Stimulation of ^{125}I-Transferrin Binding and ^{59}Fe Uptake in Rat Adipocytes by Vanadate: Treatment Time Determines Apparent Tissue Sensitivity

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Vanadium compounds have been documented to stimulate a number of insulin biological effects in vitro and in vivo. We previously demonstrated stimulation of glucose transport and insulin-like growth factor-II (IGF-II) binding in rat adipocytes. These actions are associated with translocation of glucose transporters and IGF-II receptors from an intracellular compartment to the plasma membrane. The transferrin receptor is also recruited to the plasma membrane in response to insulin. Freshly isolated rat adipocytes were incubated with vanadate and insulin at 37°C, and after treating the cells with KCN to inhibit further receptor movement, diferric ^{125}I-transferrin binding was assayed. Vanadate stimulated a dose- and time-dependent increase in ^{125}I-transferrin binding, reaching maximum (~threefold) stimulation at 1 mmol/L after a 4-hour incubation. This was equivalent to the maximum insulin effect that was obtained with 10^{-8} mol/L after 30 minutes. A similar degree of stimulation was achieved with 0.1 mmol/L vanadate after 8 hours of exposure. Dose-response data showed that the apparent sensitivity to vanadate was time-dependent and increased with the duration of exposure (EC_{50}: 30 minutes, 1 mmol/L; 3 hours, 0.35 mmol/L). Scatchard analysis of ^{125}I-transferrin binding showed that both insulin and vanadate increased receptor binding capacity with no effect on receptor affinity. Total cellular transferrin receptor content measured by immunoblotting with monoclonal anti-transferrin receptor antibody (OX-26) was not altered by insulin or vanadate, consistent with receptor translocation. Assessment of ^{59}Fe uptake from ^{59}Fe-labeled diferric transferrin showed that vanadate augmented ^{59}Fe uptake in a dose-dependent manner to an extent similar to insulin, demonstrating the functional activity of the receptors (percent of control: 10^{-8} mol/L insulin, 175% ± 23.8%, $P < .02$; 0.3 mmol/L vanadate, 188% ± 17.3%, $P < .01$). We conclude that vanadate mimics insulin to augment cell surface transferrin receptors and increase Fe uptake in rat adipocytes. The time-dependent apparent increase in sensitivity is consistent with the effectiveness of very low concentrations of vanadate in vivo after several days of administration, and suggests a requirement for vanadate entry into cells to mediate this biological response.

VANADIUM COMPOUNDS have been found to be effective to decrease blood glucose concentrations in a number of rodent models of diabetes mellitus.[1-9] In two recent studies in human subjects with diabetes mellitus, glycemic control was improved by oral administration of vanadyl sulfate[10] and sodium metavanadate,[11] and such compounds have been proposed as potential therapeutic agents.[12] In vitro studies have also documented a number of insulin-mimetic effects, particularly in regard to glucose transport and metabolism.[13-20] The mechanism of action of vanadate or vanadyl compounds is not clear. Although a number of studies have documented inhibition of phosphoprotein tyrosine phosphatase (PTP) activity by vanadate,[21-23] enhanced tyrosine phosphorylation and activation of the insulin receptor, a transmembrane protein tyrosine kinase, have not been uniformly observed.[2,7,15,24-28] Shisheva and Schechter[28,29] reported the activation of an intracellular cytosolic tyrosine kinase in rat adipocytes by vanadate and suggested that this alternate tyrosine kinase may be the vanadate response signal transducer. It has also been suggested that vanadate may mediate its effects on glucose metabolism by direct inhibition of glucose-6-phosphatase[30,31] and fructose-2,6-bisphosphatase.[32,33] Indeed, there are some reports that vanadate does not mimic certain actions of insulin such as stimulation of protein synthesis[17] and glycogen synthesis[34] in skeletal muscle, methylaminoisobutyric acid uptake in cultured rat L6 muscle cells,[35] suppression of phosphoenolpyruvate carboxykinase mRNA in H35 hepatoma cells,[36] and activation of glycogen synthase in isolated rat hepatocytes.[37]

To examine the effect of vanadate on an acute insulin biological response that is unrelated to glucose metabolism, its action on transferrin receptors and iron (Fe) uptake in rat adipocytes was determined. Insulin has been demonstrated to stimulate accumulation of Fe in adipocytes by increasing the binding and uptake of diferric transferrin.[38,39] The stimulation of translocation of transferrin receptors in adipocytes from an intracellular compartment to the plasma membrane by insulin[38-40] is similar to that described for the recruitment of glucose transporters.[41,42] Furthermore, the stimulation of an increase in cell surface transferrin receptors by other growth factors acting via tyrosine kinase receptors such as insulin-like growth factor-I (IGF-I), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF)[43,44] supports the involvement of tyrosine phosphorylation in the signaling mechanism. In this study, we report that vanadate stimulates a dose- and time-dependent increase in ^{125}I-transferrin receptor binding capacity and a corresponding increase in ^{59}Fe uptake in rat adipocytes that is similar in magnitude to that induced by insulin. However, the time course and dose-response demonstrate that vanadate acts much more slowly than insulin, resulting in an increase in apparent tissue sensitivity as a function of the duration of exposure.

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MATERIALS AND METHODS

Materials

Vanadate, bovine serum albumin ([BSA] A4378), and HEPES were obtained from Sigma (St Louis, MO). Collagenase (type I) was from Worthington (Mississauga, Ontario, Canada). Dulbecco's modified Eagle's medium (DMEM) was from GIBCO (Grand Island, NY). Transferrin was from Boehringer Mannheim (Indianapolis, IN). ^{125}I-transferrin (specific activity, 897 to 1,280 Ci/mmol) was purchased from Amersham (Oakville, Ontario, Canada), and $^{59}FeCl_3$ was from Dupont NEN (Boston, MA). Insulin was a kind gift from Eli Lilly & Co (Indianapolis, IN). Anti–transferrin receptor monoclonal antibody (OX-26)[38] was a kind gift from Dr R. Davis (University of Massachusetts, Worcester, MA).

Preparation of Adipocytes

Male Sprague-Dawley rats weighing 180 to 200 g (Charles River, St Constante, Quebec, Canada) were killed and adipocytes were prepared with minor modifications of previously described procedures.[45] Epididymal fat pads were removed and placed into 50-mL polypropylene centrifuge tubes containing DMEM supplemented with 25 mmol/L HEPES and 3% BSA, pH 7.5. Collagenase 1 to 2 mg/mL was added, and the tubes were incubated in a shaking water bath at 37°C for 30 to 45 minutes. At the end of incubation, the adipocytes were filtered through nylon mesh (1,000 μmol/L), centrifuged, and washed twice in DMEM and 1% BSA, pH 7.5, without collagenase and twice in Krebs-Ringer-HEPES (KRH) buffer (120 mmol/L NaCl, 6 mmol/L KCl, 1.2 mmol/L $MgS0_4$, 1 mmol/L $CaCl_2$, and 25 mmol/L HEPES) supplemented with 3% BSA, pH 7.5. The cells were resuspended in KRH-3% BSA buffer with the indicated concentrations of insulin and vanadate for various times at 37°C.

^{125}I-Transferrin Binding

At the end of incubations with insulin and vanadate, the fat cells (1.5×10^6 cells/mL) were washed, resuspended in KRH-3% BSA binding buffer, and treated with 2 mmol/L KCN for 5 minutes at 22°C to inhibit the internalization of ligand-receptor complexes.[38,46] The fat cells were then incubated with 0.1 nmol/L diferric ^{125}I-transferrin in the presence of 0 to 67 nmol/L unlabeled transferrin for 30 minutes at 22°C. Preliminary experiments demonstrated that equilibrium binding was observed under these conditions. The adipocytes were then separated by oil flotation, and the cell-associated radioactivity was measured with a gamma counter. Nonspecific binding was determined in the presence of 67 nmol/L unlabeled diferric transferrin and subtracted from total binding to yield specific binding.

Immunoblotting

At the end of incubations with insulin or vanadate as already described, adipocytes were solubilized in 1% Nonidet P-40 (NP-40) solubilization buffer (50 mmol/L HEPES, 100 mmol/L NaCl, 50 mmol/L NaF, 1 mmol/L EGTA, 1 mg/mL benzamidine, 1 TIU/mL aprotinin, 1 mg/mL bacitracin, and 1 mmol/L phenylmethylsulfonyl fluoride, pH 7.4). Solubilized total cell lysates (85 μg protein) were applied to nitrocellulose membranes (Amersham) using a slot-blot apparatus under vacuum. Membranes were blocked with 5% BSA in blocking buffer (50 mmol/L Tris, 150 mmol/L NaCl, and 0.05% Tween 20) for 30 minutes and then incubated with OX-26 monoclonal antibody as previously described.[38] Detection was performed using anti-mouse horseradish peroxidase–antibody conjugate and ECL (Amersham) according to the instructions of the manufacturer. Membranes were exposed to Kodak (Eastman Kodak, Rochester, NY) XAR film, and the intensity of the bands was assessed using laser densitometry.

^{59}Fe Uptake by Adipocytes

^{59}Fe-diferric transferrin was first prepared as previously described.[47] Briefly, transferrin was first treated with sodium ascorbate to remove unlabeled Fe. Iron-saturated transferrin (5 mg) was dissolved in 0.5 mL 0.1-mol/L Tris HCl, pH 8.0, 4 mg sodium ascorbate was added, and 0.5 mol/L sodium phosphate, pH 5.1, was then added to obtain a final pH of 5.8. After incubation at 37°C for 30 minutes, the sample was passed over an Econo-Pac 10 DG column (Bio-Rad, Richmond, CA) equilibrated with 0.25 mol/L Tris HCl and 10 μmol/L $NaHCO_3$ pH 8.0. One hundred microliters of $^{59}FeCl_3$ (1 mCi/mL) was added to 500 μL 100-mmol/L disodium nitrilotriacetate, and this solution was combined with the iron-free transferrin solution and incubated at room temperature for 1 hour. The [^{59}Fe]transferrin solution was then passed over an Econo-Pac 10 DG column equilibrated with 0.15 mol/L NaCl/0.02 mol/L Tris HCl, pH 7.4, to remove nitrilotriacetate and unincorporated ^{59}Fe to yield ^{59}Fe-transferrin (specific activity, 31,000 to 41,000 cpm/μg transferrin).

After incubation of the adipocytes with and without the concentrations of insulin and vanadate for the various times as indicated, the cells were washed and resuspended in binding buffer to which 150 nmol/L ^{59}Fe-diferric transferrin was added at 22°C. At the times indicated (30 to 180 minutes), 0.3-mL aliquots of cell suspension were removed from the incubation and the adipocytes were isolated by oil flotation. Radioactivity associated with the cells was measured by scintillation counting. Uptake remained linear over 3 hours under these conditions, and rates of uptake were determined as previously described.[38]

Data Analysis

Results are presented as the mean ± SE. Comparisons were determined by two-tailed paired Student t tests or ANOVA, and differences are considered significant at P less than .05.

RESULTS

Effect of Vanadate on ^{125}I-Transferrin Binding

Exposure of rat adipocytes to vanadate resulted in a time-dependent increase in ^{125}I-transferrin binding (Fig 1). Maximum stimulation of ^{125}I-transferrin binding by 10 nmol/L insulin was observed after 30 minutes of exposure. A higher concentration of insulin, 10^{-7} mol/L, and an earlier time point, 15 minutes, did not result in greater stimulation (data not shown). Previous studies showed that insulin acts rapidly, achieving a maximum effect within 2 minutes.[38,39] In contrast, the response to vanadate was slow, particularly at the lower concentrations. Thus, 1.0 mmol/L vanadate had a maximum effect to augment ^{125}I-transferrin binding at 4 hours that was similar in extent to that stimulated by insulin at 30 minutes (Fig 1). Moreover, with 0.1 mmol/L vanadate, this level of stimulation was observed only after 8 hours of exposure. After peak levels were attained, there appeared to be a gradual time-dependent decrease in binding. This return toward basal levels after maximum stimulation by vanadate also appeared to be slower than that obtained with insulin (Fig 1). It should be noted that in all studies adipocytes were greater than 90% viable by trypan blue exclusion and remained intact. To avoid toxicity, the concentration of vanadate used determined the maximum duration of exposure. In control experiments, adipocytes exposed to these concentrations of vanadate for the times indicated also maintained active glucose transport (data not shown).

The time-course study suggested that lower concentrations of

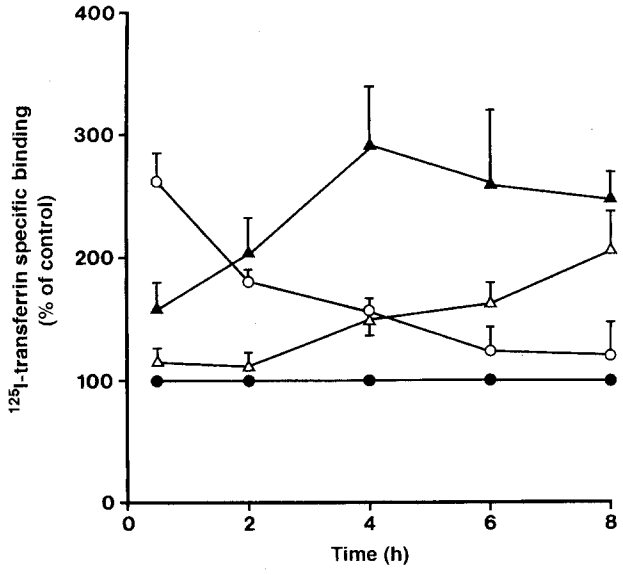

Fig 1. Time course of insulin and vanadate stimulation of diferric ^{125}I-transferrin binding to rat adipocytes. Adipocytes were isolated and incubated with and without insulin or vanadate for the times indicated at 37°C. The cells were then cooled to 22°C and fixed with 2 mmol/L KCN for 5 minutes, and ^{125}I-transferrin binding was measured. Insulin 10 nmol/L (○), vanadate 0.1 mmol/L (△), vanadate 1.0 mmol/L (▲), and buffer alone (●). Results are the mean ± SE of 4 separate experiments and are expressed as % of control. ^{125}I-transferrin specific binding to control adipocytes was 0.74% ± 0.11% per 5 × 10^5 cells. The maximum stimulation by vanadate was similar to that of insulin but required a longer duration of exposure.

vanadate (≤100 μmol/L) could achieve a similar maximum stimulation of transferrin binding as higher concentrations (1 to 5 mmol/L) but required a longer exposure time. Thus, dose-response curves were generated at two different times, after 30 minutes and 3 hours of incubation with vanadate. After 30 minutes of exposure, the EC_{50} for stimulation by vanadate was 1 mmol/L, and it decreased to 0.35 mmol/L after 3 hours (Fig 2). Furthermore, while a very low concentration of vanadate, 0.033 mmol/L, had no significant effect up to 8 hours, after 18 hours of exposure, ^{125}I-transferrin binding was stimulated to 135% ± 6.2% of control levels ($P < .02$).

The increase of transferrin binding to adipocytes induced by insulin and to fibroblasts by other growth factors is associated with an increase in receptor binding capacity.[38,43] This reflects a recruitment or translocation of receptors from an intracellular to a plasma membrane compartment. To compare the effects of vanadate, binding competition experiments were performed on cells fixed with KCN to specifically assess cell surface receptors. Analysis of the binding curves by the Scatchard method[48] showed that vanadate acted in a manner similar to insulin to increase receptor binding capacity and did not significantly alter receptor affinity after either a 30-minute or 3-hour exposure (Fig 3 and Table 1). This was also the case after 18 hours of exposure to 0.033 mmol/L vanadate (receptor binding capacity, 126.4% ± 4.3% of control, $P < .01$; affinity, 106.5% ± 9.2% of control, NS; n = 4, data not shown).

To confirm that vanadate did not alter total cellular transferrin receptor content, adipocytes were solubilized in 1% NP-40 buffer as already described after 30 minutes of exposure to 10^{-7} mol/L insulin and 5 mmol/L vanadate, as well as after 4 hours of exposure to 1 mmol/L vanadate. Immunoblotting with monoclonal anti–transferrin receptor antibody showed that these treatments did not alter the amount of total cellular transferrin receptors (Fig 4). These data are consistent with an insulin-mimetic effect of vanadate to stimulate receptor translocation from an intracellular pool to the plasma membrane.

Effect of Vanadate on ^{59}Fe Uptake

The functional capacity of cell surface transferrin receptors induced by vanadate was assessed by measurement of ^{59}Fe uptake. Insulin significantly increased ^{59}Fe uptake from ^{59}Fe-labeled diferric transferrin after 30 minutes of exposure at 37°C. The enhanced rate of uptake remained linear over a subsequent 3-hour period at 22°C (Fig 5). Vanadate at 0.1 mmol/L and with a greater effect at 0.3 mmol/L also increased the rate of ^{59}Fe uptake (Figs 5 and 6). The maximum stimulation of ^{59}Fe uptake by vanadate was similar to that achieved by insulin (Fig 6). A combination of insulin with vanadate, each at a maximum stimulating concentration, did not augment ^{59}Fe uptake to a level greater than that obtained with either agent alone (data not shown).

DISCUSSION

This study demonstrates that vanadate mimics insulin to increase cell surface transferrin receptor binding capacity and transferrin-mediated Fe uptake. This biological response is associated with a translocation of transferrin receptors from an intracellular compartment to the plasma membrane,[38,39] and

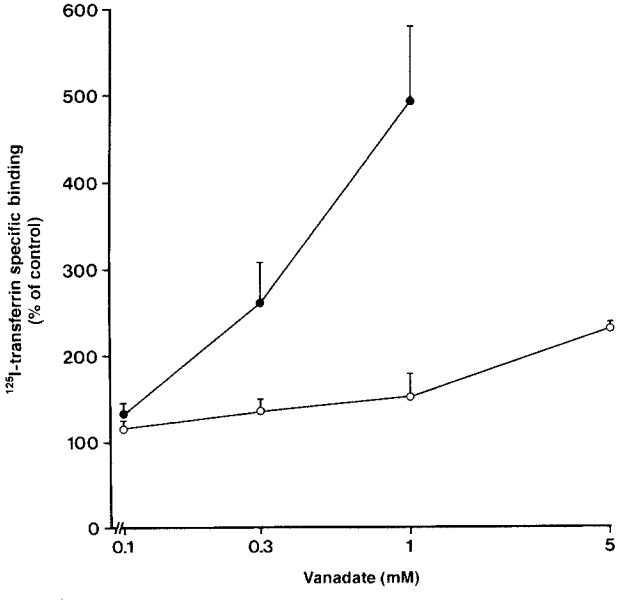

Fig 2. Dose-response of vanadate stimulation of diferric ^{125}I-transferrin binding. Adipocytes were prepared and incubated with 0 to 5 mmol/L vanadate for 30 minutes (○) and 0 to 1 mmol/L vanadate for 3 hours (●). After KCN treatment, ^{125}I-transferrin binding was measured. Results are the mean ± SE of 3 separate experiments and are expressed as % of control. Sensitivity to vanadate increased as a function of exposure time. In this set of experiments, the response of adipocytes was greater with vanadate (Fig 2 *v* Fig 1) and with insulin (not shown).

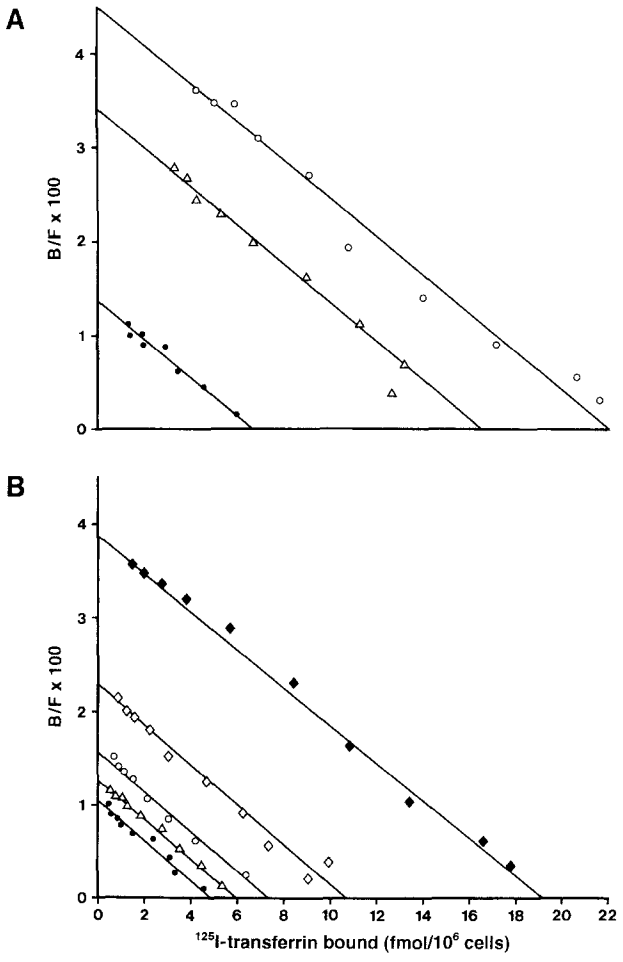

Fig 3. Effect of vanadate on diferric ^{125}I-transferrin binding by Scatchard analysis. Adipocytes were prepared and incubated (A) for 30 minutes with buffer alone (●), 10 nmol/L insulin (○), or 5 mmol/L vanadate (△) or (B) for 3 hours with buffer alone (●), 10 nmol/L insulin (○), and either 0.1 mmol/L (△), 0.3 mmol/L (◇), or 1 mmol/L (◆) vanadate at 37°C. After treatment with KCN, ^{125}I-transferrin binding was measured in the presence of 0 to 67 nmol/L unlabeled diferric transferrin. Both insulin and vanadate increased receptor binding capacity without altering affinity (see Table 1).

although the translocation mechanism is similar to that described for recruitment of GLUT 4 by insulin,[41] an increase in transferrin binding capacity is also stimulated by growth factors such as PDGF and EGF that act via tyrosine kinase receptors.[43,44] Vanadate is well documented to stimulate glucose uptake into insulin target tissues,[12-15,17,20] and in rat adipocytes the mechanism has been demonstrated to be similar to that of insulin, namely a translocation of glucose transporters to the cell surface.[49]

We previously reported that vanadate mimics insulin to increase cell surface IGF-II binding to rat adipocytes.[19,50] The IGF-II receptor is another example of a protein that is translocated in response to both insulin and vanadate.[46] Although we did not perform subcellular fractionation experiments in this study, based on the results of (1) the ^{125}I-transferrin binding competition curves in the presence of KCN, (2) the absence of any change of total cellular transferrin receptors after up to 4 hours of vanadate exposure that achieved a maximum increase in cell surface binding, (3) the similar findings with IGF-II receptors,[50] and (4) the demonstrated stimulation by vanadate of translocation of glucose transporters,[49] it is suggested that the ability of vanadate to augment cell surface transferrin receptors is mediated by a similar mechanism.

Table 1. Effect of Insulin and Vanadate on Transferrin Receptor Binding Affinity and Capacity in Rat Adipocytes (mean ± SE, n = 3)

	Incubation Time (h)			
	Affinity (K_d, nmol/L)		Capacity (fmol/10^6 cells)	
Condition	0.5	3	0.5	3
Control	0.67 ± 0.09	0.66 ± 0.04	6.2 ± 1.36	5.3 ± 1.28
Insulin 10 nmol/L	0.69 ± 0.06	0.65 ± 0.04	18.3 ± 4.82	8.6 ± 1.08
Vanadate				
0.1 mmol/L	ND	0.75 ± 0.06	ND	7.3 ± 0.29
0.3 mmol/L	ND	0.68 ± 0.04	ND	13.2 ± 2.83
1.0 mmol/L	ND	0.67 ± 0.03	ND	25.0 ± 3.51
5.0 mmol/L	0.56 ± 0.01	ND	13.3 ± 3.92	ND

Abbreviation: ND, not determined.

In a previous study using vanadate, H_2O_2, and pervanadate (H_2O_2 plus vanadate), we demonstrated a significant correlation between insulin receptor kinase activation and stimulation of IGF-II binding in rat adipocytes.[19] The increase of cell surface transferrin receptor binding capacity and Fe uptake, which are cellular effects stimulated by insulin and by other growth

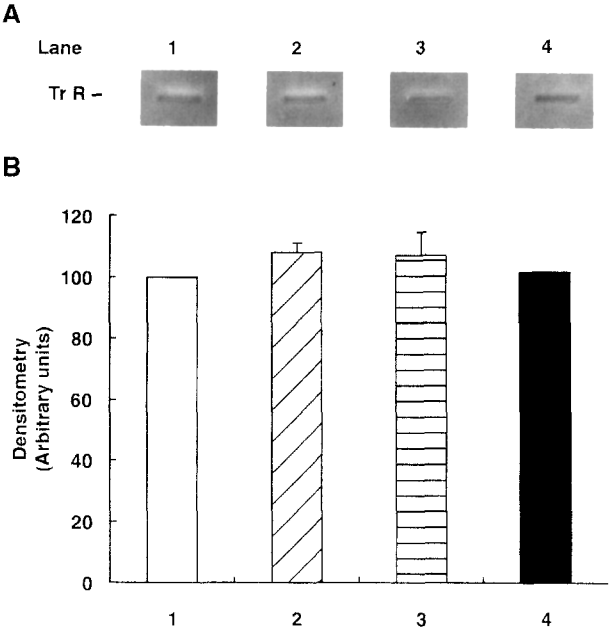

Fig 4. Effect of vanadate and insulin on total cellular transferrin receptor content. Adipocytes were isolated and incubated in the absence (lane 1) and presence of 10^{-7} mol/L insulin (lane 2) or 5 mmol/L vanadate (lane 3) for 30 minutes and with 1 mmol/L vanadate for 4 hours (lane 4) at 37°C. At the end of incubations, the cells were washed and solubilized in 50 mmol/L HEPES buffer containing 1% NP-40. Equal amounts of cell lysate (85 μg protein) were applied to nitrocellulose using a slot-blot apparatus and immunoblotted with anti-transferrin receptor antibody. (A) Representative autoradiogram from 1 experiment. (B) Mean ± SE of 3 separate experiments performed in duplicate. There was no effect of insulin or vanadate on the total complement of cellular transferrin receptors.

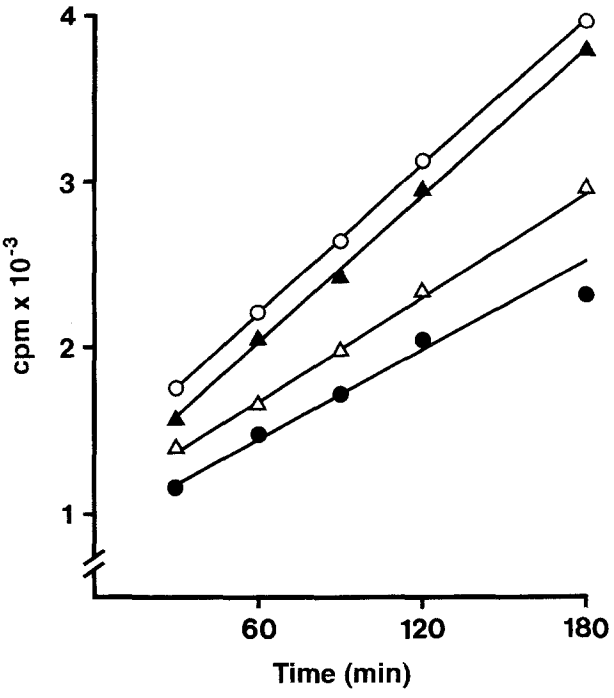

Fig 5. Time course of ^{59}Fe uptake from ^{59}Fe-labeled diferric transferrin. Adipocytes were isolated and preincubated without (●) and with 10 nmol/L insulin (○) or 0.1 mmol/L (△) or 0.3 mmol/L (▲) vanadate for 30 minutes at 37°C. The cell suspension was cooled to 22°C, and 150 nmol/L ^{59}Fe-diferric transferrin was added. Aliquots (300 μL) of adipocytes were removed at various times after 30 to 180 minutes, the cells were separated by centrifugation through oil, and cell-associated radioactivity was determined. ^{59}Fe uptake was linear from 30 to 180 minutes under all conditions, and both insulin and vanadate stimulated the rate of uptake. Results shown are from 1 representative experiment. Each point is the mean of triplicate determinations. Similar results were obtained in 5 separate experiments.

factors, also supports the concept that enhancement of tyrosine phosphorylation is the predominant mechanism by which vanadate exerts these actions. We have previously demonstrated stimulation of insulin receptor tyrosine phosphorylation in intact adipocytes exposed to vanadate.[45] However, the role of the insulin receptor tyrosine kinase versus other cellular tyrosine kinases in mediating the biological effects of vanadate remains controversial.[51]

One additional consideration specific to the study of transferrin receptors and Fe uptake is the fact that after administration to mammals, vanadium is found bound to transferrin in the serum.[52] Thus, it has been suggested that the cellular entry of vanadium may be mediated, in part, via the transferrin-receptor complex. It should be noted that in our binding experiments the incubations with vanadate were performed in the absence of any added transferrin, and subsequent binding studies with ^{125}I-transferrin were performed in the absence of vanadate, ie, after washing the cells, and in the presence of KCN to prevent receptor movement. In the case of ^{59}Fe uptake studies, washing the cells free of vanadate after preincubation but before measurement of ^{59}Fe uptake yielded the same results as when vanadate was maintained in the medium during ^{59}Fe uptake. One might have expected a decrease in ^{59}Fe uptake if a significant binding of vanadate to transferrin resulted in displacement of the labeled ^{59}Fe. This apparent lack of transferrin binding is perhaps not surprising, since transferrin binds the reduced form, vanadyl (IV), but not vanadate (V), which was used here.[52,53] After injection into dogs[54] or rats,[53] there is a delay of 30 hours to 4 days, respectively, until injected vanadate is found maximally bound to transferrin, suggesting a slow rate of reduction. Using electron-spin resonance spectroscopy to detect vanadyl (IV), Degani et al[55] reported that after addition of vanadate (V) to adipocytes for several hours, all vanadyl (IV) was cell-associated without any detectable in the medium. Thus, although vanadyl complexation with transferrin did not appear to affect these in vitro studies, its potential importance in vivo in human subjects receiving vanadyl sulfate or sodium metavanadate will require further study.

An important and unique aspect of the biological response to vanadate is its time course. Thus, the dose-response to vanadate of the stimulation of transferrin binding was dynamic, determined at least in part by the duration of exposure. The longer the exposure time, the greater the apparent sensitivity, which was manifested by a shift to the left of the dose-response curve (Fig 2). It should be noted that the same maximum response could be achieved at lower concentrations after longer incubation times.

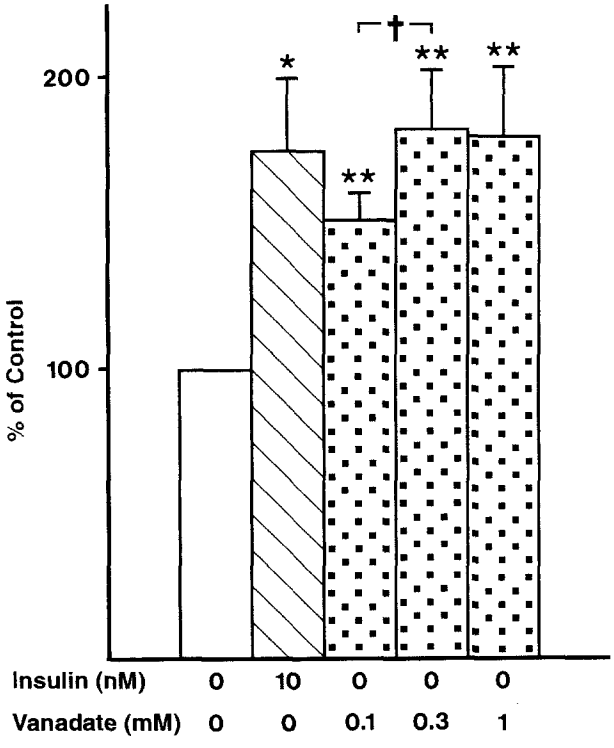

Fig 6. Stimulation of ^{59}Fe uptake in rat adipocytes by insulin and vanadate. Adipocytes were isolated and then exposed to the indicated concentrations of insulin or vanadate for 30 minutes at 37°C followed by cooling to 22°C and incubation with 150 nmol/L ^{59}Fe-labeled diferric transferrin. ^{59}Fe uptake was determined. Data are the mean ± SE of 5 separate experiments and are expressed as % of control. The rate of uptake of control cells was 4.14 ± 0.97 cpm/min or 12 × 10^5 molecules/cell/h, similar to that reported by Davis et al[38] at 9 × 10^5 molecules/cell/h. *$P < .02$; **$P < .01$ *v* control; †$P < .05$, 0.1 mmol/L *v* 0.3 mmol/L vanadate.

We have recently observed similar results for glucose transport stimulation (manuscript in preparation). The reason(s) for this relatively slow onset of action and apparent time-dependent increase in sensitivity is not completely clear. The data suggest that this phenomenon may depend on the slow rate of entry of vanadate into cells. Shechter et al[56] demonstrated that the apparent sensitivity to vanadyl was enhanced in adipocytes by facilitating its entry into the cells by hydrophobic carrier ligands. The rate of cell membrane permeation may also explain the slow response to the glucose-lowering effect of vanadate in vivo in rodent models of diabetes.[1-8] In addition, the animals are sensitive to very low circulating vanadate levels (10 to 15 μmol/L)[2] in comparison to the concentrations typically used in vitro. The demonstration in this study of the relationship between the sensitivity to vanadate and the duration of exposure is consistent with and may explain these observations. The findings in rodents have been recently confirmed in studies of human subjects with diabetes mellitus, who responded with a decrease in blood glucose at very low circulating vandate concentrations.[10,11] Furthermore, the slow time course of action and relatively small degree of stimulation of tyrosine kinase activity both in vitro[19,45] and in vivo[57,58] by vanadate have likely contributed to the difficulty of documenting whether the mechanism of action to mediate a particular biological response is related to PTP inhibition.

In summary, this study demonstrates for the first time that vanadate increases the number of cell surface transferrin receptors and augments Fe uptake in rat adipocytes. These biological responses are also stimulated by insulin, and both agents achieve the same maximum response. The time course of stimulation by vanadate is slower than that of insulin and depends on the concentration of vanadate used. This results in an apparent increase in the sensitivity to vanadate as the exposure time is prolonged. These data are consistent with a sensitivity to low concentrations of vanadate after long-term treatment in vivo. The results suggest that slow and variable cellular entry into different tissues may explain, in part, the discrepancies in the sensitivity to vanadate among various studies.

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