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Rectal Absorption of Gly^{1}-α^{1-18} Adrenocorticotropin Amide

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Plasma corticosteroids level was determined after rectal administration of Gly^{1}-α^{1-18} ACTH amide to hypophysectomized rat. When the aqueous solution of the peptide was administered, the corticosteroidogenic response was detected in 5 min and reached its maximum in 15 to 30 min after the administration. The half maximum effective dose from dose response curve was estimated to be 40 μg/kg. The extent of absorption of the peptide was markedly influenced by suppository bases as well as the nonionic surfactants added to the bases. Polyoxyethylene(20)cetyl ether (BC-20TX) enhanced the absorption from both hydrophobic and hydrophilic bases while BC-7 stimulated that from only hydrophobic bases.

Keywords——Gly^{1}-α^{1-18} ACTH amide; hypophysectomized rat; rectal absorption; plasma corticosteroids; suppository base; nonionic surfactant

The advantages of rectal administration of drugs have been thoroughly documented by Lowenthal,[2] who emphasized the efficient absorption of readily metabolizable compounds that bypass the portal circulation. This is accomplished by absorption through the hemorrhoidal vein entering the general circulation rather than drainage to portal system. Controled absorption of drugs has also been demonstrated using suitable suppository bases of inclusion of surfactants.[3,4] Although rectal absorption of several drugs, such as analgesics, antispasmodics and local anesthetics, has been reported,[5,6] there is little information on peptides. It seemed therefore of value to investigate the uptake of a readily metabolizable peptide by the rectum and the effect of suppository bases or surfactants on its absorption. Gly^{1}-α^{1-18}-ACTH amide is the known shortest chain length peptide with corticotropic activity comparable to natural ACTH,[7] and since elimination of one amino acid residue resulted in marked reduction of the activity,[8] the entire structure is supporsed to be necessary for the activity. The present investigation was undertaken to study the absorption of Gly^{1}-α^{1-18}ACTH amide from rat rectum and the resultant change in plasma corticosteroids levels. The action of aqueous solution or suppositories of the peptide as well as the effect of nonionic surfactants on absorption was investigated.

Experimental

Chemicals——Gly^{1}-α^{1-18} ACTH amide was provided by Dr. Inouye of our laboratory. Porcine ACTH, grade II, was purchased from Sigma and purified by carboxy methyl cellulose (CMC) column chromatography. Polyoxyethylene(20)cetyl ether (BC-20TX), polyoxyethylene(7)cetyl ether (BC-7), and polyoxyethylene sorbitan mono-oleate (Tween 80) were obtained from Nikko Chem. Co. Tripalmitin and Tridecane were the products of Nippon Fine Chem. Co.

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Preparation of Suppositories——Suppositories were formulated to include 2.5 to 40 μg of Gly^1-α^{1-18} ACTH amide in a 100 mg suppository base by melting method. When cacao butter, triglyceride or polyethylene glycol (PEG) was used as the base, a quantity of Gly^1-α^{1-18} ACTH amide was thoroughly mixed with the molten mass of the base in a mortar then poured into a polyethylene tube of 3 mm diameter. After cooling on ice, the mold was removed and the mass was cut out to give a 100±2 mg cylinder. Two percent of nonionic surfactant was included, in some preparation, in the molten mass prior to mixing the peptide. The compositions of triglyceride and PEG suppository base are 9:1 mixture of tridecane and tripalmitin, and 2:3 mixture of PEG 6000 and PEG 1500, respectively. When glycerinated gelatin (gly. gelatin) suppository was prepared, Gly^1-α^{1-18} ACTH amide was dissolved in an aliquot of warmed glycerin (final concentration 70%), which was then mixed with gelatin solution at 70°. Surfactants were previously suspended in the gelatin. The suppositories thus obtained were stored overnight at 15° and, before administration, they were allowed to stand for 1 hour at 25° except for those made of cacao butter.

Administration of Gly^1-α^{1-18} ACTH Amide and Plasma Corticosteroids Assay——Hypophysectomized rat, fasting overnight, weighing 120—140 g were used. Under light anesthesia, a 0.1 ml aliquot of Gly^1-α^{1-18} ACTH amide in saline was administered to the rat rectum and the anus was closed with surgical adhesive, Aron Alpha A (Sankyo Co.). Before administration of the aqueous sample, a 3×5 mm paraffin cylinder slightly coated with cacao butter was inserted 2.5 cm into the rectum to prevent the sample from flowing up to the colon. At a predetermined time, blood samples (0.5 ml) were collected from the abdominal aorta, and concentration of 11-hydroxycorticosteroids (11-OHCS) in the plasma was determined by fluorometry, ext. at 475 nm and emit. at 525 nm.[9] The value shown in Table and Fig. is the mean of 5 rats and standard error (S.E.) was also calculated. It was noted that the plasma levels of 11-OHCS elicited by Gly^1-α^{1-18} ACTH amide varied daily to some extent due to the variation in sensitivity of the rats. However, the relative response to varying peptide concentration were comparable to each other and were reproducible.

***In Vitro* Dialysis of Suppository**——A cylindrical model suppository (7×22 mm), weighing 1.0 g and containing 1.0 mg of Gly^1-α^{1-18} ACTH amide, was prepared as described above. The sample was put into a visking tube, both ends were tied, then immersed into 20 ml saline at 37° with stirring. At 30 min intervals, 4 ml aliquots were pipetted out, and 4 ml saline was supplemented. The amount of Gly^1-α^{1-18} ACTH amide passed through the membrane into the saline was analyzed by fluorometry, ext. at 295 nm and emit. at 350 nm. The blank value which was obtained with a preparation not containing the peptide was small and was substracted.

Incubation of Gly^1-α^{1-18} ACTH Amide with Isolated Rectum——A rectum of hypophysectomized 240 g male rat, fasting overnight, was isolated, washed with Ringer solution, ligated at one end and 2.5 mg of Gly^1-α^{1-18} ACTH amide in 0.25 ml Ringer was introduced. The other end was ligated, then it was immersed in 10 ml Ringer at 37°. After incubation for 30 min, the inner fluid was poured out and the inside was rinsed with 0.25 ml of Ringer. The experiment was duplicated and all of the fluid obtained was combined, then 0.9 ml aliquot was applied on a Sephadex G-25 column (1.2×50 cm) eluted with 1 N AcOH. The optical density at 280 nm, fluorescence, and fluorescence produced by reaction with fluorescamine (ext. 365 nm, emit. 470 nm) of the eluate, 4 ml per tube, was analyzed. As a reference, a segment (10 cm) of rat small intestine was isolated, washed ligated at one end, incubated with 5 mg of Gly^1-α^{1-18} ACTH amide in 0.5 ml Ringer at 37° for 30 min. The content and wash was analyzed in the same way. Aliquots of the fractionated eluate from the column were also used for the determination of corticotropic activity.

Results

Absorption of Gly^1-α^{1-18} ACTH Amide Aqueous Solution

Rectal absorption of Gly^1-α^{1-18}ACTH amide was examined by the adrenal stimulation of hypophysectomized male rats. As shown in Table I, uptake of the peptide started within 5 min and maximum stimulation was attained approximately 20 min after administration of 7 μg of Gly^1-α^{1-18}ACTH amide. Fig. 1 shows a dose response curve 30 min after the administration, where the half maximum effective dose of the peptide was found to be about 40 μg/kg. Incubation of Gly^1-α^{1-18}ACTH amide with isolated rectum and following purification by Sephadex G-25 column chromatography revealed that more than 90% of the peptide remained unchanged after 30 min of incubation and a little amount of small molecules supporsed to be oligopeptides were accumulated. On the other hand, as much as 5 mg of Gly^1-α^{1-18}ACTH amide was hydrolyzed to oligopeptides and amino acids within the ligated small

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Table I. Plasma 11-OHCS Level after Rectal Administration of 7 μg Gly^1-α^{1-18}ACTH Amide in Aqueous Media

Time after administration (min)	Plasma 11-OHCS (μg/100 ml)
0	4.1± 0.8
5	10.6± 3.2
10	25.7± 5.9
15	62.4± 3.7
30	66.6±14.0
60	33.8± 4.2
90	15.4± 5.1
120	4.7± 1.3

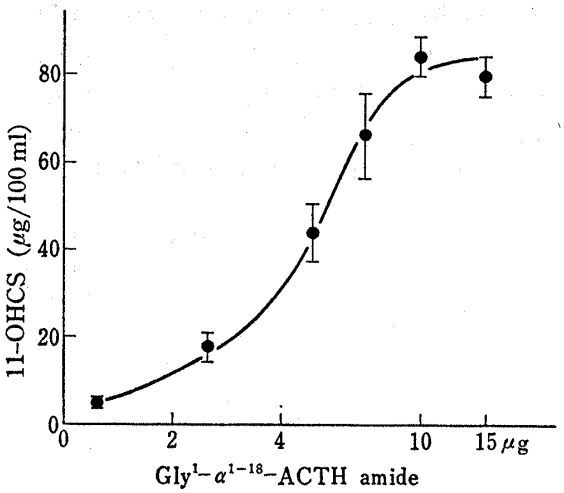

Fig. 1. Dose Response Curve of rectally administered Gly^1-α^{1-18}ACTH Amide

Various amounts of Gly^1-α^{1-18} ACTH amide in 0.1 ml saline was administered to rat rectum and plasma 11-OHCS level 30 min after the administration was determined.

intestine. Therefore, although the extent of digestion of the peptide in rectum was much smaller than that of the intestine, the enzymic degradation of rectally administered Gly^1-α^{1-18}ACTH amide at the locus can not be ruled out.

Administration of Gly^1-α^{1-18} ACTH Amide Suppositories

Samples were prepared with hydrophobic suppository bases; cacao butter, triglyceride or hydrophilic bases; PEG, gly·gelatin. The former bases melt at body temperature, while the latter dissolve into body fluid; the mechanism of drug release is substantially different. The data shown in Table II indicate that absorption of the peptide is significantly influenced

Table II. Plasma 11-OHCS Level (μg/100 ml) 30 min after Administration of Gly^1-α^{1-18}ACTH Amide in Various Suppository Bases

Suppository base	Gly^1-α^{1-18}ACTH amide (μg)			
	5	10	20	40
Cacao butter		26.1±13.3	81.0±4.9	
Triglyceride			3.4±0.8	27.5±11.5
PEG			16.8±6.7	30.0±15.1
Gly·gelatin	79.2±4.5	78.4±11.0		

by the type of suppository base. The dose response curve with 2.5—30 μg peptide in gly·gelatin, the most effective suppository base, was similar to that of aqueous solution, and the half maximum effective dose was estimated to be 40 μg/kg (data not shown). Although the order of biological efficacy of suppository was gly·gelatin, cacao butter, PEG, and triglyceride, it seemed to be inconsistent with the apparent melting or dissolution properties. When the model suppositories in the visking tubes were immersed in saline at 37°, those of cacao butter and triglyceride melted within 5 and 10 min, respectively, while complete dissolution of PEG or gly·gelatin required about 30 min. An attempt to compare the release rate of Gly^1-α^{1-18}-ACTH amide from suppositories by means of visking tube failed because gelatin interacted with the membrane to prevent the passage of the peptide. Since gly·gelatin suppository contained as much as 70% of glycerin, the possibility of direct participation of glycerin in the peptide absorption was anticipated. However, addition of 70% of glycerin to Gly^1-α^{1-18}ACTH amide in saline solution (7 μg/0.1 ml) did not produce any effect on its absorption.

Effect of Surfactant on Absorption of Gly^1-α^{1-18} ACTH Amide

The effect of 2% of anionic surfactant, BC–20TX (H.L.B. 13.9) and BC–7 (H.L.B. 8.8), on absorption of the peptide from various suppository bases was examined. The experimental procedures were as described in Table II, except that the plasma 11-OHCS level was determined 60 min after the administration of suppositories. As shown in Table III, BC–20TX

Table III. Plasma 11-OHCS Level (μg/100 ml) 60 min after Administration of Gly^1-α^{1-18}ACTH Amide in Various Suppository Bases with or without 2% Surfactant

Surfactant	Suppository base			
	Cacao butter[a)]	Triglyceride[b)]	PEG[c)]	Gly·gelatin[d)]
—	7.9±2.4	5.3±1.3	18.7± 5.7	22.4± 8.8
BC-20TX	77.8±2.4	81.3±5.3	68.9±11.6	72.4± 6.0
—	4.4±3.5	3.4±1.2	15.7± 5.4	25.0±12.8
BC-7	60·0±3.6	81.1±3.5	16.0± 3.5	25.3±11.2

The amount of Gly^1-α^{1-18} ACTH amide in suppository was *a*) 10 μg, *b*) 30 μg, *c*) 20 μg and *d*) 5 μg.

enhanced the absorption significantly both with hydrophobic and hydrophilic bases. BC–7, on the other hand, stimulated only with hydrophobic ones. The effect of BC–20TX on dissolution rate of hydrophilic bases was little judging from the visking tube method experiment, however, much faster emulsification of the bases by the surfactant was clearly found: the emulsion would be penetrate the rectal membrane more easily. It was also assumed that BC–20TX or Tween 80 had some direct effect on the membrane as well as the diffusion promotion of the peptide in the suppository base. To clarify this possibility, the effect of the surfactants on rectal absorption of the peptide from aqueous media was examined. When 5 μg of Gly^1-α^{1-18}ACTH amide in saline was administered, the plasma 11-OHCS level after 30 min was 25.2±3.8, while the value was increased to 45.2±3.9 or 50.2±6.7 upon addition of 2% BC–20TX or Tween 80, respectively. Brief microscopic investigation with 30-fold magnification revealed that the surface of the rectum was unaffected by the treatment with 10% BC–20TX.

Discussion

Absorption of macromolecules such as trypsin or chymotrypsin by the gastric tract has been extensively investigated.[10,11] Although absorption of such large peptides were confirmed both *in vivo* and *in vitro*, the permeation of intact macromolecule through the membrane is rather ambiguous. Recently, enzymic digestion of D-Ser, Lys^{17,18}-α^{1-18}ACTH amide in small intestine was reported in detail.[12] Since adrenal stimulation of Gly^1-α^{1-18}ACTH amide requires the entire molecule,[8] the quick corticotropic response after rectal administration of the peptide proved in the present study indicates the absorption of intact peptide from rat rectum. The concept was supported by the finding of weak peptidase activity in the locus. We also examined the rectal absorption of porcine ACTH, the entity of which is more than double that of Gly^1-α^{1-18}ACTH amide, and found that porcine ACTH was as effective as Gly^1-α^{1-18}ACTH amide. The half maximum effective dose of Gly^1-α^{1-18}ACTH amide was about 40 μg/kg, the value was approximately 100 times that obtained by intraveous injection of the peptide. However, it was noted that the half life of the corticosteroidogenesis by rectal

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administration was about 30 min which was 10- to 15-fold longer than that of intravenous injection. The bioavailability of rectally administered Gly^{1}-α^{1-18}ACTH amide was strongly influenced by the surfactants added to suppository bases as well as the variety of the bases. BC–20TX enhanced the absorption both with hydrophobic and hydrophilic bases, while BC–7 showed the stimulatory effect on hydrophobic bases. BC–20TX, the H.L.B. of which is 13.9, is supporsed to act directly on the membrane as well as to emulsify the hydrophobic bases to facilitate the absorption. Although Nissin reported that nonionic surfactants did not produce any damage to the gastrointestinal mucosa while anionic surfactants produced severe haemorrhage,[13] further investigation is necessary to elucidate the stimulatory effect of nonionic surfactants on absorption of the peptide.

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