Biochimica et Biophysica Acta, 979 (1989) 311–315
Elsevier

BBA 74345

The effect of vanadate on glucose transport and metabolism in rat small intestine

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(Received 20 September 1988)

Key words: Glucose transport; Vanadate; (Rat small intestine)

The effect of sodium orthovanadate on the absorption, transmural transport and metabolism of glucose was studied by perfusion of isolated loops of rat jejunum in vitro. The presence of 1 mM vanadate in the serosal medium diminished absorption from 539 ± 19 ($n = 12$) to 246 ± 19 ($P < 0.001$) μmol/h per g dry weight and transmural transport from 333 ± 17 to 14 ± 19 ($P < 0.001$) μmol/h per g dry weight, whereas glucose utilisation was unaffected. The rate of release of lactate into the serosal medium was also diminished from 168 ± 14 to 75 ± 5 μmol/h per g dry weight ($P < 0.001$). The observed rates were linear with respect to time and vanadate was effective within 5 min. In contrast, the rate of release of lactate into the luminal perfusate was strongly enhanced. Moreover, the progress curve showed a positive transient with an apparent lag time of 18.0 ± 0.3 min, during which the rate increased to a value 9.2-times that of the control. Under the final steady-state conditions, the ratio of mucosal to serosal lactate production was 5.2 ± 0.2 compared with 0.25 ± 0.06 for the control, so that the effect of vanadate was to reverse the vectorial disposition of lactate. The concentration dependence of the effect of vanadate on absorption and metabolism was similar to that observed for the inhibition by vanadate of Na^+/K^+-ATPase activity in mucosal homogenates. The results are discussed in terms of the dissipation of transmembrane Na^+ gradients as a result of the inhibition of the Na^+/K^+-ATPase.

Introduction

Vanadium is a trace element that exerts a number of effects on energy metabolism in mammalian systems. In particular, its pentavalent form (vanadate) is a powerful inhibitor of the membrane-bound Na^+/K^+-ATPase [1,2]. Moreover, it exerts specific effects on glucose metabolism, having a stimulatory action on glucose oxidation and transport [3,4] and on glycogen synthesis in rat adipocytes [5].

Glucose crosses the intestine by two routes; after absorption across the brush-border membrane, it is either transported across the basolateral membrane unchanged or it is metabolised predominantly to lactate for subsequent hepatic gluconeogenesis, the latter route being marginally more important in quantitative terms [6–11]. Many transport processes, including glucose absorption and lactate reabsorption across the brush-border membrane, are driven by transmembrane Na^+ gradients generated by the Na^+/K^+-ATPase of the basolateral membrane [12,13]. We have therefore studied the effect of vanadate on these processes and their relationship.

Methods

Measurement of intestinal absorption and metabolism. Female Wistar rats (220–250 g) were fed ad libitum on a standard laboratory chow (Bantin & Kingman Ltd., Hull, U.K.) with free access to water: they were then maintained on glucose (0.5% w/v) in the drinking water for a period of 48 h prior to the experiment.

Glucose absorption, transmural transport and metabolism were studied in isolated jejunal loops in vitro using a preparation modified from that described by Fisher and Parsons [14]. The abdomen of an anaesthetised rat (Sagatal, 0.1 ml/100 g body weight) was opened by midline incision and a 20 cm loop of jejunum starting at a point 5 cm below the Ligament of Treitz was defined by two incisions across half the diameter of the intestine. The loop was cleaned by flushing with a modified Krebs-Henseleit [15] buffer (37°C) containing NaCl (118 mM), KCl (4.74 mM), KH_2PO_4 (1.2 mM), $CaCl_2$ (1.25 mM) and $NaHCO_3$ (24.8 mM) gassed with a 95% O_2/5% CO_2 mixture to pH 7.4. The loop was cannulated, connected to the

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luminal perfusion circuit and the perfusion commenced immediately: only then was the loop removed from the rat by cutting away the associated mesentery and excess intestine. The perfused jejunum was rinsed briefly in modified Krebs-Henseleit buffer to remove any excess blood and placed in the serosal chamber of the perfusion apparatus. The luminal perfusate (50 ml) and serosal medium (40 ml) both consisted of modified Krebs-Henseleit buffer and were well oxygenated in their respective reservoirs by the use of gas-lifts (95% O_2/5% CO_2). The luminal perfusate was recirculated at a rate of 25 ml/min [16] and was also segmented with gas at a rate of 3 ml/min to minimise the effect of unstirred layers [17]. After preperfusion for 5 min to wash out any remaining blood, the serosal medium was replaced with one containing 5 mM glucose and the luminal perfusate was made 5 mM in glucose by the addition of 250 μl of a stock solution (1 M) to the luminal reservoir. Sodium orthovanadate (BDH Ltd., Poole, U.K.) was added from a concentrated stock solution at the same time as the glucose to give the the stated concentration on either the luminal or serosal side of the intestine as indicated. Samples (0.1 ml) were then taken every 5 min for a period of 45 min from both sides of the intestine for the analysis of glucose and lactate [18].

In this preparation, glucose is absorbed from the lumen across the brush-border membrane, a part of it is then utilised by the tissue and the remainder is transported across the basolateral membrane into the serosal medium. Under normal conditions, as in control experiments, about 80% of the lactate appears in the serosal medium with the remainder escaping into the luminal perfusate. Absorption was therefore measured by the rate of disappearance of glucose from the luminal perfusate, transmural transport by its rate of appearance in the serosal medium and tissue utilisation by their difference under steady-state conditions. Total lactate production was given by the sum of the steady-state rates of appearance in both compartments: the conversion of glucose to lactate was given by (0.5-times total rate of lactate production) expressed as a percentage of the rate of glucose utilisation. Rates were expressed in μmol/h per g dry weight.

The absorption and transmural transport of galactose (5 mM) were measured using D-[1-^{14}C]galactose (7 mCi/mmol; Amersham International, Amersham): samples for scintillation counting were dissolved in 1 ml of Optiphase 'MP' (Fisons plc, Loughborough).

Assay of ATPase activity. The activity of mucosal ATPases was measured by a modification of the method of Fujita et al. [19]. Scrapes of jejunal mucosa were homogenised in 50 vol (ml/g) of 100 mM Tris-HCl buffer, containing 5 mM $MgCl_2$, 1 mM EGTA, 100 mM NaCl and 10 mM KCl, using a Polytron homogeniser at setting 8 for 30 s. After preincubation with 0.075% Triton X-100 to expose all the ATPase sites [20], the homogenate was assayed for ($Mg^{2+} + Na^+ + K^+$)-ATPase activity by the addition of 40 μl to 1 ml of incubation medium containing 3 mM ATP and vanadate at the stated concentration. After 30 min at 37 °C, the reaction was terminated by the addition of 30% trichloroacetic acid and the free phosphate liberated was assayed by the reduction of molybdate [21]. The ATPase activity was expressed in μmol P_i/mg protein per h. Na^+/K^+-ATPase activity was determined by the difference in activity observed in the presence and absence of 4 mM ouabain.

Statistical analysis of results. Results are given as mean $\pm$ 1 S.E. for the indicated number of observations. Statistical significance was assessed using the Student's *t*-test.

Results

The presence of 1 mM vanadate in the serosal medium caused strong inhibition of the rates of glucose absorption (539 ± 19 to 246 ± 19 μmol/h per g dry weight, $P < 0.001$), transmural transport (333 ± 17 to 14 ± 19 μmol/h per g dry weight, $P < 0.001$) and lactate release into the serosal medium (168 ± 14 to 75 ± 5 μmol/h per g dry weight, $P < 0.001$): the time courses of the latter two processes were similar to those for glucose absorption in that vanadate was effective within 5 min and a steady state was maintained for a further 40 min (Fig. 1A). In sharp contrast, vanadate very strongly increased the rate of lactate release into the mucosal medium (Fig. 1B). Moreover, there was a pronounced lag phase during which the rate of lactate release slowly increased to a steady state that was finally achieved at about 30 min. The apparent lag time, taken for the sake of simplicity as the intercept of the steady-state rate on the time axis, was 18.0 ± 0.3 min ($n = 4$). The final steady-state rate of mucosal lactate release was about 9.2-times greater than that observed in the absence of vanadate. Indeed, the effect of vanadate was so great as to reverse completely the vectorial disposition of lactate: in the absence of vanadate, the ratio of mucosal to serosal lactate release was 0.25 ± 0.06 ($n = 12$), whereas in its presence the ratio was 5.2 ± 0.2 ($n = 4$). Total lactate production was increased to 2.2-times that of control values and the conversion of glucose to lactate was increased from 56 ± 7 to 104 ± 12% ($P < 0.001$). The dependences of the final steady-state rates of absorption, transmural transport and metabolism on serosal vanadate concentration are summarised in Figs. 2A and B.

The absorption and transmural transport of galactose were effectively abolished by the presence of 1 mM vanadate in the serosal medium: in the absence of vanadate, the rates of absorption and transmural transport were 187 ± 11 ($n = 4$) and 178 ± 37 ($n = 5$) μmol/h per g dry weight, whereas in its presence the corre-

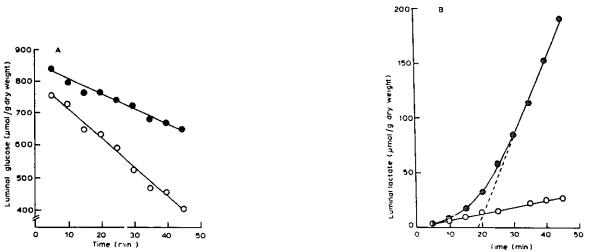

Fig. 1. Representative time courses of a single perfusion. (A) The absorption of glucose from the luminal perfusate. (B) The release of lactate into the luminal perfusate; the intercept of the steady-state rate (dashed line) on the time axis is taken as the apparent lag time for the transient observed in the presence of vanadate (see text). Control in the absence of vanadate (○) and 1 mM vanadate in the serosal medium (●).

sponding values were 9 ± 32 and 3 ± 14 μmol/h per g dry weight (both $P < 0.001$).

Experiments at a concentration of 1 mM showed that vanadate in the luminal perfusate was slightly more effective in inhibiting glucose absorption than when in the serosal medium (187 ± 13 ($n = 4$) and 246 ± 19 ($n = 4$) μmol/h per g dry weight, respectively; $P < 0.05$). In contrast, vanadate in the luminal perfusate was much less effective in stimulating mucosal lactate production than when in the serosal medium (222 ± 25, $n = 4$ and 389 ± 31 ($n = 4$) μmol/h per g dry weight, respectively; $P < 0.01$).

The total ($Mg^{2+} + Na^+ + K^+$)-ATPase activity in homogenates of jejunal mucosa was 28.7 ± 2.1 ($n = 3$) μmol P_i/mg protein per h and the Na^+/K^+-ATPase activity was 17.5 ± 1.5 ($n = 3$) μmol P_i/mg protein per h. The majority of the inhibitory effect of vanadate

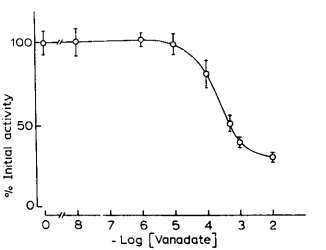

Fig. 3. The dependence of ($Mg^{2+} + Na^+ + K^+$)-ATPase activity in mucosal homogenates on vanadate concentration. The activity at a given vanadate concentration is expressed as a percentage of the initial activity in the absence of vanadate.

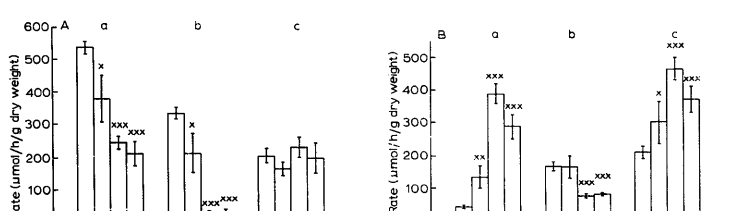

Fig. 2. (A) The dependence of the rates of absorption (a), transmural transport (b) and utilisation of glucose (c) on vanadate concentration. (B) The dependence of the rates of lactate release into the luminal perfusate (a) and serosal medium (b) and of the total rate of lactate production (c) on vanadate concentration. From left to right within each 4-bar histogram block, the concentrations of vanadate in the serosal medium were 0 (control), 0.1 mM, 1.0 mM and 10.0 mM. For control measurements $n = 12$ and for experimental measurements $n = 4$; experimental values significantly different from the corresponding control are indicated as: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

occurred predominantly over the range of 0.01 mM to 1 mM and the maximal extent of inhibition at 10 mM was 70.5 ± 2.7% (Fig. 3). This figure was comparable to the 60.8 ± 2.7% inhibition ($n = 3$) observed in the presence of 4 mM ouabain, implying that most of the inhibitory effect of vanadate was exerted on the Na^+/K^+-ATPase.

Discussion

Vanadate is an inhibitor of the Na^+/K^+-ATPase in mammalian tissues [1], including that of the basolateral membrane of rat intestinal epithelial cells (Fig. 3; [22]), and is readily taken up to inhibit the enzyme on the cytoplasmic side of the plasma membrane [2]. The inhibition by 1 mM vanadate in the serosal medium of glucose absorption from the luminal perfusate and the rapidity of the effect (Fig. 1A) can therefore be explained in terms of the Na^+ gradient hypothesis [12] as being caused by the inhibition of the Na^+/K^+-ATPase with the consequent dissipation of the associated Na^+ gradient that drives Na^+/glucose cotransport across the brush-border membrane. The concentration dependence of vanadate inhibition of glucose absorption (Fig. 2A) closely paralleled that of the inhibition of Na^+/K^+-ATPase in mucosal homogenates (Fig. 3), with maximal inhibition being observed at concentrations of 1 mM and above and partial inhibition at 0.1 mM, thus supporting the idea that the majority of effects are caused by dissipation of transmembrane Na^+ gradients. Consistent with this view, 1 mM vanadate also caused the almost total abolition of the absorption of galactose, which is poorly metabolised, and of alanine [22]: both are absorbed by Na^+-dependent processes [23,24]. Transport of glucose across the basolateral membrane occurs by facilitated diffusion [25]. Thus, in the absence of any change in glucose utilisation, the observed inhibition of the transmural transport of glucose is consequent upon the inhibition of glucose absorption (Fig. 2A).

Any explanation of the changes in lactate release into luminal perfusate and serosal medium must be tentative, if only because of a general lack of detailed information about the relevant transport processes. It has been reported that lactate is transported as the anion across the basolateral membrane by Na^+-independent facilitated diffusion [26]. Thus it appears that the rapid inhibition of lactate release into the serosal medium by vanadate cannot be linked directly to the dissipation of Na^+ gradients. However, an indirect connection seems likely. Na^+ exported across the basolateral membrane by the Na^+/K^+-ATPase is partially reabsorbed by the Na^+/H^+ antiport [27]. Thus destruction of the Na^+ gradients would result in rapid decrease in pH [28], especially in the microclimate of the basolateral membrane, and so inhibit lactate transport.

It has been suggested that lactate is released into the luminal perfusate by diffusional leak across the brush-border membrane in the form of the free acid [29] and it is to be expected that such a diffusional leak would be countered by a specific transport system; indeed, a Na^+/lactate cotransporter in the brush-border membrane has been described in detail for rabbit small intestine [13], although its presence specifically in jejunum has been questioned [30]. The existence of the Na^+/lactate transport system in rat small intestine has also been reported [26]. This system would be inhibited by the dissipation of the Na^+ gradients, as would also the Na^+/H^+ antiport system present in the brush-border membrane [31], together with that in the basolateral membrane. The resulting acidification of the cell [28] would then result in intracellular accumulation of lactic acid that would not be reabsorbed after release into the lumen. If the apparent K_m for release were to be relatively high compared with the basal cell concentration of lactate, as might be expected since under basal conditions some 80% of lactate is transported into the serosal medium (Fig. 2B), then there would be a significant lag during which intracellular accumulation of lactate occurred before the final steady-state rate of lactate release was achieved in the presence of vanadate (Fig. 1B). As noted earlier, vanadate stimulated total lactate production 2.2-fold and increased the conversion of glucose to lactate from 56% to 104%. Since the latter value did not significantly exceed 100% and glucose utilisation was not significantly enhanced, the increase in lactate production was presumably caused by the inhibition of pyruvate oxidation.

The ratio of mucosal to serosal lactate production is very sensitive to inhibition of the Na^+/K^+-ATPase. Measurement of the vectorial disposition of lactate should therefore prove useful as a preliminary test of whether inhibitors of glucose absorption act at the level of the Na^+/K^+-ATPase or the glucose transporter.

Acknowledgment

We gratefully acknowledge financial support from The Wellcome Trust. We thank Mr. N.L. Beach for the ATPase assays and Dr. J.G. Hastewell for helpful discussions.

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