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Acetate uptake by intestinal brush border membrane vesicles

A J M Watson, E A Brennan, M J G Farthing, P D Fairclough

Abstract

The mechanism of acetate absorption in the small intestine is not yet established. One possible mechanism is by carrier mediated Na^+-acetate cotransport since acetate, like glucose, stimulates intestinal Na^+ and water absorption in vivo. Uptake of radioactive carbon acetate by small intestinal brush border membrane vesicles was not saturable or Na^+ dependent and did not respond to osmotic shrinkage of the vesicles. This suggests that acetate binds to the membranes but is not transported into the intravesicular space and argues against carrier mediated Na^+ acetate cotransport. These results are consistent with acetate absorption by a non-mediated diffusion and suggest that the stimulation of water and Na^+ absorption by acetate in vivo is largely due to osmotic forces and solvent drag.

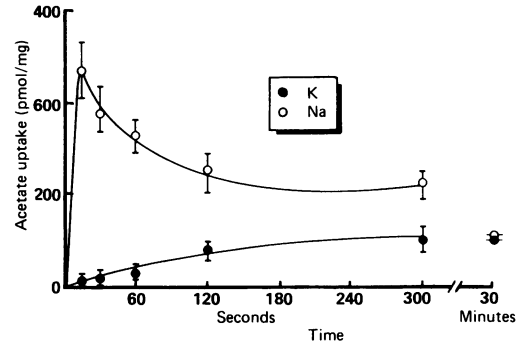

Figure 1: Effect of KCl and NaCl on uptake of acetate (0·2 mmol/l) by rabbit kidney cortical brush border membrane vesicles. Vesicles loaded with 200 mmol/l mannitol and 20 mmol/l HEPES-Tris at pH 7·4 were incubated at 37°C in medium containing either 100 mmol/l KCl or NaCl plus 20 mmol/l HEPES-Tris pH 7·4 or 0·2 mmol/l ^{14}C Na-acetate. The results shown are the mean of nine uptake experiments. The bars indicate mean (SEM).

Short chain fatty acids, including acetate, stimulate Na^+ absorption in the small intestine of rat and man.[1,2] In mammalian colon short chain fatty acids are probably absorbed by non-ionic diffusion and the related Na^+ absorption may take place by an Na^+/H^+ exchange mechanism; what happens in the small intestine is unclear.[3] The short chain fatty acids, propionate and butyrate, are known to be taken up by rabbit renal proximal tubular brush border membrane vesicles by two Na^+ dependent mechanisms – an electroneutral system in the pars convoluta and an electrogenic system in the pars recta.[4] The possibility that acetate transport in the small intestine take place via an Na^+ coupled system has not been examined in brush border membrane vesicles. This paper reports our studies on this topic.

Methods

Brush border membrane vesicles were prepared from whole intestine of male Wistar rats, and from ileum and kidney cortex of 2–3 kg New Zealand White rabbits by calcium precipitation and differential centrifugation (MSE Mistral 4L) as previously reported.[5] Vesicles were suspended in vesicle buffer containing 200 mmol/l mannitol and 20 mmol/l hydroxyethylpiperazine ethanesulphonic acid (HEPES)/Tris pH 7·4, rehomogenised, and collected by centrifugation at 43 000 *g* for 20 minutes. The pellet was then resuspended in vesicle buffer to yield a vesicle protein concentration of approximately 10 mg/ml. The protein concentration was measured by the method of Lowry *et al*, using bovine serum albumin as standard.[6] Sucrase was measured by the method of Dahlquist,[7] alkaline phosphatase by the method of Eicholz,[8] and Na^+-K^+-adenosine triphosphatase (ATPase) by the coupled enzyme method of Scharmschmidt *et al*.[9]

The uptake of radioactive ^{14}C acetate, ^{14}C glucose, and ^{22}Na (Amersham) was studied by a standard rapid filtration method.[10] A 50 µl aliquot of vesicle suspension was added to 250 µl of reaction mixture containing the radiolabelled substrate (usually 1 µCi per reaction) plus the appropriate salts and buffers as described in the legends to the figures. The reaction was followed by dilution of 50 µl samples of the reaction mixture into 1 ml of buffered 'stop' solution (100 mmol/l KCl, 20 mmol/l HEPES/Tris, pH 7·4, unless otherwise stated) at 4°C, followed by rapid filtration of the mixture on a 0·45 µm Millipore (HAWP) filter. The filters were washed with two 5 ml aliquots of 'stop' solution at 4°C. Vesicle

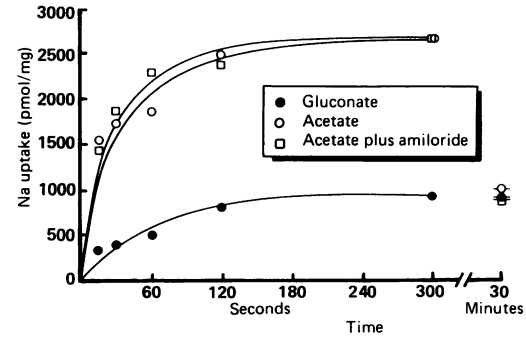

Figure 2: Effect of acetate on 1 mmol/l Na^+ uptake in rabbit kidney cortical brush border membrane vesicles. Vesicles loaded with 200 mmol/l mannitol and 20 mmol/l HEPES-Tris pH 7·4 were incubated at 37°C in a medium containing Na_2SO_4 1 mmol/l, K gluconate or K acetate 100 mmol/l, and 20 mmol/l HEPES-Tris pH 7·4. Amiloride (1 mmol/l) was added as appropriate. 0·5 µCi of ^{22}Na was used per time point. Stop solution contained 200 mmol/l mannitol and 20 mmol/l HEPES-Tris pH 7·4 and 0·1 mmol/l amiloride. The results shown are the mean of three uptake experiments.

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associated radioactivity was measured by liquid scintillation spectroscopy (LKB 1210 Ultrabeta liquid scintillation counter) after dissolution of the filter in 10 ml of a solution of ethylene glycol monomethyl ether 300 ml, toluene 900 ml, and diphenoxazole 7·2 g. Results are expressed as the mean of three experiments in each case. All experiments were performed in duplicate and repeated on preparations from at least three different animals. All chemicals used were obtained from BDH or Sigma and were of the highest available purity. Preliminary experiments indicated that brush border membrane vesicle (rat intestine and rabbit kidney) associated radioactivity was stable for one to six hours when incubated in 100 mmol/l KCl and 20 mmol/l HEPES/Tris, and 0·2 mmol/l ^{14}C-acetate. As β oxidation produces two carbon units of acetyl-COA which are completely oxidised by the Krebs cycle, we would expect radioactivity to be lost as CO_2 if appreciable catabolism occurred.

Results

ENZYME ENRICHMENT

In rat intestinal brush border membrane vesicles enrichment of alkaline phosphatase was mean (SEM) 12·6 (1·0) fold (n=25), and that of sucrase was 14·3 (0·9) fold (n=6). Enrichment of the basolateral membrane marker Na^+-K^+-ATPase was 1·5 (1·0) fold (n=6).

In rabbit ileal brush border membrane vesicles, alkaline phosphatase enrichment was mean (SEM) 12·8 (2·7) fold and Na^+-K^+-ATPase was 1·1 (0·3) fold (n=6). These results are comparable with those of others[5 11 12] and show reasonable separation of brush border from basolateral membranes.

CONTROL EXPERIMENTS

In rabbit kidney vesicles in the presence of Na^+ there was a transient accumulation of acetate five times greater than equilibrium. No such accumulation was noted when K^+ was substituted for Na^+ (Fig 1). Na^+ uptake was enhanced by an inwardly directed gradient of acetate (100 mmol/l), but not by equimolar concentration of gluconate (Fig 2). Thus we have been able to show an

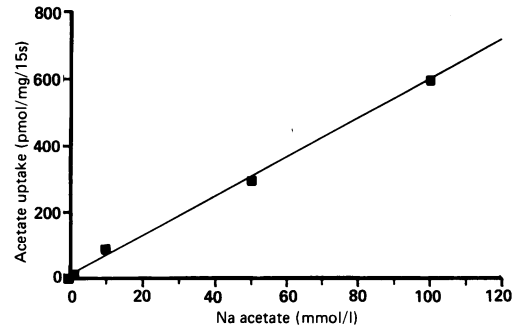

Figure 4: Effect of extravesicular acetate concentration on acetate uptake. Vesicles loaded with 200 mmol/l mannitol and 20 mmol/l HEPES-Tris pH 7·4 were incubated at 37°C in a medium containing KCl substituted with the appropriate concentration of Na acetate so that the combined concentration was 100 mmol/l, plus 20 mmol/l HEPES-Tris pH 7·4 and 0·1 mmol/l ^{14}C-Na acetate. Uptake time was 15 seconds. Results are expressed as the mean of three experimental uptake experiments.

Na^+/acetate coupled transport system as expected.[4] The stimulation of Na^+ uptake by acetate was not amiloride sensitive suggesting that Na^+/H^+ exchange does not participate in this process in rabbit kidney brush border membrane vesicles (Fig 2).

In rat and rabbit small intestinal brush border membrane vesicles glucose uptake was enhanced, with accumulation above equilibrium in the presence of Na^+ compared with K^+. It was further enhanced by thiocyanate compared with Cl^- showing the well known features of glucose transport in vesicles (data not shown).[10]

ACETATE UPTAKE BY SMALL INTESTINAL BRUSH BORDER MEMBRANE VESICLES

In contrast to the results in the control experiments, acetate uptake by rat small intestinal brush border membrane vesicles was similar in presence of inwardly directed Na^+ and K^+ gradients. There was no accumulation above equilibrium in either jejunal or ileal brush border membrane vesicles (Fig 3), and no evidence of saturation of uptake over a range of acetate concentrations from 0·01 to 100 mmol/l (Fig 4). An inwardly directed proton gradient (external pH 5·5, internal 7·4) increased acetate uptake only by 1·5 times. Similar results were obtained

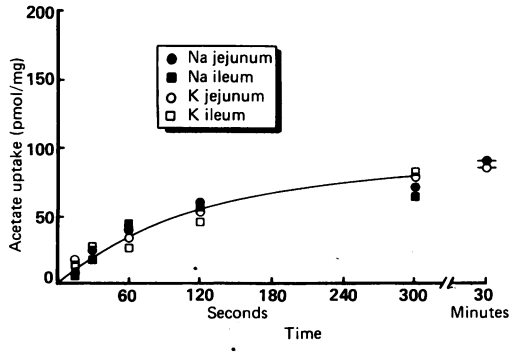

Figure 3: Effect of KCl or NaCl on acetate uptake by rat jejunal or ileal brush border membrane vesicles. Vesicles loaded with 200 mmol/l mannitol and 20 mmol/l HEPES-Tris pH 7·4 were incubated at 37°C in a medium containing either 100 mmol/l KCl or NaCl with 20 mmol/l HEPES-Tris pH 7·4 and 0·2 mmol/l ^{14}C- Na acetate. The results shown are the mean of three uptake experiments.

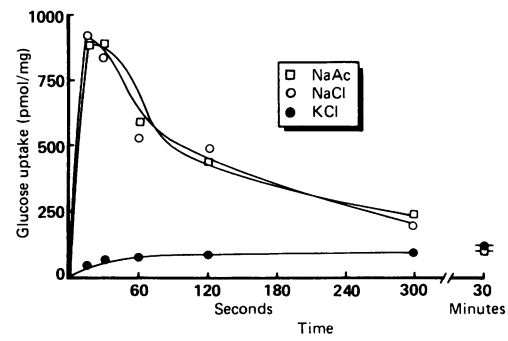

Figure 5: Effect of KCl, NACl, or Na^+-acetate on D-glucose uptake by rat small intestinal brush border membrane vesicles. Vesicles loaded with 200 mmol/l mannitol and 20 mmol/l HEPES-Tris pH 7·4 were incubated at 37°C in a medium containing either 100 mmol/l KCl, NaCl, or Na acetate with 20 mmol/l HEPES-Tris pH 7·4 and 0·1 mmol/l ^{14}C D-glucose (0·5 μCi for each time point). The results shown are the mean of three experiments.

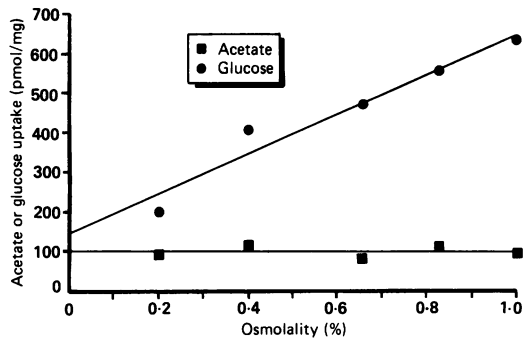

Figure 6: Effect of medium osmolality on equilibrium uptake of acetate and glucose. Vesicles loaded with 200 mmol/l mannitol and 20 mmol/l HEPES-Tris pH 7·4 were incubated at 37°C in a medium containing varying concentrations of cellobiose (47–533 mmol/l), 20 mmol/l HEPES-Tris pH 7·4, 100 mmol/l NaCl, and 0·2 mmol/l ^{14}C-Na acetate ^{14}C D-glucose. The incubation period was 90 minutes. The results shown are the mean of three experiments.

using rabbit ileal brush border membrane vesicles (results not shown). Na^+ dependent glucose uptake was used as a marker of membrane potential in a study in which vesicles were incubated in medium containing either Na^+ acetate or NaCl (100 mmol/l each); glucose uptake was unaltered by acetate (Fig 5), suggesting that under these circumstances acetate does not cause an increase in membrane potential any greater than chloride; this should be contrasted with the effect of the membrane permeant anion thiocyanate on glucose.[10] Attempts to stimulate acetate uptake from 0·01 mM acetate in the incubation medium by preloading the vesicles with 100 mmol/l unlabelled acetate showed no stimulation of uptake (no counter transport). Incubation of brush border membrane vesicles in media of differing osmolality showed that glucose uptake was into an osmotically sensitive space, whereas there was no evidence of internalisation of acetate (Fig 6).

Discussion

Previous work has suggested carrier mediated transport by the finding of saturation of short chain fatty acid transport and competitive inhibition.[13] Such data, however, are also compatible with diffusion and have to be balanced against the studies of Sallee and Dietschy who found no saturation of short chain fatty acid absorption in rat jejunum.[14] In addition, the correlation of short chain fatty acid absorption with chain length (and therefore lipid solubility) and the enhancement of short chain fatty acid uptake by hydrogen ions strongly suggest that these are absorbed in the undissociated form.[15 16] Nevertheless, no previous work has directly addressed the question of whether there is Na^+ dependent acetate absorption.

Despite our finding of both Na^+ dependent acetate transport in renal brush border membrane vesicle and Na^+ dependent electrogenic glucose transport in intestinal brush border membrane vesicle, we have been unable to show carrier mediated transport of acetate into intestinal vesicles in either rat or rabbit intestine. The observation that glucose is taken up into an osmotically sensitive space agrees with other studies.[10 12] Acetate, however, seems not to enter the intravesicular space, since uptake was unaffected by vesicle volume. The acetate associated with the brush border membrane vesicle is presumably bound to or dissolved in the lipid membrane; this binding is unaffected by Na^+ or K^+ and only minimally affected by a pH gradient.

In vivo, acetate acts as a stimulus to Na^+ and water absorption, presumably acting like fructose, which stimulates water absorption osmotically, with Na^+ absorption occurring passively by entrainment in the fluid flow.[17] It is possible that an acetate transport was destroyed in the preparation of the brush border membrane vesicles, though this seems unlikely since we were easily able to show the Na^+ dependent acetate carrier in renal brush border membrane vesicles. We must conclude that brush border membrane vesicles prepared by the methods described in this paper show no evidence of carrier mediated transmembrane transport of acetate and thus the data are consistent with intestinal absorption of acetate by diffusion alone. These conclusions are similar to those resulting from our own in vivo studies of small intestinal acetate absorption in rats.[18]

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