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Lanthanide-stimulated glucose and proline transport across rabbit intestinal brush-border membranes

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Trivalent cations of the lanthanide series ($La^{3+} \rightarrow Yb^{3+}$) stimulated uptake of proline or glucose in rabbit small intestinal brush-border membrane vesicles. The lanthanides stimulated uptake to an extent greater than Al^{3+}, choline, and in many cases, Na^+. A time-course of Er^{3+}-stimulated glucose uptake gave initial rates and overshoots greater than Na^+ stimulation. The best activators were Sm^{3+}, Eu^{3+} and Tm^{3+}, which stimulated proline initial uptakes by 400–600%, and stimulated glucose uptake by 120–150%, compared to Na^+. The best lanthanide cotransport activators possessed high third ionization potentials.

Sodium-stimulated glucose and amino acid transport are hallmark phenomena of brush-border membranes from epithelial cells [1–4]). The original dogma of strict Na^+-dependency [5,6] was recently shaken by an observation in E.M. Wright's laboratory [7], with a more complete follow-up study [8] describing lanthanide substitution for Na^+ on the kidney brush border proline cotransporter of rabbit. In this paper, we demonstrate lanthanide-stimulation of both the glucose and proline contransporters in brush borders from rabbit small intestine.

Intestinal brush-border membrane vesicles (BBMV) were prepared from rabbit jejunum by a standard Ca^{2+} aggregation procedure [9]. Final BBMV suspensions containing 430 mM mannitol in 10 mM Hepes-Tris (pH 7.5) were stored in liquid nitrogen [10] until needed. Transport measurements were conducted at 22°C using a rapid mix/filtration technique described previously [9], with the appropriate zero-time blanks. For the present study, we developed a special 'stop buffer' required for rapid filtration of lanthanides through the cellulose mixed ester 0.45 μm filters (Gelman type GN-6). The ice-cold stop buffer was: 210 mM choline chloride and 5 mM EDTA in 0.05 M citric acid/0.1 M Na_2HPO_4 (pH 2.6). Lanthanide (III) chloride salts were 'gold label' formulations (> 99.997%) from Aldrich Chemical Company. All other reagents were obtained from Sigma Chemical Company. The solutions were manipulated in plastic containers. L-[2,3,4,5-^{3}H]Proline and D-[6(n)-^{3}H]glucose were obtained from Amersham or New England Nuclear.

An extended timecourse of ion-activated glucose uptake in rabbit intestinal BBMV's is shown in Fig. 1. The inwardly directed Er^{3+} concentration gradient resulted in an initial rapid uptake of glucose, an overshoot which peaked at about 1 min, and an eventual decline to an equilibrium value at 120 min. In the presence of Na^+, the uptake time course was similar, although attenuated in absolute value for the initial uptake

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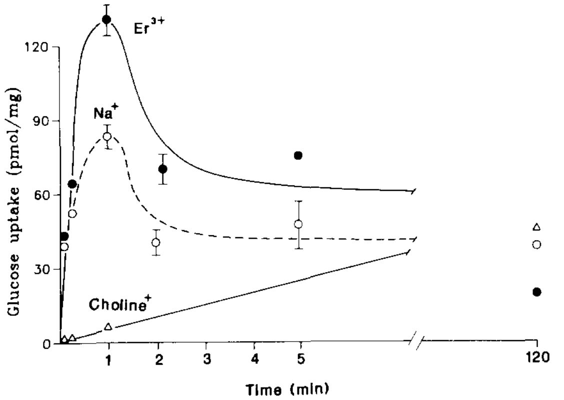

Fig. 1. Glucose uptake time course. The uptake of 12.5 μM glucose into rabbit intestinal membrane vesicles was measured under isosmotic conditions in the presence of initial *cis* 100 mM choline chloride, NaCl, or $ErCl_3$. Data represent the mean ± S.E. ($n = 3$).

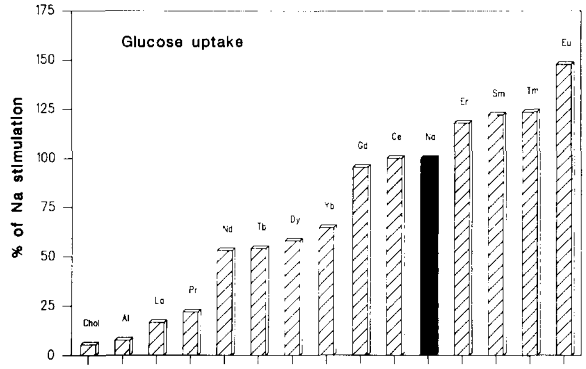

Fig. 2. Lanthanide stimulation of rabbit intestinal D-glucose uptake. Total 12.5 μM glucose uptake was measured during 5 s in the presence of various chloride salts. BBMV protein (50 μg) was mixed with uptake buffer to give final *cis* concentrations of [^{3}H]glucose, 25 mM choline chloride, 25 mM of the appropriate test cation (lanthanide, choline, Al^{3+}, or Na^+), 25 mM Hepes-Tris adjusted to pH 7.5, and sufficient mannitol to maintain an osmolality of 434 ± 1 mosmM. Results represent the percent of total uptake in the presence of Ln^{3+} compared to total uptake in the presence of Na^+. The height of each bar represent the average of three determinations.

and overshoot. Substituting choline for Na^+ or Er^{3+} resulted in an attenuated initial uptake, an absence of overshoot, and an equilibrium value common with Er^{3+} and Na^+. A proline uptake timecourse was similar (data not shown), although we were unable to demonstrate the phenomenon of lanthanide-stimulated overshoot exceeding the magnitude of the Na^+-stimulated overshoot. For subsequent experiments, we report total uptake rates measured only during the initial 5 s uptake period as stimulated by 25 mM salts.

Cation-stimulated uptake of D-glucose in rabbit intestinal BBMV is shown in Fig. 2. Total glucose uptake was increased in the presence of Na^+ compared to choline$^+$ (organic monovalent cation) or Al^{3+} (control for trivalent inorganic cations). Furthermore, all trivalent lanthanide cations (henceforth collectively abbreviated Ln^{3+}) stimulated total glucose uptake, compared to choline or Al^{3+}. Several Ln^{3+} ions stimulated glucose uptake greater than Na^+.

Cation-stimulated uptake of L-proline is shown in Fig. 3. As with glucose, many Ln^{3+} ions stimulated total proline uptake, compared to choline or Al^{3+}, and in several cases, greater than Na^+. Comparing the data of Figs. 2 and 3, Ln^{3+} ions generally stimulated proline uptake to a greater extent than they stimulated glucose uptake. For example, Eu^{3+}, Tm^{3+} and Sm^{3+} stimulated proline uptake in the range of 400–600%, but the same lanthanides stimulated glucose uptake in the range 120–150%, compared to Na^+.

Our intestinal brush-border glucose and proline transport results generally agree with observations of rabbit kidney lanthanide/proline cotransport [8]. We have preliminary data (Stevens and Cerda, unpublished data) which demonstrate similar Ln^{3+} stimulation of proline and glucose cotransporters in human intestinal BBMV's. Collectively, these studies suggest that the phenomenon of Ln^{3+}

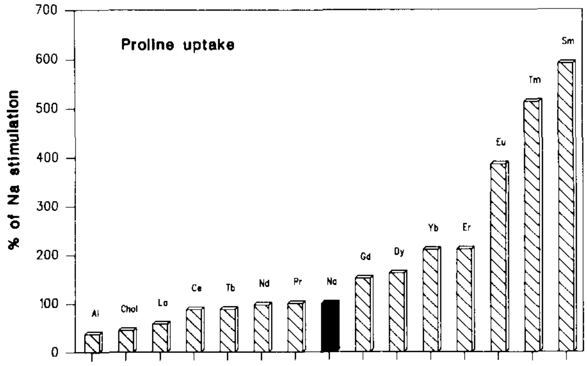

Fig. 3. Lanthanide stimulation of rabbit intestinal L-proline uptake. Transport of 12.5 μM [^{3}H]proline was measured under conditions described in Fig. 2.

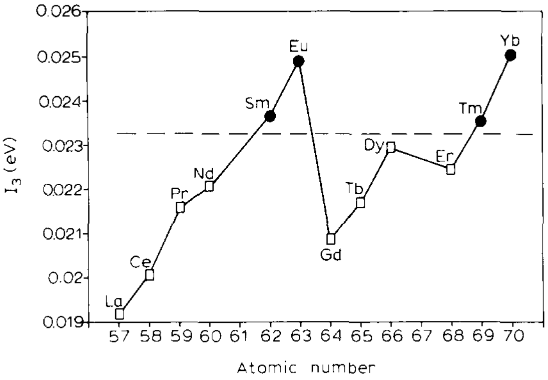

Fig. 4. Third ionization potentials (I_3) of the lanthanides. Values expressed in eV are based on data from Johnson [11] for ionization energies of the $4f^n \rightarrow 4f^{n-1}$ processes. Ln^{3+} ions with I_3 values exceeding about 0.023 eV (above dashed line) were particularly strong activators of proline and glucose cotransporters (see Figs. 2 and 3).

stimulation may extend to all epithelial sodium/organic solute cotransporters.

The data of Figs. 2 and 3 may offer clues about Ln^{3+} ion interactions with cotransporter activator binding site(s). The degree of transport stimulation relative to Na^+ stimulation (Figs. 2 and 3) was related to the third ionization potentials of Ln^{3+} ions [11], as depicted in Fig. 4. The drop between Eu^{3+} and Gd^{3+} (Fig. 4) reflects an electronic characteristic of the lanthanide series: the outer orbitals prescribe integrated interactions of ligand-field effects plus steric binding effects, due to the lanthanides' unique hydration properties and coordination numbers [11–14]. Coordination numbers may vary from 6 to 9, and the effective ionic radii generally increase by 0.05 Å with each coordination number [15]. The same general pattern depicted in Fig. 4, including the Eu/Gd break, is characteristically observed in the formation of complexes between Ln^{3+} ions and a variety of bioorganic ligands [12,14]; stronger binding generally occurs for Sm^{3+}, Eu^{3+}, Tm^{3+} and Yb^{3+}, compared to La^{3+} and Ce^{3+}, for example. In the case of both proline and glucose cotransporters, the best activators were Sm^{3+}, Eu^{3+} and Tm^{3+} (Figs. 2 and 3). Accordingly, these ions may be grouped as having sufficient ionization energy ($>$ 0.023 eV) to affect proper interaction with the cotransporter activator binding site(s). Lanthanides of Figs. 2 and 3 which possessed smaller third ionization potentials (e.g., La^{3+}) did not stimulate uptake as effectively. Yb^{3+} appears to be an exceptional case of sub-maximal transport activation with a proper ionization potential, possibly explained by the small effective ionic radius at all coordination numbers, compared to the considerably larger radii of either Eu^{3+} or Sm^{3+} [15]. The rankings of Figs. 2 and 3 could not be correlated with singular factors such as atomic number, effective ionic radius [15], or partial molal volume [13]; the lack of these simple trends suggested that the Ln^{3+} stimulation may be due to the multiple factors relating to third ionization potentials. The reader is directed elsewhere for a more complete treatment of the coordination chemistry of Ln^{3+} complexes with water and bioorganic molecules [11,12,14].

Detailed studies of Ln^{3+} interactions with epithelial cotransporters remain to be fully exploited. The recently cloned Na^+/glucose cotransporter of rabbit intestine brush border [16] will likely prove to be indispensible in assessing the true mechanism of cation-dependent organic solute transport. Useful techniques could include, for example, X-ray diffraction of Na^+-bound vs. Ln^{3+}-bound purified cotransporter protein, magnetic resonance shifts associated with Gd^{3+} bound to cotransporter, and fluorescence studies of Tb^{3+} energy transfer near the cotransporter tyrosine residue(s) of ion activator site(s) [17].

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References

1 Stevens, B.R., Kaunitz, J.D. and Wright, E.M. (1984) Ann. Rev. Physiol. 46, 417–433.
2 Hopfer, U., Nelson, K., Perotto, J. and Isselbacher, K.J. (1973) J. Biol. Chem. 248, 25–32.
3 Semenza, G., Kessler, M., Hosang, M., Weber, J. and Schmidt, U. (1984) Biochim. Biophys. Acta 779, 343–379.
4 Stevens, B.R. and Wright, E.M. (1987) J. Biol. Chem. 262, 6546–6552.
5 Crane, R.K., Miller, D. and Bihler, I. (1961) in Membrane Transport and Metabolism (Kleinzeller, A. and Kotyk, A., eds), pp. 439–449, Czechoslovakian Academy of Sciences, Prague.
6 Schultz, S.G. and Curran, P.F. (1970) Physol. Rev. 50, 637–718.
7 Schell, R.E. and Wright, E.M. (1985) J. Physiol. 360, 95–104

8 Birnir, B., Hirayama, B. and Wright, E.M. (1987) J. Membr. Biol. 100, 221–227.

9 Stevens, B.R., Ross, H.J. and Wright, E.M. (1982) J. Membr. Biol. 66, 213–225.

10 Stevens, B.R., Wright, S.H., Hirayama, B., Gunther, R., Ross, H., Harms, V., Nord, E., Kippen, I. and Wright, E.M. (1982) Membr. Biochem. 4, 271–282.

11 Johnson, D.A. (1969) J. Chem. Soc. (A) 1969, 1525–1528.

12 Nieboer, E. (1975) in Structure and Bonding (Dunitz, J.D., ed.), pp. 1–47, Springer-Verlag, New York.

13 Millero, F.J. (1971) Chem. Rev. 71, 147–176.

14 Angelici, R.J. (1973) in Inorganic Biochemistry, Vol 1 (Eichhorn, G.L., ed.), pp. 63–73, Elsevier, Amsterdam.

15 Martin, R.B. and Richardson, F.S. (1979) Q. Rev. Biophys. 12, 181–209.

16 Hediger, M.A., Coady, M.J., Ikeda, T.S. and Wright, E.M (1987) Nature 330 (6146), 379–381.

17 Peerce, B.E. and Wright, E.M. (1986) Proc. Natl. Acad. Sci USA 83, 8092–8096.