

Effect of enkephalin and naloxone on gastric acid and serum gastrin and pancreatic polypeptide concentrations in humans

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SUMMARY The effects of a synthetic enkephalin analogue with prolonged opioid activity, D-ala-2-enkephalin (ala-enk) and naloxone given alone or in combination, on vagally, pentagastrin- and histamine-induced gastric secretion and plasma hormonal responses to vagal stimulation have been studied in healthy subjects. D-ala-2-enkephalin reduced basal gastric acid and pepsin secretion, and caused a dose-dependent inhibition of gastric secretory responses to modified sham-feeding and pentagastrin but not to histamine. It increased serum gastrin concentration and suppressed plasma pancreatic polypeptide response to modified sham-feeding. Naloxone alone at lower dose levels did not affect gastric secretion and plasma hormonal concentrations but at higher doses it reduced both basal and modified sham-feeding-induced secretion. When combined with ala-enk it reversed in part gastric secretory and plasma hormonal changes induced by this peptide during modified sham-feeding and pentagastrin stimulation. These results indicate that (1) stable enkephalin analogue inhibits basal and vagally or pentagastrin-induced gastric secretion, and affects plasma hormonal response to vagal stimulation, at least in part, *via* activation of opioid receptors and (2) endogenous opioid substances may be involved in the stimulation of gastric secretion in man.

Previous studies have shown the presence of immunoreactive counterparts or enkephalin throughout the gastrointestinal tissue, especially in the antrum and duodenum.^{1,2} Methionine-enkephalin analogue (FK 33-824) given in a small dose was found to increase pentagastrin-induced gastric acid secretion³ and the blockade of opioid receptors with the narcotic antagonist agent naloxone was reported to decrease both basal and meal stimulated secretion in humans.⁴ These results suggest that endogenous opioids may be involved in the stimulation of gastric secretion in man.

On the other hand, morphine⁴ and potent peripheral opioid agonists such as loperamide⁵ were reported to inhibit basal or submaximal pentagastrin and meal-stimulated gastric acid secretion. These results could be explained by the multiplicity and heterogeneity of opioid receptors⁶ or that morphine

and other opioid agonists have actions other than opioid receptor stimulation such as the inhibition of acetylcholine release from cholinergic endings in the stomach.⁷⁻⁹ This anticholinergic effect could account for the inhibition of vagally-induced gastric secretion observed in dogs,⁸⁻¹⁰ but not examined so far in man.

This study was designed to compare the effects of the stable enkephalin analogue, D-ala-2-enkephalin (ala-enk), and naloxone given alone or in combination on basal, vagally, pentagastrin- and histamine-induced gastric secretion and on basal and vagally stimulated plasma gastrin and pancreatic polypeptide concentrations in healthy subjects.

Methods

SUBJECTS

This study was performed on 18 male volunteers who gave informed consent. All subjects were in good physical health and did not complain of any

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gastrointestinal problems. Their average age was 21 years (range 19–25 years) and weight 67 kg (range 57–79 kg).

Each study was performed after an overnight fast. A nasogastric tube was first positioned under fluoroscopic control with the tip in the distal antrum. Residual gastric contents were discarded and then gastric aspiration was started using a vacuum pump at a negative pressure of about 20 mm Hg. The suction was interrupted every two minutes and air was injected down the tube to ensure its constant patency. Gastric juice was collected in 15 minute samples, the volume of gastric aspirate was recorded and the acid and pepsin contents were determined as described previously.¹¹

EXPERIMENTAL DESIGN

Several series of secretory tests were performed using modified sham-feeding alone, combined with intravenous infusion of ala-enk or naloxone, or combined with naloxone plus ala-enk. In addition, the secretory tests using pentagastrin or histamine alone or in combination with ala-enk and naloxone were performed.

Modified sham-feeding was performed during a 15 minute period using an appetising meal as described previously.¹² The meal was prepared in a separate building so that the subjects could not see or smell the food until the time of sham-feeding. Each subject was trained in preliminary study not to swallow food. In addition, phenol red was added to the meal used in sham-feeding and none was found in the gastric aspirates. The infusion of naloxone, ala-enk or in combination was started after collection or two 15 minute basal samples and 30 minutes before the beginning of modified sham-feeding and continued during and after this procedure (Fig. 1). Naloxone (Narcan, Endo Laboratories, Inc, Garden City, NY) was given in a dose of 40 $\mu\text{g}/\text{kg}/\text{h}$ and ala-enk in a dose of 20 $\mu\text{g}/\text{kg}/\text{h}$. In four subjects, dose response studies with ala-enk or naloxone were performed on a separate day, both basal and modified sham-feeding-induced secretion being measured without and with doses of ala-enk 2.5, 5, and 10 $\mu\text{g}/\text{kg}/\text{h}$ or doses of naloxone 20, 40 and 80 $\mu\text{g}/\text{kg}/\text{h}$. For the comparison, maximal acid output in response to pentagastrin (2 $\mu\text{g}/\text{kg}/\text{h}$) was obtained in each subject. The sum of the two highest consecutive 15 minute outputs was used to express the maximal response to pentagastrin.

In another series of tests performed in six subjects, pentagastrin (2 $\mu\text{g}/\text{kg}/\text{h}$) or histamine dihydrochloride (20 $\mu\text{g}/\text{kg}/\text{h}$) was infused intravenously for 180 minutes. When the secretory rate reached a well sustained plateau, ala-enk (20 $\mu\text{g}/\text{kg}/\text{h}$) or naloxone (40 $\mu\text{g}/\text{kg}/\text{h}$) was added for 60

minutes. In control tests, pentagastrin or histamine alone, without ala-enk or naloxone, was given for the duration of the experiment. Acid and pepsin outputs during the last 30 minute period of the infusion of ala-enk, naloxone or their combination and the corresponding period when pentagastrin or histamine alone (control) was administered were used for the comparison.

Drugs used in this study were administered alone or in combination in a random order on separate test days. The doses were based on our previous study on dogs. Ala-enk was synthesised by solid phase method and used in the pure form.

HORMONE MEASUREMENT

In all tests, except those with pentagastrin and histamine, venous blood samples were obtained from a peripheral vein for measurement of serum gastrin and plasma pancreatic polypeptide concentrations. The hormone concentrations were measured by radioimmunoassay according to the methods described previously.^{11 12}

STATISTICAL EVALUATION OF RESULTS

Results are expressed as the mean \pm SEM. Differences in mean values were tested for significance by paired *t* test and *p* values of <0.05 were considered statistically significant.

Results

In control tests with modified sham-feeding, the peak acid outputs occurred in the second and third 15 minute periods after the beginning of sham-feeding and reached about 55% of maximum pentagastrin stimulated acid output. The mean pepsin output attained about 82% of the pentagastrin maximum. Ala-enk resulted in a significant reduction in basal and modified sham-feeding-induced acid and pepsin outputs. The mean 30 minute peak acid response to modified sham-feeding in tests with ala-enk at a dose of 20 $\mu\text{g}/\text{kg}/\text{h}$ was only about 27% of pentagastrin maximum and only about half of that attained by modified sham-feeding alone. This reduction in acid response to modified sham-feeding by ala-enk was observed throughout the modified sham-feeding tests and at the end of the experiment acid secretion was only about 50% of that observed in control experiments. Pepsin secretion in tests with ala-enk fell similarly as acid secretion and was roughly about 60% of that recorded in control tests with modified sham-feeding alone (Figs 1 and 2). Smaller doses of ala-enk given to four subjects resulted in less pronounced inhibitory effects on acid output. The per cent reduction in the initial 30 minute peak acid

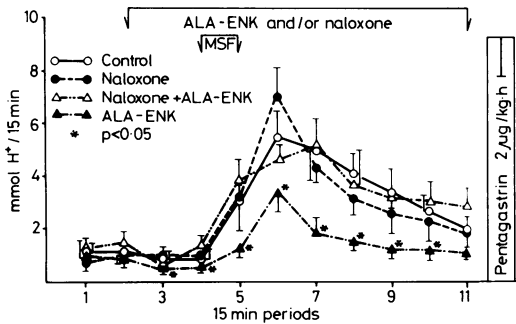


Fig. 1 Effects of ala-enk, naloxone or their combination on basal and MSF-induced gastric acid secretion in eight healthy subjects.

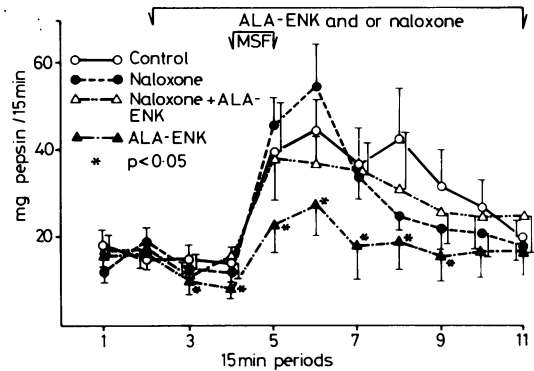


Fig. 2 Pepsin outputs in tests as in Fig. 1.

responses to modified sham-feeding by doses of 2.5, 5.0, and 10.0 $\mu\text{g}/\text{kg}/\text{h}$ ala-enk was about 15, 37, and 45%, respectively (Table 1).

Naloxone given in a dose of 20 or 40 $\mu\text{g}/\text{kg}/\text{h}$ before, during and after the modified sham-feeding did not significantly affect basal or modified sham-feeding-induced acid or pepsin secretion. When administered in a larger dose (80 $\mu\text{g}/\text{kg}/\text{h}$), naloxone produced significant reduction in both basal and modified sham-feeding-induced gastric secretion (Table 2). Naloxone (40 $\mu\text{g}/\text{kg}/\text{h}$) combined with ala-enk prevented almost completely the fall in acid and pepsin secretion under basal conditions and in response to modified sham-feeding.

Serum or plasma hormonal changes in response to modified sham-feeding alone or in combination with ala-enk plus naloxone are shown on Table 3. Basal serum gastrin was 31 ± 7 pmol/l and modified sham-feeding caused a slight and statistically insignificant rise in serum gastrin. Ala-enk resulted in a small but

significant increase in basal serum gastrin from 28 ± 5 to 37 ± 4 pmol/l but again modified sham-feeding did not result in any further significant rise in serum gastrin. Naloxone alone had no effect on basal or modified sham-feeding stimulated serum gastrin concentration but when combined with ala-enk it prevented the increase in serum gastrin observed in tests with ala-enk alone. Basal pancreatic polypeptide concentration was 29 ± 8 pmol/l and after modified sham-feeding it rapidly and significantly rose to a peak of 67 ± 11 pmol/l occurring in the second 15 minute period after the start of modified sham-feeding and then decreased to a level not significantly different from the control value. Ala-enk significantly reduced the pancreatic polypeptide response to modified sham-feeding and the combination with naloxone rendered a pancreatic polypeptide response to modified sham-feeding similar to that observed in control experiments. Naloxone alone did not affect the plasma pancreatic

Table 1 Effects of various doses of ala-enk on basal and peak gastric acid and pepsin outputs in response to modified sham-feeding (MSF) in four healthy subjects

Dosage of ala-enk ($\mu\text{g}/\text{kg}/\text{h}$)	Basal		MSF	
	H^+ mmol/30 min	Pepsin mg/30 min	H^+ mmol/30 min	Pepsin mg/30 min
0	2.63 ± 0.35	39.71 ± 8.21	12.72 ± 2.18	93.52 ± 19.33
2.5	2.15 ± 0.45	34.81 ± 8.18	10.15 ± 1.93	65.32 ± 12.14
5.0	1.82 ± 0.41	35.21 ± 7.32	7.45* ± 1.05	59.35* ± 9.13
10.0	1.52* ± 0.23	27.31* ± 4.22	6.51* ± 0.88	48.22* ± 7.18

* Significance ($p < 0.05$) decrease below control level.

Table 2 Effects of various doses of naloxone on basal and peak gastric acid and pepsin outputs in response to MSF in four healthy subjects

Dosage of naloxone ($\mu\text{g}/\text{kg}/\text{h}$)	Basal		MSF	
	H^+ mmol/30 min	Pepsin mg/30 min	H^+ mmol/30 min	Pepsin mg/30 min
0	2.18 ± 0.24	36.84 ± 9.42	14.38 ± 3.10	108.34 ± 24.66
20	1.94 ± 0.32	34.12 ± 8.46	13.62 ± 4.11	96.24 ± 18.34
40	1.88 ± 0.26	36.62 ± 4.12	12.84 ± 3.42	90.30 ± 17.12
80	1.49* ± 0.31	26.12* ± 2.14	9.17* ± 2.02	78.62 ± 16.33

* Significance ($p < 0.05$) decrease below control level.

Table 3 Serum gastrin and plasma PP concentrations (Mean + SEM) under basal state and in response to MSF in eight subjects with and without ala-enk, naloxone or ala-enk + naloxone

	Serum gastrin (pmol/l)							Plasma PP (pmol/l)						
	Time (min)							Time (min)						
	B	-30	-15	+15†	+30	+60	+90	B	-30	-15	+15†	+30	+60	+90
Control	31	29	31	35	36	30	29	29	31	34	67	61	52	42
MSF alone	± 7	± 5	± 6	± 7	± 7	± 4	± 5	± 8	± 6	± 6	± 11	± 9	± 7	± 9
MSF + ala-enk	28	39	49*	49*	50*	53*	47*	31	33	24*	38*	32*	30*	29
	± 5	± 7	± 4	± 5	± 8	± 7	± 5	± 7	± 6	± 4	± 6	± 5	± 6	± 7
MSF + naloxone	32	29	30	36	34	31	36	34	35	33	75	74	63	45
	± 6	± 4	± 3	± 4	± 7	± 3	± 7	± 7	± 9	± 5	± 12	± 11	± 9	± 9
MSF + naloxone + ala-enk	29	34	32	39	41	37	33	30	31	37	58	59	50	49
	± 3	± 7	± 4	± 6	± 8	± 5	± 6	± 8	± 4	± 5	± 12	± 13	± 8	± 12

* Significance (p<0.05) change from control value.

† Period when sham-feeding was performed.

polypeptide response to modified sham-feeding.

The effects of ala-enk and naloxone on pentagastrin-induced acid and pepsin responses are shown in Figs 3 and 4. Ala-enk given in a standard dose (20 µg/kg/h) during pentagastrin (2 µg/kg/h) stimulation produced significant inhibition of acid and pepsin outputs by about 32% and 30% of the control level, respectively. Upon the withdrawal of ala-enk administration both acid and pepsin output almost immediately returned to the control values. Naloxone (40 µg/kg/h) given together with ala-enk partly reversed the inhibitory effect of this peptide and raised acid pepsin outputs significantly above that obtained with ala-enk alone. Naloxone alone without ala-enk did not significantly affect gastric acid or pepsin response to pentagastrin.

In tests with histamine (20 µg/kg/h), acid outputs

reached levels not significantly different from those obtained with pentagastrin in the same subjects. The secretory rate was well sustained and neither ala-enk nor naloxone had any influence on this secretion (Table 4).

Ala-enk in a dose of 20 µg/kg/h caused a transient cutaneous flushing in the head region and a moderate degree of dryness in the mouth. There was a significant increase in pulse rate from basal level of 74±5 to 88±4 per minute. Mean blood pressure did not change markedly throughout the study. No symptoms were observed when lower doses of ala-enk or naloxone were administered.

Discussion

This study shows that enkephalin in man inhibits

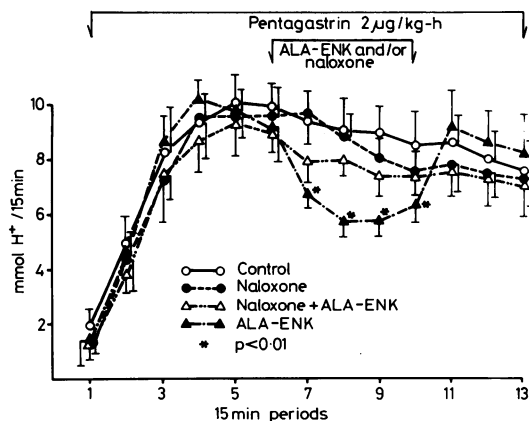


Fig. 3 Effects of ala-enk, naloxone or their combination on pentagastrin-induced acid secretion in six healthy subjects.

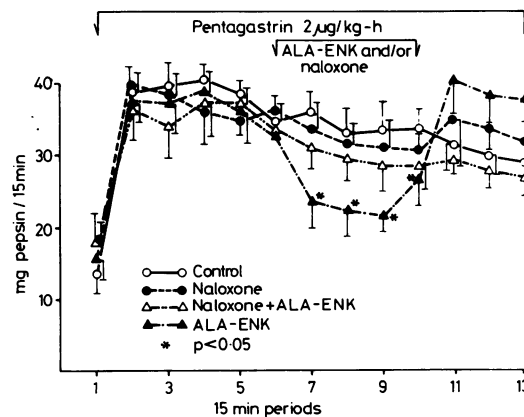


Fig. 4 Pepsin outputs in tests as in Fig. 3.

Table 4 Effects of ala-enk alone or in combination with naloxone on histamine-induced gastric acid secretion in six healthy subjects

Treatment	H+ mmol/30 min	Pepsin mg/30 min
Histamine alone (control)	22.36±3.20	67.54±14.20
Histamine + ala-enk	20.12±2.18	63.44±17.2
Histamine + naloxone	19.27±2.62	52.51±12.8
Histamine + ala-enk + naloxone	21.32±3.12	58.10±10.10

gastric secretion and affects plasma hormone concentrations induced by vagal stimulation and pentagastrin but not by histamine. These effects can be reversed, at least in part, by the administration of naloxone, an opioid receptor antagonist. To our knowledge this is the first attempt to compare the effects of enkephalin on gastric secretion induced by various stimulants and to determine the action of this opioid peptide on plasma hormonal responses to cephalic stimulation in man.

Our results related to the inhibition by enkephalin of gastric secretory response to vagal excitation in humans remain in good agreement with previous findings in dogs. Magee⁹ reported that morphine inhibits gastric acid and pepsin secretion induced by 2-deoxy-D-glucose (2-DG). Anderson *et al.*¹⁰ and we¹¹ confirmed that methionine-enkephalin can also suppress gastric response to vagal stimulation induced by 2-DG or sham-feeding. These effects have been explained by the suppression of acetylcholine release from the cholinergic endings as opioids are known to inhibit this release in every tissue tested so far.^{7 8 14} The peripheral 'anticholinergic' action could explain the reduction in gastric acid secretion and plasma pancreatic polypeptide concentration as well as the increase in serum gastrin response to modified sham-feeding observed in our study after the administration of ala-enk. Actually, similar changes in plasma hormones were previously described in man after injection of morphine⁴ during postprandial state or anticholinergic agents^{12 13} such as atropine or pirenzepine during vagal excitation.

The anticholinergic effect might also contribute to the observed inhibition by ala-enk of pentagastrin-induced secretion because of the well established synergism and interaction between vagal (cholinergic) and hormonal (gastrin) stimulation of the oxyntic glands.¹⁵ This is supported by the fact that atropine can reduce pentagastrin-induced gastric secretion in man indicating an important role of cholinergic innervation in this stimulation.¹⁶ Histamine-induced gastric secretion which represents direct excitation of the oxyntic glands,

was not affected by ala-enk.

Our present finding that ala-enk inhibits pentagastrin-induced secretion and does not affect histamine-stimulated secretion in man apparently disagrees with our previous studies in dogs¹⁷ in which opioids augmented both pentagastrin- and histamine-induced secretion. This might be explained by marked species differences and perhaps the release of histamine by opioids^{18 19} in the canine but not in human gastric mucosa and an increase in the gastric mucosal blood flow¹⁸ in the dog but not in man. In addition, the effect of opioid peptide may depend on the dose used. As shown by Olsen *et al.*,³ a lower dose of enkephalin (0.1 µg/kg/h) stimulated, whereas a higher dose (1 µg/kg/h) had the opposite effect. The doses of ala-enk used in our study (2.5–20 µg/kg/h) exhibited only inhibitory effects on modified sham-feeding or pentagastrin-induced secretion. Another possibility is the activation of different receptor sites by opioids and the multiplicity of actions of opioids in various species. The existence of multiple types of opioid receptors has been well documented in various *in vitro* studies.⁶ Morphine and the synthetic enkephalin analogue FK 33-824 appear to activate µ-receptors, whereas methionine- and leucine-enkephalin and some enkephalin analogues including ala-enk used in our study act *via* δ-receptors. This heterogeneity of opioid receptors could explain the difference in the action of ala-enk and FK 33-824 on pentagastrin-induced secretion between our study and that reported by Olsen *et al.*³

The receptor heterogeneity might also explain the differences in the action of naloxone on gastric secretion in different species or in the same species under various experimental conditions. Naloxone preferentially antagonises µ-receptors but shows relatively small affinity for δ-receptors. The failure of naloxone given at lower doses to affect gastric secretion under basal conditions and following vagal, pentagastrin, or histamine stimulation does not necessarily exclude the role of endogenous opioids in the control of gastric secretion because methionine-enkephalin which has been detected in the gastric mucosa is typical δ-agonist. At higher doses sufficient to inactivate δ-receptors, naloxone is capable of inhibiting both basal and stimulated secretion thus supporting the previous notion^{4 16} that endogenous opioid peptides may contribute to the stimulation of oxyntic glands. Further studies including isolated human oxyntic cells are required to determine whether enkephalins affect gastric acid secretion by direct activation of opioid receptors of these cells or through some other indirect mechanisms.

The action of opioids and opioid receptor

antagonists on gastric secretion and plasma hormones induced by central vagal activation as provoked by modified sham-feeding may not entirely depend upon the peripheral action of these compounds but may also involve the central nervous system, particularly limbic structures and the hypothalamus. These brain areas playing an important role in the vagally-induced gastric secretion,²⁰ exhibit the presence of various opioid receptors, especially of μ - and δ -types.⁶ Natural enkephalins given intravenously are quickly degraded because of their high susceptibility to enzymatic destruction in the blood. In contrast, enkephalin analogues such as ala-enk, in which the glycine residue is replaced by D-alanine, show greater resistance to enzymatic degradation than natural enkephalin.^{21 22} They can pass the blood-brain barrier, which prevents natural enkephalin from entering the brain, and may act on its centres^{2 23} involved in vagal activation by modified sham-feeding. The same applies to naloxone which may enter the brain and antagonise central opioid receptors.²⁴ This study did not distinguish between central and peripheral effects of opioids and their antagonists but recent studies with loperamide,⁵ which does not cross the blood-brain barrier but reduces gastric secretion suggests that opiates may be involved in the regulation of gastric secretion by acting mainly at a peripheral site.

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