Influence of Cholecalciferol (Vitamin D₃) on the Initial Kinetics of the Uptake of Calcium by Rat Small-Intestinal Mucosa

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The uptake of Ca²⁺ by isolated small-intestinal mucosa from vitamin D-depleted and -repleted rats was analysed for the effects of vitamin D on initial kinetics. The rapid association of Ca²⁺ with the tissue, which is complete within 1 min, was unaffected by the vitamin, whereas the subsequent, linear, uptake was significantly increased. Neither the tissue space accessible to inulin nor the permeability to thiourea was influenced by vitamin D treatment.

Administration of cholecalciferol (vitamin D₃) to vitamin D-depleted rats causes increased Ca²⁺ absorption by the small intestine (Schacter & Rosen, 1959) and the formation of large amounts of a calcium-binding protein (Kallfelz *et al.*, 1967). On the basis of evidence accumulated, mainly but not exclusively in the chick, it has been suggested that the calcium-binding protein is directly responsible for the vitamin D-induced increase in intestinal Ca²⁺ transport (Wasserman *et al.*, 1969; Wasserman & Corradino, 1971). The location of the calcium-binding protein in relation to the mucosal cell should therefore yield information about the probable site at which the protein acts to facilitate Ca²⁺ transport. Immunofluorescent studies in the chick showed the calcium-binding protein to be located both in the surface coat and on the microvillar membranes of goblet and epithelial cells (Taylor & Wasserman, 1970), although on homogenization of mucosal cells the protein appeared in the supernatant fraction (Kallfelz *et al.*, 1967).

If the calcium-binding protein facilitates the uptake of Ca²⁺ while being itself located at such surface sites its effects should be readily demonstrable on the initial kinetics of Ca²⁺ uptake. Initial Ca²⁺ uptake by intestinal mucosa from normal rats can be resolved into two distinct components: a rapid adsorption complete within 1 min of incubation, and a subsequent, linear, component (O'Donnell & Smith, 1973). The object of the present work was to determine the effects of vitamin D on these two phases of Ca²⁺ uptake, with preparations of small-intestinal mucosa from vitamin D-deficient and -repleted rats.

Materials and methods

Lister hooded weanling rats were maintained on a rachitogenic diet (Ca, 1%; P, 0.25%) until a weight range of 100–130 g was attained. An intraperitoneal injection of 50000 i.u. of cholecalciferol (Duphaphar;

Philips-Duphar, Amsterdam, The Netherlands) was given in 0.05 ml of arachis oil 48 h before the experiment. Equal numbers of control rats were given oil alone. Food but not water was withheld for 24 h before the experiment.

Assessment of the vitamin D status of the rats by X-ray examination of leg joints was kindly performed by Dr. E. M. Cruickshank of the Dunn Nutritional Laboratory, Cambridge. Rats showed evidence of mild rickets in a 'feathered' appearance of the epiphysis 3 weeks after commencement of the diet. The serum total calcium concentration determined at this time by atomic absorption spectrophotometry (Pye Unicam SP.90) was 4.21 ± 0.14 m-equiv./l (21) (mean $\pm$ S.E.M. of 21 animals). Vitamin D-treated rats had a mean serum total calcium concentration of 4.81 ± 0.15 m-equiv./l (21).

Animals were killed for experiment by decapitation, and a segment of intestine extending 7 cm distal to the pylorus was rapidly excised, cut longitudinally and mounted so that the mucosal surface only was superfused by a medium containing (final concentrations) 117 mM-NaCl, 5 mM-KCl, 25 mM-Tris-HCl buffer, 1.0 mM-CaCl₂ and 15 mM-glucose, final pH 7.4. This medium was maintained at 37°C and gassed with pure O₂. Ca²⁺ uptake after 5 min equilibration was determined by introducing medium containing traces of ⁴⁵Ca²⁺ and [³H]inulin (The Radiochemical Centre, Amersham, Bucks., U.K.). In other experiments, by the same procedure, the uptake of thiourea (as [¹⁴C]thiourea) was determined in place of Ca²⁺ uptake. A tissue surface of area 0.174 cm² was bathed by 0.2 ml of radioactive medium. At 1, 3 or 5 min incubation the influx was terminated by a rapid wash with ice-cold iso-osmotic mannitol. The segments of intestine were then leached in dilute HNO₃, and the ⁴⁵Ca and ³H radioactivities were determined by liquid-scintillation spectrometry.

Inulin space was calculated as $\mu\text{l}/\text{cm}^2$; ⁴⁵Ca radioactivity associated with space accessible to inulin,

estimated from the $^{45}\text{Ca}/^3\text{H}$ ratio in the incubation medium, was subtracted for each individual tissue sample. Ca^{2+} uptake was calculated from at least six randomly distributed loci along each segment of intestine, for any one set of conditions, and expressed as $\text{nmol of Ca}^{2+}/\text{cm}^2$ of tissue surface.

Results and discussion

Inulin spaces increased in the course of an incubation, but did not differ in intestines from vitamin D-treated and control rats, rising from 2.83 to $6.68 \mu\text{l}/\text{cm}^2$ and from 3.09 to $6.54 \mu\text{l}/\text{cm}^2$ respectively as the incubation time increased from 1 to 5 min. This distribution of inulin is similar to that found with intestines taken from normal rats weighing 200 g (O'Donnell & Smith, 1973). It indicates that neither depletion nor repletion with the relatively large amounts of vitamin D used in the present experiments had any significant effect on the gross topography of the mucosal surface. The administration of the vitamin likewise did not alter the uptake of thiourea (at concentration 1 mM); mean rates ($\pm\text{S.E.M.}$) were 1.64 ± 0.12 and $1.72 \pm 0.07 \text{ nmol}/\text{cm}^2$ per min in vitamin D-depleted and -repleted rats respectively. This lack of effect on general permeability of mucosa was also found in the chick (Adams & Norman, 1970).

The time-courses of Ca^{2+} uptake at Ca^{2+} concen-

tration 1 mM are compared for both kinds of rat in Fig. 1(a). Rapid adsorption of Ca^{2+} can be gauged either by generating regression lines and comparing the intercepts or by direct comparison of total 1 min uptake values. The first method gives purely theoretical values for the immediate binding of Ca^{2+} to the surface coat of the intestinal mucosa, and the 1 min uptake values give experimentally determined overestimates of the same phenomenon, the amount of the overestimate depending on the rate at which Ca^{2+} penetrates the tissue. Treatment with vitamin D changed neither the theoretical values for intercepts (2.14 ± 0.35 and $1.65 \pm 0.55 \text{ nmol}/\text{cm}^2$ for control and vitamin D-treated rats respectively) nor the 1 min uptake values (3.75 ± 0.17 and $4.13 \pm 0.16 \text{ nmol}/\text{cm}^2$ respectively).

The second component of uptake, which was linear between 1 and 5 min of incubation, was, however, changed significantly in the treated animals. Expressed as nmol/cm^2 [mean $\pm\text{S.E.M.}$ (no. of observations)] the value at 3 min for the intestine from vitamin D-treated rats was 9.31 ± 0.50 (21) whereas the corresponding control value was 6.69 ± 0.26 (21) ($P < 0.01$); at 5 min the respective values were 13.94 ± 0.63 (21) and 9.99 ± 0.40 (21) ($P < 0.01$). The rates calculated from these values were 2.48 ± 0.16 and $1.56 \pm 0.10 \text{ nmol}/\text{cm}^2$ per min, the rate in the vitamin D-depleted rat being significantly less than that in the repleted animal. The rate for vitamin

Fig. 1. Effects of vitamin D on the linear and the rapid components of initial uptake of Ca^{2+} by mucosal cells of rat small intestine

(a) Uptake of Ca^{2+} (as ^{45}Ca) by intestinal mucosa of vitamin D-deficient ($\circ$) and -repleted rats ($\bullet$). Uptake was corrected for inulin space. Each point is the mean ($\pm\text{S.E.M.}$) of 34–44 replicate determinations. The intercepts on the ordinate are theoretical points generated by treating uptake as being linear from 1 to 5 min. The Ca^{2+} concentration was 1 mM. (b) Variation of rapid (1 min) uptake of Ca^{2+} by intestinal mucosa of vitamin D-deficient ($\circ$) and -repleted ($\bullet$) rats as a function of the concentration of Ca^{2+} . Each point gives the mean of five or six determinations.

D-treated rats is approximately twice that found in older rats on a normal diet (O'Donnell & Smith, 1973).

All the previous work had been carried out with 1 mM- Ca^{2+} in the medium, and it may be that this is not the optimal concentration to see a vitamin D-mediated effect on surface binding. Martin & deLuca (1969) estimated the K_m of mucosal influx, assuming simple Michaelis-Menten kinetics for the overall process, to be about 2 mM, and an estimate of 1.25 mM was arrived at by studies on transfer across whole intestine (Walling & Rothman, 1969), but the binding affinity of calcium-binding protein for Ca^{2+} has been quoted as $2.6 \times 10^5 \text{ M}^{-1}$ (Wasserman *et al.*, 1968). We therefore measured the 1 min uptakes of Ca^{2+} by intestines taken from vitamin D-depleted and -repleted rats, using media containing Ca^{2+} ranging in concentration from 0.04 to 9 mM. The results obtained are shown in Fig. 1(b). Ca^{2+} uptake increased as the external concentration was raised from 0.04 to 9 mM. Treatment with vitamin D had no effect on the 1 min uptake of Ca^{2+} for any of the concentrations of Ca^{2+} used. There is no obvious saturation of uptake measured over this concentration range of Ca^{2+} .

If the rapid association of Ca^{2+} with mucosal tissue discernible by this procedure represents a measure of adsorption on the surface, then, since this was unaffected by vitamin D while cellular influx was increased, it appears unlikely that the immediate

association of Ca^{2+} with surface-coat sites is a significant element in enhancement of mucosal uptake. The rapidity with which a subsequent enhancement is seen suggests, nevertheless, that vitamin D-mediated action is at a site adjacent to the microvillar membrane.

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