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Absorption of a vasopressin analogue, 1-deamino-8-D-arginine-vasopressin (dDAVP), in a human intestinal epithelial cell line, CaCO-2

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Summary

The intestinal absorption of the metabolically stable vasopressin analogue dDAVP was studied in human intestinal epithelial (CaCO-2) cells grown on permeable supports. The parent peptide arginine-vasopressin (AVP) was used as a control peptide. Both peptides displayed linear kinetics and were absorbed at very slow rates across CaCO-2 monolayers. The apparent permeability coefficients were in the range of $4–14 \times 10^{-8}$ cm/s. No degradation of dDAVP and AVP was observed during the incubation period. The absorption of dDAVP was concentration independent, indicating that the transport was mediated by a passive permeation. The low permeability coefficients indicate that the absorption rate across CaCO-2 monolayers is similar to that of colonic epithelium in vivo.

Introduction

dDAVP is a more potent and stable analogue of the antidiuretic hormone arginine-vasopressin (AVP) (Zaoral, 1985). When administered orally the bioavailability of this analogue is comparatively low, about 1% in man (Vilhardt and Lundin, 1986a). AVP is rapidly degraded by lumenal enzymes and is therefore absorbed to a very low extent after oral administration (Vilhardt and Bie, 1983). It has been shown that dDAVP is absorbed at different rates from different parts of the intestine in experimental animals (Lundin and Vilhardt, 1986). Thus, dDAVP is absorbed at a higher rate from the distal than the proximal part of the small intestine while the absorption from colonic epithelium is negligible.

It is still not known how dDAVP is absorbed across epithelia. In vitro experiments using everted intestinal sacs from rats indicate passive transfer of the peptide across the intestinal mucosa (Vilhardt and Lundin, 1986b). A more detailed analysis of the absorption of dDAVP could be obtained if the transport over isolated intestinal epithelial monolayers could be studied. In the present study, the absorption of dDAVP across a human intestinal epithelial cell line, CaCO-2 was investigated.

CaCO-2 cells differentiate spontaneously in cell culture to polarized monolayers with a morpho-

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logical appearance similar to that of small intestinal enterocytes (Neutra and Louvard, 1989). The monolayers have well developed tight junctions and the microvilli contain enzyme activities similar to those found in small intestinal epithelium (Rousset et al., 1985). In addition, CaCO-2 cells show polarized transport of various nutrients and specifically bind and endocytose cobalamin-intrinsic factor – a transport process specific for distal ileal epithelial cells (Dix et al., 1990). All these properties indicate that the CaCO-2 cells are similar to ileal enterocytes. However, in some aspects CaCO-2 cells are more similar to colonic epithelium. For instance, the transepithelial electric resistance (TER) of CaCO-2 monolayers is higher than that of small intestinal epithelium (Artursson, 1990). Thus, CaCO-2 cells have properties that are similar to both small intestinal and colonic epithelium. Studies on the absorption of dDAVP in the CaCO-2 model should therefore not only give information on the mechanisms of peptide absorption, but also indicate if CaCO-2 monolayers should be used as a model epithelium for the small intestinal or colonic epithelial barrier to peptide absorption.

Materials and Methods

Cells

CaCO-2 cells (Fogh et al., 1977) were obtained from the American Cell Culture Collection (Rockville, MD). The cells were cultivated on polycarbonate filters (Nucleopore or Costar cell culture inserts; mean pore diameter 0.45 μm) as described elsewhere (Artursson, 1990). Cells of passage number 85–95 were used throughout. The cell culture procedure is comparable to that recently reported for cultivation on cellulose filters (Wilson et al., 1990) but differs from that described for polycarbonate filters (Hidalgo et al., 1989), since the addition of extracellular matrix components, such as collagen, has been excluded. The integrity of the monolayers was routinely checked by measurements of transmembrane resistance and by determination of the permeability of the two hydrophilic markers [^{3}H]mannitol and [^{14}C]polyethylene glycol (mol. wt. 4000) (Artursson, 1990). The transmembrane resistance of 2–4-week-old monolayers of CaCO-2 cells was approx. 300 Ω cm^{-2} and the permeability to [^{3}H]mannitol and [^{14}C]polyethylene glycol was 0.05 and 0.02% h^{-1} of the dose, respectively.

Peptides

AVP and dDAVP were synthesized by solid-phase methods (Ferring AB, Malmö, Sweden). Growth hormone-releasing factor (1–29) amide (GRF(1–29)NH_2) was obtained from KABI (Stockholm, Sweden). Radioiodinated dDAVP was supplied by Ferring (spec. act. 1800 Ci/mmol). ^{3}H-labelled dDAVP (spec. act. 30 Ci/mmol) was a gift from Dr Anna-Lena Ungell (AB Hässle, Mölndal, Sweden) and ^{3}H-labelled AVP (spec. act. 60 Ci/mmol) was supplied by NEN (Du Pont, Nemours, F.R.G.). The radiochemical purity was 99%.

Drug transport studies

A suitable concentration of radiolabelled or unlabelled dDAVP or AVP was added to the apical or basolateral chamber and samples were withdrawn at predetermined intervals. GRF(1–29)NH_2 (3.3 μmol/l) was added to the apical chamber for assessment of the metabolic capacity of CaCO-2 cells. The radioactive samples were counted in a liquid scintillation counter. The unlabelled samples were analyzed by HPLC and RIA (see below). The resistance of the monolayers was checked at the end of each experiment. Apparent permeability coefficients (P_{app}) were calculated according to (Schoenwald and Huang, 1983):

$$P_{app} = (dQ/dt)(1/AC_0)$$

where dQ/dt is the permeability rate, C_0 the initial concentration in the donor chamber and A the surface area of the monolayer.

Analyses of peptides

All peptides present in incubation media were extracted using Sep-Pak^R octadecasilyl cartridges (Waters, Milford, CT, U.S.A.) as previously described (La Rochelle et al., 1980) prior to HPLC or RIA analyses.

AVP, dDAVP and ^{125}I-dDAVP were analyzed by high-performance liquid chromatography (HPLC) using isocratic elution conditions (Lundin et al., 1989). Unlabelled AVP and dDAVP were also measured using specific radioimmunoassay methods described previously (Lundin et al., 1985).

Analyses of GRF(1–29)NH_2 were performed using a HPLC system consisting of a Vydac pH-stable RPC_8 column (25 × 0.46 cm), Spectra Physics SP 8700 pump, SP 4270 integrator, SP 8780 autosampler and autoinjector, and Spectroflow 783 UV detector. The flow rate was 1 ml/min with UV detection at 220 nm. The mobile phase consisted of (A) 25 : 50 : 25 0.1% trifluoroacetic acid (TFA)/distilled water/acetonitrile (ACN) and (B) 25 : 75 TFA/ACN. The peptide was eluted using a linear gradient with an ACN content of 25% at $t = 0$ min increasing to 35% at $t = 20$ min. The dried extracts were dissolved in 200 μl of 0.08% TFA and 10-μl aliquots were injected into the system.

Results

Octanol-water distribution determination showed that radiolabelled dDAVP and AVP are hydrophilic peptides (Table 1). Their permeability coefficients across CaCO-2 cells were very high. Iodine labelling increases peptide hydrophobicity but this does not influence peptide transport as much as the increase in molecular weight. [^{3}H]dDAVP was transported about 3-times faster than [^{125}I]dDAVP. The rate of [^{3}H]dDAVP transport from the apical to basolateral chamber was identical to that in the reverse direction (Fig. 1). No inhibition of peptide transport could be demonstrated by adding increasing amounts of unlabelled dDAVP to the apical compartment (Fig. 2).

dDAVP and AVP were also characterized for possible enzymatic degradation by CaCO-2 cells. When incubated in apical medium for a period of 4 h, no loss of immunoreactive peptide occurred (Table 2). Samples were also withdrawn from the 4 h incubates and analyzed by HPLC. No chromophoric metabolites were found and the peptides eluted as did synthetic standards (Fig. 3). However, when [^{125}I]dDAVP from the basolateral medium was fractionated on HPLC it was found that free ^{125}I constituted 27.8% of the total radioactivity. This amount of ^{125}I is included in the permeability data for [^{125}I]dDAVP listed in Table 1. The linear peptide GRF(1–29)NH_2 incubated

TABLE 1

Molecular weights, logarithms of octanol/water partition coefficients (D), apparent transport rates (P_{app}) of labelled dDAVP and AVP

	Molecular weight	Log D	P_{app} (cm/s)
[^{125}I]dDAVP	1194	−1.40	$4.1 \pm 0.1 \times 10^{-8}$
[^{3}H]dDAVP	1071	−1.95	$12.9 \pm 1.1 \times 10^{-8}$
[^{125}I]AVP	1208	−2.15	ND
[^{3}H]AVP	1084	−2.73	$14.3 \pm 0.9 \times 10^{-8}$

ND, not determined. P_{app} values are given ± SD, $n = 3$.

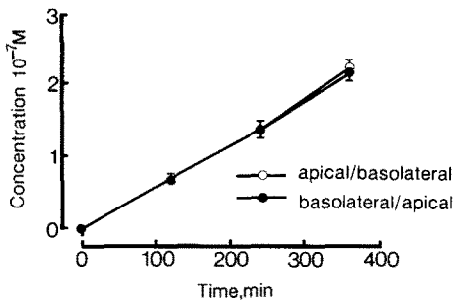

Fig. 1. Transport across CaCO-2 cells of [^{125}I]dDAVP from apical to basolateral chamber and in the reverse direction. Values are given ± SD, $n = 6$.

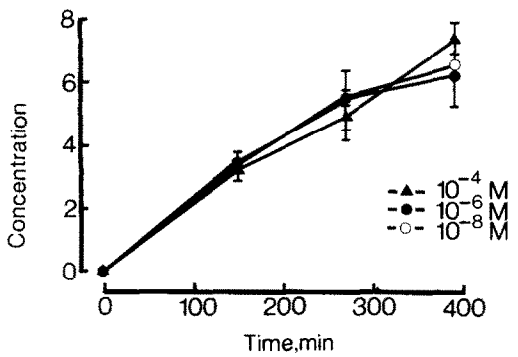

Fig. 2. Transport across CaCO-2 cells of [^{3}H]dDAVP in the presence of various concentrations of unlabelled peptide. Values are given ± SD, $n = 6$.

TABLE 2

Concentrations of immunoreactive (ir) AVP and dDAVP incubated for various time periods with CaCO-2 cells (values expressed ± SD (n = 3) and not corrected for extraction losses)

	Time (min)			
	0	5	60	240
ir AVP				
(μmol/l)	0.60±0.08	0.57±0.11	0.67±0.02	0.49±0.07
ir dDAVP				
(μmol/l)	0.82±0.07	0.81±0.04	0.93±0.05	0.82±0.08

for 4 h was extensively degraded by the brush-border enzymes of the CaCO-2 cells. HPLC analysis revealed that this peptide was degraded, yielding two visible metabolites (Fig. 3).

Discussion

Compounds with such low lipid solubilities (log *D* values) as dDAVP and AVP do not partition significantly into cell membranes. Instead, they are mainly absorbed by the paracellular route (Pappenheimer and Reiss, 1987; Krugliak et al., 1989). Thus, the log D values of dDAVP and AVP indicate that the transcellular route is of limited importance in the absorption of these peptides. This is also evident from the low permeability coefficients for dDAVP and AVP (Table 1). These were comparable to those of mannitol and polyethyleneglycol, two compounds that are known to be absorbed across intestinal epithelium by the paracellular route (Dawson, 1977; Madara et al., 1986; Pappenheimer and Reiss, 1987; Krugliak et al., 1989). Although the ^{125}I-labelled peptides are more hydrophobic than the ^{3}H-labelled ones, it appears that the effect of the increased molecular weight has a greater influence on transport rate. This is in line with observations by McMartin et al. (1987) who found a strong relationship between molecular weight and oral and nasal absorption for a large number of peptides. The transport rate of [^{3}H]dDAVP was identical from both apical and basolateral compartments and no inhibition of this rate could be demonstrated by the presence of unlabelled peptide in the apical compartment. Together with previous observations using everted intestinal sacs (Vilhardt and Lundin, 1986b), these results strongly suggest that dDAVP is absorbed by passive permeation, most likely by the paracellular route.

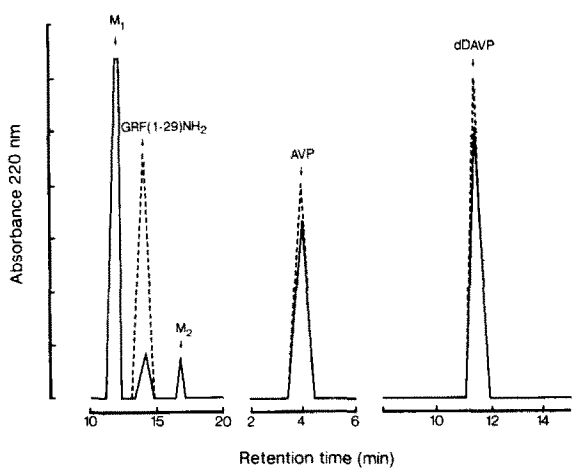

Fig. 3. HPLC chromatograms showing elution patterns of AVP, dDAVP and GRF(1–29)NH_2 after incubation with CaCO-2 cells for a 4 h period. Elution positions of reference peptides incubated without CaCO-2 cells are indicated by hatched lines. M_1 and M_2 indicate positions of products formed when incubating GRF(1–29)NH_2.

In vivo studies have shown that only very small amounts of dDAVP are absorbed in colonic epithelium (Lundin and Vilhardt, 1986). Similarly, very low absorption rates were obtained for dDAVP and AVP in the CaCO-2 model as compared to those obtained in vitro using everted intestinal sacs (Vilhardt and Lundin, 1986b). These results indicate that the permeability of the CaCO-2 monolayers is more similar to colonic than small intestinal epithelium. This is also supported by the finding that low molecular weight hydrophilic drugs (that are well absorbed in the small intestine but not at all in the colon) are absorbed at very slow rates across CaCO-2 monolayers (Artursson, 1990). An alternative route for dDAVP uptake would be by endocytosis. However, this is less likely since the non-specific endocytotic capacity of CaCO-2 cells appears to be too low to give permeability coefficients of 4–14 × 10^{-8} cm/s. These permeability coefficients correspond to the absorption of 0.03–0.1% of the dose per h while the permeability to horseradish per-

oxidase (HRP; a macromolecule that is transported across CaCO-2 cells by the endocytotic route) in CaCO-2 monolayers was 0.0001% (Wilson et al., 1990). However, it should be noted that HRP is metabolised by CaCO-2 cells. The absorption of the peptides by an endocytotic route therefore cannot be completely ruled out.

Both dDAVP and AVP remained stable when incubated with CaCO-2 cells. Earlier studies by ourselves (Lundin et al., 1989) and others (Matuszewska et al., 1988) showed that dDAVP remained intact after incubation with ileal mucosal strips while AVP was completely degraded. This is not in agreement with the results in the CaCO-2 model. However, AVP is sensitive to the activity of pancreatic enzymes while dDAVP is not. These enzymes may still remain in the tissue even though well rinsed ileal tissue was used. That considerable enzymatic activity was present in the CaCO-2 cells was shown by the extensive degradation of GRF(1–29)NH_2. This peptide has been shown to be degraded by plasma dipeptidyl peptidase IV (Kubiak et al., 1989). This enzyme is also present in the brush-border membrane of CaCO-2 cells (reviewed in Neutra and Louvard, 1989) and may account for some of the observed degradation.

In conclusion, dDAVP is absorbed by CaCO-2 monolayers by a non-saturable, non-polar process. The absorption proceeds at a very slow rate and is comparable to that of other hydrophilic compounds that are absorbed by the paracellular route in intestinal epithelium. Thus, it is likely that dDAVP is absorbed by passive permeation by the paracellular route in CaCO-2 monolayers.

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References

Artursson, P., Epithelial transport of drugs in cell culture. I: A model for studying the passive diffusion of drugs over intestinal absorptive (CaCO-2) cells. *J. Pharm. Sci.*, 79 (1990) 476–482.

Dawson, D., Na and Cl transport across the isolated turtle colon: Parallel pathways for transmural ion movement. *J. Membr. Biol.*, 37 (1977) 213–233.

Dix, C.J., Hassan, I.F., Obray, H.Y., Shah, R. and Wilson, G., The transport of vitamin B12 through polarised layers of CaCO-2 cells. *Gastroenterology*, 98 (1990) 1272–1279.

Fogh, J., Fogh, J.N. and Orfeo, T., One-hundred and twenty seven cultured human tumor cell lines producing tumors in mice. *J. Natl. Cancer Inst.*, 59 (1977) 221–226.

Hidalgo, I.J., Raub, T.J. and Borchardt, R.T., Characterization of the human colon carcinoma cell line (CaCO-2) as a model system for intestinal epithelial permeability. *Gastroenterology*, 96 (1989) 736–749.

Krugliak, P., Hollander, D., Ma, T.Y., Tran, D., Dadufalza, U.D., Katz, K.D. and Le, K., Mechanisms of polyethyleneglycol permeability of perfused rat intestine. *Gastroenterology*, 97 (1989) 1164–1170.

Kubiak, T.M., Kelly, C.R. and Krabill, L.F., In vitro metabolic degradation of a bovine growth hormone-releasing factor analog Leu27-hGRF(1–29)NH_2 in bovine and porcine plasma. *Drug Metab. Disp.*, 17 (1989) 393–397.

La Rochelle, F.T. jr., North, F.T. and Stern, P., The use of a new extraction of arginine vasopressin from blood using octadecasilylsilica. *Pflügers Arch.*, 387 (1980) 79–81.

Lundin, S., Melin, P. and Vilhardt, H., Use of three specific radioimmunoassays in measuring neurohypophysial hormone content and plasma concentrations of vasopressin, oxytocin and dDAVP in rats after prolonged infusion of dDAVP. *Experientia*, 41 (1985) 933–935.

Lundin, S. and Vilhardt, H., Absorption of 1-deamino-8-D-arginine vasopressin from different regions of the gastrointestinal tract in rabbits. *Acta Endocrinol.*, 112 (1986) 457–460.

Lundin, S., Bengtsson, H.-I., Folkesson, H.G. and Weström, B.R., Degradation of [Mercaptopropionic acid[1], D-arginine[8]]-vasopressin (dDAVP) in pancreatic juice and intestinal mucosa homogenate. *Pharmacol. Toxicol.*, 65 (1989) 92–95.

Madara, J.L., Barenberg, D. and Carlson, S., Effects of cytochalasin D on occluding junctions of intestinal absorptive cells: Further evidence that the cytoskeleton may influence paracellular permeability and junctional charge selectivity. *J. Cell. Biol.*, 102 (1986) 2125–2136.

Matuszewska, B., Liversidge, G.G., Ryan, F., Dent, J. and Smith, P.L., In vitro study of intestinal absorption and metabolism of 8-L-arginine vasopressin and its analogues. *Int. J. Pharm.*, 46 (1988) 111–120.

McMartin, C., Hutchinson, L.E.F., Hyde, R. and Peters, G.E., Analysis of structural requirement for the absorption of drugs and macromolecules from the nasal cavity. *J. Pharm. Sci.*, 76 (1987) 535–540.

Neutra, M. and Louvard, D., In Matlin, K.S. and Valentich, J.D. (Eds.), *Modern Cell Biology*, vol. 8: Functional epithelial cells in culture, A.R. Liss, New York, 1989, pp. 363–398.

Pappenheimer, J.R. and Reiss, K.Z., Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. *J. Membr. Biol.*, 100 (1987) 123–136.

Rousset, M., Laburthe, M., Pinto, M., Chevalier, G., Rouyer-Fessard, C., Dussaulx, E., Trugnan, G., Boige, N., Brun, J.-L. and Zweibaum, A., Enterocytic differentiation and glucose utilization in the human colon tumor cell line CaCO-2: Modulation by forskolin. *J. Cell. Physiol.*, 123 (1985) 377–385.

Schoenwald, R.D. and Huang, H.-S., Corneal penetration behavior of β-blocking agents. I: Physiochemical factors. *J. Pharm. Sci.*, 72 (1983) 1266–1271.

Vilhardt, H. and Bie, P., Antidiuretic response in conscious dogs following peroral administration of vasopressin and its analogues. *Eur. J. Pharmacol.*, 93 (1983) 201–204.

Vilhardt, H. and Lundin, S., Biological effect and plasma concentration of dDAVP after intranasal and peroral administration to humans. *Gen. Pharmacol.*, 17 (1986a) 481–483.

Vilhardt, H. and Lundin, S., In vitro intestinal transport of vasopressin and its analogues. *Acta Physiol. Scand.*, 126 (1986b) 601–607.

Wilson, G., Hassan, I.F., Dix, C.J., Wiliamson, I., Mackay, M., Woodcock, S. and Artursson, P., Transport and permeability properties of human CaCO-2 cells: An in vitro model of the intestinal epithelial cell barrier. *J. Controlled Release*, 11 (1990) 25–40.

Zaoral, M., Vasopressin analogs with high and specific antidiuretic activity. *Int. J. Peptide Protein Res.*, 25 (1985) 561–575.