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Report

Rectal Absorption Enhancement of Des-Enkephalin-γ-Endorphin (DEγE) by Medium-Chain Glycerides and EDTA in Conscious Rats

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The stability of the neuroleptic peptide des-enkephalin-γ-endorphin (DEγE; Org 5878) in the rectal lumen and the rectal bioavailability of DEγE were investigated in conscious rats. Furthermore, the influence of peptidase inhibition, peptidase saturation, and absorption enhancement on DEγE bioavailability were evaluated. Na_2EDTA (0.25%, w/v) prolonged the degradation half-life of DEγE in the ligated colon from 33 ± 7 to 93 ± 45 min. Without adjuvant, tritium-labeled DEγE was absorbed from the rat rectum to a very low extent (0–4%). After administration of an excess of unlabeled DEγE or with Na_2EDTA, comparable results were obtained. The medium-chain glyceride preparation MGK markedly enhanced the rectal DEγE bioavailability, up to 8–20%, which was further increased to 10–44% by coadministration of Na_2EDTA. No substantial influence of varying the rectal delivery rate was observed. The results suggest that absorption enhancement and enzyme inhibition both are essential for effective increase of rectal peptide bioavailability.

KEY WORDS: absorption enhancement; neuropeptides; des-enkephalin-γ-endorphin; medium-chain glycerides; EDTA; peptidase inhibition.

INTRODUCTION

Research in pharmacology has led to the discovery of peptides with pharmacological activity (1,2). Because of recent advances in peptide chemistry and biotechnology, relatively large amounts of these peptides have become available and are currently under investigation as potential therapeutic agents. Successful application of peptides in human pharmacotherapy is, however, determined not only by their pharmacological action per se, but also by their ability to reach the site of action. This means that the pharmaceutical formulation, route of administration, and presystemic and systemic degradation are important issues to be considered. From a practical point of view, oral administration is the route of first choice. Unfortunately, this is unsuitable for delivery of peptides and proteins, because of rapid and extensive breakdown in the gastrointestinal (GI) tract by peptidases and because of poor transport of peptides containing more than three amino acids across the mucosa (3–5). Consequently, the oral bioavailability of oligopeptides is generally less than 5% (6). Much attention is therefore now being directed toward alternative delivery routes for peptides and proteins, e.g., nasal, rectal, buccal, and vaginal administration (4). Although rectal delivery is poorly accepted in several countries, it may potentially be an important route for peptide administration. The rectal cavity is easily accessible (7), and the high local drug concentration gives rise to a high driving force for absorption. Additional advantages are the avoidance of first-pass elimination (8) and the relatively low enzymatic activity (4,9). This has been reported to be advantageous for the delivery of tetragastrin (10) and Gly^1-α^{1-18} ACTH amide (11). In spite of these favorable circumstances, the rectal bioavailability of peptide drugs is generally still very low, e.g., less than 1% for the nonapeptide leuprolide in women (12), 5–16% for the tetrapeptide tetragastrin in rats (10), and about 1% for the tripeptide thyrotropin-releasing hormone (TRH) in humans (13). For the polypeptide insulin a pharmacological availability of 5.3% was observed, using suppositories in rabbits (14).

The reported poor rectal bioavailability of peptide drugs is probably caused by poor mucosal transport and by presystemic elimination. Attempts are therefore made to enhance their absorption by coadministration with absorption promoting agents. Surfactants, for instance, proved to enhance rectal uptake of interferon (15), Gly^1-α^{1-18} ACTH amide (11) and leuprolide (16) in rats. Sodium salicylate in mineral oil appeared to enhance the colonic bioavailability of methionyl-human growth hormone in rats from 0.2 to 9.5% (17). The rectal bioavailability of [$Asu^{1,7}$]-eel calcitonin from polyacrylic acid aqueous gel base in rats amounted to 0.8%, relative to i.m. delivery (18). In dogs the rectal bioavailability of insulin was enhanced to 18.6–43.7%, compared to i.m.

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injection, by DL-phenylalanine-ethylacetoacetate, which proved to be a more potent enhancer than polyoxyethylene-9-laurylether (19). In the same species the rectal uptake of insulin proved to be promoted by the medium-chain glyceride preparation MGK (20).

In spite of coadministration with absorption enhancers, the rectal bioavailability of peptide drugs is still relatively low. Effective presystemic breakdown, e.g., in intestinal lumen and in the mucosal membranes, probably prevents high bioavailability values. Thus it is conceivable that additional coadministration of peptidase inhibitors could inhibit degradation in the lumen and gut wall and enable a larger amount of intact peptide to be absorbed. This should result in enhanced bioavailability, provided that other routes of presystemic metabolism, such as hepatic first-pass elimination and metabolism in the absorbing cell and in blood, are not dominant.

The objective of the present study was to evaluate the influence of the metalloenzyme inhibitor EDTA (21) on the luminal breakdown of the neuroleptic dodecapeptide des-enkephalin-γ-endorphin (DEγE) in the rat rectum, as well as to investigate the enhancing effect of the absorption promoter MGK (20,22) and EDTA on the rectal bioavailability of DEγE in rats. Due to the time-consuming assay of DEγE, several experiments were performed as pilot studies in a limited number of rats, in order to detect major trends.

MATERIALS AND METHODS

Peptides, Chemicals, and Drug Solutions

DEγE (Org 5878) and [^{3}H-Lys^9]DEγE were generously supplied by Organon, Oss, The Netherlands. Three batches of the tritium-labeled product were used, their specific activities ranging from 41 to 45 Ci/mmol. MGK was a gift from Nikko Chemicals Co., Ltd., Tokyo, and contained glycerol (8%, w/v), octanoic acid (3%, w/v), glyceryl monooctanoate (57%, w/v), glyceryl dioctanoate (29%, w/v), and glyceryl trioctanoate (3%, w/v). Phenol red was obtained from Merck, Darmstadt, F.R.G. EDTA disodium salt (2 aq) was supplied by J. T. Baker, Deventer, The Netherlands. All other reagents used were of analytical grade.

Stability Studies. For stability studies in the ligated rectum solutions were used, containing DEγE (0.04%, w/v) in 154 m*M* KCl solution, with or without Na_2EDTA (0.25%, w/v). To correct for water absorption all solutions contained phenol red (0.028%, w/v) as unabsorbable internal standard (23).

Bioavailability Studies. For i.v. administration a solution containing [^{3}H-Lys^9]DEγE (1 mCi/ml; 30 μg/ml) in saline was used. For rectal delivery without adjuvant a solution of [^{3}H-Lys^9]DEγE containing 3 mCi/ml (90 μg/ml) in water was used. Solutions with adjuvant also contained 93% (v/v) MGK, unlabeled DEγE (0.8%, w/v), Na_2EDTA (0.25%, w/v), or combinations of these adjuvants.

Animals

Male Wistar rats of laboratory breed were used, weighing 175 to 200 g. The rats were deprived of food for 16 hr prior to the experiments. Water was provided ad libitum.

Drug Administration and Sampling

Stability Studies. Under light ether anesthesia a stainless-steel rectal delivery device (24) was ligated in the rectum. After laparotomy the rectum was ligated at a distance of 5 cm from the anus. Then the abdomen was closed by suturing, and a recovery period of 2 hr was allowed. Through the rectal delivery device 500 μl of solution was introduced as a bolus into the ligated lumen of the freely moving rat.

At regular intervals after administration 50-μl samples of drug solution were drawn from the ligated rectum of the freely moving animals through the delivery system. Samples were collected in polypropylene vials, containing 50 μl of ice-cold water, and were stored immediately at −20°C until analysis.

Bioavailability Studies. For i.v. infusion a polyvinylchloride cannula (length, 125 cm; i.d., 0.5 mm; o.d., 1.0 mm; Talas, Ommen, The Netherlands), filled with drug solution, was introduced into the right jugular vein under light ether anesthesia. For rectal administration a polyvinylchloride cannula filled with drug solution was connected to the rectal delivery device (24). Under light ether anesthesia the device was ligated in the rectum, allowing administration in the rectum at a distance of 2 mm from the anus. For blood sampling, a polyvinylchloride cannula (length, 50 cm; i.d., 0.5 mm; o.d., 1.0 mm), filled with heparin in saline (200 IU/ml), was introduced into the right carotid artery. After insertion the cannulas were pulled s.c., emerging on the nape of the neck, and were suspended above the animal, allowing free movement. After a recovery period of 2 hr the cannula containing drug solution was connected to an infusion pump (Braun, Melsungen, F.R.G.) and 200 μl of solution was delivered by bolus delivery in 24 sec or by zero-order infusion in 32 min.

Blood samples of 100 or 200 μl were taken at regular intervals after starting drug delivery into the jugular vein or into the rectum, respectively. After each sampling the cannula was filled with heparinized saline to prevent clotting. Blood samples were immediately precipitated in 2 ml of chilled trichloroacetic acid (4%, w/v), containing 20 μg of unlabeled DEγE as internal standard (25). Following centrifugation for 10 min at 1500*g*, the samples were stored at 4°C until extraction, which was performed the next day.

Drug Assay and Data Analysis

Stability Studies. Fifteen to forty microliters of each collected sample was injected on a liquid chromatographic system, consisting of a Sykam S 1000 solvent delivery system (Sykam, Gauting, F.R.G.), a Sykam S 2110 low-pressure gradient mixer, a Sykam S 2000 HPLC controller, a Rheodyne 7125 injector (Chrompack, Middelburg, The Netherlands), an Ultrasphere-5 ODS column (0.46 × 25 cm; Beckman Instruments, Berkeley, Calif.), a Shimadzu SPD-2A spectrophotometric detector (Shimadzu, Kyoto, Japan), operating at 210 nm, and a BD 40 pen recorder (Kipp & Zonen, Delft, The Netherlands). The mobile phase consisted of 0.125 *M* NaH_2PO_4 with 0.0125 *M* sodium octanesulfonate in water, adjusted to pH 2.1 using concentrated H_3PO_4 (A) and acetonitrile–water (2:1, v/v) (B). A:B was maintained at 62:38 (v/v) during the elution (26). Solvents were degassed continuously using helium (Hoekloos, Schiedam, The Neth-

erlands). A flow rate of 1 ml/min was used. The height of the recorded peaks of DEγE and phenol red was measured manually.

The peak height ratio of DEγE versus phenol red was followed in time and the half-life of DEγE disappearance was calculated using ordinary least-squares regression analysis.

Bioavailability Studies. [^{3}H-Lys^9]DEγE in trichloroacetic acid extracts was assayed according to Verhoef and van den Wildenberg (25). Collected supernatant was purified on Sep-Pak cartridges (Millipore Waters, Millford, Conn.), prewashed with 5 ml of methanol, 5 ml of distilled water, and 5 ml of 0.01 *M* NH_4OAc (pH 4.2 using glacial acetic acid), consecutively. Interfering compounds were eluted with 4 ml of 0.01 *M* NH_4OAc (pH 4.2 using glacial acetic acid) and with 3 ml of 0.01 *M* NH_4OAc:CH_3OH (7:3, v/v), respectively. Finally, [^{3}H-Lys^9]DEγE and DEγE were eluted with 3 ml of 0.01 *M* NH_4OAc:CH_3OH (4:6, v/v). Fractions containing labeled and unlabeled DEγE were evaporated to dryness at 50°C under a stream of nitrogen and were stored at −20°C until HPLC analysis.

Before analysis, the residue was dissolved in 100 μl of distilled water, and 75 μl was injected on the liquid chromatographic system as used for the stability studies. [^{3}H-Lys^9]DEγE-containing fractions of 500 μl were collected in counting minivials under the guidance of the recorded peak of unlabeled DEγE. Three milliliters of Emulsifier Scintillator 299 (United Technologies Packard, Downers Grove, Ill.) was added and radioactivity was measured using a scintillation counter (Packard Tri-Carb 4640, United Technologies Packard). After summation of the radioactivities of the individual fractions, the amount of eluted [^{3}H-Lys^9]DEγE thus obtained was corrected by the external standard method, using the peak height of unlabeled DEγE as reference. Calibration was performed with known amounts of DEγE.

The area under the individual [^{3}H-Lys^9]DEγE blood concentration–time curve (AUC) was calculated from $t = 0$ to maximal concentration with the linear trapezoidal rule and, after maximal concentration, to the last sampling point using the logarithmic trapezoidal rule (27). Intravenous curves were extrapolated using the individual elimination rate constants. Curves obtained after rectal delivery were extrapolated using the mean elimination rate constant of i.v. infusion, because of an irregular pattern of the apparent elimination phase on rectal administration. Systemic clearance of [^{3}H-Lys^9]DEγE was calculated as 100/AUC, AUC being the total area under the curve (% dose · min/ml). Bioavailabilities were calculated as $AUC_{rect}/AUC_{iv,mean}$, AUC_{rect} referring to the AUC after rectal delivery and $AUC_{iv,mean}$ referring to the mean AUC after i.v. infusion.

RESULTS

Intact DEγE disappeared from the ligated rectal lumen with a mean half-life of 33 ± 7 min (SD). Coadministration of 0.25% (w/v) Na_2EDTA prolonged the half-life of DEγE to 93 ± 45 min (Fig. 1).

A mean systemic clearance of 60 ± 39 ml/min and a mean elimination half life of 1.2 ± 0.6 min were found after i.v. infusion of labeled DEγE (Fig. 2). Rectal delivery of

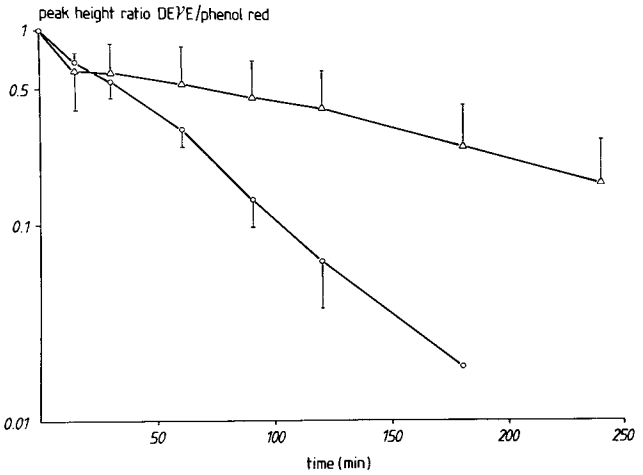

Fig. 1. Mean peak height ratios of DEγE versus phenol red in rectal luminal contents after bolus administration of 500 μl of a solution containing DEγE (0.04%, w/v) and phenol red (0.028%, w/v) without (○; $N = 6$) and with (△; $N = 6$) Na_2EDTA (0.25%, w/v) into a ligated section of rat colon.

labeled DEγE without adjuvant gave rise to very low blood concentrations (Fig. 3) and AUC values (Fig. 4) of the intact labeled compound, corresponding to bioavailabilities of 0 to 4%. Bolus delivery with an excess of unlabeled DEγE or rectal infusion with EDTA resulted in comparably low AUC values (Fig. 4) and bioavailabilities of 2–7 and 0–1%, respectively. An increase in bioavailability was seen after infusion as well as after bolus delivery with MGK, resulting in values of 8 to 20% (Figs. 3 and 4). Bolus delivery of [^{3}H-Lys^9]DEγE with a combination of MGK and unlabeled DEγE gave rise to bioavailabilities comparable to those seen with coadministration of MGK alone (Fig. 4). Combination of labeled DEγE with MGK and EDTA caused a strong increase in blood concentrations, with bioavailability values of 10 to 44% (Figs. 3 and 4).

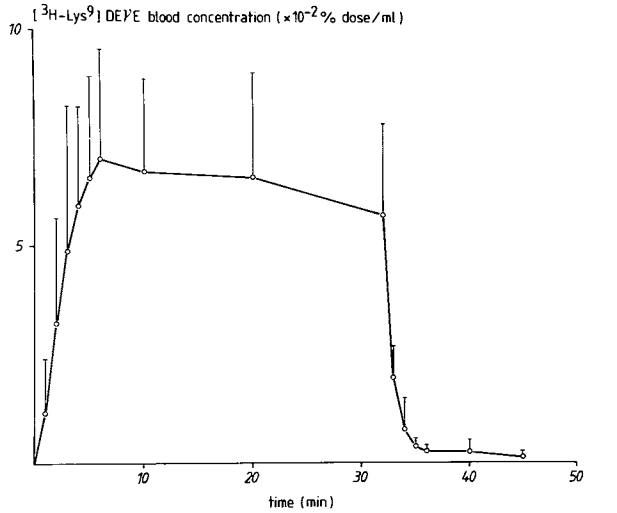

Fig. 2. Mean blood concentrations of [^{3}H-Lys^9]DEγE ± SD after i.v. infusion of 200 μCi (6 μg) of [^{3}H-Lys^9]DEγE in conscious rats ($N = 6$).

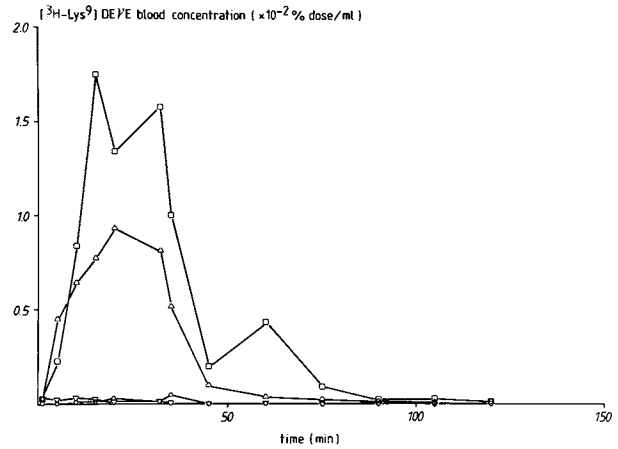

Fig. 3. Mean blood concentrations of [^{3}H-Lys^9]DEγE after rectal infusion of 600 μCi (18 μg) of [^{3}H-Lys^9]DEγE without adjuvant (○; N = 4), with Na_2EDTA (0.25%, w/v; ▽; N = 2), with MGK (93%, v/v; △; N = 2), and with MGK + Na_2EDTA (□; N = 5).

DISCUSSION

The relatively high bioavailability of 10 to 44% following rectal delivery of [^{3}H-Lys^9]DEγE with the combination MGK/EDTA (Fig. 4) indicates that, in order to achieve substantial rectal bioavailability of a peptide drug, both absorption enhancement and enzyme inhibition are prerequisites. Effective enzymatic degradation prior to the absorption phase causes rapid disappearance of intact DEγE from the intestinal lumen, as indicated by the prolonged half-life of intact DEγE in the presence of the metallopeptidase inhibitor EDTA (Fig. 1). Although EDTA inhibited DEγE degradation in the ligated rectum, the systemic bioavailability of labeled DEγE, rectally delivered with EDTA, remained very low (Figs. 3 and 4). Apparently, DEγE is not able to pass the rectal mucosa if no absorption promoter is present. The mucosal penetration of DEγE proved to be enhanced by coadministration of MGK, EDTA increasing the effectiveness of

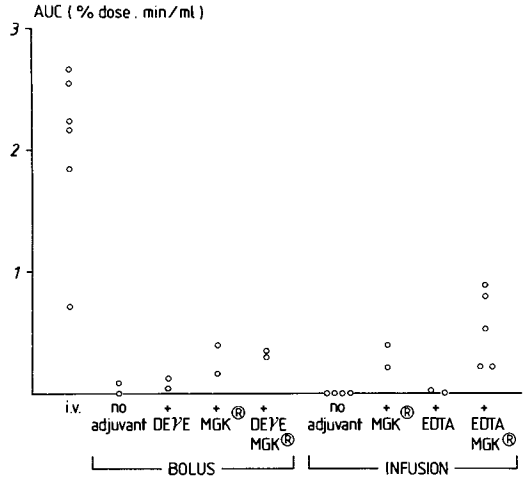

Fig. 4. Individual AUC values of intact [^{3}H-Lys^9]DEγE after i.v. infusion and after rectal administration as a bolus or as an infusion without any adjuvant, with unlabeled DEγE (0.8%, w/v), MGK (93%, v/v), or Na_2EDTA (0.25%, w/v) or with combinations of these adjuvants.

MGK by peptidase inhibition. A 90-fold excess of unlabeled DEγE appeared to be ineffective in further enhancing the bioavailability of bolus-delivered [^{3}H-Lys^9]DEγE in MGK (Fig. 4), probably indicating that this amount of peptide is not high enough to saturate local peptidases.

It cannot be excluded that the strong increase in bioavailability, as exhibited by the combination MGK/EDTA, is due to mutual synergistic effects of the two adjuvants on their absorption-promoting properties, apart from any effect on peptidase activity. Nevertheless, the results of the effects of EDTA on the half-life of DEγE in the rectal lumen suggest that, besides absorption enhancement, peptidase inhibition may play a role as well.

It has been shown that the rate of rectal delivery modifies the enhancing action of MGK on rectal cefazolin absorption (28). However, in the present study there were no indications for an important influence of delivery rate on DEγE absorption. Thus, it is conceivable that transmucosal passage and presystemic elimination outweigh delivery rate as important variables for DEγE bioavailability.

Further research will focus on the applicability of various peptidase inhibitors as well as on other absorption promoters for enhancing rectal peptide bioavailability.

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