Enhanced Intestinal Absorption of Oxytocin Peptide Analogues in the Absence of Pancreatic Juice in Pigs

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Purpose. The present investigation was done to study the intestinal absorption of three oxytocin peptide analogues and to elucidate the role of pancreatic juice on their absorption.

Methods. In conscious chronically catheterized pigs (6–8 weeks of age) plasma concentration of the peptides, [Mpa[1], D-Tyr(Ethyl)[2], Thr[4], Orn[8]]-oxytocin (F314), [Mpa[1], D-Tyr(Ethyl)[2], Val[4], D-Arg[8]]-oxytocin (CAT), and [Mpa[1], D-Tyr(Ethyl)[2], Thr[4], Orn[8], desGly[9], carba[6]]-oxytocin (F327) after intraduodenal administration, during presence or diversion of the pancreatic juice via a pancreatic duct catheter, were determined by radioimmunoassay. The stability of the peptides to degradation was determined in vitro by incubation with activated pancreatic juice, chymotrypsin or trypsin, followed by reversed phase HPLC analyses.

Results. All peptides were absorbed with a bioavailability of about 0.5% in the presence of pancreatic juice, but increased to 1.0%, 2.1%, and 13.5% for F314, CAT, and F327, respectively, when the pancreatic juice was diverted from the intestine. After incubation with pancreatic juice 95% of F314, 98% of F327, and 100% of CAT was found intact. When incubated with trypsin CAT remained intact while F314 and F327 were degraded by 54% and 46%, respectively. Incubation with purified chymotrypsin did not degrade the test peptides.

Conclusions. The results indicate that the increased absorption of peptides observed under conditions of diverted pancreatic juice cannot only be explained by the absence of pancreatic enzymes, but also by changed absorptive properties in the gastrointestinal tract.

KEY WORDS: absorption; intestine; pancreatic juice; peptides; pig.

INTRODUCTION

Oral delivery and the subsequent intestinal absorption is a preferred route for drug administration. For peptide drugs, however, this route has been of limited value because the intestines provides a hostile environment.

The intestinal uptake of short peptides up to tetrapeptides takes place predominantly by active transport mechanisms in the epithelial cell brush-border membranes (1,2), while larger peptides are considered to be passively transported via both paracellular and transcellular routes (3,4). The extent of absorption of the latter peptides are interpreted to be a consequence of their physico-chemical properties, e.g., size, charge, lipophilicity, and resistance to enzymatic degradation. In the absence of pancreatic juice, e.g. in pancreatic juice insufficiency, the intestinal permeability has shown to increase (5).

The purpose of the present investigation was to study the intestinal absorption and the bioavailability of the three oxytocin peptide analogues having structural similarities to the earlier thoroughly studied nonapeptide 1-deamino-8-D-arginine vasopressin (dDAVP) (6–9). In order to elucidate the role of pancreatic juice on peptide absorption, we utilized a pig model with chronic pancreatic duct and duodenal catheters enabling conditions where pancreatic juice was present, or entirely diverted.

MATERIALS AND METHODS

Peptides

Three oxytocin analogues, the nonapeptides F314 and CAT and the octapeptide F327, were synthesized by Ferring Research Institute AB (Malmö, Sweden) with a chromatographic purity ≥99% (Table I).

Animals

The studies were carried out on seven 6–8 weeks old (12 ± 4 kg body wt.) purebred weaned Swedish Landrace pigs (*Sus scrofa*) obtained from Dept. of Agricultural Biosystems and Technology, Lund, Sweden. The pigs were kept in separate cages with free access to a standard pig diet (Växfor, Lantmännen, Stockholm, Sweden) and water.

Surgical Procedures

Overnight fasted animals were sedated with azaperon (Stresnil, Janssen Pharmaceutica, Beerse, Belgien), 2 mg kg^{-1} body wt. intramuscularly, and anesthesized with ketamin (Ketalar, Parke-Davis, Barcelona, Spain), 10 mg kg^{-1} body wt., and sodium pentobarbital, 10 mg kg^{-1} body wt., intravenously. The animals were surgically fitted with a pancreatic duct catheter and a duodenal reenterant T cannula (10). The pancreatic duct catheter was connected to the duodenal cannula and maintained so between the experiments to reintroduce the pancreatic juice to the intestine. A catheter was also implanted in the right jugular vein for blood sampling.

Peptide Administration Experiments

The three peptides were separately administered in three randomized experiments to the unanesthetized overnight fasted pigs. The peptides were dissolved in NaCl (0.9%) and given (a) intravenously (3.0 nmol kg^{-1}; n = 3–4), (b) intraduodenally (150 nmol kg^{-1}; n = 3–4) with pancreatic juice re-entered into the duodenum, and (c) intraduodenally (150 nmol kg^{-1}; n = 3–4) with pancreatic juice entirely diverted 16 h before peptide administration.

After peptide administration, blood samples of 2.5 ml were withdrawn between 0–240 min into ice chilled tubes containing EDTA (10 mg, Sigma Chemical Co, St Louis, Missouri, USA) and aprotinin (3,000 KIU, Traskolan, Polfa, Jelenia Góra, Poland). The blood was centrifuged and

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Table I. Structure, Molecular Weight (Da), and Relative Lipophilicity (log K) of the Three Oxytocin Analogues: The Nonapeptides F314 and CAT, and the Octapeptide F327.

Peptide		Mw	log K
F314	[Mpa^1, D-Tyr(Ethyl)^2, Thr^4, Orn^8]-oxytocin	993	0.68
CAT	[Mpa^1, D-Tyr(Ethyl)^2, Val^4, D-Arg^8]-oxytocin	1024	1.08
F327	[Mpa^1, D-Tyr(Ethyl)^2, Thr^4, Orn^8, desGly^9, carba^6]-oxytocin	919	0.61

plasma samples were stored at −20°C. Pancreatic juice was collected during the experiments when it was diverted, and stored at −20°C for the in vitro experiments.

Peptide Stability Experiments

The peptides (50 μl of a 1.0 mM solution) were incubated in vitro with pancreatic juice (50 μl) in 0.2 M Tris-HCl buffer containing 0.05 M $CaCl_2$, pH 7.8 (150 μl) in triplicates at 37°C. The pancreatic juice had been activated by preincubation with 0.12 mg ml^{-1} enterokinase (E-1256, Sigma Chemical Co) 20 min at 37°C, and was determined to contain 5 mg ml^{-1} protein, using the Lowry method (11), and a trypsin activity of 3 U ml^{-1} (12). The incubations were stopped at 0 and 60 min by boiling for 5 min, whereafter they were centrifuged for 10 min (1,500 × g) and the supernatants were analysed by HPLC.

The peptides (1.0 mM final concentration) were also incubated with purified bovine α-chymotrypsin (50 U mg^{-1}, Sigma Chemical Co) or trypsin (10,000 U mg^{-1}, Sigma Chemical Co) at 1.0 mg ml^{-1} final enzyme concentrations in a volume of 30 μl comprising 0.025 M Tris, 0.065 M NaCl, 0.0025 M $CaCl_2$, pH 7.8 at 37°C. The reaction was stopped by the addition of 2% (w/v) of $ZnSO_4$ in 50% methanol, after which the vials were centrifuged for 5 min (10,000 × g) and the supernatants were analysed by HPLC.

Analyses

High Performance Liquid Chromatography (HPLC)

Analyses of the peptides after enzyme incubations were performed by reversed phase HPLC. The HPLC system consisted of a Kontron pump 420 + 422, an autosampler 460, a detector 432, and a Kromasil C8 column (Hichrom Ltd, England). The sample (30 μl) was injected and the peptide was eluated, using isocratic elution with 35% acetonitrile in 65% of 0.1% trifluoroacetic acid, at a flow rate of 1.0 ml min^{-1} and monitored at 220 nm. The peak areas of each sample was compared with the respective peptide reference incubated in the same way, but omitting the enzyme. The pancreatic juice did not contain any endogenous peaks at the elution positions of the respective peptides. The detection limit of the HPLC assay was 0.1 μg (3 μM) with a coefficient of variation <3% in the 5–10 μg (170–330 μM) range.

Radioimmunoassay (RIA)

Plasma concentrations of the peptides were determined by two different RIA's.

An antiserum (K8620; 13) raised against the N-terminal part of F314 and F327 was used for the assay of these peptides. Plasma samples (≤30 μl) were analysed without prior extraction in a final volume of 400 μl RIA-buffer (0.1 M Na-phosphate, pH 7.6, 50 mM NaCl, 0.1% human serum albumin, 0.02% Na-azide, 0.01% Triton X-100), containing the antiserum at a final dilution of 1:150,000 and 1–2 fmol tracer ([^{125}I]Tyr^{10}-F314). Following incubation overnight at 4°C, the free and bound fractions of the tracer were separated by centrifugation subsequent to the addition of charcoal in RIA-buffer supplemented with 2.5% human normal plasma. The antiserum crossreacted 100% with F314 and 85% with F327 for the RIA method used. Since the antisera were raised against the N-terminus of the peptides, crossreactivity with metabolites modified in the C-terminus are possible.

CAT was measured similarly, but an antiserum raised against the C-terminus of [D-Arg^8]-vasopressin was used (14), which crossreacted by 22.6% with CAT.

The lowest detectable amounts of F314, F327, and CAT were 1.2, 1.5, and 9.9 fmol/assay tube, respectively, corresponding to plasma concentrations of 40, 50, and 330 pM, respectively, and the inter- and intra-assay coefficient of variation were approximately 10%, 5%, and 10% at 30 fmol/assay tube, respectively.

Calculations

The kinetic behavior of the peptides was estimated by use of the computer program, MK MODEL (Biosoft, Cambridge, UK; 15). The area under the concentration vs. time curve (AUC) during 240 min was calculated by the trapezoidal method, and plasma clearance (Cl_p) was calculated by the relationship:

$$Cl_p = \text{Dose}/\text{AUC}$$

The elimination rate constants (L_z) and plasma half-life ($T_{1/2}$) were determined, and the apparent volumes of distribution (V_d) were calculated using:

$$V_d = Cl_p/L_z$$

The bioavailability (F) was calculated according to:

$$F = (\text{Dose}_{i.v.} \times \text{AUC}_{i.d.})/(\text{Dose}_{i.d.} \times \text{AUC}_{i.v.})$$

The relative lipophilicity, log K, of the peptides was appraised using the relation:

$$\log K = \log (t_r - t_0)/t_0$$

Where t_r refers to retention time of the peptide analogue and t_0 is the retention time of the solvent in the reversed phase HPLC analyses.

The data were statistically compared using Mann-Whitney U test where $P < 0.05$ indicated a significant difference between the medians of two samples.

RESULTS

The plasma concentration vs. time curves obtained after intravenous administration of the peptides indicated a two-compartment system (Figure 1). The plasma half-life ($T_{1/2}$) was longer ($P < 0.05$) for the octapeptide F327 and its plasma

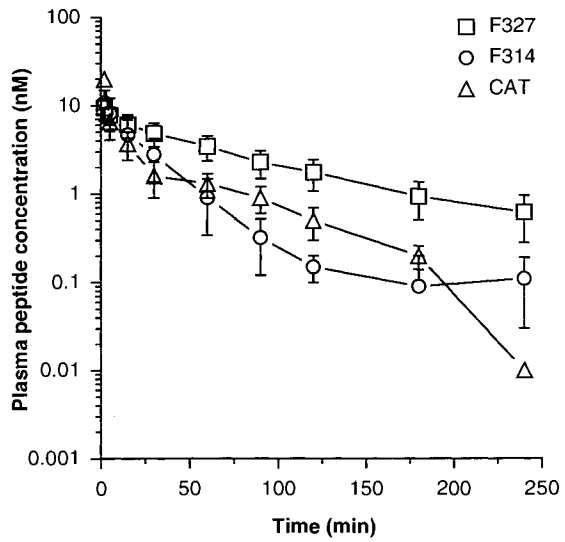

Fig. 1. Plasma concentration curves (nM) of the three oxytocin analogues: F327, F314, and CAT, after intravenous administration. Values given as mean ±SD; n = 3–4.

clearance (Cl_p) was slower ($P < 0.05$) than those of F314 and CAT (Table II).

After intraduodenal peptide administration, when the pancreatic juice was re-entered, the peptides were found in the blood plasma with peaks after 15–30 min (Figure 2a). The bioavailability was found to be 0.67, 0.52 and 0.38% of the dose given for the three peptides F314, F327 and CAT, respectively (Figure 3).

Diversion of pancreatic juice from the intestines augmented the absorption of the peptides after their intraduodenal administration (Figure 2b). The plasma concentrations were after 15 min. about 200 times higher for F327 in the absence of pancreatic juice than when it was present. For F314 and CAT, the corresponding values were about 3 times higher. Thus, diverting the pancreatic juice increased the bioavailability of CAT by a factor of 5 ($P = 0.020$) and of F327 by a factor of 10 ($P = 0.021$), while for F314 the increase was insignificant (Figure 3).

Peptide incubation with activated pancreatic juice showed that of the initial peptide concentration, 95% of F314, 98% of F327, and 100% of CAT remained intact after 60 min, as determined by HPLC analysis (Table III). No peptide degradation was observed after 60 min incubation

Table II. Pharmacokinetic Parameters Plasma Clearance (Cl_p), Plasma Half-Life ($T_{1/2}$), and Volume of Distribution (V_d) of the Three Oxytocin Analogues, F314, F327, and CAT, after Intravenous Administration[a]

Peptide	Cl_p (L h^{-1} kg^{-1})	$T_{1/2}$ (h)	V_d (L kg^{-1})
F314	0.77 ± 0.24	0.49 ± 0.12	0.26 ± 0.01
CAT	0.96 ± 0.22	0.56 ± 0.04	0.16 ± 0.01†
F327	0.29 ± 0.12*	1.06 ± 0.29*	0.35 ± 0.12

[a] Shown are mean values ± S.D. (n = 3–4).
* Indicates a statistical difference ($P < 0.05$) compared with F314 and CAT, while † compared with F314 and F327.

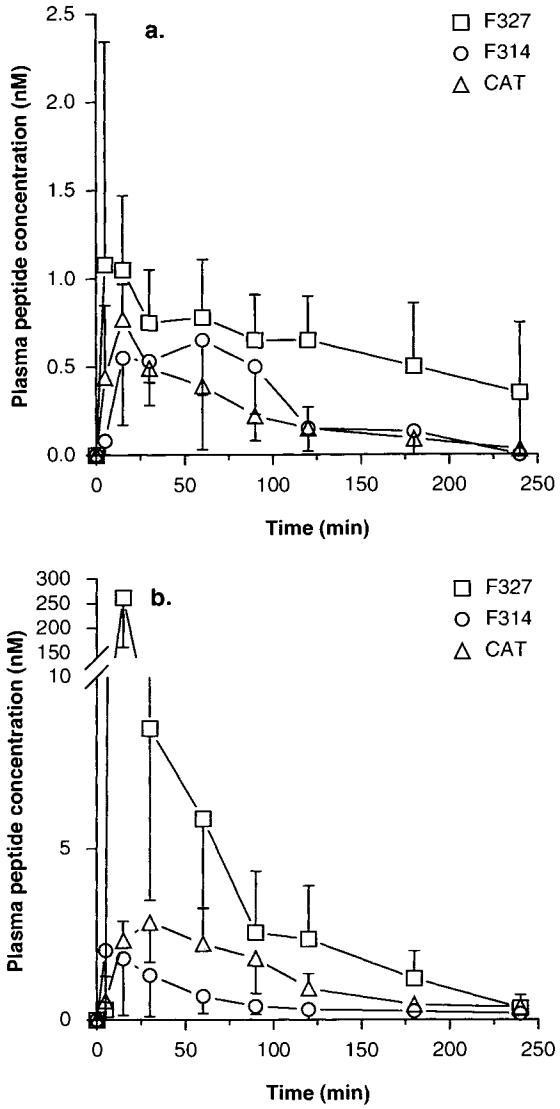

Fig. 2. Plasma concentration (nM) of the three oxytocin peptide analogues: F327, F314, and CAT, after intraduodenal administration (a) in the presence of pancreatic juice or (b) when the pancreatic juice was diverted from the intestine. Values presented as mean ± SD; n = 3–4.

with purified chymotrypsin. Incubation with purified trypsin did not result in any decomposition of CAT, but a considerable breakdown of both F314 and F327 was seen, since 54% and 46%, respectively, was found intact after 60 min (Table III).

DISCUSSION

In this study we have used a large animal model with conscious pigs, implanted with chronic catheters enabling the complete diversion of pancreatic juice, to study the influence of pancreatic juice on the absorption of peptides after duodenal administration.

The pharmacokinetic data showed that the three oxytocin analogues were cleared from plasma at different rates despite their structural similarity. A number of possible explanations may be forwarded. A plausible explanation is a

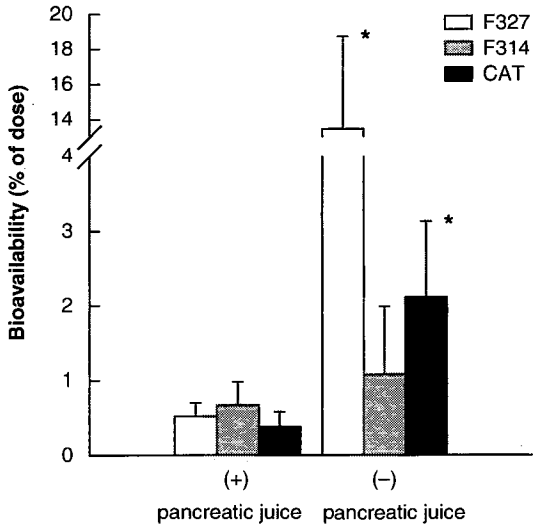

Fig. 3. Bioavailability (% of dose) of the three oxytocin analogues: F327, F314, and CAT, presented as mean ± SD, n = 3–4, where * indicates a significant difference ($P < 0.05$) in bioavailability between the peptides in the presence and in the absence of pancreatic juice.

difference in the route of excretion, either via the kidneys or the liver, dependent upon the extent of their lipophilicity (14,16). Peptides of higher lipophilicity are mainly eliminated via the liver, followed by either hepatic degradation or excretion into the bile, whereas the highly hydrophilic [^{3}H] dDAVP was cleared faster by the kidney (17).

In the present study, however, the most hydrophilic peptide F327 showed the slowest plasma clearance, which makes predictions of plasma clearance difficult to generalize. This low plasma clearance of F327 might be a consequence of its carba group in position 6, making it more stable. Moreover, the analogues may be degraded differently in the circulation or in the tissues. However, this possibility is less likely since CAT and the structurally related vasopressin analogue, dDAVP, was found chromatographically intact in blood (14).

The intestinal absorption of all three oxytocin analogues in the presence of pancreatic juice were about 0.51% of the dose given, which is somewhat higher than that of the structurally similar peptide, dDAVP, having a bioavailability of 0.11% in the pig (13).

The intestinal absorption of all the peptides was improved after the diversion of pancreatic juice, plausibly indicating a susceptibility of the peptides to pancreatic enzyme degradation. However, no decomposition of CAT was observed when incubated with pancreatic juice as well as with pure enzymes, and F314 and F327 were only partly degraded, especially in pancreatic juice. The presence of ornithine in F314 and F327 probably renders these peptides susceptible to trypsin degradation.

Thus, in the absence of any enzymatic degradation of CAT and the rather low susceptibility of F314 and F327 to pancreatic juice degradation other explanations for the increased peptide absorption must be sought. One factor could be that as a consequence of the diversion of pancreatic juice from the intestine, the mucosal hydrolysis of proteins, initiated by pancreatic proteases, is considerably decreased. This might result in an increased absorption of intact peptides due to a decreased peptide hydrolysis at the brush-border surface (18). The possibility of degradation by brush-border microvilli enzymes was not investigated for F314, CAT, and F327, but dDAVP has been shown to be completely stable when incubated with purified brush-border membrane (19). Altered gastrointestinal hormone levels is another possible consequence after diversion of pancreatic juice (20) that might alter peptide absorption across the intestine. Another explanation for enhanced peptide absorption when the pancreatic juice was diverted could be some factor in the pancreatic juice acting directly or indirectly on the gastrointestinal tract epithelium.

The structure of the oxytocin analogues was found to be of importance with respect to their bioavailability. A general conclusion is that there was no obvious correlation between the peptide susceptibility to enzymatic degradation and its bioavailability. The enhanced absorption of the oxytocin analogues when the pancreatic juice was diverted, may instead by a manifestation of changed absorptive properties in the gastrointestinal tract.

Table III. Amount of Intact Peptide Remaining After Incubation with Activated Pancreatic Juice, Chymotrypsin, and Trypsin, Respectively, for 60 min

	Intact peptide (%)		
Peptide	Pancreas juice	Chymotrypsin	Trypsin
F314	95	100	54
CAT	100	100	100
F327	98	100	46

[a] Data presented as % intact peptide of initial amount determined by reversed phase HPLC analysis.

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