Absorption of 1-deamino-8-D-arginine vasopressin from different regions of the gastrointestinal tract in rabbits

Stefan Lundin1,2 and Hans Vilhardt3

Departments of Zoophysiology1, University of Lund, S-223 62 Lund, Sweden and Ferring Pharmaceuticals2, S-200 62 Malmö, Sweden and Department of Medical Physiology3, University of Copenhagen, DK-2200 Copenhagen N, Denmark

Abstract. By using a specific radioimmunoassay for 1-deamino-8-D-arginine vasopressin (DDAVP), plasma concentrations were measured in rabbit plasma at various times after intra-gastrointestinal injections. Five anatomically distinct regions were employed to administer the peptide, namely: the stomach, the duodenum, the mid-part of the ileum, the ileo-coecal junction and the mid-part of the colon. In each case immunoreactive DDAVP could be demonstrated in plasma. The highest concentrations were observed after duodenal and ileo-coecal administration. Absorption was rapid; peak levels were reached after 10–20 min.

It has been shown that the vasopressin analogue, 1-deamino-8-D-arginine vasopressin (DDAVP) is absorbed from the gastrointestinal tract in quantities sufficient to induce an antidiuretic effect in diabetes insipidus rats (Kinter & Beeuwkes 1982), water loaded conscious dogs (Vilhardt & Bie 1983) and hydrated human volunteers (Vilhardt & Bie 1984). After peroral administration of DDAVP in rats (Lundin et al. 1985) and dogs (Lundin & Vilhardt, in press), immunoreactive material was detected in plasma through the use of a newly developed radioimmunoassay (RIA). In both of these studies it was demonstrated that almost all plasma immunoreactivity coeluted with standard DDAVP when subjected to high-performance liquid chromatography (HPLC). This finding lends support to the assumption that undegraded peptide is transported through the intestinal mucosa. In an extension of this work we have now studied the in vivo absorption of DDAVP from different gastrointestinal sites in rabbits to locate areas of preferential uptake.

Materials and Methods

Animal experiments

Male rabbits weighing between 1.5 and 3 kg of the 'Swedish Look' strain were used. Standard pellet diet and water allowed ad libitum. The animals were anesthetized with an sc injection of Inactin® (Byk, FRG), followed by repeated iv injections. The abdomen was opened at proper locations for intra-gastrointestinal administrations. For this purpose a Venflon® catheter, gauge 22 (Viggo, Helsingborg, Sweden) was introduced intraluminally with a minimum of handling. When DDAVP was administered intragastrically a non-traumatic ligature was tied proximal to the pylorus. The catheter was inserted so that no visible blood vessels were damaged. The following sites were employed for peptide administration: the stomach, duodenum, 'mid'-ileum, ileo-coecal junction and 'mid'-'colon. The trachea was cannulated and a PE90 catheter was inserted in the carotid artery for collection of blood. No injections were made during the first 30 min after surgery. DDAVP (Ferring Pharmaceuticals, Malmö, Sweden) was dissolved in physiologic saline and administered in a dose of 30 nmol/kg body weight. Blood samples (2 ml) were taken in heparinized polypropylene tubes at 0, 10, 20, 30, (45) and 60 min after the drug injection. The blood samples were kept on ice and centrifuged at 800 x g for...
15 min at 4°C. The plasma was separated and kept stored at −60°C until analyzed.

**Radioimmunoassay**

Analysis of plasma DDAVP was performed with a specific RIA method (Lundin et al. 1985). In the present investigation the method was modified so that the extraction step was omitted. The incubation mixture therefore consisted of 200 µl standard (range 0.25–128 fmol/tube), 50 µl [¹²⁵I]DDAVP and 200 µl antiserum. Unknown plasma samples were added instead of standards diluted in rabbit plasma. Otherwise all dilutions were made with phosphate buffer, containing 0.1% HSA and 0.2% neomycin sulphate, adjusted to pH 7.5. The antiserum, designated ADA’6 is highly specific and cross-reacts less than 0.01% with arginine vasopressin and oxytocin. It is used at a final dilution of 1:150 000. After an incubation period of 24 h, bound and free peptide were separated by the addition of 1 ml plasma-coated charcoal. Iodination of DDAVP was accomplished with the iodogen method (Salecinski et al. 1979) followed by purification of tracer using an HPLC system (Waters Inc, Milford, CT, USA). This procedure produces tracers with specific activities > 1000 Ci/m mole. Assay sensitivity, defined as the amount of unlabelled DDAVP required to displace tracer by 2 standard deviations, was 5 pmol/l under equilibrium conditions. The intra- and inter-assay coefficients of variation were 7.5 and 14%, respectively. The amount of standard displacing antibody-bound tracer by 50%
(B$_{20}$) was found to be 25.4 ± 1.9 fmol/tube (n = 10). To assess the identity between DDAVP as diluted in the standard curve and a plasma sample obtained 1 h after intraduodenal injection, serial dilutions of the latter was prepared and compared to the standard curve for possible parallelism.

**Calculations**

The experimental data were subjected to statistical evaluation using analysis of variance (ANOVA). Data are expressed as means ± standard error (±SEM).

**Results**

RIA standard curves of DDAVP diluted in rabbit-plasma were parallel to those diluted in buffer, although the sensitivity was reduced by a factor of four. When serial dilutions of a plasma sample obtained from animals injected with the peptide was compared with the standard curve, a parallel relationship was found.

Intra-gastrointestinal injections of DDAVP at all sites except for the colon led to rapid appearance of the peptide in plasma. Already after 10 min the DDAVP concentrations were maximal and remained elevated during the observation period (Fig. 1). ANOVA revealed that only the ileo-coecal (P < 0.01) and the duodenal (P < 0.01) concentration curves were significantly elevated in relation to the zero level.

**Discussion**

The results of the present study show that immunoreactive DDAVP is absorbed from the gastrointestinal tract in rabbits. Transmucosal transport of the peptide, however, seems to occur at different rates depending on which site it is administered. Maximal plasma levels were reached already after 10 min.

Absorption of small peptides (mostly di-tetra peptides) has been discussed mainly in the context of nutrition (Matthews 1975). These small molecules seem to be absorbed largely by active processes. The fast absorption of DDAVP observed in this study is in agreement with our earlier findings using the everted sac intestine of the rat where DDAVP was transported through the mucosa at a considerably higher rate than vasopressin (unpublished results). Also, deamination of the molecule renders it more resistant to enzymatic degradation and increases its lipophilicity (Sawyer et al. 1974; Lauson, 1974). These features would seem to facilitate transport by passive diffusion. We have reason to believe that most of the DDAVP immunoreactivity appearing in plasma is in an intact form since in previous peroral studies with DDAVP (Lundin et al. 1985), plasma extracts subjected to HPLC fractionation and subsequent RIA showed identical retention times for extracted peptide and standard. In addition, the parallelism observed between standard DDAVP and serial dilutions of a plasma sample obtained after intraduodenal injection in this study suggests immunoidentity.

The reasons for the differences in absorption at anatomically distinct sites are as yet obscure. A progressive fall in permeability from the duodenum to the terminal ileum, possibly reflecting a reduced number of pores, has been demonstrated (Loehry et al. 1973). The surface area of the intestine also decreases down the length of the small intestine (Fisher & Parsons 1950). Taken together, this would explain why DDAVP absorption is lower in the ileum than in the duodenum but does not explain the increased absorption observed just proximal to the ileo-coecal valve. Possibly, degradation by intestinal and pancreatic enzymes is less pronounced in the very distal part of the small intestine leaving more intact DDAVP for absorption. The continuous absorption of DDAVP from this region may indicate a prolonged transit time in these animals. Possibly, intestinal motility was reduced due to anaesthesia. For the same reason it cannot be excluded that regional blood flow at the sites of administration may have been altered.

The present findings are in contrast to observations with another biologically active peptide, TRH, where in vivo absorption was negligible from the distal part of the ileum. Transmucosal TRH transport, however, seems to occur mainly by active mechanisms as described by Yokohama et al. (1984). Absorption of DDAVP from the stomach and colon was low. The greatly reduced surface area due to lack of villi in these locations may be a likely explanation for the poor absorption. The extent of enzymatic degradation of DDAVP in the stomach is difficult to evaluate. However, the low rate of absorption of the peptide from this region is probably not due to
degradation since DDAVP is not metabolized by the principal gastric peptidase, pepsin, and the peptide is relatively stable at acidic pH (Thorn 1959).

Acknowledgments
We are grateful to Ms. Lise-Lotte Carlsten for skilled technical assistance and Dr. Claus Rerup for help with statistical evaluations.

References

Received November 13th, 1985.
Accepted February 2nd, 1986.