A stable peroxovanadium compound with insulin-like action in human fat cells

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Summary Aqueous solutions of peroxovanadium (pV) compounds are potent insulin-mimics in various types of cell. Since chemical instability is a problem with these agents, we studied the insulin-like action in human fat cells of a stable pV complex, bpV(pic). It enhanced ¹⁴C-U-glucose uptake in a dose-dependent manner by approximately twofold which was slightly less than the effect of insulin (approximately threefold). The pV complex did not alter cell-surface insulin binding and submaximal concentrations did not influence cellular sensitivity to insulin action on glucose uptake. The bpV(pic) inhibited the lipolytic effect of isoprenaline to the same extent as insulin; however, when the cGMP-inhibitable low-K_m phosphodiesterase (cGI-PDE) was blocked with the specific inhibitor OPC 3911, the antilipolytic effect of insulin, but not that of bpV(pic), was completely prevented. Moreover, when lipolysis was stimulated by the non-hydrolysable cAMP analogue N⁶-monobutyryl cAMP, bpV(pic), in contrast to insulin, main-

tained an antilipolytic effect. These findings indicate that bpV(pic) exerts its antilipolytic effect not only through cGI-PDE activation, similar to the effect of insulin, but also by means of other mechanisms. The tyrosine kinase activity of insulin receptors from human placenta was not altered by the pV compound itself, whereas bpV(pic) clearly enhanced insulin-stimulated activity. In contrast, in situ tyrosine phosphorylation of the insulin receptor β -subunit as well as that of several other proteins was clearly increased in cells which were treated with bpV(pic), whereas vanadate only amplified insulin-stimulated tyrosine phosphorylation. In conclusion, bpV(pic) exerts powerful insulin-like effects in human fat cells and may be a new and potentially useful agent in the management of insulin-resistant states. [Diabetologia (1996) 39: 235–242]

Key words Adipocytes, insulin, vanadate, peroxovanadate, glucose uptake, lipolysis, tyrosine kinase.

Vanadium is a trace element which is essential for most plant and animal species [1]; it is found in small amounts in various tissues [2]. Vanadium salts, e.g. vanadyl sulphate, sodium metavanadate or orthovanadate are well-known insulin-mimics [3]; this has been shown in cellular studies in vitro for glucose up-

take and metabolism, antilipolysis and glycogen synthase activation [3–6]. Moreover, the insulin-like action of vanadate has also been demonstrated in vivo, and studies have shown that vanadate treatment improves glucose homeostasis in diabetic rats [7, 8]. One possible mechanism involved may be the ability of vanadate to enhance insulin receptor tyrosine kinase activity [6, 9] which, in turn, is attributed to phosphotyrosine phosphatase (PTP) inhibition [10]. However, other studies suggest that at least some of the insulin-like effects of vanadate occur independently of the tyrosine kinase [5, 11].

Hydrogen peroxide (H₂O₂) can exert insulin-like effects [12] and since intracellular H₂O₂ production is enhanced by insulin it has been proposed as a

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Abbreviations: pV, Peroxovanadium; bpV(pic), potassium bisperoxopicolinatooxovanadate; cGI-PDE, cGMP-inhibitable low-k_m phosphodiesterase; EC₅₀, half-maximal concentration; PTP, phosphotyrosine phosphatase; sp act, specific activity; WGA, wheat-germ agglutinin.

second messenger in insulin signalling [13, 14]. An aqueous solution of hydrogen peroxide and vanadate generates peroxovanadates (pV), which are more potent as insulin-mimics than either agent by itself [15, 16]. pV compounds are far more potent PTP inhibitors than vanadate, and, as a consequence, can enhance insulin receptor tyrosine kinase activity [15–18]. We recently reported that pV solutions, but not vanadate, display insulinomimetic properties in human adipocytes [19]. This is in contrast to rat adipocytes where both agents are effective [15, 16]. Thus, pV is potentially interesting for the treatment of insulin-resistant states in humans. However, one problem with aqueous pV solutions is their chemical instability [15]. Therefore, the insulin-like action in human fat cells of a well-characterized stable pV compound, potassium bisperoxopicolinatooxovanadate, bpV(pic) [18], was investigated.

Subjects, materials and methods

Materials. Human monocomponent insulin and mono[¹²⁵I]tyr-A₁₄-insulin (specific activity [sp act] 200–300 µCi/µg) were purchased from Novo (Copenhagen, Denmark). [¹⁴C]U-D-glucose (sp act 246 µCi/µmol) and ECL chemiluminescence kit were from Amersham (Amersham, Bucks., UK) and [³²P]ATP (sp act 3 Ci/µmol) was from Du Pont (Geneva, Switzerland). Collagenase, bovine serum albumin (fraction V), wheat-germ agglutinin (WGA), isoprenaline, N⁶-monobutyl cAMP, Glu⁸⁰-Tyr²⁰, Ro-1724 and protease inhibitors were from Sigma (St. Louis, Mo., USA). The OPC 3911 was a kind gift from Dr. P. Belfrage (Lund, Sweden). Medium 199 was obtained from Statens Bakteriologiska Laboratorium (Stockholm, Sweden) and sodium orthovanadate (Na₃VO₄) from BDH Chemicals (Poole, Dorset, UK). A monoclonal mouse antiphosphotyrosine antibody was purchased from UBI (Lake Placid, N. Y., USA). The bpV(pic) was synthesized and purified as described previously [18] by Dr. J. Ng, Department of Chemistry, McGill University (Montreal, Canada).

Adipose tissue biopsies. Biopsies (3–5 g) of subcutaneous adipose tissue were removed from non-diabetic subjects (*n* = 22) undergoing elective abdominal surgery for non-malignant disease. The biopsies were obtained immediately after induction of anaesthesia and placed in a vessel containing medium 199 at 37 °C. The study was approved by the ethics committee of Göteborg University.

Isolation of adipose cells. Dissected tissue pieces (~5 mg) were incubated at 37 °C in medium 199 containing 4 % bovine serum albumin and 0.8 mg/ml collagenase in a shaking water bath. After approximately 50 min, liberated cells were filtered through a nylon mesh and washed 4 times in fresh medium containing albumin. Cell size and number were measured as described previously [20]. The average fat cell volume was 0.53 ± 0.04 nl/cell (mean $\pm$ SEM, calculated from the average cell volume of each of the 22 patients).

¹⁴C-U-glucose uptake. Cellular uptake of ¹⁴C-U-glucose was measured during a 1-h incubation of cells at 37 °C in glucose-free medium 199 at a lipocrit of 3–5 %. Following pre-incubation for 15 min in the absence or presence of insulin, bpV(pic) and other agents as indicated, 0.86 µmol/l ¹⁴C-U-glucose was

added. After 1 h the cells were separated from the incubation medium, put into scintillation fluid and radioactivity was counted. The cellular clearance of medium glucose was calculated according to the formula:

$$\frac{(\text{cpm cells})}{(\text{cpm medium}) \times (\text{cell number}) \times s}$$

and taken as an index of the rate of glucose uptake [21]. Cell-associated radioactivity obtained when the tracer was added immediately before separation from medium was subtracted to correct for extracellularly trapped radioactivity (9.1 ± 1.0 % of total radioactivity in non-stimulated cells).

Lipolysis. Isolated cells at a lipocrit of 1–3 % were incubated at 37 °C with or without isoprenaline, insulin or bpV(pic) at the indicated concentrations for 1 h. The incubation was stopped by centrifuging the cells through silicone oil. The glycerol content of the incubation medium was analysed [22] and taken as an index of lipolysis.

Partial purification of insulin receptors from human placenta. Human placental membranes were obtained essentially as previously reported [23] and were solubilized in 1 % Triton X-100. Following centrifugation at $9,000 \times g$ for 10 min, the supernatant was applied to a WGA agarose column and allowed to recirculate 3 times [24]. After careful washing, the purified insulin receptors were eluted with 0.3 mol/l N-acetylglucosamine in 4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid (Hepes) buffer. Insulin binding was determined by adding 0.034 pmol ¹²⁵I-insulin to 100 µl eluate. After 18 h at 4 °C, the receptors were precipitated with polyethyleneglycol using IgG as a carrier and specific binding was measured.

Insulin receptor tyrosine kinase activity. Triplicate samples (40 µl) of the eluate containing purified insulin receptors were incubated for 15 min at 20 °C with 2 mmol/l MnCl₂ in the presence or absence of bpV(pic), vanadate and insulin as indicated. Unlabelled ATP (15 µmol/l) was then added and after an additional 15 min Glu⁸⁰-Tyr²⁰ (2.5 mg/ml) together with [³²P]ATP (~10⁶ cpm) plus unlabelled ATP (maintaining the total ATP concentration at 15 µmol/l) were added to a final volume of 100 µl. The incubations were then continued for another 15 min. Aliquots (75 µl) were spotted on Whatman 3-MM papers (Whatman International, Maidstone, Kent, UK), washed with 10 % trichloroacetic acid containing 10 mmol/l Na₄P₂O₇ and then once with 95 % ethanol followed by 95 % acetone. Radioactivity was determined by scintillation counting, and the background obtained from samples without Glu⁸⁰-Tyr²⁰ was subtracted.

Detection of phosphotyrosine-containing proteins in whole cell lysates by immunoblotting. Adipose cells were pre-incubated for 5 min at 37 °C in medium 199 in the presence or absence of bpV(pic) and vanadate as indicated and the incubation was then continued for another 10 min with or without addition of insulin (1000 µU/ml). Then, 400 µl of cells per sample were centrifuged through dinonylphthalate and were lysed with 0.4 ml of a solution (Lysis Medium) containing (in mmol/l) 25 Tris-HCl, pH 7.4, 0.5 EGTA, 25 NaCl, 1 sodium orthovanadate, 10 NaF, 0.2 leupeptin, 1 benzamidine, and 0.1 4-(2-Aminoethyl)-benzene sulphonylfluoride (Calbiochem, La Jolla, Calif., USA) 1 % NP-40 and rocked for 40 min at 4 °C. The solubilized proteins were separated on a 10 % SDS-polyacrylamide gel, transferred to a nitrocellulose membrane, and immunoblotted with monoclonal anti-phosphotyrosine antibody according to the recommendations of the manufacturer. An ECL chemiluminescence kit with a secondary, horseradish-

Fig. 1. Effect of bpV(pic) (●) and H₂O₂ (▲) on glucose uptake in human adipocytes. Cells were incubated for 15 min at 37°C with the indicated concentrations of bpV(pic) or H₂O₂ after which ¹⁴C-U-glucose was added. After 60 min cell-associated and total medium radioactivity was measured. Data are from one representative experiment of three. Insert: Effects of insulin and bpV(pic). Cells were pre-incubated for 15 min at 37°C with or without 1000 μU/ml insulin and 0.1 mmol/l bpV(pic), respectively. Data are means ± SEM of six separate experiments

peroxidase-labelled antibody (Amersham), was employed and the bands were visualized on an autoradiography film.

Statistical analysis

Unless otherwise indicated, statistical significance of differences was tested with Student's two-tailed *t*-test for paired observations. Data are expressed as means ± SEM.

Results

¹⁴C-U-glucose uptake. The compound bpV(pic) stimulated ¹⁴C-U-glucose uptake in human adipocytes in a dose-dependent manner. The maximum effect was approximately twofold and occurred at a bpV(pic) concentration of approximately 0.1 mmol/l (Fig. 1). The effect of insulin was somewhat more pronounced (*p* < 0.05) with an approximately threefold increase in glucose uptake (Fig. 1, insert). The dose-response curve for bpV(pic) was bell-shaped with a complete return to basal glucose uptake at very high concentrations (Fig. 1). However, the range of effective concentrations covered at least three orders of magnitude. The EC₅₀ (concentration producing half-maximal effect) for bpV(pic) was 0.026 ± 0.006 mmol/l (*n* = 6).

Fig. 2. Effect of insulin on glucose uptake in the presence (▲) or absence (●) of 0.005 mmol/l bpV(pic). Cells were pre-incubated with the indicated concentrations of insulin and glucose uptake was measured as in Figure 1. Data are from one representative experiment of three

For comparison, the effect of H₂O₂ alone was also studied. The maximal effect of this agent was smaller (*n* = 3, *p* < 0.05) than that of pV (~50% increase in ¹⁴C-U-glucose uptake) and required a much higher concentration, approximately 10 mmol/l (Fig. 1). Above this concentration, there was no additional effect of H₂O₂ (data not shown).

Next, the possible effects of bpV(pic) on insulin sensitivity were studied. Submaximal concentrations (0.005 and 0.01 mmol/l, respectively) of bpV(pic) added together with insulin at various concentrations did not affect the dose-response curve for insulin (Fig. 2). Only at very low insulin concentrations, was the intrinsic effect of bpV(pic) demonstrable. At higher insulin concentrations the effect of bpV(pic) was negligible and insulin sensitivity was unaffected (Fig. 2). Furthermore, no additive or inhibitory effects were seen when high concentrations of insulin (1000 μU/ml) and bpV(pic) (0.1 mmol/l) were combined (data not shown).

Sodium orthovanadate at several different concentrations (from 0.5 to 8 mmol/l) did not exert any effect on glucose uptake (data not shown). However, when added together with bpV(pic), an inhibitory interaction was seen. Vanadate decreased bpV(pic)-stimulated glucose uptake in a dose-dependent manner (Fig. 3). In contrast, no such inhibition was exerted by vanadate when combined with insulin (data not shown).

Preincubating cells with bpV(pic) for 20 min at different concentrations (0.01–0.5 mmol/l) did not produce any change in subsequent cell surface ¹²⁵I-insulin binding (performed at 16°C in the presence of KCN as described previously [25]) (data not shown). Neither was ¹²⁵I-insulin binding at 37°C for 1 h altered by the concomitant presence of bpV(pic) (data not shown).

Fig. 3. Effect of vanadate on bpV(pic)-stimulated glucose uptake. Cells were pre-incubated with 0.1 mmol/l bpV(pic) together with the indicated concentrations of sodium orthovanadate. Data are from one representative experiment of three and are expressed as percent of maximal increase (produced by bpV(pic) alone)

Fig. 4. Antilipolytic effect of insulin (A) and bpV(pic) (B). Cells were incubated for 60 min with isoprenaline (1 μ mol/l) and insulin or bpV(pic) at the indicated concentrations in the absence (●) or presence of OPC 3911 (10 μ mol/l, ■) or Ro-1724 (50 μ mol/l, ▲). Then, glycerol release was assessed. Data are from one representative experiment repeated 4 times

Table 1. Tyrosine kinase activity of WGA-purified insulin receptors from human placenta – effects of insulin, bpV(pic) and vanadate

Insulin	–	bpV(pic) (0.05 mmol/l)	bpV(pic) (0.5 mmol/l)	Vanadate (1 mmol/l)
		Activity	(% of basal)	
–	100	109 $\pm$ 10	127 $\pm$ 19	121 $\pm$ 13
10 ³ μ U/ml	204 $\pm$ 32 ^b	261 $\pm$ 47 ^{b,c}	389 $\pm$ 68 ^{a,c}	248 $\pm$ 74 ^a
10 ⁵ μ U/ml	240 $\pm$ 38 ^b	300 $\pm$ 63 ^{a,c}	479 $\pm$ 73 ^{a,c}	ND

Samples of eluate from WGA-columns were incubated at 20°C with insulin and other agents as indicated for 15 min followed by the addition of ATP and then, after another 15 min, addition of ³²P-ATP (total ATP concentration 15 μ mol/l) and Glu⁸⁰-Tyr²⁰ (see Materials and Methods). Activity is expressed as percent of non-stimulated activity and was assessed as phosphate incorporation in Glu⁸⁰-Tyr²⁰ in relation to amount of receptors measured by ¹²⁵I-insulin binding. Data are means $\pm$ SEM of seven to ten separate experiments. Statistical significance of differences was assessed with the Wilcoxon signed rank test. ^a $p < 0.05$; ^b $p < 0.01$, vs no addition. ^c $p < 0.05$ vs insulin alone at the indicated concentration. ND, Not determined

Lipolysis. When lipolysis was stimulated with 1 μ mol/l isoprenaline (by 10–50-fold, data not shown) both insulin and bpV(pic) exerted equal, pronounced antilipolytic effects (Fig. 4A, B). The dose-response curve for the antilipolytic effect of bpV(pic) was shifted approximately fourfold to the right compared to the effect on glucose uptake, (Fig. 4B vs Fig. 1), and the EC₅₀ for the antilipolytic effect was 0.11 $\pm$ 0.02 mmol/l ($n = 4$). In contrast, the opposite relationship was found for these two effects of insulin, i.e., a higher sensitivity to the effect on lipolysis as compared to glucose uptake (for example Fig. 4A vs Fig. 2).

As shown in Figure 4A, the antilipolytic effect of insulin was completely blocked by the cilostamide derivative OPC 3911, a selective inhibitor of the low-K_m cGMP-inhibitable phosphodiesterase (cGI-PDE) [26, 27]. In contrast, this agent at a maximally effective concentration (10 μ mol/l) did not affect the magnitude of the antilipolytic effect of pV (Fig. 4B), whereas it tended to decrease cellular sensitivity to the pV compound. Inhibition of the so-called cAMP-specific phosphodiesterase (PDE) with Ro-1724 [27] did not alter the antilipolytic effect of pV (Fig. 4B). Furthermore, lipolysis stimulated with the non-degradable cAMP analogue N⁶-monobutyl cAMP [28] was not affected by insulin, whereas bpV(pic) at high concentrations (5–10 mmol/l) exerted a pronounced antilipolytic effect ($n = 3$, $p < 0.05$, data not shown).

Insulin receptor tyrosine kinase activity and protein tyrosine phosphorylation. Partially purified insulin receptors were obtained from human placenta in order to ensure sufficient quantities. The presence of insulin in the receptor preparation dose-dependently stimulated tyrosine kinase activity assessed by phosphate incorporation into Glu⁸⁰-Tyr²⁰, whereas bpV(pic)

Fig. 5. Effect of insulin (1000 μ U/ml), bpV(pic) (0.1 mmol/l) and vanadate (4 mmol/l) on tyrosine phosphorylation of lysates of human adipocytes. Intact cells were pre-incubated for 15 min at 37 °C with or without vanadate and bpV(pic). During the final 10 min of the pre-incubation, insulin was added when indicated. Then, the cells were lysed and anti-phosphotyrosine immunoblots were performed as described in "Materials and Methods". Data are from one representative experiment of three. The indicated numbers refer to protein standards with different molecular weights (kDa). Insulin receptor β -subunit approximately 97 kDa

alone, did not produce any effect (Table 1). However, when added together with insulin, bpV(pic) amplified the kinase activity. Vanadate (1 mmol/l) did not consistently influence basal or insulin-stimulated activity in this assay.

In situ tyrosine phosphorylation was assessed in lysates of human adipocytes. Insulin receptor β -subunit autophosphorylation of insulin-treated fat cells was amplified when bpV(pic) or vanadate was also present (Fig. 5). bpV(pic) alone, but not vanadate, enhanced insulin receptor β -subunit (~97 kDa) tyrosine phosphorylation, as well as tyrosine phosphorylation of several other proteins. In contrast, vanadate only amplified insulin-stimulated tyrosine phosphorylation, mainly at the insulin receptor β -subunit (Fig. 5).

Discussion

Vanadate and H_2O_2 in combination act synergistically to produce a powerful insulin-like effect. This is attributed to pV compounds formed by the two agents [15]. However, aqueous pV solutions are unstable [15] and, thus, unsuitable as therapeutic agents in insulin-resistant states. This study investigated the insu-

lin-like action in human adipocytes of a well-characterized stable pV compound. The data show that this agent, bpV(pic), is at least as effective as the previously studied combination of vanadate and H_2O_2 [19]. The effect of bpV(pic) on glucose uptake was nearly as pronounced as the effect of insulin itself, whereas the antilipolytic response to these two agents did not differ.

With glucose uptake a biphasic dose-response curve for bpV(pic) was found. The range of effective concentrations was, however, much wider than for the combination of vanadate and H_2O_2 (approximately three orders of magnitude compared to one, respectively [19]). It is not clear why glucose uptake returns towards basal levels at high pV concentrations. Previously, we speculated that a competitive interaction with vanadate formed by ligand dissociation from pV solutions may be involved [19]. However, bpV(pic) is stable and not expected to decompose. This was also supported by the finding of a biphasic curve even when a much shorter incubation period was used; total time 35 instead of 75 min (data not shown). Furthermore, another pV compound, bpV(phen), which is even more stable than bpV(pic) ($t_{1/2}$ in solution 10 vs 8.5 days, respectively [18]) also displayed a biphasic effect on glucose uptake (data not shown). An alternative explanation for biphasic dose-response curves is that pV complexes, while shown to be specific phosphotyrosine phosphatase inhibitors [16, 18] at high concentrations may also inhibit serine/threonine phosphatases [18]. Such an effect of pV may lead to enhanced serine/threonine phosphorylation of the insulin receptor itself, the GLUT 4 glucose transporter and/or protein kinase A, leading to a decreased rate of glucose transport. In contrast, the antilipolytic action of bpV(pic) was not biphasic. This may also be explained by an increased serine phosphorylation and subsequent activation of the cGMP inhibitable phosphodiesterase leading to an inhibition of lipolysis [29, 30]. However, further studies are clearly required to corroborate this hypothesis.

Insulin sensitivity, assessed as insulin-stimulated glucose uptake, was not affected by submaximal bpV(pic) concentrations. This differs from results of vanadate treatment in rat adipocytes [25]. This discrepancy may, at least partly, be due to the lack of effect of bpV(pic) on insulin binding in human adipocytes. Vanadate, on the other hand, enhances insulin binding and insulin sensitivity in rat adipocytes [25, 31]. Thus, the present results suggest that, in human adipocytes, pV compounds exert their effects mainly at the post-binding level.

The inhibitory action of vanadate on bpV(pic)-stimulated glucose uptake is likely to be due to a competition between vanadate and bpV(pic) at critical post-receptor sites. Cytosolic tyrosine kinases may be involved, since vanadate has been reported

to exert its insulin-like effects in rat fat cells via such a kinase [32]. Other possible explanations for the inhibitory effect of vanadate on bpV(pic) action include competition for a common transmembrane transport mechanism (B.I. Posner et al., unpublished data) leading to a decrease in cellular uptake of bpV(pic) as well as an inhibitory effect of the major intracellular form of vanadate, vanadyl, on insulin receptor kinase activity [33].

The antilipolytic effect of bpV(pic) displayed differences from that of insulin. First, the dose-response curve for bpV(pic) was shifted approximately four-fold to the right compared to its effect on glucose uptake. Insulin displayed the opposite relationship, i.e., a greater sensitivity for antilipolysis than for glucose uptake. Second, the selective inhibitor of the cGI-PDE, OPC 3911, impaired the sensitivity but not the responsiveness to the antilipolytic action of bpV(pic). In contrast, insulin's antilipolytic effect was completely abolished, which is consistent with previous findings [29, 34] and documents the key role of cGI-PDE activation for the antilipolytic effect of insulin in fat cells. Taken together, these findings indicate that partially divergent pathways are involved in the actions of insulin and bpV(pic) on lipolysis. The effect of insulin to stimulate cGI-PDE leads to an enhanced degradation of intracellular cAMP [29, 34] which, in turn, reduces the rate of lipolysis. Although activation of cGI-PDE also seems to be one important mechanism for the effect of bpV(pic), additional mechanisms must be involved. This is also supported by the finding that lipolysis stimulated by N^6 -mbcAMP, a cAMP-analogue which is resistant to degradation by cGI-PDE [28, 34], was inhibited by high concentrations of bpV(pic) but not by insulin. This is at variance with our previous work [19] in which we found no effect of the aqueous pV solution on N^6 -mbcAMP-stimulated lipolysis. However, it is not clear which concentrations of pV were attained in that study but they were probably much lower than those presently employed. In harmony with our present findings, it has previously been reported that vanadate can stimulate cGI-PDE through activation of tyrosine kinases and protein kinase C in a manner different from that of insulin [35].

The basic mechanisms for pV action are, as discussed above, not fully established. The possibility that the peroxide component per se plays a critical role should be taken into account. In addition, previous work has shown that insulin can activate an H_2O_2 -generating system, and that H_2O_2 is capable of producing insulin-like effects in human adipocytes [14]. Vanadate and, thus, also pV compounds could possibly act in a similar manner by activating NADH oxidase which promotes H_2O_2 formation [13, 36]. The present data, however, clearly argue against H_2O_2 as the sole mediator for the action of bpV(pic). The effect of H_2O_2 on glucose uptake was

not as marked as that of bpV(pic), and, furthermore, much higher concentrations (~100-fold) were needed to exert a maximal effect. A more likely mechanism for the action of bpV(pic) on human fat cells is its ability to enhance insulin receptor tyrosine kinase activity [15–18]. Previous work has shown that this is not due to a direct effect of pV on the insulin receptor [37] but rather to its ability to inhibit PTP associated in situ with the insulin receptor [17, 18]. This results in receptor autophosphorylation and activation, albeit at a slower rate than that seen following insulin administration [18, 37]. The present data support this concept since bpV(pic) alone had no significant effect on partially purified insulin receptors. Its ability to augment the effect of insulin may reflect the presence of some PTP activity in the preparations of partially purified insulin receptors used in this study. The effect of bpV(pic) in intact cells to augment insulin receptor β -subunit phosphorylation as well as tyrosine phosphorylation of several other proteins in situ, even in the absence of insulin, probably reflects the presence of PTPs in the intact cell which play a major role in the regulation of the receptor phosphorylation state [37]. This may also suggest an ongoing, constitutive phosphorylation/dephosphorylation process in intact cells, as opposed to the partially purified receptor preparation where insulin may be required to induce a significant receptor phosphorylation. These findings are also consistent with previous results with vanadate as well as pV in rat adipocytes [15, 16, 25, 37]. The inability of bpV(pic) as well as vanadate (U. Smith, unpublished data) to alter insulin sensitivity in human fat cells with respect to glucose uptake indicates that under the employed conditions an enhanced insulin-stimulated receptor autophosphorylation and kinase activity does not further amplify the insulin signal. This implies a large spare kinase activity of the insulin receptor.

Although the mechanisms involved are not fully established, it is clear that bpV(pic), a stable pV compound, is a powerful insulin-mimic in human fat cells. Ongoing in vivo studies indicate that this agent is effective in tissues other than fat, e.g., liver and skeletal muscle (J.-F. Yale et al., unpublished data). Moreover, pV administered i.p. was recently shown to produce a rapid insulin-like response in diabetic rats in vivo [38]. Further investigations are warranted to elucidate the mechanisms underlying the metabolic effects of pV compounds and also to assess the efficacy of these agents in human and animal states of insulin resistance.

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