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THE RELATIONSHIP BETWEEN THE TRANSPORT OF GLUCOSE AND CATIONS ACROSS CELL MEMBRANES IN ISOLATED TISSUES

XI. THE EFFECT OF VANADATE ON ^{45}Ca-EFFLUX AND SUGAR TRANSPORT IN ADIPOSE TISSUE AND SKELETAL MUSCLE

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(1) The effects of vanadate of hexose transport, ^{45}Ca-exchange and (Na^+, K^+)-contents have been characterized in isolated adipose tissue and skeletal muscles of the rat. (2) In whole epididymal fat pads, vanadate (0.5–5.0 mM) markedly stimulated the uptake of 2-deoxy[^{14}C]glucose as well as the efflux of 3-*O*-[^{14}C]methylglucose. (3) Within the same concentration range, vanadate induced an early increase in ^{45}Ca-washout from preloaded fat pads. The maximum increases in the fractional losses of 3-*O*-[^{14}C]methylglucose and ^{45}Ca were significantly correlated ($P < 0.001$, $r = 0.98$). (4) In extensor digitorum longus and soleus muscles, vanadate (0.5–5.0 mM) stimulated the efflux of 3-*O*-[^{14}C]methylglucose and this effect was preceded by a rise in the washout of ^{45}Ca. The maximum increases in the fractional losses of 3-*O*-[^{14}C]methylglucose and ^{45}Ca were significantly correlated ($P < 0.005$, $r = 0.98$). (5) In extensor digitorum longus and soleus muscles, vanadate increased K^+-contents and decreased Na^+ contents. (6) The stimulation of ^{45}Ca-washout presumably reflects an increase in the cytoplasmic Ca^{2+} level, brought about by an inhibitory effect of vanadate on the Ca^{2+}-sensitive ATPase of the sarcoplasmic or the endoplasmic reticulum. As demonstrated for most other insulin-like agents (Sørensen, S.S., Christensen, F. and Clausen, T. (1980) Biochim. Biophys. Acta 602, 433–445), the stimulating effect of vanadate on glucose transport appears to be associated with or mediated by a rise in the cytoplasmic Ca^{2+} level.

Introduction

It was recently demonstrated that vanadate inhibits the Ca^{2+}-activated ATPase of isolated sarcoplasmic reticulum [1–3]. This would indicate that in the intact cell, vanadate might interfere with the clearance of Ca^{2+} from the cytoplasm, and, as a further implication, induce stimulation of cellular Ca^{2+}-sensitive processes. Several studies have shown that a variety of factors and conditions, which induce an increase in the cytoplasmic Ca^{2+} level, give rise to an activation of the glucose transport system in muscle cells, adipocytes and a number of other cell types (for reviews, see Refs. 4–6; for a dissenting view, see Ref. 7). Therefore, it seemed reasonable to expect that vanadate via an effect on cellular calcium distribution, should stimulate glucose transport. Indeed, two laboratories have reported that vanadate induces a pronounced increase in the transport and metabolism of glucose in free fat cells [8–10].

The present study was undertaken with the purpose of detecting possible effects of vanadate on calcium exchange in muscle and adipose tissue, and, in particular, to determine whether this had any relation to the insulin-like action of vanadate on glucose transport. Since vanadate is a potent inhibitor of the (Na^+, K^+)-activated ATPase [11] and interferes with the active (Na^+, K^+)-transport in erythrocytes [12], its

effects on (Na^+, K^+)-contents were also assessed. The results show that vanadate stimulates the uptake and efflux of non-metabolized sugars in whole epididymal fat pads, extensor digitorum longus and soleus muscles of the rat. This effect is associated with an early rise in the washout of ^{45}Ca from preloaded tissues, indicating that vanadate induces a mobilization of Ca^{2+} from cellular pools into the cytoplasm.

Methods

Experiments with epididymal fat pads

Washout experiments were performed essentially as described in earlier reports [13,14]. Whole epididymal fat pads were obtained from fed Wistar rats weighing 100–120 g and following a wash, loaded for 60 min in polyethylene counting vials containing 3 ml of buffer with ^{45}Ca (2 μCi/ml) or 3-*O*-[^{14}C]-methylglucose (1 mM and 2 μCi/ml). Hereafter, the fat pads were washed out in a series of counting vials containing 2 ml of unlabelled buffer. At the end of washout, the radioactivity remaining in the tissue was determined, and by counting the radioactivity released into the washout vials, the fractional loss could be calculated as described earlier [13]. Sugar influx was determined by incubating whole epididymal fat pads in vials containing 2 ml of buffer with 2-deoxy[^{14}C]glucose (0.1 μCi/ml and 0.1 mM). Following 60 min of incubation, the tissues were blotted, weighed and homogenized in 2 ml of 5% trichloroacetic acid. After centrifugation for 10 min at 2 000 × *g*, the clear interphase was withdrawn for counting. The uptake was calculated on the basis of the specific activity of the incubation medium and expressed as nmol/g wet weight per h. The intracellular accumulation was calculated by deducting the amount of sugar confined to the sucrose space as measured in parallel experiments. The K^+-contents were determined by flame photometry of the trichloroacetic acid extract. All fat pad experiments were performed at 37°C, and the Krebs-Ringer bicarbonate buffer used contained 1% of dialyzed bovine serum albumin.

Experiments with extensor digitorum longus and soleus muscles

The procedures for the preparation and incubation of these muscles have been described in detail elsewhere [15,16]. Muscles weighing 25–35 mg were obtained from fed Wistar rats in the weight range 60–70 g and incubated in Krebs-Ringer bicarbonate buffer. All experiments were performed at 30°C, and in order to ensure adequate oxygenation, the muscles were kept agitated by continuous aeration with a mixture of 95% O_2 and 5% CO_2; The methods for the measurements of the uptake and the washout of 3-*O*-[^{14}C]methylglucose and ^{45}Ca as well as (Na^+, K^+)-contents have earlier been described [15,17]. Experimental details are given in the legends to figures and tables.

Chemicals, isotopes and hormone

All chemical used were of analytical grade. Bovine serum albumin was used after dialysis against distilled water for 24 h at 4°C, followed by neutralization with NaOH. Albumin, 3-*O*-methyl-D-glucose and 2-deoxy-D-glucose were purchased from the Sigma Co, St. Louis, MO, U.S.A., cytochalasin B was a product of Aldrich-Europe, Beerse, Belgium. ^{45}Ca (1 000 mCi/mmol) was obtained from the Danish Atomic Energy Commission Isotope Laboratory, Risø, Denmark. 3-*O*-[^{14}]Methyl-D-glucose (59 mCi/mmol), 2-deoxy-D-[1-^{14}C]glucose (57 mCi/mmol) and [U-^{14}C]sucrose (477 mCi/mmol) were products of The Radiochemical Centre, Amersham, U.K. Insulin was a gift from Nordic Insulin.

Results

Experiments with whole epididymal fat pads

Table I shows the effects of vanadate and insulin on the uptake of 2-deoxy[^{14}C]glucose in whole epididymal fat pads. In the concentration range 0.5–5.0 mM, vanadate induces a highly significant increase in the total amount of ^{14}C-activity accumulated in the tissue during a 60-min incubation. Since a fraction of the total uptake is confined to the extracellular compartment, the results were corrected on the basis of [^{14}C]sucrose space determinations. It then appears that vanadate produces more than 6-fold increase in the penetration of 2-deoxy[^{14}C]glucose into the tissue compartment not available to [^{14}C]sucrose, i.e., an effect exceeding that obtained with a supramaximal concentration of insulin (10 mU/ml).

Since this effect might reflect stimulation of the phosphorylation as well as the transport of the sugar,

TABLE I

EFFECT OF VANADATE AND INSULIN ON THE UPTAKE OF 2-DEOXY[^{14}C]GLUCOSE IN ISOLATED WHOLE EPIDIDYMAL FAT PADS OF THE RAT

Groups of five fat pads were incubated for 60 min in 3 ml of Krebs-Ringer bicarbonate buffer containing 0.1 mM 2-deoxy-[^{14}C]glucose (0.1 μCi/ml) without or with the additions indicated. Hereafter, the fat pads were blotted, weighed and homogenized in 2 ml of 5% trichloroacetic acid. After centrifugation, aliquots of the clear supernatant below the fat cake were counted and the amount of 2-deoxy[^{14}C]glucose taken up calculated on the basis of the specific activity of the incubation medium. After correction for the amount taken up into the tissue compartment available to [^{14}C]sucrose, the mean intracellular accumulation was expressed as nmol/g tissue wet weight ± S.E. The number of observations is given in parentheses.

Additions	Penetration of 2-deoxy[^{14}C]-glucose into the intracellular space	Significance of difference *P*
Controls	57 ± 4 (10)	
Vanadate (0.5 mM)	141 ± 23 (10)	<0.005
Vanadate (2.0 mM)	386 ± 28 (10)	<0.001
Vanadate (5.0 mM)	318 ± 23 (10)	<0.001
Insulin (10 mU/ml)	217 ± 20 (10)	<0.001

the following experiments were performed using the nonphosphorylated sugar 3-*O*-methylglucose. Earlier studies have demonstrated that the washout of 3-*O*-[^{14}C]methylglucose from preloaded whole epididymal fat pads can be inhibited by phlorizin and stimulated by insulin or a number of insulin-like agents [13,17–19]. It seems reasonable to assume, therefore, that changes in the fractional loss of 3-*O*-[^{14}C]methylglucose during washout reflects alterations in the transport of the sugar across the plasma membrane.

From Fig. 1 (lower panel) it can be seen that vanadate (2.0 mM) induces a marked stimulation of 3-*O*-[^{14}C]methylglucose efflux, which can almost entirely be suppressed by cytochalasin B (10 μg/ml). This indicates that the effect is not the outcome of a non-specific leakage of the plasma membrane. Vanadate also stimulates the washout of ^{45}Ca (Fig. 1, upper panel). This effect appears to be somewhat earlier in onset than that on sugar efflux, and, in contrast, it was not affected by the addition of cytochalasin B.

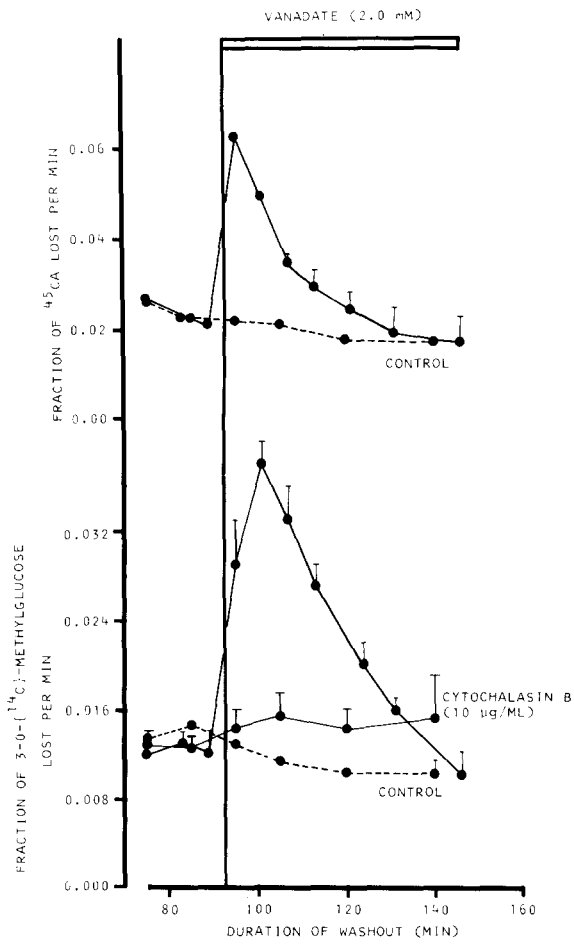

Fig. 1. Effect of vanadate and cytochalasin B on the washout of ^{45}Ca and 3-*O*-[^{14}C]methylglucose from whole epididymal fat pads. Fat pads were loaded by incubation for 60 min in Krebs-Ringer bicarbonate buffer containing 1% dialysed bovine serum albumin and either 1.27 mM ^{45}Ca (2 μCi/ml) or 1 mM 3-*O*-[^{14}C]methylglucose (2 μCi/ml). They were then transferred through a series of plastic counting vials containing 2 ml of unlabelled buffer without or with the additions indicated. At the end of washout, the radioactivity retained in the tissue and released into each vial was determined. The fraction of radioactivity released during each time interval was calculated as described elsewhere [13]. Each curve represents the mean of three to six observations with the vertical bars indicating the S.E. where this exceeds the size of the symbols. In one group of fat pads, cytochalasin B (10 μg/ml was added together with the vanadate.

In the concentration range 0.1–5.0 mM, vanadate stimulated the washout of both ^{45}Ca and 3-*O*-

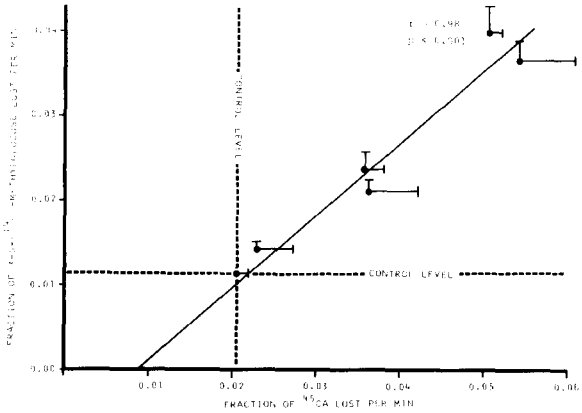

Fig. 2. Relationships between the fractional loss of ^{45}Ca and 3-*O*-[^{14}C]methylglucose induced by vanadate. The fractional loss of the two isotopes was determined as described in the legend to Fig. 1. Following 90 min of washout, vanadate (0.1–5.0 mM) was added. The fractional loss of ^{45}Ca as determined within the first 10 min after the addition of vanadate is plotted against the fractional loss of 3-*O*-[^{14}C]-methylglucose measured from 10 to 20 min after the onset of exposure to the same concentration of vanadate. Each point represents the mean of three to seven observations, with bars indicating the S.E., where this exceeds the size of the symbols. The full-drawn line is constructed by the method of least squares. The two dashed lines represents the control levels for the fractional loss of ^{45}Ca and 3-*O*-[^{14}C]methylglucose, respectively.

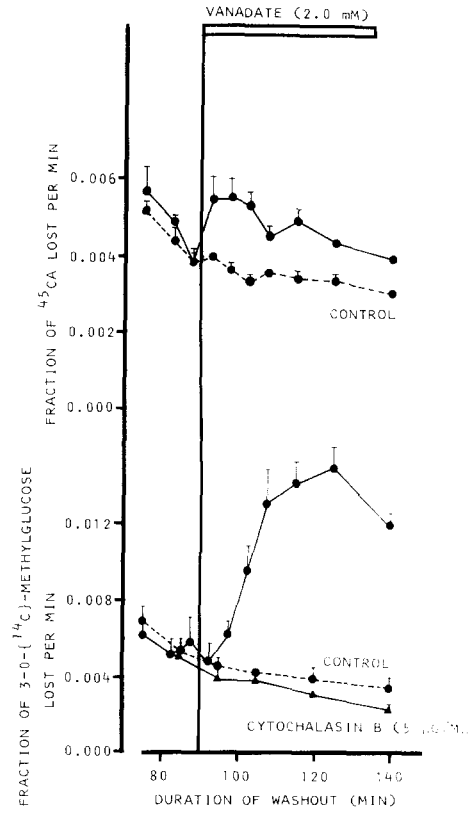

Fig. 3. Effect of vanadate and cytochalasin B on the washout of ^{45}Ca and 3-*O*-[^{14}C]methylglucose from extensor digitorum longus muscles. Muscles were loaded by incubation for 60 min in Krebs-Ringer bicarbonate buffer containing either 1.27 mM ^{45}Ca (2 μCi/ml) or 1 mM 3-*O*-[^{14}C]methylglucose (2 μCi/ml). They were then transferred through a series of tubes containing 2 ml of unlabelled buffer without or with the additions indicated. At the end of washout, the radioactivity retained in the tissue and the amount released into each tube were determined. The fraction of radioactivity released during each time interval was calculated as described elsewhere [13]. Each curve represents the mean of three to six observations with the vertical bars indicating the S.E. where this exceeds the size of the symbols. In one group of muscles, cytochalasin B (5 μg/ml) was added together with vanadate.

[^{14}C]methylglucose. When the maximum values for the fractional losses of the two isotopes obtained at each concentration were plotted against each other (Fig. 2), it appeared that they were significantly correlated ($r = 0.98, P < 0.001$).

Experiments with isolated intact muscles

Both in soleus and extensor digitorum longus muscles vanadate (0.5–5.0 mM) produced a highly significant increase in the fractional loss of ^{45}Ca and 3-*O*-[^{14}C]methylglucose. In all instances, the rise in ^{45}Ca washout clearly preceded the stimulation of 3-*O*-[^{14}C]methylglucose efflux. This is illustrated in Fig. 3, which shows the effect of 2 mM vanadate on extensor digitorum longus muscles. Like in the experiments with fat pads, cytochalasin B (5 μg/ml) suppressed the rise in sugar efflux without causing any significant change in the effect of vanadate on the washout of ^{45}Ca.

The relationship between the washout of ^{45}Ca and 3-*O*-[^{14}C]methylglucose is illustrated in Figs. 4 and 5, which compare the maximum increases in the two parameters obtained with vanadate concentrations ranging from 0.1 to 5.0 mM. Regression analysis shows that for both extensor digitorum longus (Fig. 4) and soleus muscles (Fig. 5), there is a highly significant correlation between the peak values of the

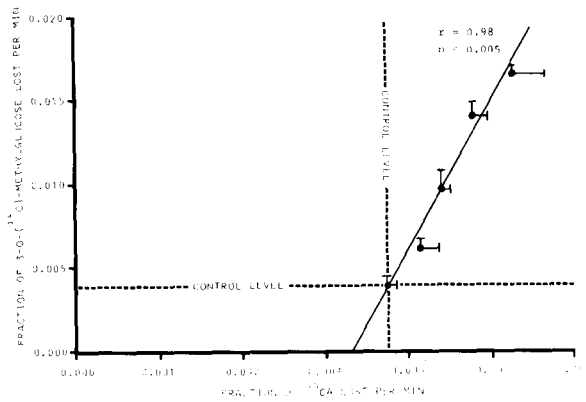

Fig. 4. Relationships between the fractional loss of ^{45}Ca and 3-*O*-[^{14}C]methylglucose from extensor digitorum longus muscles. The fractional loss of the two isotopes was determined as described in the legend to Fig. 3. Following 90 min of washout vanadate (0.1–5.0 mM) was added. The fractional loss of ^{45}Ca as determined within the first 10 min after the addition of vanadate is plotted against the fractional loss of 3-*O*-[^{14}C]methylglucose measured from 20–40 min after the onset of exposure to the same concentration of vanadate. Each point represents the mean of three to eleven observations with bars indicating the S.E. where this exceeds the size of the symbols. The full-drawn line is constructed by the method of least squares. The two dashed lines represent the control levels for the fractional loss of ^{45}Ca and 3-*O*-[^{14}C]-methylglucose, respectively.

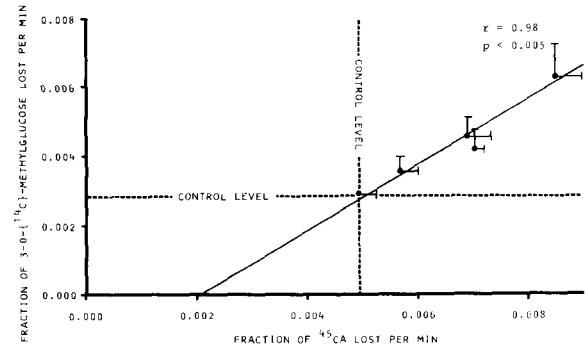

Fig. 5. Relationships between the fractional loss of ^{45}Ca and 3-*O*-[^{14}C]methylglucose from soleus muscles. The fractional loss of the two isotopes was determined as described in the legend to Fig. 3. The effects of vanadate (0.1–5.0 mM) were determined and correlated as described in the legend to Fig. 4. Each point represents the mean of three to eight observations with bars indicating the S.E. where this exceeds the size of the symbols.

fractional losses produced by vanadate. Whereas extensor digitorum longus muscles show a pronounced increase in sugar transport and a relatively modest rise in ^{45}Ca-washout, the reverse seems to be the case for soleus muscles.

In order to assess the role of extracellular calcium, the effect of vanadate (2.0 mM) on the washout of ^{45}Ca and 3-*O*-[^{14}C]methylglucose from extensor digitorum longus muscles was determined using a buffer from which calcium had been omitted and EGTA (0.5 mM) was added so as to obtain a further lowering of the extracellular Ca^{2+} level. Under these conditions vanadate induced virtually the same rise in the fractional losses of ^{45}Ca and 3-*O*-[^{14}C]methylglucose as in the standard buffer (data not presented).

From Table II it can be seen that vanadate also stimulates the uptake of 3-*O*-[^{14}C]methylglucose in extensor digitorum longus muscles. Like in the efflux experiments, this effect is less pronounced than that

TABLE II

EFFECT OF VANADATE AND INSULIN ON THE UPTAKE OF 3-*O*-[^{14}C]METHYLGLUCOSE IN ISOLATED RAT EXTENSOR DIGITORUM LONGUS MUSCLES

Groups of five muscles were incubated for 60 min in 3 ml of Krebs-Ringer bicarbonate buffer containing 0.1 mM 3-*O*-[^{14}C]methylglucose (0.1 μCi/ml) without or with the additions indicated. Hereafter, the muscles were blotted, weighed and homogenized in 4 ml of 5% trichloroacetic acid. After centrifugation, aliquots of the supernatant were counted and the amount of 3-*O*-[^{14}C]methylglucose taken up calculated on the basis of the specific activity of the incubation medium. After correction for the amount taken up into the tissue compartment available to [^{14}C]sucrose, the mean intracellular accumulation was expressed as nmol/g tissue wet weight ± S.E. The number of observations is given in parentheses.

Additions	Penetration of 3-*O*-[^{14}C]-methylglucose into the intracellular space	Significance of difference *P*
Controls	150 ± 17 (10)	
Vanadate (0.1 mM)	185 ± 9 (10)	<0.10
Vanadate (0.5 mM)	188 ± 17 (10)	n.s.
Vanadate (2.0 mM)	260 ± 18 (10)	<0.001
Vanadate (5.0 mM)	310 ± 32 (5)	<0.001
Insulin (100 mU/ml)	445 ± 11 (5)	<0.001

TABLE III

EFFECT OF VANADATE ON (Na^+, K^+)-CONTENTS IN RAT EXTENSOR DIGITORUM LONGUS AND SOLEUS MUSCLES

Experimental conditions as described in the legend to Table II. The (Na^+, K^+)-contents of the muscles were determined by flame photometry of trichloroacetic acid extracts. The results are given as means ± S.E. with the number of observations in parentheses. n.s., not significant.

Additions	K^+-contents (μmol/g wet wt.)	P	Na^+-contents (μmol/g wet wt.)	P
Extensor digitorum longus muscles				
Controls	92.5 ± 1.7 (15)		44.5 ± 0.9 (14)	
Vanadate (0.1 mM)	96.0 ± 1.7 (10)	n.s.	39.5 ± 1.6 (10)	<0.01
Vanadate (0.5 mM)	99.5 ± 1.2 (10)	<0.01	39.9 ± 1.2 (10)	<0.01
Vanadate (2.0 mM)	98.1 ± 1.5 (10)	<0.05	44.0 ± 1.9 (10)	n.s.
Vanadate (5.0 mM)	99.0 ± 1.6 (10)	<0.02	46.7 ± 1.9 (10)	n.s.
Soleus muscles				
Controls	76.0 ± 1.4 (8)		58.7 ± 2.1 (8)	
Vanadate (0.1 mM)	83.5 ± 1.5 (8)	<0.005	52.9 ± 1.7 (8)	<0.05
Vanadate (0.5 mM)	83.0 ± 2.1 (8)	<0.02	46.6 ± 1.8 (8)	<0.001

exerted by insulin at a supramaximal concentration (compare with Ref. 15).

Since it is known that vanadate inhibits (Na^+, K^+)-activated ATPase as well as the active (Na^+, K^+)-transport in erythrocytes and other cell types [11,12], its effect on (Na^+, K^+)-contents was determined. Following 60 min of incubation with vanadate in the concentration range 0.1 to 5.0 mM, neither fat pads nor muscles showed any significant decrease in K^+-content. On the contrary, in both extensor digitorum longus and soleus muscles, vanadate produced a significant increase in K^+-content and decrease in Na^+-content (Table III).

Discussion

The present results confirm and extend previous studies [8–10] in demonstrating an insulin-like action of vanadate on the transport of non-metabolized sugars in intact adipose tissue as well as soleus and extensor digitorum longus muscles. Both the effective dose-range (0.1–5.0 mM) and the relatively late onset of the effect are in good agreement with the observations on free fat cells. However, the maximum effect of vanadate on 3-*O*-methylglucose transport is somewhat smaller than that exerted by a supramaximal concentration of insulin (for comparison, see Refs. 13 and 15).

The major new observation is that vanadate induces a stimulation of ^{45}Ca-washout from preloaded tissues. Since this effect was also seen in a calcium-free medium containing 0.5 mM EGTA, it is unlikely to be the result of increased Ca^{2+}-Ca^{2+} exchange across the plasma membrane or a release of calcium bound to the outer surface of the sarcolemma. A stimulation of Ca^{2+} efflux mediated by plasma membrane Ca^{2+}-ATPase may also be excluded in view of the observations that this enzyme is inhibited by vanadate [20, 21]. The efflux of calcium is to a large extent determined by the concentration of free Ca^{2+} available for transport in the cytoplasm [22,23]. It seems reasonable to assume, therefore, that the effect of vanadate on the fractional loss of ^{45}Ca reflects a rise in the cytoplasmic Ca^{2+} level. Vanadate inhibits the Ca^{2+}-ATPase involved in the accumulation of calcium into the sarcoplasmic reticulum, and since this organelle system is of major importance for the clearing of Ca^{2+} from the cytoplasm, vanadate may cause a marked net release of Ca^{2+} into the cytoplasm. The observations that vanadate induces vasoconstriction indicates that also in other cell types, the compound may increase cytoplasmic Ca^{2+} [24,25].

The effect of vanadate on ^{45}Ca-washout was seen within the same concentration range shown to stimulate 3-*O*-methylglucose efflux, and there was a highly significant correlation between the maximum increases in the two parameters. A similar close correlation was already demonstrated [17] for the effects on soleus muscles of five different insulin-like agents

(*p*-chloromercuriphenylsulfonic acid, H_2O_2, 2,4-dinitrophenol, trypsin and hyperosmolar medium). As shown for these and several other glucose transport stimuli, the effect of vanadate on ^{45}Ca-washout precedes or coincides with the increase in 3-*O*-[^{14}C]-methylglucose efflux. In the extensor digitorum longus and soleus muscles, there was a clear cut time-lag between the two effects. These results lend further support to the idea that a rise in the cytoplasmic Ca^{2+} ion concentration can elicit activation of the glucose transport system (for reviews, see Refs. 4–6).

In agreement with data obtained in experiments with free fat cells [8,9], vanadate did not interfere with the maintenance of cellular Na^+/K^+-gradients. In the fat pads, vanadate caused no significant change in K^+-content, and in the extensor digitorum longus and soleus muscles, the intracellular K^+/Na^+-concentration ratio was clearly increased. This could be the result of vanadate mimicking the action of membrane stabilizors in inhibiting the passive fluxes of Na^+ and K^+ across the plasma membrane [26]. However, no such effects of vanadate have been described. Another possibility is suggested by the observation that vanadate stimulates the ouabain-sensitive ^{86}Rb-uptake in cultured rat heart muscle cells [27]. A stimulation of the active (Na^+, K^+)-transport seems paradoxical in view of the pronounced direct inhibitory effect of vanadate on the isolated (Na^+, K^+)-ATPase. However, at the relatively high concentration of ATP found in the cytoplasm, the affinity of the (Na^+, K^+)-ATPase for vanadate is considerably reduced [11]. Vanadate was found to stimulate isolated adenylate cyclase [28,29], but in intact cells, only a relatively modest rise in cyclic AMP-content was produced [30]. Cyclic AMP stimulates the active (Na^+, K^+)-transport in rat skeletal muscle [31], and it should be explored whether the observed effect of vanadate on (Na^+, K^+)-contents is related to adenylate cyclase activation in rat skeletal muscle.

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