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The insulin-like effect of sodium vanadate on adipocyte glucose transport is mediated at a post-insulin-receptor level

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Sodium vanadate has several insulin-like effects. To determine whether vanadate acts via the insulin receptor, I investigated the effect of vanadate on glucose transport (2-deoxyglucose uptake) in adipocytes that had been treated to decrease the number of insulin receptors. (1) Trypsin (100 μg/ml) caused > 95% loss of ^{125}I-insulin binding and rendered glucose transport resistant to both insulin and an anti-insulin-receptor antibody. However, vanadate caused an 8-fold increase in the transport rate [EC_{50} (concn. giving 50% of maximum effect) 0.2 mM] in both control and trypsin-treated cells, demonstrating that the insulin receptor does not have to be intact for vanadate to stimulate glucose transport. (2) Insulin receptors were depleted by treatment of adipocytes with insulin (100 ng/ml) in the presence of Tris (which blocks receptor recycling). A 2 h treatment caused 60% loss of receptors, and a shift to the right in the dose–response curve for insulin stimulation of glucose transport (EC_{50} 0.3 ng of insulin/ml in controls, 1.2 ng/ml in treated cells). The response to vanadate was again unaffected. Treatment with insulin for 4 h caused a 67% decrease in insulin binding and, in addition to the rightward shift in the insulin dose–response curve, a decrease in basal and maximal transport rates (which cannot be explained by decreased insulin receptor number). The EC_{50} of vanadate was again equal in control and treated cells, but glucose transport in the presence of a maximally effective concentration of vanadate (1 mM) was decreased. I conclude that the effect of vanadate on glucose transport is independent of the insulin receptor. Induction of a post-receptor defect (which may be a decrease in the total number of cellular glucose transporters) by prolonged exposure to insulin decreases the potency of a maximally effective concentration of vanadate. The findings demonstrate that vanadate stimulates glucose transport by an effect at a level distal to the insulin receptor.

INTRODUCTION

Sodium vanadate has a wide range of metabolic effects (for review see Ramasarma & Crane, 1981). Among these effects are several that coincide with those of insulin. For example, vanadate stimulates glucose transport and oxidation in adipocytes (Dubyak & Kleinzeller, 1980; Clausen *et al.*, 1981) and glucose incorporation into glycogen in muscle and hepatocytes (Clausen *et al.*, 1981; Tolman *et al.*, 1979), and increases the activity of adipocyte glycogen synthase (Tamura *et al.*, 1983, 1984). These insulin-like effects suggest that vanadate might be a useful tool in studies of the mechanism of insulin action.

Insulin is known to act via binding to a specific cell-surface receptor. Little is understood of the mechanisms involved in coupling insulin binding to its biological actions. However, there is evidence that a reaction catalysed by an insulin-receptor-associated, tyrosine-specific, protein kinase is involved in mediating at least some of the actions of insulin (Kasuga *et al.*, 1982; Petruzelli *et al.*, 1982; Haring *et al.*, 1982; Kahn *et al.*, 1985). It has been demonstrated that vanadate, like insulin, increases the kinase activity of adipocyte insulin receptors (Tamura *et al.*, 1984). It was proposed that increased kinase activity of the receptor is responsible for insulin-like actions of vanadate. However, the role of the insulin-receptor kinase in stimulation of glucose metabolism has been questioned (Simpson & Hedo, 1984; Pilch *et al.*, 1985). Therefore, in the present work, I have specifically addressed the question of whether the effect of vanadate on adipocyte glucose transport is mediated via the insulin receptor. The approach was to deplete the cells of insulin receptors and then to determine the effect of vanadate on glucose transport rates. Two completely different techniques were utilized to deplete adipocytes of insulin receptors: (1) treatment with the proteolytic enzyme trypsin, and (2) incubation with insulin under conditions where receptor recycling is inhibited (i.e. in the presence of Tris). I demonstrate that sodium vanadate is an equally potent stimulator of glucose transport after either treatment regime. The findings demonstrate that vanadate acts at a site distinct from the insulin receptor and hence that its effect on receptor phosphorylation is not involved in stimulation of glucose transport.

MATERIALS AND METHODS

Chemicals

Pig insulin was generously given by Dr. Mary Root of Eli Lilly and Co. (Indianapolis, IN, U.S.A.). Bovine serum albumin (type CRG-7) was purchased from Armour Pharmaceutical Co. (Kankakee, IL, U.S.A.). Collagenase (type CLS) was from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.). Silicone oil (density 0.99 g/ml) was purchased from A. H. Thomas (Philadelphia, PA, U.S.A.). 2-Deoxy-D-[1-^{3}H]glucose was from New England Nuclear Corp. (Boston, MA, U.S.A.). 2-Deoxy-D-glucose, Hepes, Tris, trypsin (type XI), soya-bean trypsin inhibitor and phloretin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sodium orthovanadate was purchased from

Fisher Scientific (Houston, TX, U.S.A.). Anti-insulin-receptor antibodies were raised by immunizing hens with human liver membranes. The IgG was semi-purified from egg yolks (Stuart *et al.*, 1986).

Preparation of isolated adipocytes

Male Sprague–Dawley rats, weighing 190–230 g, were killed by cervical dislocation and epididymal fat-pads were removed. Adipocytes were liberated by a modification of the method of Rodbell (1964), by incubating the fat-pads at 37 °C for 1 h in a buffer containing 137 mM-NaCl, 5 mM-KCl, 4.2 mM-$NaHCO_3$, 1.3 mM-$CaCl_2$, 0.5 mM-KH_2PO_4, 0.5 mM-$MgCl_2$, 0.4 mM-$MgSO_4$, 20 mM-Hepes, pH 7.4, plus bovine serum albumin (40 mg/ml), collagenase (1.5 mg/ml) and 5 mM-glucose. Cells were filtered through 1000 μm nylon mesh, centrifuged (25 *g* for 90 s) and washed three times in the appropriate incubation buffer.

Glucose transport assay

Uptake of 2-deoxyglucose was used as an index of the rate of glucose transport (Olefsky, 1978). Adipocytes (approx. 300000 cells in 1 ml) were incubated with shaking for 45 min at 37 °C in the same buffer as described above except that the bovine serum albumin concentration was 10 mg/ml, and there was no collagenase or glucose. Additions were as described in the text and Figure legends.

At the end of the incubation period, 2-deoxy-D-[1-^{3}H]glucose (0.2 μCi) and unlabelled 2-deoxyglucose were added (total concn. 0.1 mM). The assays were terminated 3 min later by transferring 200 μl samples of the cell suspension to plastic micro-tubes containing silicone oil (100 μl) and centrifuging for 30 s in a Beckman Microfuge. The tubes were cut through the oil layer with a razor blade, and the radioactivity in the cell pellet was measured in a liquid-scintillation counter. In each experiment, deoxyglucose uptake was also determined in the presence of 0.3 mM-phloretin. Phloretin completely inhibits specific transport of deoxyglucose, so that cell-associated radioactivity in the presence of phloretin forms a measure of the amount of sugar trapped in the extracellular water space of the cell layer (Gliemann, 1985). All data of 2-deoxyglucose uptake have been corrected for this factor.

Trypsin treatment

Adipocytes were incubated in the same buffer as for the glucose transport assay, together with trypsin (100 μg/ml unless otherwise indicated) and 5 mM-glucose (Green & Olefsky, 1982). After 10 min at 37 °C (with shaking at 140 rev./min), soya-bean trypsin inhibitor was added at twice the concentration of trypsin, to stop the reaction, and the cells were washed three times.

Insulin-binding assay

Insulin was iodinated by the chloramine-T method and purified on a column of Sephadex G-50 (Freychet *et al.*, 1971). The specific radioactivity of the iodinated insulin was approx. 150–180 μCi/μg.

Adipocytes were incubated in a buffer similar to that described for the glucose-transport assay, but containing 5 mM-glucose and of pH 7.6. Binding of ^{125}I-insulin (0.3 ng/ml) was determined after 2.5 h at 16 °C, as previously described (Marshall *et al.*, 1981; Green *et al.*, 1984). All data have been corrected for non-specific binding, which was determined in the presence of a large excess of unlabelled insulin (50 μg/ml).

Down-regulation of insulin receptors

Adipocytes suspended in a buffer similar to that used for the insulin-binding assay, but containing 30 mM-Tris instead of Hepes, were incubated at 37 °C in the absence or presence of insulin (100 ng/ml) for 2 or 4 h as indicated. They were then washed three times in the Tris buffer, pH 7.0, and the receptor-bound insulin was allowed to dissociate at pH 7.0 for 1 h at 37 °C. It has been demonstrated previously that all receptor-bound insulin and any insulin internalized (including subsequently generated degradation products) is effectively dissociated or released by this procedure (Marshall & Olefsky, 1980). After the 1 h dissociation, the cells were washed and resuspended in the buffer for measurement of insulin binding or glucose transport as described above.

RESULTS

Effects of trypsin on insulin binding and glucose transport

As previously reported (Kono & Barham, 1971*b*; Green & Olefsky, 1982), exposure of adipocytes to trypsin resulted in a dramatic decrease in subsequently measured specific binding of ^{125}I-insulin. This effect was maximal at trypsin concentrations above 30 μg/ml (Fig. 1). Subsequent experiments were therefore performed in cells treated with 100 μg of trypsin/ml.

Trypsin itself is known to have insulin-like activities, including stimulation of glucose transport (Kono & Barham, 1971*a*; Kikuchi *et al.*, 1981). The aim of the present study was to investigate the ability of vanadate, and other agents, to stimulate glucose transport in trypsin-treated cells. Therefore it was important to allow the glucose transport rate to recover from the stimulatory effect of trypsin. To determine the time period necessary for complete deactivation of the trypsin effect, adipocytes

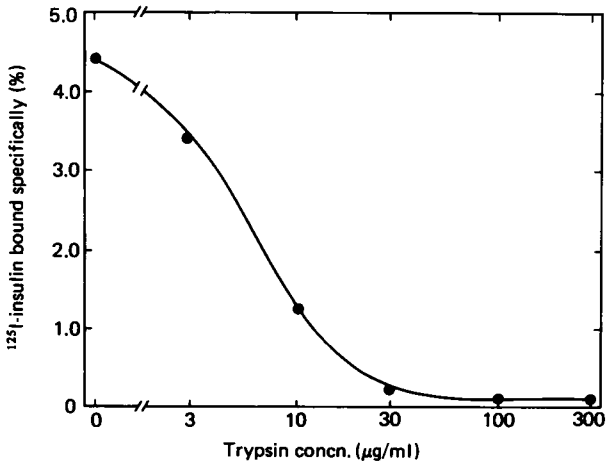

Fig. 1. Effect of trypsin on insulin binding to adipocytes

Adipocytes were treated with trypsin, at the concentrations indicated, for 10 min at 37 °C. Soya-bean trypsin inhibitor was added (final concn. twice that of trypsin) and the cells were washed three times. Specific binding of ^{125}I-insulin was determined as described in the Materials and methods section.

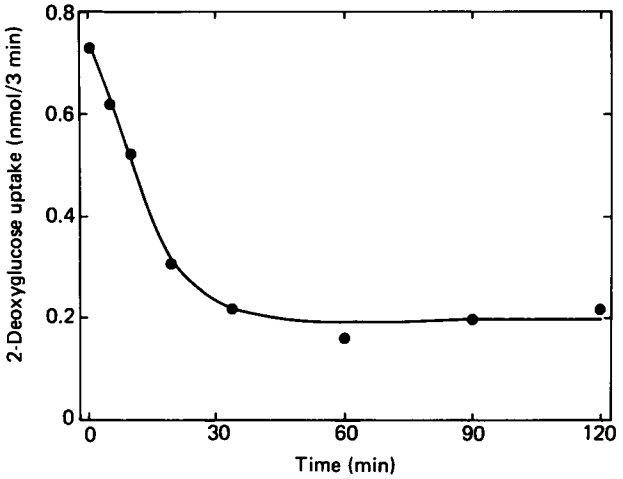

Fig. 2. Deactivation of glucose transport from the stimulatory effect of trypsin

Adipocytes were treated with trypsin (100 μg/ml) for 10 min. At $t = 0$, soya-bean trypsin inhibitor was added (200 μg/ml) and 2-deoxyglucose uptake rate was measured (see the Materials and methods section) at the times indicated. The basal uptake rate (i.e. before trypsin treatment) was 0.19 nmol/3 min.

Table 1. ^{125}I-insulin binding to adipocytes at intervals after trypsin treatment

Adipocytes were treated with trypsin (100 μg/ml) as described in the Materials and methods section. They were then washed, resuspended and incubated at 37 °C. At the times indicated, samples of the cells were taken, and ^{125}I-insulin binding was determined at 16 °C as described in the Materials and methods section.

	^{125}I-insulin bound specifically (%)	
Time (h)	Control	Trypsin-treated
0	4.10	0.16
1	4.25	0.21
2	4.48	0.27
3	4.62	0.32
4	4.83	0.31

were incubated with trypsin (100 μg/ml) for 10 min, and then the reaction was stopped with soya-bean trypsin inhibitor (Fig. 2). Immediately after trypsin treatment ($t = 0$ in Fig. 2), transport was increased approx. 4-fold above the basal rate. After addition of the soya-bean trypsin inhibitor, the transport rate rapidly decreased, returning to basal values after about 1 h. The half-time for deactivation was approx. 12 min.

Since measurements of glucose transport in trypsin-treated cells were to be performed after a recovery period, it was important to determine whether the insulin-binding capacity of the cells also recovered. Adipocytes were treated with trypsin, washed, and then incubated at 37 °C for 0–4 h before measurement of ^{125}I-insulin binding (Table 1). Trypsin-treated adipocytes had a 96% decrease in binding capacity as compared with controls. The binding capacity of the treated cells increased slowly, but, even after a 4 h recovery period,

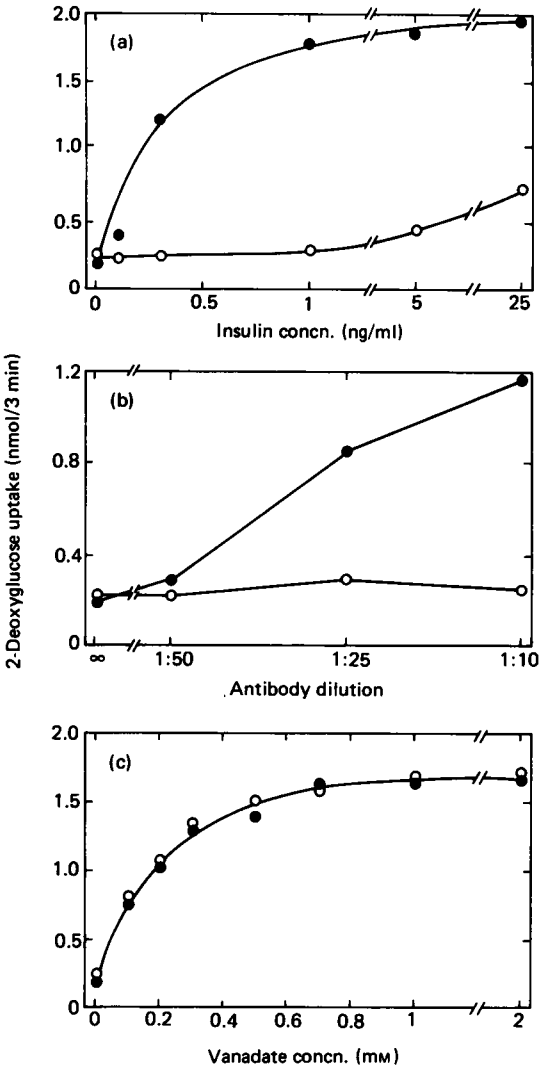

Fig. 3. Glucose transport in untreated and trypsin-treated adipocytes

Adipocytes were incubated without (●) or with (○) trypsin (100 μg/ml) as described in the Materials and methods section. They were then incubated at 37 °C for 1.5 h to allow the transport rate to return to basal values, washed again, and incubated with insulin (*a*), a serum containing antibodies to the insulin receptor (*b*) or sodium vanadate (*c*). After a further 45 min, 2-deoxyglucose uptake rate was determined over a 3 min period.

binding was 94% lower than in the controls. Interestingly, binding also increased in the untreated adipocytes. This latter phenomenon has been reported by other workers (Marshall *et al.*, 1984).

Effect of vanadate in trypsin-treated adipocytes

Adipocytes were treated with trypsin to destroy insulin receptors and then washed and incubated at 37 °C for 90 min to allow the glucose transport rate to return to basal values. The cells were then incubated with various concentrations of insulin, an insulin-receptor antibody or sodium vanadate, and glucose transport was measured (Fig. 3).

Insulin increased the rate of glucose transport approximately 10-fold in the untreated cells (Fig. 3*a*),

with a half-maximally effective insulin concentration of approx. 0.3 ng/ml. The trypsin-treated cells, however, were almost totally unresponsive to insulin at physiological concentrations. High concentrations of insulin (above approx. 5 ng/ml) did stimulate glucose transport somewhat, even though > 95% of the insulin receptors were destroyed in these cells. This is consistent with the well-established concept of 'spare receptors' for insulin (see the Discussion section).

An antibody to the insulin receptor (raised in chickens) also stimulated glucose transport in untreated cells (Fig. 3*b*), as has been described for many anti-insulin-receptor antibodies (Van Obberghen & Kahn, 1981). However, like insulin, the antibody was unable to stimulate glucose transport in the trypsin-treated cells. Again, this is consistent with the marked destruction of insulin receptors in the trypsin-treated cells. Finally, treatment of the trypsin-treated adipocytes with a second exposure to trypsin did not re-stimulate glucose transport (results not shown).

In contrast with insulin, trypsin and anti-receptor antibody, sodium vanadate was an equally potent stimulator of glucose transport in the trypsin-treated adipocytes as in the untreated cells (Fig. 3*c*). In both groups of cells maximal stimulation was approx. 8-fold, in the presence of 1 mM-vanadate, and the dose–response curves were superimposable. Half-maximal stimulation was seen at a vanadate concentration of approx. 0.2 mM.

The experiments described above demonstrate that vanadate stimulates glucose transport in adipocytes depleted of insulin receptors by treatment with trypsin. This suggests that vanadate stimulates glucose transport by a mechanism independent of the insulin receptor. However, an alternative explanation is that trypsin only destroys the binding function, and that vanadate acts on a trypsin-resistant region of the receptor. To distinguish between these two possibilities, I utilized a second technique to deplete adipocytes of insulin receptors: insulin-induced down-regulation.

Effect of vanadate after insulin-induced down-regulation of insulin receptors

Previous studies have demonstrated that insulin induces internalization of insulin receptors into adipocytes (Green & Olefsky, 1982; Berhanu *et al.*, 1985). Under normal physiological conditions the receptors are recycled back to the cell surface (Marshall *et al.*, 1981). However, the buffer Tris inhibits the recycling process, such that, when adipocytes are incubated with insulin in a Tris-buffered medium, insulin receptors are rapidly lost from the cell surface and degraded intracellularly (Marshall *et al.*, 1981; Green & Olefsky, 1982).

In the experiment shown in Fig. 4, adipocytes were suspended in a Tris-buffered medium and incubated for 2 h in the absence or presence of insulin (100 ng/ml). The cells were then washed, bound insulin was allowed to dissociate, the cells were washed again, and both insulin binding and glucose transport were measured (see the Materials and methods section for details). The 2 h exposure to insulin resulted in a 60% decrease in insulin binding to the cells (Fig. 4*b*, inset). This was accompanied by a marked shift to the right in the dose–response curve for insulin's effect on glucose transport, as would be expected (Fig. 4*a*). There was little or no effect on the maximally insulin-stimulated rate. In contrast with insulin, vanadate was equally able to stimulate glucose

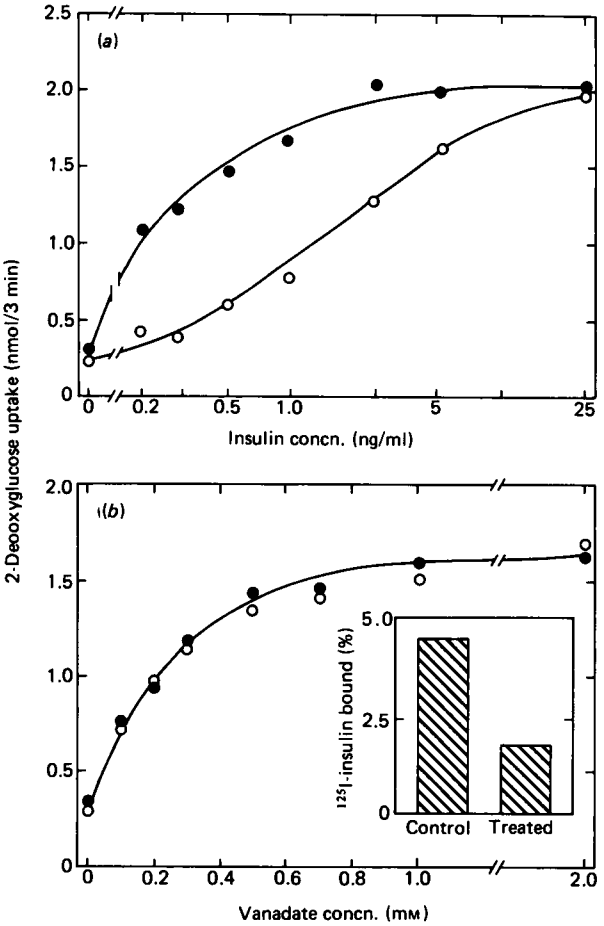

Fig. 4. Effect of insulin or vanadate on glucose transport after a 2 h preincubation with insulin

Adipocytes were suspended in Tris-buffered medium either without or with insulin (100 ng/ml) for 2 h. They were then washed, incubated at pH 7.0 for 1 h, washed again, and incubated with insulin (*a*) or vanadate (*b*) at the concentrations indicated. After 45 min, 2-deoxyglucose uptake rate was measured: ●, control cells; ○, insulin-treated cells. The inset shows specific binding of ^{125}I-insulin to the control and insulin-treated cells, measured at 16 °C (see the Materials and methods section for details).

transport in the down-regulated and in the control cells (Fig. 4*b*). That is, a 60% decrease in the number of insulin receptors on the cells did not affect the ability of vanadate to stimulate transport. This is further evidence that vanadate acts at a post-receptor level.

More prolonged exposure of adipocytes to insulin in the presence of Tris (4 h) has been reported to cause a decrease in the rate of maximally insulin-stimulated glucose transport, as well as a shift to the right in the dose–response curve (Marshall & Olefsky, 1980; Garvey *et al.*, 1985). The decrease in the maximally stimulated rate has been attributed to post-insulin-receptor changes (Garvey *et al.*, 1985). Fig. 5 shows effects of insulin and vanadate in cells that had been treated with insulin for 4 h in a Tris-buffered medium. Insulin treatment caused a loss of approx. 67% of cell-surface insulin receptors under these conditions (Fig. 5*b*, inset). This was accompanied by a marked decrease in the basal and maximally insulin-stimulated glucose transport rates

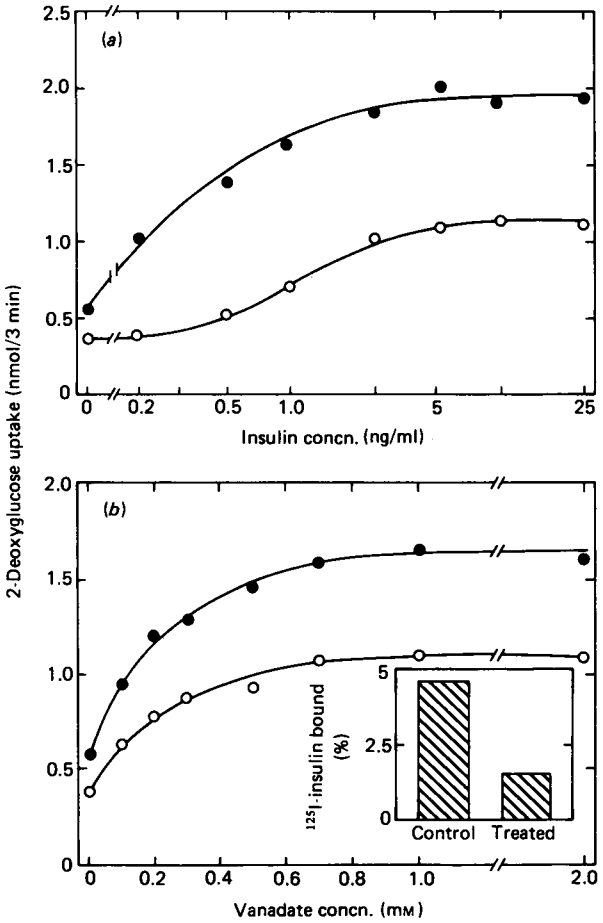

Fig. 5. Effect of insulin or vanadate on glucose transport after a 4 h preincubation with insulin

Adipocytes suspended in a Tris-buffered medium were incubated without or with insulin (100 ng/ml) for 4 h. Other details are as in Fig. 4: ●, controls; ○, insulin-treated cells. Rates of glucose transport in the treated cells were significantly lower than in controls at each insulin concentration (including basal) at the 5% level or less (by Student's *t* test). The inset shows specific binding of ^{125}I-insulin to the control and insulin-treated cells, measured at 16 °C (see the Materials and methods section for details).

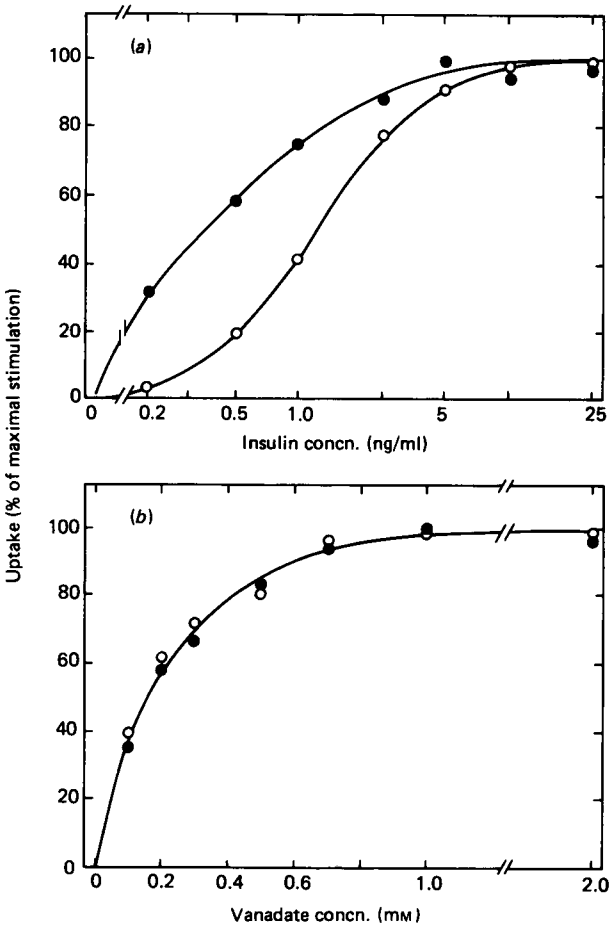

Fig. 6. Glucose transport in adipocytes treated with insulin for 4 h

The data from Fig. 5 have been re-plotted as percentages of maximal stimulation by insulin (*a*) or vanadate (*b*) in control (●) and insulin-treated (○) adipocytes.

(Fig. 5*a*), indicating post-receptor changes or changes in the number of glucose transporters (see the Discussion section). Interestingly, the 4 h insulin treatment also decreased the ability of a maximally effective concentration of vanadate to stimulate glucose transport (Fig. 5*b*). To assess more accurately the dose–response relationships of insulin and vanadate to glucose transport in these cells, the data from Fig. 5 have been normalized, i.e. expressed as percentages of maximal insulin or vanadate effects (Fig. 6). By this analysis it is possible to determine the effect of receptor loss, after correction for the post-receptor effects of insulin exposure. As before, there is a marked shift to the right in the insulin dose–response curve as a result of the loss of insulin receptors (Fig. 6*a*). However, after correction for the post-receptor change, vanadate was equally potent at stimulating glucose transport in the insulin-treated cells and in the controls (Fig. 6*b*). This indicates that the cells have normal sensitivity to vanadate, despite a marked receptor loss. These findings further support the conclusion that loss of insulin receptors from adipocytes does not alter the ability of vanadate to stimulate glucose transport. However, the insulin-induced impairment of maximal transport rates (i.e. the post-receptor defect) is not overcome by vanadate. This indicates that vanadate acts at a level distal to the insulin receptor, but before the level of the insulin-induced post-receptor alterations.

DISCUSSION

Previous studies (Tamura *et al.*, 1983, 1984) have demonstrated that vanadate, like insulin, increases the ability of the insulin receptor to phosphorylate both itself and an exogenous substrate (histone H2b). It was suggested that this effect of vanadate on the insulin receptor is responsible for its insulin-like activity. I have specifically addressed the question of whether the insulin receptor is indeed involved in the stimulatory effect of vanadate on glucose transport. The experimental approach was to treat adipocytes to destroy insulin receptors and then assess the effect of vanadate on 2-deoxyglucose uptake. Insulin receptors were destroyed either by treatment of adipocytes with trypsin or by exposure of

the cells to insulin in the presence of Tris. Both of these techniques effectively depleted the cells of insulin receptors, but did not alter the ability of vanadate to stimulate glucose transport. This demonstrates that the insulin receptor is not involved in the mechanism by which vanadate stimulates transport.

Trypsin treatment of adipocytes digested cell-surface insulin receptors, and destroyed the ability of insulin to stimulate glucose transport. Furthermore, a chicken antibody to the insulin receptor, which, like many anti-insulin-receptor antibodies (Van Obberghen & Kahn, 1981), stimulates glucose transport in adipocytes, was ineffective in the trypsin-treated cells. Finally, a second exposure to trypsin did not re-stimulate glucose transport. These findings demonstrate that exposure to trypsin destroys the ability of those agents that normally interact with the insulin receptor to stimulate glucose transport. In contrast, sodium vanadate was fully able to stimulate glucose transport in the trypsin-treated cells. This strongly suggests that vanadate stimulates glucose transport in adipocytes via a mechanism that does not involve interaction with the insulin receptor. However, from the trypsin data alone it is possible that vanadate acts via a trypsin-insensitive region of the receptor, as described below.

The insulin receptor is a tetrameric glycoprotein consisting of two α-subunits and two β-subunits joined by disulphide bonds (for review see Pessin *et al.*, 1985). The α-subunits are thought to bind insulin, and there is evidence that the β-subunits play a role as effectors. Thus it has been demonstrated that the β-subunit has associated with it a tyrosine-specific protein kinase activity. Furthermore, the activity of this kinase is increased by insulin. These findings have led to speculation that the biological actions of insulin are mediated via activation of this kinase.

Treatment of adipocytes with trypsin destroyed the insulin-binding capacity of the insulin receptor, suggesting that the α-subunit is destroyed by trypsin, or at least that the insulin-binding region of the subunit is altered. Therefore the finding that vanadate stimulates glucose transport in trypsin-treated cells suggests that vanadate does not work by interaction with the α-subunit. The β-subunit of the receptor is thought to be also exposed at the cell surface (Hedo *et al.*, 1981). However, if the β-subunit remains intact after trypsin treatment, vanadate could act via this subunit. This would be consistent with the view that the insulin-like actions of vanadate are a result of increased phosphorylation of the β-subunit of the insulin receptor (Tamura *et al.*, 1984). To address the question of the involvement of the β-subunit in the action of vanadate, we used a second technique to deplete insulin receptors, namely chronic incubation with insulin in the presence of Tris. Previous studies have shown that, in the presence of insulin, adipocyte insulin receptors are internalized, but can be recycled to the cell surface (Marshall *et al.*, 1981). Hence, during a relatively short-term incubation with insulin (less than 12 h) there is no net loss of insulin receptors from the cell surface (Marshall *et al.*, 1981, 1984). However, Tris inhibits recycling, such that, in a Tris-buffered medium, insulin results in a rapid decrease in the insulin-binding capacity of the adipocytes. The decrease in insulin-binding capacity under these conditions is due to endocytosis and intracellular degradation of the receptors (Green & Olefsky, 1982). Furthermore, it is clear that both the α- and β-subunits are internalized and degraded under these conditions (Berhanu *et al.*, 1985).

A 2 h incubation of adipocytes with insulin, in a Tris-buffered medium, caused a loss of approx. 60% of specific binding of ^{125}I-insulin. As would be expected, this caused a marked shift to the right in the dose–response curve for the effect of insulin on glucose transport, but little or no decrease in the maximally stimulated rate. This is consistent with previous studies (Marshall & Olefsky, 1980; Garvey *et al.*, 1985) and with the well-established concept of 'spare receptors' for insulin action (Kono & Barham, 1971*a*). However, vanadate was an equally potent stimulator of glucose transport in the 2 h-insulin-treated cells as in the untreated cells. This provides strong evidence that the stimulatory effect of vanadate on glucose transport is not mediated at the level of either the α- or β-subunit of the insulin receptor.

As in previous reports (Marshall & Olefsky, 1980; Green & Olefsky, 1982; Garvey *et al.*, 1985), 4 h exposure to insulin caused only a slightly more pronounced receptor loss than the 2 h treatment (67% versus 60%). However, in addition to the rightward shift in the dose–response curve, 4 h treatment with insulin results in a decrease in the basal and maximally stimulated transport rates. Since the magnitude of the receptor loss under these conditions does not fall below the number of 'spare receptors' on the cells (less than 10% of the receptors present on adipocytes have to be occupied for maximal stimulation), and since the basal rate decreases, this decreased rate has been attributed to changes at a post-receptor level. Indirect evidence suggests that the nature of this post-receptor change is a decrease in the total number of functional glucose transporters (Garvey *et al.*, 1985). In contrast with that for insulin, there was no rightward shift in the curve for the effect of vanadate on glucose transport in the cells exposed to insulin for 4 h. Again, this is consistent with the view that vanadate acts at a post-insulin-receptor level. Interestingly, the rate of glucose transport in the presence of a maximal concentration of vanadate was decreased by the 4 h insulin treatment. Thus vanadate is not able to stimulate transport fully in the cells displaying a decrease in insulin action at the post-receptor level. This demonstrates that vanadate stimulates glucose transport via a step distal to the insulin receptor but before the insulin-induced post-receptor defect. This is consistent with the view that the post-receptor defect is due to a decrease in the total number of cellular glucose transporters.

Tamura *et al.* (1984) demonstrated convincingly that vanadate, like insulin, increases phosphorylation and protein kinase activity of the insulin receptor. The present findings demonstrate that these effects of vanadate on the insulin receptor are not involved in stimulation of glucose transport. Interestingly, there is also strong evidence that the stimulatory effect of insulin on glucose transport is not mediated via its effects on phosphorylation of the insulin receptor. Firstly, Simpson & Hedo (1984) have reported an anti-insulin-receptor antibody that stimulates glucose transport, but has no effect on receptor phosphorylation. Secondly, Pilch *et al.* (1981, 1985) have reported that treatment of adipocytes with elastase causes cleavage of the β-subunit and impaired kinase activity, but has no effect on the ability of insulin to stimulate glucose oxidation. The current findings further support the view that insulin-receptor

kinase activity is not required for stimulation of glucose transport, and suggest that phosphorylation may be involved in other actions of both insulin and vanadate.

It is possible that vanadate works by increasing phosphorylation of a non-insulin-receptor protein involved in regulation of glucose transport. Thus it is known that vanadate is a potent inhibitor of membrane phosphotyrosyl-protein phosphatase (Swarup *et al.*, 1982). There have been several other mechanisms proposed which could account for effects of vanadate, including inhibition of $Na^+ + K^+$-ATPase and inhibition of the $Ca^{2+} + Mg^{2+}$-ATPase/Ca^{2+}-transport system (Delfert & McDonald, 1985). However, the current findings do not distinguish between these possibilities. Further study is required to elucidate the mechanism of vanadate action, and this information could provide valuable insight into the mechanism of insulin action.

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REFERENCES

Berhanu, P., Green, A. & Olefsky, J. M. (1985) Methods Diabetes Res. **1**, part C, 25–41

Clausen, T., Anderson, T. L., Sturup-Johansen, M. & Petkova, O. (1981) Biochim. Biophys. Acta **646**, 261–267

Delfert, D. M. & McDonald, J. M. (1985) Arch. Biochem. Biophys. **241**, 665–672

Dubyak, G. R. & Kleinzeller, A. (1980) J. Biol. Chem. **255**, 5306–5312

Freychet, P., Roth, J. & Neville, D. M., Jr. (1971) Biochem. Biophys. Res. Commun. **43**, 400–408

Garvey, W. T., Olesky, J. M. & Marshall, S. (1985) J. Clin. Invest. **76**, 22–30

Gliemann, J. (1985) Methods Diabetes Res. **1**, part C, 105–118

Green, A. & Olefsky, J. M. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 427–431

Green, A., Bustillos, D. P. & Misbin, R. I. (1984) Diabetes **33**, 1045–1050

Haring, H. U., Kasuga, M. & Kahn, C. R. (1982) Biochem. Biophys. Res. Commun. **108**, 1538–1545

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Hedo, J. A., Kasuga, M., Van Obberghen, E., Roth, J. & Kahn, C. R. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 4791–4795

Kahn, C. R., White, M. F., Grigorescu, F., Takayama, S., Haring, H. U. & Crettaz, M. (1985) in Molecular Basis of Insulin Action (Czech, M. P., ed.), pp. 67–93, Plenum Press, New York

Kasuga, M., Karlsson, F. A. & Kahn, C. R. (1982) Science **215**, 185–187

Kikuchi, K., Schwartz, C., Creacy, S. & Larner, J. (1981) Mol. Cell. Biochem. **37**, 125–130

Kono, T. & Barham, F. W. (1971*a*) J. Biol. Chem. **246**, 6204–6209

Kono, T. & Barham, F. W. (1971*b*) J. Biol. Chem. **246**, 6210–6216

Marshall, S. & Olefsky, J. M. (1980) J. Clin. Invest. **66**, 763–772

Marshall, S., Green, A. & Olefsky, J. M. (1981) J. Biol. Chem. **256**, 11464–11470

Marshall, S., Garvey, W. T. & Geller, M. (1984) J. Biol. Chem. **259**, 6376–6384

Olefsky, J. M. (1978) Biochem. J. **172**, 137–145

Pessin, J. E., Mottola, C., Yu, K.-T. & Czech, M. P. (1985) in Molecular Basis of Insulin Action (Czech, M. P., ed.), pp. 3–29, Plenum Press, New York

Petruzelli, L. M., Ganguly, S., Smith, C. J., Cobb, M. H., Rubin, C. S. & Rosen, O. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 6792–6796

Pilch, P. F., Axelrod, J. D., & Czech, M. P. (1981) in Current Views on Insulin Receptors (Andreani, D., DePirro, R., Lauro, R., Olefsky, J. & Roth, J., eds.), pp. 255–260, Academic Press, London

Pilch, P. F., Shia, M. A. & Rubin, J. B. (1985) in Molecular Basis of Insulin Action (Czech, M. P., ed.), pp. 95–100, Plenum Press, New York

Ramasarma, T. & Crane, F. L. (1981) Curr. Top. Cell. Regul. **20**, 247–301

Rodbell, M. (1964) J. Biol. Chem. **239**, 375–380

Simpson, I. A. & Hedo, J. A. (1984) Science **223**, 1301–1304

Stuart, C. A., Pietrzyk, R. A. & Prochnow, P. A. (1986) Diabetes **35**, Suppl. 1, 172A (abstract)

Swarup, G., Cohen, S. & Garbers, D. (1982) Biochem. Biophys. Res. Commun. **107**, 1104–1109

Tamura, S., Brown, T. A., Dubler, R. E. & Larner, J. (1983) Biochem. Biophys. Res. Commun. **113**, 80–86

Tamura, S., Brown, T. A., Whipple, J. H., Fujita-Yamaguchi, Y., Dubler, R. E., Cheng, K. & Larner, J. (1984) J. Biol. Chem. **259**, 6650–6658

Tolman, E. L., Barris, E., Burns, M., Pansini, A. & Partridge, R. (1979) Life Sci. **25**, 1159–1164

Van Obberghen, E. & Kahn, C. R. (1981) Mol. Cell. Endocrinol. **22**, 277–293