Differences in Transport Rate of Oxytocin and Vasopressin Analogues Across Proximal and Distal Isolated Segments of the Small Intestine of the Rat

Stefan Lundin,[1,4] Niclas Pantzar,[2] Anja Broeders,[3] Mats Ohlin,[3] and Björn R. Weström[2]

Received January 9, 1991; accepted April 15, 1991

The transmural intestinal passage of some oxytocin and vasopressin analogues (oxytocin, OT; [Mpa[1], D-Arg[8]]vasopressin, dDAVP; [Mpa[1], Tyr (OMe)[2], carba[6]]oxytocin, carbetocin; [Mpa[1], D-Tyr (OEt)[2], Thr[4], Orn[8]]vasotocin, antocin II; [Mpa[1], D-Tyr (OEt)[2], Thr[4], desPro[7]Orn[8]Gly[9]NH_2]tocinoic acid-$NH(CH_2)_3NH_2$, desPOG-antocin II-$NH(CH_2)_3NH_2$) was studied using isolated proximal and distal segments in the rat. All peptides (measured as peptide-like immunoreactivity) displayed a higher transport rate across distal intestinal segments as determined by radioimmunoassay (RIA). The smallest peptide, des POG-antocin II-$NH(CH_2)_3NH_2$, was transported at the fastest rate. No correlation of lipophilicity with transport rate was observed. Determination of the amount of peptide remaining in the mucosal media at the end of the incubation period by HPLC did not reveal any visible degradation products. However, the large difference in transport rate between [^{3}H]OT and immunoreactive OT indicates mucosal metabolism of this peptide. [^{3}H]dDAVP was distributed in a larger mucosal volume than the extracellular space marker [^{3}H]inulin, indicating tissue uptake, but was too low (<100% of buffer concentration) to make an active transport mechanism likely. The differences in peptide transport rates between proximal and distal intestinal segments are most likely due to a higher distal paracellular permeability despite a decreased absorptive surface area at this region.

KEY WORDS: oxytocin; vasopressin; rat; *in vitro;* intestine; peptide absorption.

INTRODUCTION

The possibility of using peptides and proteins as therapeutic agents is currently receiving attention (1). The greatest obstacle to their use in therapy is the difficulty of delivery by other than the intravenous route (2). One peptide currently used in therapy and effective upon both intranasal and oral delivery is the vasopressin analogue dDAVP ([Mpa[1], D-Arg[8]] vasopressin) (3,4). This peptide is therefore well suited as an experimental tool in permeability studies on biological membranes. In a previous study where dDAVP was administered to different parts of the gastrointestinal tract in rabbits, differences in the local absorption was observed, with the highest absorption taking place in the most distal (ileocecal) part of the small intestine (5). These regional differences could be due to a number of factors such as variations in intestinal transit time, enzymatic activity, and blood flow, but the permeability of the distal small intestine could also be higher. In order to obtain further information on the transport characteristics of dDAVP as well as other peptides belonging to the vasopressin and oxytocin family with different physicochemical properties, we performed transport experiments using isolated segments from both proximal and distal small intestine of the rat.

MATERIALS AND METHODS

Chemicals

The molecular structure and abbreviations for the investigated peptides are shown in Fig. 1. All unlabeled peptides with a chromatographic purity >98% were obtained from Ferring AB, Malmö, Sweden. [^{3}H]dDAVP (sp act, 16 mCi/μmol) was obtained from Axis Research AS, Oslo, Norway; [^{3}H]oxytocin (sp act, 30–60 mCi/μmol) was purchased from NEN, Du Pont de Nemours/Dreieich, Germany; and [^{3}H]inulin (sp act, 1.05 Ci/mmol) was obtained from Amersham International, UK.

Methods

Animals

Sprague–Dawley rats (Alab, Solna, Sweden) were kept in polycarbonate cages on chopped wood under a 12-hr day–night rhythm at 20°C with a mean relative humidity of 50% and had free access to rat chow (R3 EWOS, Södertälje, Sweden) and tap water. Male and female rats weighing 200–500 g were fasted overnight. The abdominal cavity was opened under diethyl ether anesthesia and the small intestine, starting at the end of the duodenum, was removed and immediately placed in incubation medium at room temperature.

Transport Studies

For the everted sac experiments three segments (5 cm) were cut from the proximal and distal parts of the small intestine, respectively. The segments were everted, using a stainless-steel rod, and washed in incubation buffer. The distal end of each segment was ligated to a cone-shaped tip of a lid covering the organ bath and the proximal end was closed by ligation. The organ bath was filled with 8.5 ml incubation medium containing (m*M*): 110 NaCl, 5.5 KCl, 3.0 $CaCl_2$, 1.4 KH_2PO_4, 29.0 $NaHCO_3$, 5.7 Na pyruvate, 7.0 Na fumarate, 5.7 Na glutamate, 13.4 glucose. Albumin (0.01%) was added to the medium to prevent adsorption. The medium (pH 7.4) was gassed with 5% CO_2 in O_2 and kept at 37°C. The intestinal sacs were filled with 0.5 ml incubation medium at the serosal side. At the start of the experiments (t = 0 min), within 30 min from the induction of anesthesia, the peptides were added to the mucosal medium at a concentration of 1 μ*M*.

At 5, 15, 30, and 45 min an aliquot (10 μl) was taken from the serosal medium and acidified with 100 μl 0.1 *M*

[1] Department of Clinical Pharmacology, Lund University, S-221 85 Lund, Sweden.
[2] Department of Animal Physiology, Lund University, Lund, Sweden.
[3] Ferring Pharmaceuticals, Malmö, Sweden.
[4] To whom correspondence should be addressed.

0724-8741/91/1000-1274$06.50/0

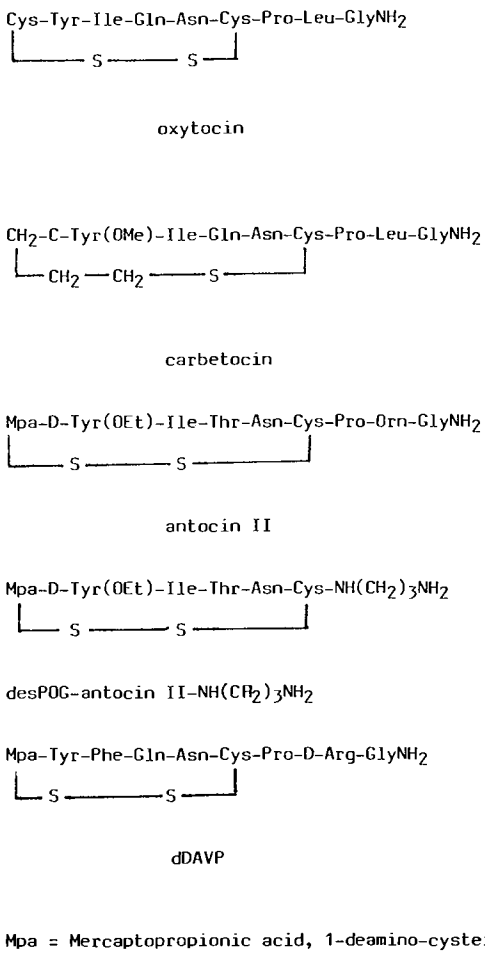

Fig. 1. Molecular structures of and abbreviations used for the neurohypophyseal hormone analogues used in the present study.

HCl. The samples were stored frozen at −20°C until peptide analyses.

Experiments were also performed on intestinal segments which were cut along the mesenterium (3 cm) and mounted in modified Ussing chambers (diffusion cells) (6) (Precision Instrument Design, Los Altos, CA, USA). After tissue mounting the assembled cells were placed in an aluminum block heater at a temperature of 37°C, filled with 4 or 5 ml of incubation medium on the serosal and mucosal reservoir, and gassed with 5% CO_2 in O_2. The exposed surface area was 2.06 cm^2. Six cells, containing three segments from the proximal and distal small intestine, respectively, were used in a single experiment. dDAVP and OT were added to the mucosal side at a concentration of 10 μM. Serosal samples (10 μl) were removed at 30, 60, 90, 120, and 150 min and acidified with 100 μl of 0.1 *M* HCl and stored frozen at −20°C until analyses. [^{3}H]dDAVP and [^{3}H]OT were added to the mucosal medium (1.84 μCi/ml, 11.7 n*M*) and incubated for 60 min, whereafter appropriate sample volumes from both reservoirs were removed for liquid scintillation counting. Apparent permeation coefficients (P_{app}) were calculated according to the formula

$$P_{app} = \frac{dO}{dt} \times \frac{1}{AC_0} \quad (cm \times sec^{-1})$$

where dQ/dt represents the permeability rate, A the exposed area (not corrected for regional surface area variation), and C_0 the initial concentration in the mucosal chamber.

The diffusion coefficient, K, was determined for each peptide using the relationship

$$K_{unknown} = K_{reference} \times \left(\frac{MW_{reference}}{MW_{unknown}}\right)^{1/3} (cm^2 \times sec^{-1})$$

K (9.14×10^{-6} $cm^2 \times sec^{-1}$ at 37°C) and MW (182.18) for mannitol were used as reference data.

The viability of both the everted sac and the diffusion-cell method was checked by evaluating the intestinal transport of [^{3}H]methyl-D-glucose as previously described (6,7).

Mucosal Distribution of [^{3}H]dDAVP

The method described by Kerchner and Geary (8) was used in a slightly modified form. [^{3}H]dDAVP was incubated in the diffusion cells as described above. After 60 min the tissue segments were washed with buffer, removed, weighed, blotted on filter paper, and placed on objective glasses, followed by fast-freezing in liquid N_2 and lyophilization. The mucosa was scraped off from the dried segments under a preparation microscope and weighed. The scrapings were then placed in vials and solubilized in 0.5 ml Soluen overnight. Ten milliliters of scintillation fluid (Ready Safe, Beckman, Fullerton, CA) and 4 drops of acetic acid were added to the vials before counting. The extracellular space volume (ECS) of the intestinal mucosa was determined by incubating [^{3}H] inulin (2.32 μCi/ml) in the diffusion cells at the mucosal side for 60 min. The tissue segments were then treated as above. The ratio of ECS was expressed as the amount of [^{3}H]inulin present in the dried mucosa divided by the amount in the mucosal buffer. The mucosal distribution of [^{3}H]dDAVP was determined in the same way.

Peptide Analyses

dDAVP and OT were measured by specific radioimmunoassays (RIA) as described earlier (9). Antocin II and desPOG-antocin II-$NH(CH_2)_3NH_2$ were also analyzed using a RIA method.

Rabbit antisera to antocin II were raised by immunization with antocin II conjugated to succinylated bovine serum albumin. The peptide was coupled through the amino group of ornithine to the carrier protein using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide as described (10). A total of 8 mol of peptide was coupled to each mole of carrier protein, as determined by amino acid analysis.

A radiolabeled tracer useful for radioimmunoassay was prepared by iodination (^{125}I) of Tyr^{10}-antocin II using the chloramine-T oxidation procedure (11). The monoiodinated tracer was purified by reversed-phase HPLC (10).

The assay was carried out in 0.1 *M* sodium phosphate buffer, pH 7.6, containing 0.05 *M* NaCl, 0.02% sodium azide, 0.1% human serum albumin, and 0.01% Triton X-100. Standards or samples were incubated with 1–2 fmol ^{125}I-Tyr^{10}-antocin II and antiserum K8620-1 (final dilution, $1/200{,}000$) for 40–50 hr at 4°C. Separation of bound and free

tracer was carried out by adsorption of the free fraction to plasma-coated charcoal. The supernatant was collected after centrifugation and counted in an LKB Wallac 1272 gamma counter. Standard curves were on-line computer calculated using a spline-function methodology and subsequently used to evaluate sample results. Assay sensitivity was approximately 1 fmol antocin II/assay tube. Intra- and interassay coefficients of variation were 10 and 11%, respectively, at 20 fmol antocin II/assay tube. Under these conditions, this assay shows specificity for N-terminal residues of antocin II, as expected from the structures being exposed in the immunogen. The presence of an ethyl ether derivative of tyrosine at position 2 is essential and this residue must also be of D-configuration. The C-terminal residues are not necessary for immunoreactivity. No cross-reactivity with natural pituitary peptide hormones could be detected, as outlined in Table I.

Aliquots from mucosal medium were subjected to analysis by high-pressure liquid chromatography (HPLC). The samples were extracted with octadecasilyl columns (Sep-Pak, Millipore-Waters, Milford, MA) and dried prior to HPLC (12). The samples were reconstituted in 0.1% trifluoroacetic acid (TFA) and injected into a system consisting of a Supelcosil LC-18DB column (15 × 4.6 mm), 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) 90:10 0.1% TFA in distilled water/acetonitrile (ACN) and (B) 10:90 TFA/ACN. The peptides were eluted using a linear gradient of ACN in 0.1% TFA. Peptide incubation media were also fractionated using this HPLC system to assess immunoidentity with synthetic standards. One-milliliter fractions were collected, dried, and measured by the appropriate RIA.

Determination of Lipophilicity

The peptides used for intestinal transport experiments were added to a mixture of 1 ml *n*-octanol and 1 ml 50 m*M* phosphate buffer, pH 7.4. This mixture was shaken for 10 min, followed by separation of the phases. After evaporation each phase was analyzed by either RIA or liquid scintillation counting. The ratio (*D*) of *n*-octanol/buffer concentrations of each peptide was calculated and expressed as log *D*.

Calculations

Peptide transport curves were analyzed by a two-way analysis of variance (ANOVA) as this method takes into account all variables of the data. Otherwise Students' unpaired *t* test and Wilcoxon's signed rank-order test was used. Both methods gave similar results. Statistically significant differences were considered to be present when $P < 0.05$.

RESULTS

The transport of immunoreactive (IR) OT, antocin II, desPOG-antocin II-NH$(CH_2)_3NH_2$ and carbetocin across everted intestinal segments is shown in Figs. 2a–d. The transport rates across the distal segments were significantly higher than over the proximal segments for all the peptides ($P < 0.05$) as calculated by ANOVA. OT displayed the lowest transport rate in both regions, while there was no difference ($P > 0.05$) between antocin II and carbetocin. The most permeable analogue was desPOG-antocin II-NH$(CH_2)_3NH_2$. The transport rates of the analogues calculated per centimeter of intestinal segment did not correlate with values for logarithms of partition coefficients in *n*-octanol (log *D*), al-

Table I. Cross-Reactivity of Antiserum K8620-1 for Natural Pituitary Peptide Hormones as Well as for Various Vasopressin and Vasotocin Analogues[a]

Trivial name of the peptide	Amino acid residue									Cross-reactivity (%)
	1	2	3	4	5	6	7	8	9	
Antocin II	Mpa	D-Tyr(OEt)	Ile	Thr	Asn	Cys	Pro	Orn	Gly-amide	100
Antocin II (1–8)	Mpa	D-Tyr(OEt)	Ile	Thr	Asn	Cys	Pro	Orn-amide	—	100
	Mpa	D-Tyr(OEt)	Ile	Thr	Asn	Cys	X	—	—	84
	Mpa	D-Tyr(OEt)	Ile	Val	Asn	Cys	Pro	Orn	Gly-amide	8
	Mpa	D-Tyr(OEt)	Ile	Val	Asn	Cys	Pro	D-Arg	Gly-amide	4
	Mpa	D-Tyr	Ile	Val	Asn	Cys	Pro	Orn	Gly-amide	<0.1
	Mpa	Tyr(OEt)	Ile	Val	Asn	Cys	Pro	Orn	Gly-amide	<0.1
	Mpa	Tyr(OEt)	Ile	Val	Asn	Cys	Pro	Arg	Gly-amide	<0.1
	Mpa	D-Trp	Phe	Gln	Asn	Cys	Pro	Lys	Gly-amide	<0.1
	Mpa	Tyr(OEt)	Phe	Gln	Asn	Cys	Pro	Arg	Gly-amide	<0.1
Arginine vasopressin	Cys	Tyr	Phe	Gln	Asn	Cys	Pro	Arg	Gly-amide	<0.01
Lysine vasopressin	Cys	Tyr	Phe	Gln	Asn	Cys	Pro	Lys	Gly-amide	<0.01
Arginine vasotocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Arg	Gly-amide	<0.01
Oxytocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Leu	Gly-amide	<0.01

[a] Cross-reactivity on a weight basis was determined at 50% inhibition of tracer binding. Mpa, mercaptopropionic acid; Tyr(OEt), tyrosine ethyl ether; —, deleted amino acid; *X*, $NH(CH_2)_3NH_2$. L-amino acids were used, except where indicated otherwise.

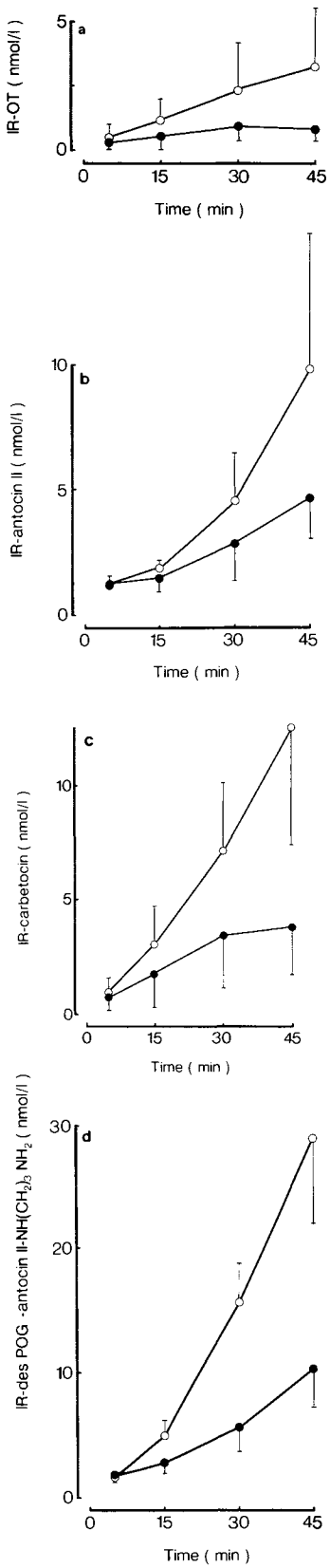

Fig. 2. (a–d) Mucosal-to-serosal transport of oxytocin and vasopressin analogues in everted intestinal sacs. Initial peptide concentration in mucosal medium was 1 μ*M*. Peptide content of serosal medium was measured as peptide immunoreactivity (IR) by use of radioimmunoassay. Values are means ± SD. Filled circles, proximal; open circles, distal. (a) $n = 12$; (b) $n = 12$; (c) $n = 9$; (d) $n = 6$.

though the least hydrophobic peptide, OT, was also the least permeable (Table II). Differences in diffusion coefficients were small (Table II).

In the Ussing chamber (diffusion-cell) experiments the intestinal transport of IR-OT and IR-dDAVP and their ^{3}H-labeled counterparts were further evaluated (Figs. 3a and b, Table III). The apparent permeation coefficients (P_{app}) for IR-dDAVP and [^{3}H]dDAVP were identical. There was, however, a significant difference in P_{app} for the transport of the labeled and unlabeled OT. The transport of [^{3}H]OT was 20- and 60-fold higher across distal and proximal segments, respectively, than that of unlabeled OT. The nondegradable polysaccharide [^{3}H]inulin was absorbed considerably slower than dDAVP and was also transported at a higher rate in distal intestinal segments ($P < 0.05$).

The mucosal media were collected after each experiment for determination of possible peptide degradation by HPLC. As seen in Table IV no differences between proximal and distal mucosal peptide concentrations were found. No detectable degradation products were observed using the present HPLC system. The mucosal media were also extracted and fractionated on HPLC for subsequent analyses by RIA. Identical elution patterns for standard peptide and extracted peptide were found. No additional immunoreactive peaks were identified (not shown).

The possibility of cellular peptide accumulation in the intestinal mucosa was assessed by comparing the content of [^{3}H]dDAVP in mucosal scrapings with that of the inert polysaccharide [^{3}H]inulin, the latter representing the ECS. After a 60-min incubation period at 37°C the ECS constituted 57.1 ± 7.2% in both proximal and distal mucosa. The mucosal distributions of [^{3}H]dDAVP were 69.6 ± 10.9 and 84.0 ± 17.9, respectively. Thus, [^{3}H]dDAVP distributed in a larger volume than ECS in distal mucosa ($P < 0.05$).

DISCUSSION

The use of *in vitro* methods to study intestinal absorption of drugs has both advantages and drawbacks. The main drawback is the relatively short experimental time period as a result of limited tissue viability. Therefore studies on the viability of the two *in vitro* systems used in the present study have been performed by measuring the active transport of methyl-D-glucose across the intestinal segments (6,7). These experiments showed that the intestinal preparations were useful for at least a 3-hr period. The present study was prompted by the finding that dDAVP was absorbed differently from various regions of the gastrointestinal tract in rabbits, where the highest uptake was observed from the ileo-cecal part (5). These differences in regional absorption could be due to a number of factors such as intestinal blood flow, transit time, enzymatic peptide degradation, and epithelial permeability. With the use of *in vitro* methods differences in intestinal epithelial enzymatic activity and permeability have to be considered.

The peptides used in this study all possess biological activities. dDAVP is used for the treatment of diabetes insipidus and oxytocin for the induction of labor, while carbetocin promotes milk ejection in pigs (13). The novel oxytocin antagonists antocin II and desPOG-antocin II-$NH(CH_2)_3NH_2$ may be of potential use to prevent premature labor (14).

Table II. Molecular Weights (MW), Logarithms of *n*-Octanol in Water (pH 7.4) Partition Coefficients (log *D*), Diffusion Coefficients (*K*), and Transport Rates Across Distal and Proximal Small Intestinal Segments of the Rat in the Everted Sac Model*

Peptide	MW (Da)	log *D*	Transport rate (fmol × cm^{-1} × min^{-1})		*K* (cm^2 × sec^{-1})
			Proximal ileum	Distal ileum	
Oxytocin (OT)	1007	−2.43	1.2	9.2	5.17×10^{-6}
Antocin II	984	−2.26	11.6	28.6	5.21×10^{-6}
DesPOG-antocin II-$NH(CH_2)_3NH_2$	783	−1.29	28.6	90.0	5.65×10^{-6}
Carbetocin	987	−1.06	6.6	31.8	5.20×10^{-6}

* Statistically significant differences ($P < 0.05$) between the transport rates across distal and those across proximal intestinal segments were found for all peptides as measured by ANOVA (see text).

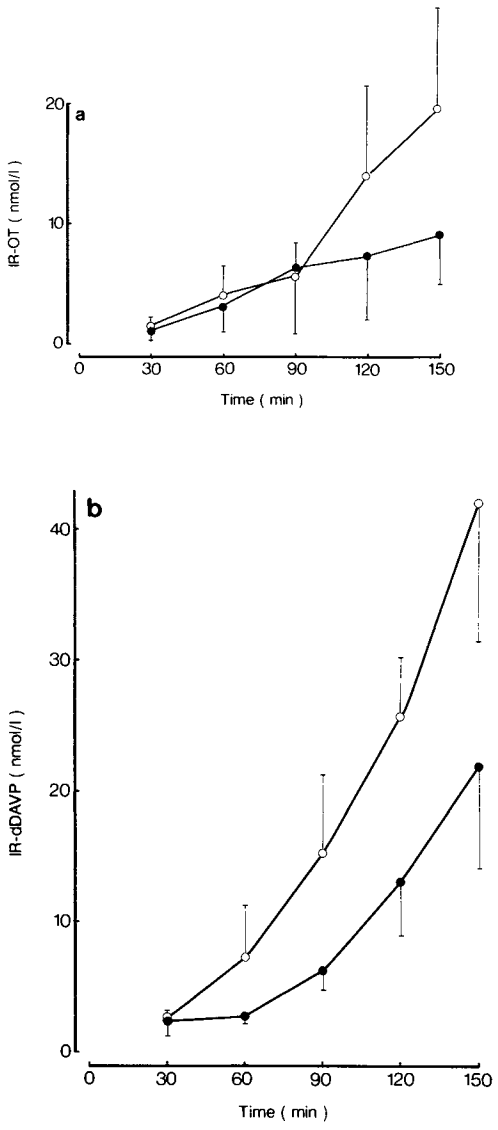

Fig. 3. (a, b) Mucosal-to-serosal transport of oxytocin (OT) and dDAVP in diffusion cells. The exposed surface area of the intestinal segment is 2.06 cm^2. Initial peptide concentration in mucosal medium was 10 μ*M* for dDAVP and 40 μ*M* for OT. Peptide content of serosal medium was measured as peptide immunoreactivity (IR) by use of radioimmunoassay. Values are means ± SD. Filled circles, proximal; open circles, distal. (a) $n = 6$; (b) $n = 8$ proximal, $n = 4$ distal.

All the peptides, measured as peptide-like immunoreactivity, were transported across the isolated intestinal segments. The transport rate was generally higher across distal than across proximal small intestine, a result in agreement with previous *in vivo* findings (5). Considering the fact that the ileal absorptive surface area decreases in the distal direction (15), this is an interesting finding. It can thus be concluded that factors such as blood flow and intestinal transit time cannot explain the observed regional differences. No correlation between peptide hydrophobicity and transport rates appeared to exist. This agrees with previous results using everted jejunal segments (16). However, simple log *D* measurements may not be suitable for complex molecules such as peptides where configuration and charge may be significant contributors to their absorptive capacity. These earlier results (16) showed that dDAVP most likely was transferred across the proximal intestinal mucosa by a passive process and were recently strengthened in studies on the absorption of dDAVP in an intestinal epithelial cell line (Caco-2) (17).

To assess the possibility that the increased distal transport rate of the peptides could be attributed to some active mechanism, the accumulation of [^{3}H]dDAVP was determined. The accumulation of [^{3}H]dDAVP in distal mucosa was significantly higher than that of the ECS marker [^{3}H]inulin, although not high enough to make a large contribution from active transport processes likely; i.e., the mucosal content of [^{3}H]dDAVP was not higher than in buffer (>100%). Trapping of dDAVP in the mucus layer could be responsible for the observed difference, but we cannot exclude the pos-

Table III. Apparent Permeation Coefficients (P_{app}) for Transport Across Proximal and Distal Small Intestine Obtained in Diffusion-Cell Experiments After Addition of the Marker Substance to the Mucosal Chamber (See Text)[a]

Substance	P_{app} (cm × sec^{-1})		*n*
	Proximal	Distal	
IR-OT	$3.35 \pm 2.73 \times 10^{-8}$	$1.35 \pm 0.53 \times 10^{-7}$	6
[^{3}H]OT	$2.09 \pm 0.19 \times 10^{-6}$	$2.60 \pm 1.14 \times 10^{-6}$	3
IR-dDAVP	$1.06 \pm 0.38 \times 10^{-6}$	$1.81 \pm 0.46 \times 10^{-6}$	8
[^{3}H]dDAVP	$1.22 \pm 0.27 \times 10^{-6}$	$1.89 \pm 0.69 \times 10^{-6}$	7
[^{3}H]Inulin	$1.48 \pm 0.25 \times 10^{-7}$	$3.12 \pm 0.13 \times 10^{-7}$	5

[a] *n* = number of experiments. Values are given as means ± SD.

Table IV. Amount of Intact Peptide Remaining After 60 min in the Mucosal Chamber of Everted Sacs Expressed as Percentage of Initial Concentration (1 μmol/L) as Determined by HPLC[a]

	Intestinal segment	
	Proximal	Distal
Oxytocin	95.8 ± 1.8	93.8 ± 1.9
Carbetocin	98.5 ± 2.3	97.7 ± 1.3
Antocin II	97.5 ± 1.0	97.0 ± 1.2
DesPOG-antocin II-$NH(CH_2)_3NH_2$	96.9 ± 1.3	98.1 ± 1.4

[a] Values are given as means ± SD (n = 3).

sibility that small amounts of peptide are taken up by the enterocytes. The log D values are most certainly too low to account for any transcellular diffusion (18). Therefore, cellular uptake by endocytosis may occur but no information on the presence of specific receptors for either arginine vasopressin (AVP) or OT on the luminal (apical) side of the epithelium is available. The renal epithelium resembles that of the small intestine by the presence of a villous structure. By use of electron microscope autoradiography, AVP was shown to be endocytosed in the renal proximal tubule (19). It is thus still an open question whether peptides such as OT and AVP can be endocytosed by the enterocyte. All evidence obtained to date favors a paracellular transport route.

It would be of interest to compare the P_{app} value for [^{3}H]dDAVP obtained in the diffusion cell model with that observed in the Caco-2 cell line (17) as this would give an indication of the validity of these models. The P_{app} values obtained in this study were 9.5 to 14.6 times higher than the P_{app} in Caco-2 cell monolayers. The mean surface area for rat intestinal epithelium was calculated to be 12–25 times larger than that in Caco-2 cells (20). The difference in P_{app} between the two models can therefore be explained almost entirely by the differences in absorptive surface area but it should be kept in mind that the Caco-2 epithelium is much tighter as reflected by a higher transepithelial resistance. Consequently the subepithelial barriers such as the basement membrane, lamina propria, and capillary wall do not seem to limit the rate of absorption of the peptide (21).

When evaluating the absorptive capacity of peptides in the gastrointestinal tract, the likelihood of enzymatic degradation also must be taken into account. Previous studies have shown that dDAVP remained intact when exposed to isolated jejunal segments (16). In the present study a hundredfold lower peptide concentration was used, thereby not allowing direct HPLC measurements of serosal medium. However, all peptides incubated in mucosal medium showed only a single peak eluting at the position of intact peptide. This does not entirely exclude the possibility of enzymatic breakdown during mucosal passage. Small hydrophilic breakdown products of dDAVP would not be retained and detected in the presently used HPLC system. No such immunoreactive fragments could be measured in the first-eluting fractions. We have investigated earlier the stability of AVP and dDAVP by incubations with pancreatic juice and intestinal mucosa homogenates (22). AVP was found to be degraded rapidly, while dDAVP remained intact after incubation with pancreatic juice. When exposed to mucosal homogenates dDAVP was degraded at its C-terminal tripeptide linear sequence, most likely through the action of a cytosolic prolylendoprotease (23). Any passage of OT and AVP analogues across intestinal epithelium by endocytosis may therefore be obscured by the degrading action of intracellular enzymes. Other studies showed that dDAVP was very slowly degraded in intestinal juice after prolonged incubations (24). Confirmation of the stability of dDAVP was obtained in this study by comparing the P_{app} for ^{3}H-labeled and unlabeled peptides, which were identical.

Information on epithelial enzymatic stability of OT is available only from incubations with renal microvilli membranes where the peptide was essentially refractory to degradation (25). Some peptide hydrolysis did occur at the Tyr^2–Ile^3 bond. The large difference between [^{3}H]OT and IR-OT transport rate indicates that the peptide undergoes degradation, giving rise to smaller ^{3}H-labeled tyrosine-containing fragments. Although this degradation of [^{3}H]OT may be rather minor, as reflected by the high amount of intact peptide remaining in the mucosal medium, the smaller fragments should be transported considerably faster than OT itself and thus add significantly to the observed P_{app}. As pointed out before (24) small amounts of pancreatic enzymes may adsorb to the intestinal mucosa despite thorough rinsing of the tissue and OT may potentially be cleaved by the action of chymotrypsin or a carboxamidopeptidase (26).

The other peptide analogues include molecular modifications which would render them more stable than OT, and future experiments should reveal their degradation pathways in the GI tract. The increased transport rate of all peptides in the distal part of the small intestine could possibly be explained by a decreased proteolytic capacity at this region. The epithelial distribution of enzymes along the small intestine is not yet fully elucidated. However, the total hydrolytic capacity seems to be higher in the ileum than in the jejunum (27). These results may be of benefit for the development of oral drug formulations with improved absorption characteristics.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Anette Persson for secretarial assistance and Inger Mattsson and Lill Ivarsson for technical assistance. This work was supported by grants from the Swedish Medical Research Council (B90-04X-08643-02B), the Crafoord Foundation, and the Swedish Natural Science Research Council.

REFERENCES

1. S. R. Bloom and G. Burnstock. *Peptides: A Target for New Drug Development,* IBC Technical Services Ltd., London, 1990.
2. J. C. Verhoef, H. E. Boddé, A. G. de Boer, J. A. Bowstra, H. E. Junginger, F. W. H. M. Merkus, and D. D. Breimer. Transport of peptide and protein drugs across biological membranes. *Eur. J. Drug Metab. Pharmacokinet.* 15(2):83–93 (1990).
3. H. Vilhardt and S. Lundin. Biological effect and plasma concentrations of dDAVP after intranasal and peroral administration to humans. *Gen. Pharmacol.* 17(4):481–483 (1986).
4. M. Hammer and H. Vilhardt. Peroral treatment of diabetes in-

sipidus with a polypeptide hormone analogue, desmopressin. *J. Pharmacol. Exp. Ther.* **234**(3):754–760 (1985).
5. S. Lundin and H. Vilhardt. Absorption of 1-deamino-8-D-arginine vasopressin from different regions of the gastrointestinal tract in rabbits. *Acta Endocrinol.* **112**:457–460 (1986).
6. G. M. Grass and S. A. Sweetana. In vitro measurement of gastrointestinal tissue permeability using a new diffusion cell. *Pharm. Res.* **5**(6):372–376 (1988).
7. S. Lundin, N. Pantzar, L. Hedin, and B. R. Weström. Intestinal absorption enhancement by sodium taurodihydrofusidate of a peptide hormone analogue (dDAVP) and a macromolecule (BSA) in vitro and in vivo. *Int. J. Pharm.* **59**:263–269 (1990).
8. G. A. Kerchner and L. A. Geary. Studies on the transport of enkephalin-like oligopeptides in rat intestinal mucosa. *J. Pharmacol. Exp. Ther.* **226**:33–38 (1983).
9. S. Lundin, P. Melin, and H. Vilhardt. 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**:933–935 (1985).
10. M. Ohlin and U. Alkner. Development of an immunoassay for glypressin, an N-terminal extended vasopressin analogue. *J. Immunoassay* **9**(1):19–36 (1988).
11. F. C. Greenwood, W. M. Hunter, and J. S. Glover. The preparation of ^{131}I-labeled human growth hormone of high specific reactivity. *Biochem. J.* **89**:114–123 (1963).
12. F. T. LaRochelle, Jr., W. G. North, and P. Stern. The use of a new extraction of arginine vasopressin from blood using octadecasilylsilica. *Pfluegers Arch.* **387**:70–81 (1980).
13. T. Barth, M. Flegel, and K. Just. Protracted milk-ejecting effect of some oxytocin analogues in rats. *Endocrinol. Exp.* **10**:67–71 (1976).
14. P. Melin and J. Trojnar. Antagonism of oxytocin analogues on the human uterus and in animal models. In S. Yoshida and L. Share (eds.), *Recent Progress in Posterior Pituitary Hormones, 1988,* Elsevier, Amsterdam, New York, Oxford, 1988.
15. R. B. Fisher and D. S. Parsons. The gradient of mucosal surface area in the small intestine of the rat. *J. Anat. (London)* **84**:272–282 (1950).
16. H. Vilhardt and S. Lundin. In vitro intestinal transport of vasopressin and its analogues. *Acta Physiol. Scand.* **126**:601–607 (1986).
17. S. Lundin and P. Artursson. Absorption of a vasopressin analogue, 1-deamino-8-D-arginine vasopressin (dDAVP) in a human intestinal epithelial cell line, Caco-2. *Int. J. Pharm.* **64**:181–186 (1990).
18. P. Krugliak, D. Hollander, T. Y. Ma, D. Tran, U. D. Dadufulza, K. D. Katz, and K. Le. Mechanisms of polyethyleneglycol permeability of perfused rat intestine. *Gastroenterology* **97**:1164–1170 (1989).
19. F. A. Carone, E. I. Christensen, and G. Flouret. Degradation and transport of AVP by proximal tubule. *Am. J. Physiol.* **253**:F1120–F1128 (1987).
20. P. Artursson. 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**:476–482 (1990).
21. M. J. Jackson. In L. R. Johnson (ed.), *Physiology of the Gastrointestinal Tract,* Raven Press, New York, 1987, pp. 1597–1621.
22. S. Lundin, H.-I. Bengtsson, H. G. Folkesson, and B. R. Weström. Degradation of [mercaptopropionic acid^{1}, D-arginine^{8}]-vasopressin (dDAVP) in pancreatic juice and intestinal mucosa homogenate. *Pharmacol. Toxicol.* **65**:92–95 (1989).
23. R. Walter. Partial purification and characterization of post-proline cleaving enzyme in enzymatic inactivation of neurohypophysial hormones by kidney preparations of various species. *Biochim. Biophys. Acta* **422**(1):138–158 (1976).
24. B. Matuszewska, G. G. Liversidge, F. Ryan, J. Dent, and P. L. Smith. In vitro study of intestinal absorption and metabolism of 8-L-arginine vasopressin and its analogues. *Int. J. Pharm.* **46**:111–120 (1988).
25. S. A. Stephenson and A. J. Kenny. Metabolism of neuropeptides: Hydrolysis of angiotensins, bradykinin, substance P and oxytocin by pig kidney microvillar membranes. *Biochem. J.* **241**:237–247 (1987).
26. R. Walter and W. H. Simmons. Metabolism of neurohypophyseal hormones: Considerations from a molecular viewpoint. In A. M. Moses and L. Share (eds.), *Neurohypophysis,* Basel, Karger 1977, pp. 167–188.
27. V. H. L. Lee. Enzymatic barriers to peptide and protein absorption. *CRC Crit. Rev. Ther. Drug Carrier Syst.* **5**:69–97 (1988).