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CALCIUM AND GLUCOSE UPTAKE IN RAT SMALL INTESTINAL BRUSH-BORDER MEMBRANE VESICLES

MODULATION BY EXOGENOUS HYPERCORTISOLISM AND 1,25-DIHYDROXYVITAMIN D-3

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The effect of exogenous hypercortisolism and 1,25-dihydroxyvitamin D-3 on small-intestinal calcium and glucose transport in the rat was studied at the level of brush-border membrane vesicles generated from isolated villous cells by a freeze-thaw procedure. At $5 \cdot 10^{-5}$ M extravesicular calcium, initial uptake rates in vesicles prepared from triamcinolone-treated adult rats were decreased by 30% after 5 days. Since calcium ionophore A23187 virtually abolished the difference in calcium uptake, triamcinolone appeared to affect calcium channel density or activity rather than intravesicular binding capacity. Kinetic analysis showed that a decrease in V_{max} of a saturable calcium transport system could entirely account for the diminished rate of vesicular calcium uptake. Calcium transport rates could be partially restored by in vivo administration of 1,25-dihydroxyvitamin D-3 at a dosage which did not affect vesicular calcium uptake in control animals. Conversely, sodium-driven glucose accumulation in brush-border vesicles from triamcinolone-treated rats was stimulated by 50–70% after 36 h and appeared insensitive to vitamin D. A specific triamcinolone action on the glucose carrier itself rather than on the driving force of the sodium gradient was indicated by (i) a similar stimulation of glucose transport under equilibrium exchange conditions and (ii) an opposite effect of triamcinolone on sodium-driven alanine transport. The triamcinolone-induced changes in calcium and glucose uptake were not accompanied by a gross alteration of membrane integrity in vitro or by major alterations in vesicular protein composition, intravesicular glucose space and sucrase or alkaline phosphatase activity. The modification of vesicular transport properties is discussed in relation to the vitamin D-antagonized inhibition of intestinal calcium uptake and the stimulation of glucose absorption in response to supraphysiologic amounts of glucocorticoids observed in intact epithelium.

Introduction

Reduction of net calcium (Ca) absorption in proximal small intestine in response to exogenous hypercortisolism has been documented in animals in vitro using everted gut sacs or Ussing chambers [1–3]. Studies monitoring fractional intestinal uptake of radioactive calcium in man showed a diminished absorption during treatment with supraphysiologic doses of glucocorticoids (GC) [4,5]. Malabsorption of Ca may contribute to the pathogenesis of GC-induced bone disease presumably by induction of a state of secondary hyperparathyroidism [5].

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Abbreviations: GC, glucocorticoids; 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D-3.

A major regulatory site in transepithelial Ca transport is the passive entry of luminal Ca across vitamin D-sensitive channels in the brush-border membrane [6,7]. According to the 'liponomic control' hypothesis, the active vitamin D metabolite 1,25-dihydroxyvitamin D-3 (1,25-$(OH)_2D_3$) would unmask cryptic calcium channels in the luminal membrane by changing the lipid annulus surrounding the channel. In these studies, duodenal brush-border membrane vesicles have been successfully applied as in vitro tools to discriminate vitamin D action at the luminal membrane from its potential effects on other epithelial components, e.g., the Ca binding protein in the cytosol [8], basolateral Ca pump [9] and the Na-Ca exchanger [10] or the Ca counter-transport system in Golgi membranes [11].

In view of the apparent antagonism between 1,25-$(OH)_2D_3$ and GC with regard to transepithelial Ca absorption, the possibility was considered that GC might likewise interfere with Ca transport across the luminal membrane. In the present study, the brush-border membrane vesicle model was chosen to monitor functional changes at the brush-border level in adult rats treated with supraphysiologic doses of GC. Effects of exogenous 1,25-$(OH)_2D_3$ on this model were also examined in order to clarify the mechanism underlying the beneficial action of vitamin D or its metabolites on intestinal calcium uptake in GC-treated animals or patients [3,4,12]. Sodium-glucose cotransport served as a marker confined exclusively to the luminal membrane [13] and as a sensitive indicator of the integrity of the vesicle membrane.

Materials and Methods

Materials. Triamcinolone (Kenacort A 40 ®) was obtained from Squibb, 1,25-$(OH)_2D_3$ for parenteral administration was kindly provided by Hoffmann La Roche, phlorizin was obtained from Fluka, D-[1-^{3}H]glucose from New England Nuclear and other radioactive isotopes were obtained from Amersham International. The Ca ionophore (A23187) was obtained from Boehringer. Other chemicals were analytical grade. Nitrocellulose filters were obtained from Sartorius, The Netherlands.

Animals. Adult male Wistar rats weighing 300–350 g and fed normal laboratory chow were injected subcutaneously with a suspension of triamcinolone acetonide, sustained release, at a dosage of 6 mg/kg (0.05 ml) 5 days prior to vesicle preparation (group II). In group III, this treatment was combined with subcutaneous administration of 300 ng/kg (0.05 ml) 1,25-$(OH)_2D_3$ 36 and 12 h prior to vesicle preparation. Group IV was pretreated with 1,25-$(OH)_2D_3$ alone and group I, the control group, was injected with equivolumes of 0.9% NaCl. In combined experiments, vesicle preparations obtained from different groups were studied simultaneously. The existence of a state of hypercortisolism after 5 days was confirmed by the finding of suppressed endogenous corticosterone levels (not shown). For the time-effect study, 6 mg/kg triamcinolone acetonide or 0.9% NaCl (both 0.05 ml) was administered subcutaneously 10, 36 and 120 h prior to vesicle preparation.

Preparation of vesicles. Brush-border membrane vesicles were prepared from the proximal half of the rat small intestine. Intestines from two rats were pooled for a single vesicle preparation. Under light ether anaesthesia, the small intestine was removed and rinsed three times with 20 ml ice-cold 0.9% NaCl. All further steps were performed at 0–4°C. First, intestinal villous cells were released by mechanical vibration, as described earlier [14]. Cells were collected by centrifugation ($160 \times g$, 5 min) and the cell pellet derived from two rats was washed three times with 40 ml buffer containing 10 mM maleate, 300 mM mannitol and 0.02% NaN_3 brought to pH 7.2 with Tris (buffer A). The method used for vesiculization of the brush-border membrane and the purification of brush-border membrane vesicles will be published in full detail elsewhere. In short, the cell pellet was resuspended in 10 ml buffer A and quickly frozen in liquid nitrogen. After 10 min, the frozen suspension was thawed slowly in ice/water for 60–90 min. Vesiculization by mechanical homogenization [15,16] was completely avoided. Released brush-border membranes were separated from other membrane fragments by differential precipitation with 10 mM $MgSO_4$ [16] and centrifugation ($3000 \times g$, 15 min). Brush-border membrane vesicles were isolated from the supernatant

by a second centrifugation step (27000 × *g*, 30 min). The crude vesicle pellet was resuspended in 20 ml buffer (pH 7.2) containing 10 mM Tris-maleate, 100 mM mannitol and 0.02 NaN_3 (buffer B). The suspension was homogenized in a Potter-Elvehjem homogenizer and the differential centrifugation procedure was repeated once. The final pellet was suspended in buffer B prior to use. The enrichment factor of the brush-border marker enzyme, sucrase, in brush-border membrane vesicles as compared to mucusal scrapings was 32 ± 5 (n = 10).

Vesicular transport. Vesicular Ca uptake was determined by preincubating 50 μl brush-border membrane vesicle suspension in buffer B (25–50 μg protein) for 1 min at 25 °C, followed by addition of an equivolume of 0.1 mM $^{45}CaCl_2$ (spec. act. 170–200 dpm/pmol) in the same buffer. Transport was stopped by addition of 2 ml ice-cold buffer B, followed by rapid filtration through nitrocellulose filters (pore size 0.45 μm). After washing four times with 2 ml ice-cold buffer B, the filter was dissolved in 5 ml scintillation fluid (Instagel, Packard) and ^{45}Ca determined in a Packard 2650 Tricarb scintillation counter. Data were corrected for blank values obtained by omitting brush-border membrane vesicles from the incubation mixture. In kinetic studies of Ca uptake, 2 mM EGTA was included in the stop buffer in order to reduce Ca binding to the vesicle exterior, which became significant at Ca levels exceeding 0.05 mM. Initial Ca-influx rates were calculated from the difference in vesicular Ca uptake at two initial time points (15 and 60 s) when uptake was virtually linear in time at all Ca concentrations tested (0.015–0.9 mM). Following preloading of the brush-border membrane vesicles for 3 min in the presence of 50 μM $^{45}CaCl_2$, ^{45}Ca-efflux was studied by diluting the incubation mixture 40-fold with buffer B containing 2 mM EGTA with or without 10 μM Ca ionophore, followed within 0–2 min by filtration and washing.

Sodium-dependent transport of glucose and alanine into brush-border membrane vesicles was determined after preincubation of the vesicle suspension (50–100 μg protein) at 25 °C for 1 min or at 37 °C for 30 min followed by 1 min at 25 °C. Transport was started by rapidly mixing a droplet of 50 μl brush-border membrane vesicle suspension with an equivolume of 0.2 M NaSCN in buffer B containing 2.6 μM D-[1-^{3}H]glucose (spec. act. $3 \cdot 10^4$ dpm/pmol) or 1.0 μM L-[2,3-^{3}H]alanine (spec. act. $8 \cdot 10^4$ dpm/pmol). Since the dissipation rate of the transmembranal Na gradient strongly affects the driving force for intravesicular glucose accumulation, the activity of the glucose carrier was also measured under isotope-equilibrium conditions as described by Hopfer and Groseclose [17]. In this way, gradient-related driving forces are completely avoided and changes in labeled glucose transport reflect alterations in glucose carrier activity. In these experiments, brush-border membrane vesicles were preincubated in the presence of 0.1 mM glucose and 0.1 M NaCl in buffer B for 30 min at 25 °C. Isotope uptake was started by mixing 50 μl brush-border membrane vesicle suspension with an equivolume of the preincubation medium containing 0.1 mM D-[1-^{3}H]glucose (spec. act. $2 \cdot 10^3$ dpm/pmol). In the case of sodium-dependent glucose uptake, transport was stopped at 0.1 min and in the case of equilibrium exchange at 10, 20, 40 and 120 s with 2 ml ice-cold buffer B containing 0.1 M NaCl and 0.5 mM phlorizin. Mixing and stopping were performed by means of a semiautomatic apparatus constructed according to Kessler et al. [18]. Following filtration on nitrocellulose filters and repeated washings (four times with 2 ml ice-cold stop buffer), the filters were processed for liquid scintillation counting. Equilibrium values for [^{3}H]glucose uptake were obtained after 60 min of incubation. In the isotope-equilibrium exchange experiments, the half-time for maximal uptake of labeled glucose ($t_{1/2}$) was estimated by interpolation on plots of $\ln(1 - \text{uptake}_t/\text{uptake}_\infty)$ vs. time, whereby uptake_t = tracer uptake at the indicated time point and uptake_∞ = uptake at equilibrium (cf. Ref. 17).

Miscellaneous. Protein concentrations were determined according to Lowry et al. [19]. Alkaline phosphatase activity was assayed at 37 °C as described by Iemhoff et al. [20]. Sucrase activity measured in 0.1% Triton X-100 was determined according to Forstner et al. [21]. Brush-border proteins were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis in principle according to Laemmli [22].

Statistical evaluation. Results from animal

groups I–IV obtained in one experiment were statistically compared with the outcome of separate experiments by a two-way analysis-of-variance test, utilising Tukey multiple confidence intervals. In experiments where only two groups were compared, the (unpaired) Student's *t*-test was applied.

Results

Characterization of vesicular calcium and glucose uptake

Some of the basic features of the Ca uptake process in brush-border membrane vesicles prepared by the freeze-thaw technique are demonstrated in Fig. 1A and can be summarized as follows:

(1) Binding of Ca to the vesicle exterior following the filter-washing procedure was apparently insignificant at the low Ca concentration in the medium (Ca_{out}) selected, since (i) 1 mM EGTA or 0.1 mM $LaCl_3$ added to the washing buffer did not affect Ca uptake values (results not shown) and (ii) excess EGTA added to the vesicle exterior at 25 °C started a slow release of Ca from ^{45}Ca-preloaded vesicles which could be accelerated substantially by addition of the Ca ionophore A23187 (see Fig. 1A, efflux experiments).

(2) Based on an osmotically active intravesicular glucose space of 1.46 μl/mg protein (Table I), equilibrium uptake of Ca at 0.05 mM Ca_{out} should be reached following transport of 0.07 nmol Ca/mg protein, provided that binding to internal Ca binding sites and the Donnan potential [23] could be neglected. However, according to Fig.

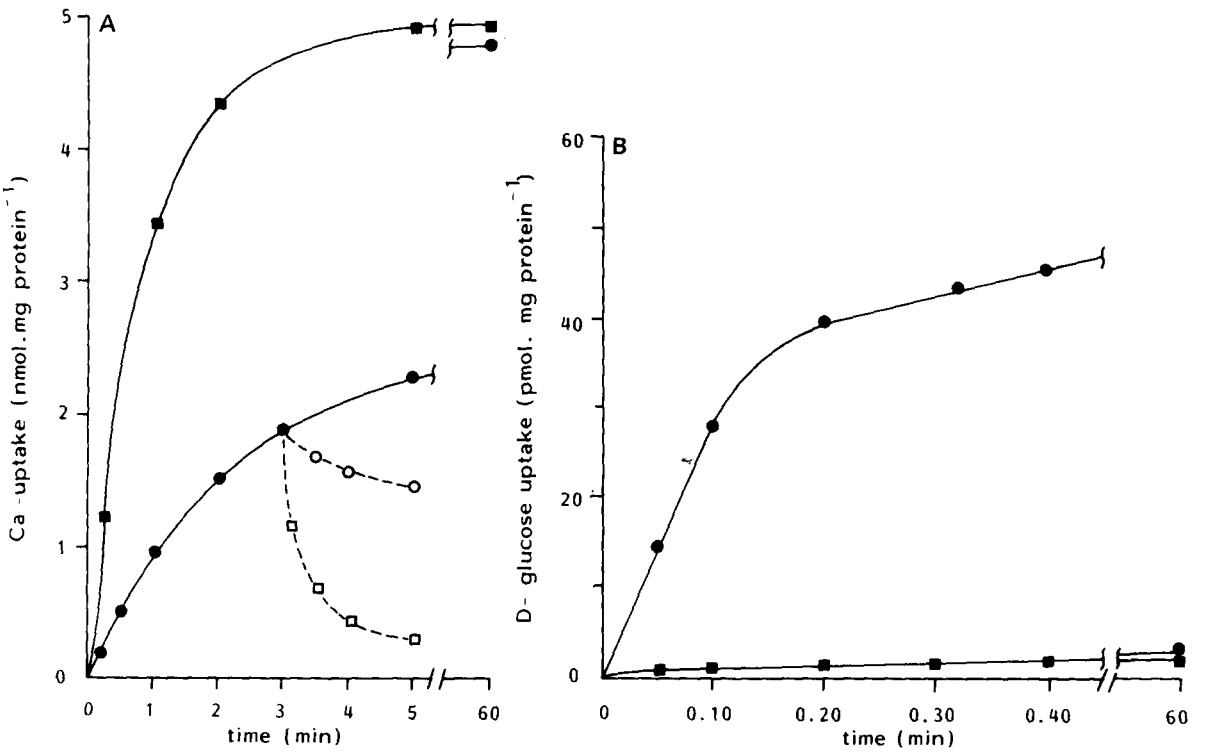

Fig. 1. (A) Time-course of Ca uptake and Ca efflux by brush-border membrane vesicles in absence (●———●, ○— — —○) and presence (■———■, □— — —□) of Ca ionophore A23178. Release of ^{45}Ca (□— — —□, ○— — —○) was started by diluting samples of the incubation mixture 40-fold in the same buffer containing EGTA (2 mM). For experimental details see Materials and Methods. (B) Time-course of glucose uptake by brush-border membrane vesicles in the presence (●———●) or absence (■———■) of a NaSCN gradient (100 mM outside, zero mM inside at $t = 0$). Equilibrium uptake was reached after 30 min of incubation. In (■———■), brush-border membrane vesicles were preincubated in the presence of 100 mM NaSCN for 30 min prior to [^{3}H]glucose addition.

1A, uptake of Ca reached a 15-fold higher value already at 1 min and a 30-fold higher value at 5 min, implying that virtually all Ca uptake reflects intravesicular binding.

(3) Preincubation of brush-border membrane vesicles in the presence of the Ca ionophore caused a 4–6 fold increase in initial rate of vesicular ^{45}Ca uptake (Fig. 1A). This level of stimulation was persistently seen within a broad concentration range of Ca_{out} (0.015–0.9 mM; data not shown). Equilibrium uptake was reached after 5 min, about 1 h earlier than found for brush-border membrane vesicles in the absence of ionophore (Fig. 1A). The finding that equilibrium values of Ca uptake in the absence or presence of ionophore were not significantly different suggests that the ionophore acts on the same class of vesicles responsible for basal Ca transport. Since the ionophore merely increases the rate of Ca influx across the vesicle membrane, without affecting Ca binding sites, Ca entry into the vesicle interior apparently functions as a rate-limiting step in the Ca uptake process, at least during the first few minutes. Therefore initial rates of vesicular ^{45}Ca uptake will primarily reflect the kinetic properties of Ca channels embedded in the microvillous membrane.

TABLE I

TRANSPORT RATES OF CALCIUM, GLUCOSE AND ALANINE AND ACTIVITIES OF BRUSH-BORDER ENZYMES IN BRUSH-BORDER MEMBRANE VESICLES FROM TRIAMCINOLONE ACETONIDE- AND 1,25-$(OH)_2D_3$-TREATED RATS

Adult Wistar rats (300 g) received triamcinolone acetonide (6 $mg \cdot kg^{-1}$) 5 days prior to vesicle preparation (group II); 1,25-$(OH)_2D_3$ (300 $ng \cdot kg^{-1}$) 12 and 36 h prior to vesicle preparation (group IV); triamcinolone acetonide as well as 1,25-$(OH)_2D_3$ (group III) or saline (group I). Ca uptake measured at 50 μM Ca_{out} (25 °C) was quantified by millipore filtration. Initial uptake rates were calculated from the difference in vesicular Ca uptake at two initial time points (15 and 60 s). Ca uptake after 5 min was measured in the presence or absence of 40 μM Ca ionophore A23178 (see Fig. 1A). Na-driven glucose and alanine uptake were measured at 0.1 min in the presence of 1.3 μM glucose or 0.5 μM alanine plus 0.1 M NaSCN after preincubation for 1 min at 25 °C or 30 min at 37 °C, as described in Materials and Methods. The $t_{1/2}$ for labeled glucose uptake was determined under isotope equilibrium-exchange conditions, as described in Materials and Methods. Intravesicular glucose space was calculated from equilibrium [^{3}H]glucose uptake at 60 min (see Fig. 1B).

Animal groups	I	II	III	IV
Triamcinolone-treated	–	+	+	–
1,25-$(OH)_2D_3$-treated	–	–	+	+
Ca uptake (initial rate) [a] (nmol/min per mg protein)	0.97 ± 0.25	0.68 ± 0.20 [d]	0.88 ± 0.16 [d]	0.93 ± 0.25
Ca uptake (5 min) [a] (nmol/mg protein)				
– ionophore	2.44 ± 0.44	1.74 ± 0.38 [d]	2.17 ± 0.33 [d]	2.53 ± 0.39
+ ionophore	4.87 ± 0.62	3.92 ± 0.79	4.03 ± 0.96	4.89 ± 0.48
Glucose transport (NaSCN gradient) [a] (pmol/0.1 min per mg protein)				
Preincubation 1 min, 25 °C	27.2 ± 8.8	45.9 ± 15.7 [d]	55.4 ± 11.8 [d]	34.8 ± 7.8
Preincubation 30 min, 37 °C	29.0 ± 14.0	42.8 ± 16.0 [d]	53.4 ± 16.6 [d]	38.4 ± 8.1
Glucose transport [b] ($t_{1/2}$ under isotope equilibrium conditions) (s)	7.8 ± 2.1	3.1 ± 1.0 [e]	n.d.	n.d.
Alanine transport (NaSCN gradient) [b] (pmol/0.1 min per mg protein)	5.61 ± 1.27	3.26 ± 0.75 [e]	n.d.	n.d.
Intravesicular glucose space [b] (μl/mg protein)	1.46 ± 0.15	1.48 ± 0.07	1.40 ± 0.11	1.47 ± 0.09
Sucrase activity [c] (U/mg protein)	2.40 ± 0.51	2.65 ± 0.65	2.70 ± 0.49	2.44 ± 0.55
Alkaline phosphatase activity [a] (mU/mg protein)	3.74 ± 1.27	3.27 ± 1.52	4.53 ± 1.74	5.85 ± 2.04 [d]

[a] Mean values ± S.D. ($n = 6$).
[b] Mean values ± S.D. ($n = 3$).
[c] Mean ± S.D. ($n = 4$).
[d] Significance of the difference (two-way analysis-of-variance): from group I ($P < 0.05$); from group II ($P < 0.05$).
[e] Significance of the difference (Student's t-test): from group I ($P < 0.05$).
n.d., not determined.

Sodium-driven glucose accumulation increased linearly with time for about 6 s (Fig. 1B). At this point, the 'overshoot' above the equilibrium value was 15–18-fold, a value similar to or better than reported for other brush-border membrane vesicle preparations [13,15]. Glucose overshoot was completely abolished if ionic gradients were dissipated by preincubating brush-border membrane vesicles with NaSCN (0.1 M) for 30 min prior to [^{3}H]glucose addition (Fig. 1B). The glucose overshoot, reflecting the activity of the Na-glucose carrier, is also a sensitive indicator of the integrity of the vesicle membrane. Monitoring vesicle stability was considered essential because in initial experiments using brush-border membrane vesicles prepared according to Kessler et al. [15] we noticed a variable loss of glucose overshoot during in vitro incubation parallelled by increased ^{45}Ca uptake and proteolysis of intravesicular proteins, e.g. actin (data not shown). These in vitro artifacts were rarely seen in vesicles prepared from triamcinolone acetonide-treated rats. By using the freeze-thaw procedure for vesicle isolation, however, such artifacts were completely avoided. This is illustrated by the persistently high levels of glucose overshoot in vesicles from groups I and II upon preincubation for 30 min at 37 °C (Table I).

Changes in vesicular Ca and glucose uptake induced by GC and 1,25-(OH)$_2$D$_3$

5 days after injection of triamcinolone acetonide, we found a significant fall in the initial Ca uptake rates (average 30%) in brush-border membrane vesicles isolated from the proximal small intestine, compared to the same preparation from saline-injected rats (cf. group II vs. group I in Table I). Addition of Ca-ionophore A23178 virtually eliminated any difference in Ca uptake (measured at $t = 5$ min) between all four groups (Table I). Since at least 95% of total Ca uptake at 5 min reflects binding to intravesicular receptor sites, the triamcinolone acetonide effect is unlikely to be the consequence of a change in intravesicular free Ca space. Moreover, the osmotically active intravesicular glucose space was unaltered by triamcinolone acetonide treatment (Table I). Table I also shows that treatment of rats with 1,25-(OH)$_2$D$_3$ alone (group IV) had no influence on vesicular Ca uptake rate compared to controls (group I). In contrast, administration of the vitamin D-3 derivative to triamcinolone acetonide-treated rats partially counteracted the triamcinolone acetonide-induced depression of vesicular Ca transport (cf. group III vs. group II).

Table I also shows a 1.7-fold increase in Na-dependent glucose uptake in brush-border membrane vesicles from triamcinolone acetonide-treated animals. Interestingly, this stimulatory effect of triamcinolone acetonide on glucose transport was not counteracted by 1,25-(OH)$_2$D$_3$ but even showed a tendency to increase further (group III vs. group II). An opposite effect of triamcinolone acetonide was found on the half-filling time ($t_{1/2}$) for vesicular glucose uptake measured under isotope equilibrium-exchange conditions (Table I), indicating that the triamcinolone acetonide-induced increase in glucose uptake was the consequence of a change in glucose carrier activity

TABLE II

TRANSPORT RATES OF CALCIUM AND GLUCOSE IN BRUSH-BORDER MEMBRANE VESICLES AS A FUNCTION OF TIME FOLLOWING THE ADMINISTRATION OF TRIAMCINOLONE

Triamcinolone acetonide (TA), sustained release, 6 mg·kg^{-1}, was injected subcutaneously at the indicated points in time. Control animals (C) received an equivolume of 0.9% saline. Na-dependent glucose transport was measured at $t = 0.1$ min as indicated in Fig. 1B. Initial Ca uptake rates were measured at 50 μM Ca_{out} as described in Materials and Methods.

In vivo exposure to triamcinolone acetonide	10 h		36 h		120 h	
	C	TA	C	TA	C	TA
Ca uptake rate (nmol/min per mg protein)	1.36 ± 0.26	1.30 ± 0.39	1.16 ± 0.26	1.02 ± 0.21	0.97 ± 0.25	0.68 ± 0.20 [a]
Glucose transport (pmol/0.1 min per mg protein)	39.3 ± 12.8	41.0 ± 11.5	30.7 ± 4.1	46.0 ± 11.3 [a]	27.2 ± 8.8	45.9 ± 15.7 [a]

[a] Significance of the difference from controls: $P < 0.05$ (Student's t-test).

rather than of a fall in Na permeability of the vesicle membrane. Moreover, Na-driven alanine accumulation was significantly depressed in brush-border membrane vesicles from triamcinolone acetonide-treated rats as compared to control vesicles (Table I).

Changes in vesicular Ca- and Na-driven glucose transport were also studied at several time points following a single injection of saline or triamcinolone acetonide (Table II). Significant changes in Na-driven glucose transport rates in triamcinolone acetonide-treated rats were detectable as early as 36 h, whereas a depression of Ca uptake became significant only at 120 h after injection of triamcinolone acetonide.

Effects of triamcinolone acetonide and 1,25-$(OH)_2D_3$ on other properties of brush-border membrane vesicles

Table I additionally shows that the specific activity of the brush-border membrane marker enzyme, sucrase, was not significantly different in brush-border membrane vesicles isolated from animal groups I–IV. The activity of alkaline phosphatase was also unaffected in triamcinolone acetonide-treated rats. As expected [24], 1,25-$(OH)_2D_3$ significantly increased the activity of this enzyme (group IV). As a trivial explanation for the triamcinolone acetonide effects on Ca and glucose transport, the possibility was also considered that triamcinolone acetonide treatment could lead to the generation or selection of a different class of vesicles no longer representative of brush-border membrane vesicles. Therefore, we analyzed the protein composition of vesicles obtained from all groups (I–IV) by SDS-polyacrylamide gel electrophoresis. Although changes in minor protein components cannot be detected accurately by this technique, the overall protein composition appeared very similar (results not shown).

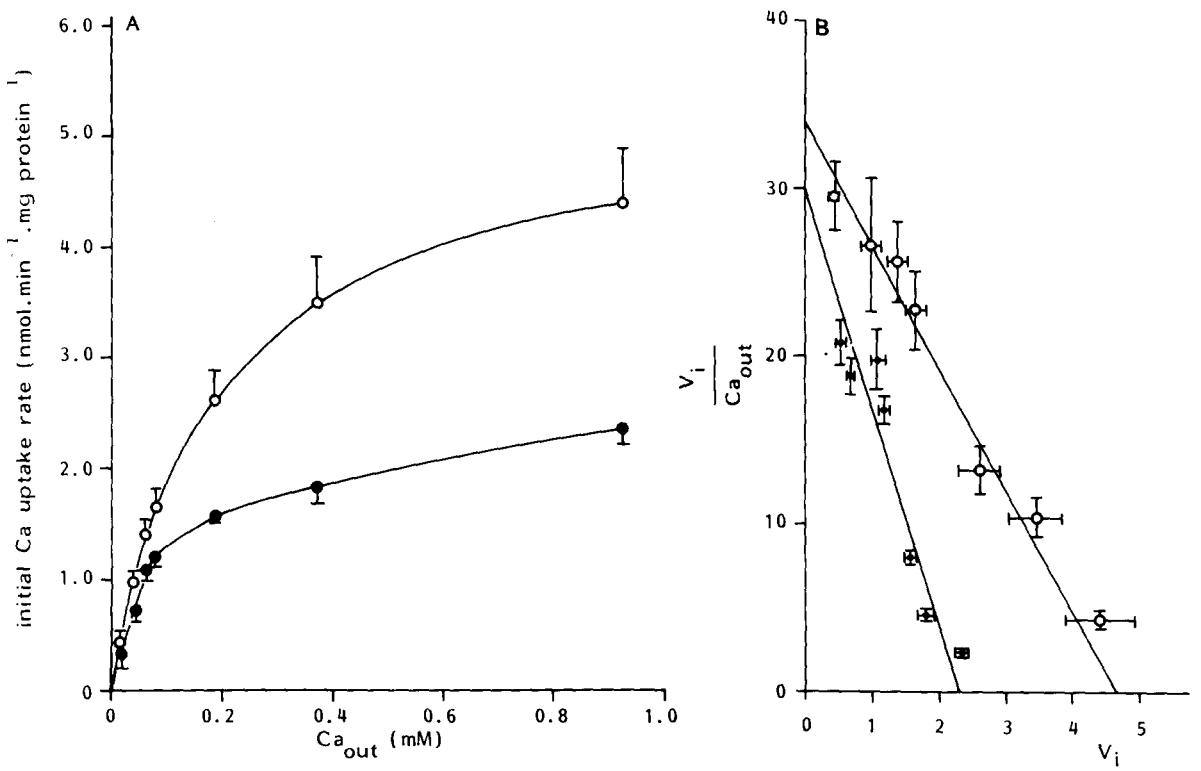

Fig. 2. (A) Initial Ca uptake rates (V_i) as a function of extravesicular Ca concentration (Ca_{out}) in brush-border membrane vesicles from control (○——○) and triamcinolone acetonide-pretreated (●——●) rats. Mean values ± S.E. are shown ($n = 4$). The difference from controls was significant over the whole range of Ca_{out} measured: $P < 0.05$ (Student's t-test). (B) The same data (mean values ± S.E.) presented in an Eadie-Hofstee plot. Linear regression analysis: the r value for control (○——○) and triamcinolone acetonide-treated (●——●) vesicles amounted to 0.99 and 0.93, respectively.

Effects of triamcinolone acetonide on kinetic properties of vesicular Ca and glucose transport

The decrease of initial Ca uptake rate in brush-border membrane vesicles from rats treated with triamcinolone acetonide for 5 days was persistently found within a broad range of extravesicular Ca concentrations (0.015–0.9 mM; Fig. 2A). Analysis of the data of four consecutive experiments in an Eadie-Hofstee plot (Fig. 2B) indicated that the decrease of Ca uptake in brush-border membrane vesicles resulted from a sharp decrease in the apparent V_{max} (mean ± S.E. = 4.7 ± 0.6 for control vs. 2.3 ± 0.05 nmol/min per mg protein for triamcinolone acetonide-treated), predominating the effect of a decrease in the K_m value of the transport system (mean ± S.E. = 138 ± 4.8 for control vs. 78 ± 4.8 μM for triamcinolone acetonide-treated).

Measurements of Na-driven [^{3}H]glucose accumulation at 6 s as a function of extravesicular glucose concentration and analysis of the data in a Lineweaver-Burk plot suggested that triamcinolone acetonide treatment shifted the apparent V_{max} value of glucose transport from 1.1 to 1.7 nmol/0.1 min per mg protein without affecting the K_m value (approx. 80 μM; data not shown). A similar K_m value has been reported for the Na-glucose symport in renal brush-border membrane vesicles [25].

Discussion

The 30% decrease in Ca uptake rate found in brush-border membrane vesicles from rats treated with GC could be due to a change in Ca channel properties, an alteration of the number or affinity of intravesicular Ca binding sites or both. The finding that the Ca ionophore A23178, which increases the Ca permeability of the vesicle membrane but does not affect intravesicular Ca buffering systems, eliminated the difference in Ca uptake between control and triamcinolone acetonide-treated vesicles, is strongly in favour of a triamcinolone acetonide effect on transmembrane Ca transport rather than on intravesicular Ca binding. Kinetic analysis of the Ca transport data showed that the triamcinolone acetonide-induced depression of vesicular Ca uptake could be ascribed completely to a fall in apparent V_{max} of the Ca transport system which is only partially compensated by a concomitant decrease in K_m. The effect is unlikely to result from a general membrane-stabilizing action of GC preventing in vitro breakdown of the membrane barrier [26], since the brush-border membrane vesicles prepared by the freeze-thaw method were extremely stable as judged by the persistence of the glucose overshoot phenomenon upon prolonged incubation at 37 °C (Table I).

The lower level of vesicular Ca transport found in triamcinolone acetonide-treated rats corresponds to reported effects of GC on intact intestinal segments in vitro [1,2]. A partial restoration of GC-suppressed Ca transport by concomitant treatment with vitamin D or 1α-hydroxylated vitamin D has also been observed with intact intestinal epithelium, both in vitro [3] and in vivo [27]. Our finding of a 1,25-$(OH)_2D_3$-correctable depression of vesicular Ca transport is not in contradiction with recent data from Shultz et al. [28], who found a normal increase of Ca uptake in brush-border membrane vesicles from vitamin D-depleted chicks treated with GC upon repletion with 1,25-$(OH)_2D_3$. Unfortunately, these authors did not present data of GC effects on brush-border membrane vesicles from untreated normal chicks, and they confined the period of treatment to 48 h.

Although the results of the brush-border membrane vesicle experiments offer a plausible explanation for the apparent decrease in segmental intestinal Ca absorption induced by exogenous hypercortisolism and for the partial reversal of this decrease by vitamin D, they do not rule out additional effects of GC on other parts of transmucosal Ca transport system, e.g., on Ca binding to cytosolic proteins, Ca pump activity or Ca-Na exchange at the basolateral membrane or on paracellular Ca transport.

The molecular mechanism involved in GC action on the Ca transport system in the intestinal brush border is not established by the present study. Depression of vitamin D activation by triamcinolone acetonide or stimulation of its catabolism are unlikely in view of the normal or even increased 1,25-$(OH)_2D_3$ serum levels found in rat [29] and man [30] during short-term hypercortisolism. However, those findings do not exclude changes in intracellular 1,25-$(OH)_2D_3$ receptor concentrations [31] or post-receptor events such as

the production of cytosolic Ca binding protein [32]. A local effect of GC in the villous cells mediated by binding to steroid receptors and affecting either a 'liponomic control' mechanism of the Ca channel (cf. Ref. 7) or the synthesis and incorporation of Ca channels into the brush-border membrane appears also unlikely in view of the rather slow response of the Ca transport system to exogenous hypercortisolism significant only after 5 days. In contrast, such a local mechanism could certainly play a role in the stimulation of vesicular glucose transport measured at 36 h following triamcinolone acetonide injection. Moreover, if GC would exert their action exclusively on intestinal crypt cells, implying a lag phase in the effects on villous cell membranes, one should except a maximal effect on microvillar Ca transport not earlier than 2–3 days after triamcinolone acetonide injection, corresponding with the transit time of rat enterocytes from crypt to villous tip [33]. We therefore suggest that the inhibitory action of triamcinolone acetonide on Ca transport either originates in the intestinal crypt cell or is secondary to effects of triamcinolone acetonide on non-intestinal tissues.

The stimulation of glucose transport in response to GC at the level of brush-border membrane vesicles has not been reported earlier, but seems to correspond in time with the GC-induced stimulation of glucose absorption reported for intact intestinal segments [34]. Charney et al. [34] have suggested a positive correlation between the activation of $(Na^+ + K^+)$-ATPase in the basolateral membrane of the enterocyte and Na, water and glucose absorption by the intact epithelium. Since brush-border membrane vesicles are devoid of this enzyme and depleted of endogenous ATP (unpublished results), such a mechanism cannot play a role in the stimulation of glucose transport at the vesicle level. On the other hand, it is more plausible to explain the increased transmucosal glucose transport observed in intact epithelium by a GC-mediated increase of glucose carrier activity in the brush-border membrane, since this system seems to function as the rate-limiting step in overall glucose transport [35].

In conclusion, the results of our vesicle studies indicate that exogenous hypercortisolism exerts a stimulatory effect on the Na-symport carrier for glucose in the brush-border membrane at 36 h followed later (120 h) by an inhibitory effect on the Ca permeability of the membrane. The active vitamin D metabolite $1,25\text{-}(OH)_2D_3$ was found capable to counteract the GC effect on Ca transport but not on glucose transport. The specific effects of GC on intestinal brush-border components not only form an interesting basis for further studies regarding the molecular mechanism underlying GC action but are also of clinical importance.

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