, part of the Forkhead box-containing protein subtype O family of transcription factors, which consists of FOXO2 and FOXO3 and FOXO6 [59], is also an important downstream effector of PKB. PKB, through serine/threonine phosphorylation, regulates FOXO activity, which results in the translocation of FOXO from the nucleus to the cytoplasm [60]. Similar to GSK-3, FOXO has also been shown to play an important role in regulating the gene expression of G6Pase and PEPCK [61-64].

Since phosphorylation of both GSK-3 and FOXO1 has been reported in response to OVCs in several different cell types, as well as in vivo diabetic models [24,46,47,50] [49,65], it may be suggested that PKB/GSK-3 pathway participates in OVC-induced suppression of gluconeogenesis and enhancement of glucose utilization and storage [26,50,66-68].

IV. Conclusions

Many OVCs have been demonstrated to exert potent insulinomimetic effects both in vitro and in vivo. The precise mechanism by which OVCs trigger these effects remain obscure, however, many of these OVCs have been reported to activate key components of the insulin signaling pathway, such as PKB and its downstream targets, GSK-3 and FOXO1. Since these signaling components play an important role in regulating glucose transport, glycogen synthesis and gluconeogenesis, it may be suggested that OVC-induced activation of this pathway may be one of the mechanisms that OVCs utilize to elicit their insulin-like properties.

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