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—Original Article—

INFLUENCE OF METHIONINE-ENKEPHALIN ANALOGUE (FK-33-824) ON THE SECRETION OF PANCREATIC HORMONES

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Summary

To determine the effects of methionine-enkephalin on secretion of pancreatic hormones, five healthy male adults were intramuscularly given 0.5 mg of FK 33-824, a methionine-enkephalin analog and blood levels of pancreatic hormones were measured at different time intervals.

FK 33-824 significantly lowered basal levels of plasma insulin (IRI) and pancreatic polypeptide and decreased IRI secretion in 75 g-OGTT. It also delayed the elevation of blood sugar level in 75 g-OGTT. No change occurred in the basal levels of plasma glucagon, somatostatin and blood sugar. These results imply that methionine-enkephalin in the pancreas and alimentary tract may act as an inhibitor on the secretion of insulin and pancreatic polypeptide and hence relates to the absorption of sugar.

Key Words: *Methionine-enkephalin, FK-33-824, Insulin, Glucagon, Pancreatic-polypeptide, Somatostatin, Blood sugar.*

Introduction

Recent studies[1-8] revealed the presence of methionine-enkephalin (M-enk), leucine-enkephalin (L-enk) and peptides derived from prepro-enkephalin[9] in the alimentary tract, including the pancreas.

To elucidate the influence of these peptides on the secretion of the pancreatic hormones, we gave FK 33-824[10-12] (FK), M-enk analog, 0.5 mg intramuscularly to healthy male adults and assessed blood levels of pancreatic hormones by radioimmunoassay.

Subjects and Methods

Five healthy Japanese men aged 24.8 ± 2.4 years (mean ± SD) volunteered to act as the subjects of this study.

These 5 individual took nothing orally after 9:00 p.m. and remained in bed. A heparinized cannula was inserted into the elbow vein for blood sampling at 9:00 a.m. the following

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morning. Blood samples were withdrawn at −15 min. and immediately before the intramuscular administration of FK 0.5 mg in physiological saline 1 ml, or the latter only, followed by blood sampling at 30, 60, 90, 120, 150, and 180 min. Under the same conditions, 75 g-OGTT was performed after 30 min. of FK i.m. and without treatment, in the same subjects. Blood samples were taken at −30 min., just before FK i.m., and the samples at 0 min. were withdrawn at 30 min. after the intramuscular administration of FK, and 75 g-glucoses were then immediately given orally. Blood samples were then taken at 15, 30, 60, 90, and 120 min. after ingestion of glucose. All blood samples were taken in ice-chilled tubes containing EDTA 2Na (EDTA 2Na 1 mg/blood 1 ml) and were immediately chilled in ice before the centrifugation at 4°C. The centrifugal plasma was frozen at −40°C until the radioimmunoassay (RIA).

Plasma insulin (IRI) was determined using commercially available RIA kits (Dainabot-Radioisotope Lab. Ltd., Tokyo, Japan). The minimum sensitive level was 5 μU/ml, with interassay variance at 7.9% and intra-assay variance at 7.2%. Plasma glucagon (IRG) was determined using the RIA-kit from Daiichi Radioisotope Lab. Ltd., Tokyo, Japan and which involved use of the antibody (OAL-123) specific to pancreatic glucagon. The minimum sensitive level was 15.6 pg/ml and the interassay variance and intra-assay variance were 3.6% and 5.3%, respectively. Plasma pancreatic polypeptide (PP) was measured by a double antibody RIA[13]. Labelled ^{125}I-bovine PP was prepared by the lactoperoxidase method. Human PP for the standard, and the rabbit human PP antiserum were a gift from Dr. R.E. Chance (Eli Lilly Co., Indianapolis, Indiana). The sensitivity of this assay was 2.5 pg/tube, interassay variance was 12% and intra-assay variance was 8%. Plasma somatostatin (SLI) was measured by RIA modified from Kronheim's methods[14]. Rabbit anti-somatostatin 14-hemocyanin (1382) (final dilution, 1:40,000) and ^{125}I-Tyr^{1}-somatostatin 14 (Protein Research Foundation, Osaka, Japan)

Table 1. Changes of pancreatic hormones, insulin, glucagon, pancreatic polypeptide, somatostatin and blood sugar by FK 33-824 (a) or physiological saline (b) injection in healthy Japanese men

a n=5 M±SE

time (min)	−15	0	30	60	90	120	150	180
IRI (μU/ml)	12.3± 1.6	11.5± 1.2	5.7± 1.5	5.1± 1.5	6.6± 2.2	5.6± 1.6	7.3± 2.2	8.5± 2.1
IRG (pg/ml)	120 ±22	104 ± 9.4	108 ±11.6	103 ±11.2	112 ±18.3	107 ±18.8	109 ±16.5	108 ±15.6
PP (pg/ml)	72.2± 8.8	79 ±13.1	42.2± 8.3	41.8± 4.6	31.8± 4.2	33.8± 5.8	36.4± 4.9	32.6± 2.7
SLI (pg/ml)	31.4± 2.3	31.4± 1.7	30.0± 1.1	30.8± 2.7	32.2± 2.8	30.6± 2.8	28.6± 1.3	30.8± 1.7
BS (mg/dl)	84.8± 4.9	76.6± 3.2	84.2± 3.4	78.2± 5.1	86.6± 5.9	85 ± 5.4	89 ± 1.6	88 ± 2.5

b n=5 M±SE

time (min)	−15	0	30	60	90	120	150	180
IRI (μU/ml)	8.6± 1.9	7.6± 1.6	7.1± 1.3	7.1± 1.6	9.6± 2.2	7.3± 1.4	8.2± 1.4	6.6± 2.1
IRG (pg/ml)	119 ±19.2	127 ±24	110 ±16.4	108 ±18	100 ±21.3	115 ±20.3	113.5±16.5	95.6±18.3
PP (pg/ml)	43 ± 4.4	38 ± 5.6	56 ±12.6	57±12	52 ± 8.0	54 ± 9.2	57 ±19.2	37 ± 5.2
SLI (pg/ml)	30.6± 5.6	32.8± 3.0	29.6± 1.8	29.2± 2.6	27.8± 2.3	31.4± 4.0	29.6± 2.7	28.8± 2.9
BS (mg/dl)	88 ± 5.5	92.4± 4.6	85.2± 2.0	81.8± 5.5	86.8± 6.0	93.6± 2.7	84.6± 2.6	90 ± 1.8

Values are means ± SEM. We gave FK 33-824 0.5 mg (a) or physiological saline (b) intramuscularly to healthy men (n=5) after blood sampling at −15 and 0 min., and then obtained more blood samples at 30, 60, 90, 120, 150 and 180 min.

Abbreviations: IRI insulin, IRG glucagon, pp pancreatic polypeptide, SLI somatostatin, BS blood sugar.

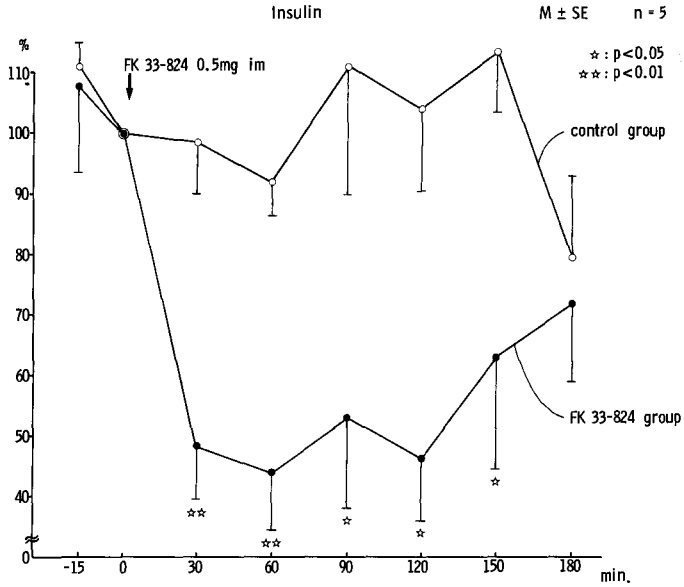

Fig. 1. Changes of insulin, taking the value at 0 min. as 100%, by FK 33-824 0.5 mg i.m. injection (●—●) (mean ± SE) (FK 33-824 group) (n=5) and physiological saline 1 ml i.m. injection (○—○) (mean ± SE) (control group) (n=5). Insulin levels significantly decreased in the FK 33-824 group at 30, 60, 90, 120 and 150 min. ☆ indicates $P<0.05$ and ☆☆ indicates $P<0.01$ significant.

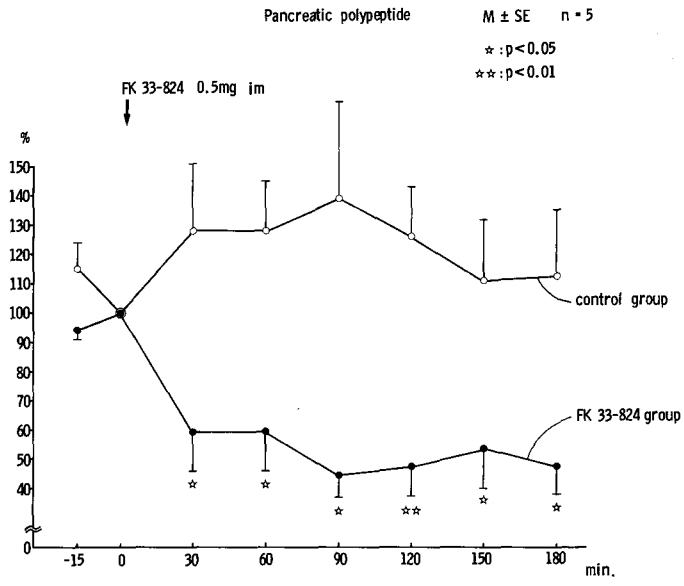

Fig. 2. Changes in the pancreatic polypeptide, taking the value at 0 min. as 100%, by FK 33-824 0.5 mg i.m. (●—●) (mean ± SE) (FK 33-824 group) (n=5) and physiological saline 1 ml i.m. (○—○) (mean ± SE) (control group) (n=5). Pancreatic polypeptide significantly decreased in the FK 33-824 group at 30, 60, 90, 120, 150 and 180 min. ☆ indicates $P<0.05$ and ☆☆ indicates $P<0.01$ significant.

were used as standards. The sensitivity of this assay was 15.6 pg/ml, interassay variance was 12% and intra-assay variance was 12%. In the statistical analysis, all data were presented in percent change ± SE taking the basal value as 100%. Significant differences were calculated by Student's t-test.

Results

Plasma IRI decreased from the basal value 11.5 ± 1.2 (mean ± SE) μU/ml to 5.1 ± 1.5 (mean ± SE) μU/ml at 60 min. after the intramuscular injection of FK 0.5 mg. Plasma PP also decreased from the basal value 79 ± 13.1 (mean ± SE) pg/ml to 31.8 ± 4.2 (mean ± SE) pg/ml at 90 min. after FK 0.5 mg i.m. injection (**Table 1**).

On the other hand, plasma IRG, SLI and blood sugar (BS) remained unchanged after intramuscular administration of FK 0.5 mg. Comparing these parameters with the control values, taking the value at 0 min. as 100%, IRI significantly decreased in the FK i.m. group at 30, 60, 90, 120 and 150 min. (**Fig. 1**), and PP at 30, 60, 90, 120, 150 and 180 min. (**Fig. 2**). No significant difference was observed between the FK i.m. group and the control group, with regard to the levels of IRG, SLI and BS (**Fig. 3**). To examine the influence of FK on the secretion of IRI, as induced by 75 g-OGTT, 75 g-OGTT was performed in two ways, i.e., pretreatment with FK 0.5 mg i.m. at 30 min. in advance of 75 g-OGTT, and without pretreatment. IRI and BS responses in both groups

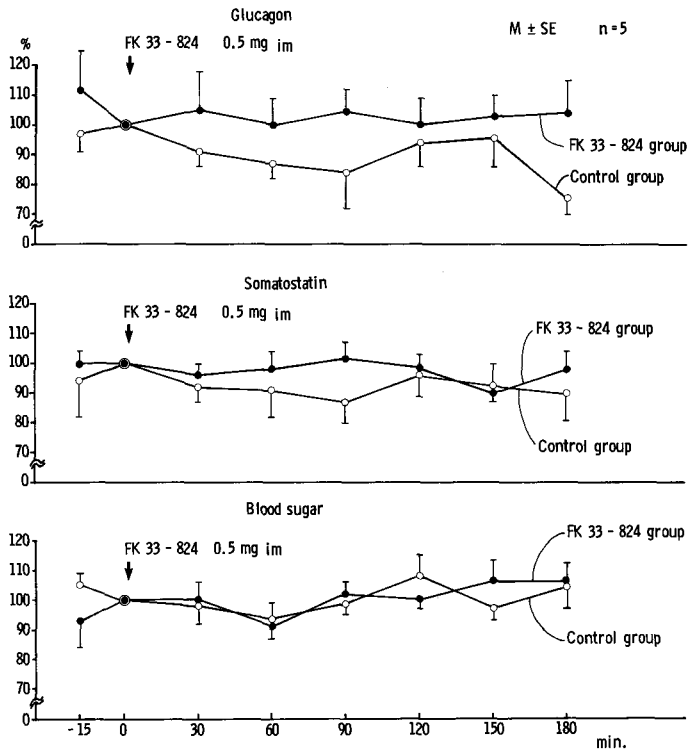

Fig. 3. Changes in glucagon, somatostatin and blood sugar, taking the value at 0 min. as 100%, by FK 33-824 0.5 mg i.m. (●—●) (mean ± SE) (FK 33-824 group) (n=5) and physiological saline 1 ml i.m. (○—○) (mean ± SE) (control group) (n=5). Glucagon, somatostatin and blood sugar in the FK 33-824 group remained unchanged, in comparison to the control group.

Table 2. Changes in insulin and blood sugar induced by 75 g-OGTT in healthy men

n=4 M±SE

Time (min)	−30	0	15	30	60	90	120
IRI (μU/ml) (FK + 75 g OGTT)	16.5 ±1.2	9.0 ±0.8	17.0 ± 1.5	32.6 ± 6.6	29.7 ± 2.6	26.0 ± 4.0	44.8 ±11.8
IRI (μU/ml) (75 g OGTT)	13.3 ±3.1	12.6 ±3.2	37.0 ±12.9	48.5 ± 9.5	49.3 ± 4.3	43.8 ± 6.3	40.8 ± 4.4
BS (mg/dl) (FK + 75 g OGTT)	97.5 ±3.9	97.8 ±3.4	104 ± 5.9	130.5 ±11.4	119 ±10.6	111 ±12.4	125.8 ± 8.8
BS (mg/dl) (75 g OGTT)	85.8 ±3.3	88.5 ±2.4	114 ±17.5	147.8 ±21.7	127.8 ± 9.9	105.5 ± 5.1	111.3 ± 8.5

Values are means ± SEM. (FK + 75 g-OGTT) indicates 75 g-OGTT with pretreatment of FK 33-824 intramuscularly to healthy men (n=4) i.e., we gave FK 33-824 0.5 mg intramuscularly after blood sampling at −30 min. and obtained blood samples at 0 min. at 30 min. after FK injection, then gave orally 75 g-glucose. Blood samples were then obtained at 15, 30, 60, 90 and 120 min. after this oral glucose intake. (75 g-OGTT) indicates 75 g-OGTT with pretreatment of physiological saline 1 ml instead of FK 33-824 intramuscularly. Blood samples were obtained at the same time course as in case of (FK + 75 g-OGTT).
Abbreviations: IRI, insulin, BS, blood sugar.
75 g OGTT: 75 g-oral glucose tolerance test.

were then compared (**Table 2**). Changes in IRI and BS levels are presented as percentages (mean ± SE), taking the respective value at −30 min. as 100% (**Fig. 4-a, b**). IRI secretion after the pretreatment with FK showed significant decreases compared with the control at 0, 15, 30, 60 and 90 min. after the oral intake of 75 g glucose (**Fig. 4-a**). Elevation of BS levels after 75 g-OGTT also showed a tendency toward inhibition (**Fig. 4-b**).

Discussion

The exact localization of enkephalin in the pancreas has not been established, although the presence of M-enk in this organ has been identified[1]. Forssmann et al. (1977)[2] reported the presence of enkephalin in pancreatic Langerhans islet cells, while Larsson (1979)[3] stated that M-enk was present in the ganglia and not in the pancreatic Langerhans islet cells.

We found a marked decrease in blood IRI levels and an inhibition of IRI secretion of 75 g-OGTT after the intramuscular administration of FK 0.5 mg to healthy volunteers. Depressive effects of FK on the basal level of IRI was thought to be its direct effect in Langerhans islet cells, because the basal level of BS did not change at all. The inhibition of IRI secretion of 75 g-OGTT after the injection of FK was also thought to be the direct effect of FK. However, because FK injection suppressed BS increase in 75 g-OGTT and the index of ΔIRI/ΔBS indicated 0.73 ± 0.11 (mean ± SE) in 75 g-OGTT or 0.78 ± 0.09 (mean ± SE) in FK + 75 g-OGTT, the possibility that the inhibition of IRI secretion of 75 g-OGTT after FK injection was caused by the suppression of BS increased in 75 g-OGTT after FK injection could not be denied. Kanter et al. (1980)[15] investigated the effect of M-enk on the secretion of IRI and IRG using pancreatic islet cultured cells of rats and reported that M-enk showed a dose-dependent inhibition of IRI and IRG secretion, in concentration ranges from 10^{-8}M to 10^{-6}M. Ogiso et al. (1983)[16] also found that FK directly inhibited IRI secretion in incubation experiments of isolated pancreatic Langerhans islet cells of rat at concentrations between 10^{-8}M and 10^{-4}M. This effect was counteracted by naloxone. According to Pozo et al. (1980)[12],

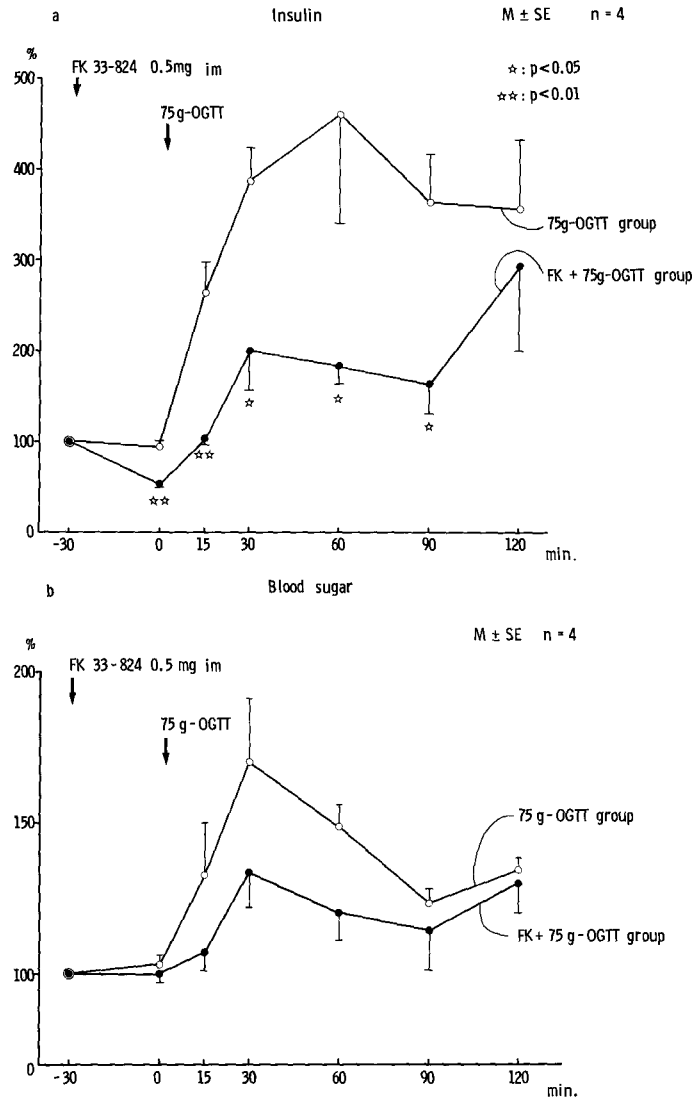

Fig. 4. a: Changes of insulin, taking the value at −30 min. as 100%, in 75 g-OGTT with pretreatment of FK 33-824 0.5 mg i.m. (●—●) (mean ± SE) (FK + 75 g-OGTT group) (n=4) and with pretreatment of physiological saline 1 ml i.m. (○—○) (mean ± SE) (75 g-OGTT group) (n=4). Insulin secretion by 75 g-OGTT significantly decreased in the FK + 75 g-OGTT group at 0, 15, 30, 60 and 90 min. ☆ indicates $P<0.05$ and ☆☆ indicates $P<0.01$ significant.
b: Changes in blood sugar, taking the value at −30 min. as 100%, in the same tests FK + 75 g-OGTT (●—●) (mean ± SE) (n=4) and 75 g-OGTT (○—○) (mean ± SE) (n=4). Blood sugar elevation by 75 g-OGTT tended to decrease in the FK + 75 g-OGTT group, in comparison to data on the control group.

plasma FK concentration after 0.5 mg i.m. is about 13 ng/ml and 14 ng/ml after 30 and 90 min. respectively, the latter representing a concentration of 2.32×10^{-8} M. The half life in the plasma is about 3 hours. Thus, our present *in vivo* results agreed with the *in vitro* data of

Kanter et al. (1983)[15] and FK seems to directly inhibit the IRI secretion from B cells in Langerhans islets. However, potential indirect action via other neurons, such as paracrine action by other hormones and anticholinergic action observed with opiates including FK, was not ruled out.

Chiba et al. (1980)[17] reported that M-enk in the concentration range between 10^{-8}M and 10^{-6}M dose dependently inhibited SLI secretion in a perfusion study in the rat stomach. In the present study, the plasma SLI did not fluctuate. Nevertheless, the pancreatic SLI level does not precisely represent the plasma SLI level, even though the latter does depend on the SLI in the gut and pancreas. As for plasma pancreatic glucagon, IRG did not fluctuate after the intramuscular administration of FK 0.5 mg, thereby suggesting that FK has little influence on IRG secretion.

Effects of FK on PP secretion were studied by Materia et al. (1981)[18] in an *in vivo* study in dogs. They found that the PP secretion induced by bombesin was inhibited by M-enk via a specific receptor. Our present *in vivo* clinical results showed that plasma PP values decreased after FK i.m. These results were supported by those of Materia et al. (1981)[18]. It is also known that the secretion of PP is inhibited by a cholinergic antagonist atropine[19], and since opiates inhibit the secretion of acetylcholine from cholinergic nerves[20,21], it is assumed that the inhibition of PP secretion by FK is due to this action.

Concerning the effect on blood sugar level, Stubbs et al. (1978)[22] reported that FK slightly lowered the blood sugar level. We found no changes in basal blood glucose levels after FK administration. 75 g-OGTT with pretreatment of FK i.m. demonstrated the inhibition of the increase in BS level at 15, 30 and 60 min., and a re-increase of BS at 120 min. was observed. M-enk inhibits the firing of neurons[23], and consequently there is a disturbance in the constriction of gut. The malabsorption of glucose from the gut may cause delayed increases in blood sugar. In this connection, fluctuation of other hormones in the gut, especially motilin, should be investigated. Finally Ipp et al. (1978)[24] and Feldman et al. (1983)[25] reported that β-endorphin accelerated the secretion of IRI and IRG in pancreatic perfusion systems and in healthy males. These findings are completely different from our present results with FK, a M-enk analogue, especially in relation to IRI secretion. It has been reported that receptors of β-endorphin and M-enk are different[23], and this probably supports our present results. It is also known that M-enk mainly acts on δ-receptors while FK acts not only on δ-receptors but also on μ-receptors[26]. Thus, although FK and M-enk do not always show the same effect, FK can probably serve as a model for the analysis of the effects of M-enk on the secretion of pancreatic hormones.

References

1) Polak JM, et al: Enkephalin-like immunoreactivity in the human gastrointestinal tract. Lancet 1: 972, 1977
2) Forssmann WG, et al: Relationship of enkephalin and endorphin immunoreactivity with D-cells and G-cells of the stomach. Acta Hepatogastroenterology 24: 488, 1977
3) Ito S, et al: Met-enkephalin-immunoreactive and gastrin-immunoreactive cells in the human and canine pyloric antrum. General Comparative Endocrinol 38: 238, 1979
4) Larsson LI: Innervation of the pancreas by substance P, enkephalin, vasoactive intestinal polypeptide and gastrin/CCK immunoreactive nerves. J Histochem Cytochem 27: 1283, 1979
5) Udin R, et al: Evidence for vagal enkephalinergic neural control of the feline pylorus and stomach. Gastroenterology 78: 492, 1980
6) Uddman R, et al: Peptidergic (enkephalin) innervation of the mammalian oesophagus. Gastroenterology 78: 732, 1980
7) Larsson LI, et al: Enkephalin/endorphin-related peptides in antropyloric gastric cells. J Histochem Cytochem 29: 1088, 1981
8) Sakamoto M, et al: Occurrence of methionine-enk-

ephalin-Arg^6-Gly^7-Leu^8, methionine-enkephalin, leucine-enkephalin and methionine-enkephalin-Arg^6-Phe^7 in human gastric antrum. J Clin Endocrinol Metab 56: 202, 1983

9) Noda M, et al: Cloning and sequence analysis of cDNA for bovine adrenal preproenkephalin. Nature 295: 202, 1982
10) Cusan L, et al: Potent prolactin and growth hormone releasing activity of more analogues of Met-enkephalin. Nature 268: 544, 1977
11) Roemer D, et al: A synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity. Nature 268: 547, 1977
12) Pozo ED, et al: Endocrine effect of a methionine-enkephalin derivative (FK 33-824) in man. Hormone Res 13: 90, 1980
13) Matsumoto M, et al: Plasma human pancreatic polypeptide response in chronic pancreatitis. Gastroenterologia Jpn 17: 25, 1982
14) Kronheim S, et al: The characterization of somatostatin-like immunoreactivity in human serum. Diabetes 27: 523, 1978
15) Kanter RA, et al: Disparate effects of enkephalin and morphine upon insulin and glucagon secretion by islet cell cultures. Diabetes 29: 84, 1980
16) Ogiso H, et al: Effects of Met-enkephalin analogue (FK 33-824) on insulin and glucagon excretion. Folia Endocrinol Jpn 59: 363, 1983 (Jpn)
17) Chiba T, et al: Effects of various gastrointestinal peptides on gastric somatostatin release. Endocrinology 106: 145, 1980
18) Materia A, et al: Effect of methionine-enkephalin and naloxone on bombesin-stimulated gastric acid secretion, gastrin and pancreatic polypeptide release in the dog. Ann Surg 196: 48, 1981
19) Taylor IL, et al: Effects of atropine and bethanechol on bombesin-stimulated release of pancreatic polypeptide and gastrin in dog. Gastroenterology 77: 714, 1979
20) Paton WDM: The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. Brit J Pharmacol 11: 119, 1957
21) Konturek SJ: Gut Hormones (edited by S.R. Bloom and J.M. Polak) 2nd ed. Churchill Livingstone, London p 432, 1981
22) Stubbs WA, et al: Hormonal and metabolic responses to an enkephalin analogue in normal man. Lancet 2: 1225, 1978
23) Chan-Palay V, et al: Inhibitory effects of motilin, somatostatin, [Leu]enkephalin, [Met]enkephalin, and taurine on neurones of the lateral vestibular nucleus: Interactions with γ-aminobutyric acid. Proc Natl Acad Sci USA 79: 3355, 1982
24) Ipp E, et al: Morphine and β-endorphin influence on the secretion of the endocrine pancreas. Nature 276: 190, 1978
25) Feldman M, et al: Beta-endorphin and the endocrine pancreas, studies in healthy and diabetic human beings. N Engl J Med 308: 349, 1983
26) Sullivan SN, et al: Inhibition of basal and stimulated gastric acid secretion by an enkephalin analogue. Am J Gastroenterol 77: 360, 1982