The Effect of Met-Enkephalin and Naloxone on Somatostatin and Insulin Secretion from the Isolated, Perfused Rat Pancreas

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SUMMARY

The effect of met-enkephalin and the opiate antagonist, naloxone, on somatostatin and insulin secretion from the isolated, perfused rat pancreas has been studied during perfusion with 300 mg/dl glucose. In response to a gradient of met-enkephalin from 0 to 10^{-5} M, release of somatostatin was inhibited at low concentrations and stimulated at high concentrations. However, both low and high concentrations of met-enkephalin stimulated insulin secretion. A gradient of met-enkephalin from 0 to 10^{-6} M caused only an inhibition of somatostatin release, whereas insulin release was stimulated.

The effects of met-enkephalin on somatostatin release were antagonized by naloxone (10^{-6} M). The met-enkephalin–induced insulin secretion was partially, but not completely, blocked by naloxone. Naloxone (10^{-6} M) alone changed the endocrine secretions by decreasing somatostatin release and by stimulating insulin release. Met-enkephalin may, therefore, be a physiologic regulator of pancreatic endocrine secretion.

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The presence of endogenous opioid peptides, such as β-endorphin and the enkephalins, has been demonstrated in the gastrointestinal tract and pancreas.[1–5] Methionine-enkephalin and leucine-enkephalin, originally isolated from brain extracts,[6] have been localized by immunohistochemistry to both endocrine-type cells in the pancreatic islet[2] and nerve fibers in the endocrine and exocrine pancreas.[3,5] In addition, pancreatic extracts have been shown to contain peptides eluting with similar characteristics to the enkephalins on high-performance liquid chromatography.[7] A functional role for the opioid peptides was suggested by studies on their effect on pancreatic endocrine secretion both in vivo[8,9] and in vitro using the isolated, perfused dog pancreas or isolated rat islets.[10,11] Increased insulin secretion in response to β-endorphin and morphine has been observed in the perfused dog pancreas.[12] Green et al.[13] showed that low concentrations of met-enkephalin or D-Ala^2-MePhe^4-Met-(O)-ol-enkephalin (DAMME) had a stimulatory effect on insulin release from isolated islets of Langerhans, while high concentrations were inhibitory. Inhibition of somatostatin release from the perfused dog pancreas occurred in response to morphine,[12] β-endorphin,[12] and met-enkephalin.[11] In addition, the opiate receptor antagonist naloxone has been reported to increase insulin release in vivo[14] and in vitro[12,15] without having any effect on somatostatin release.[11,12] Since there are inconsistencies in the published results concerning pancreatic endocrine responses to the enkephalins, this aspect has been reexamined using the isolated, perfused rat pancreas. Studies have been performed on the secretion of somatostatin and insulin in response to increasing concentrations of met-enkephalin achieved with a gradient perfusion. The gradient system has the advantages that the pancreas is exposed to a gradual increase in the peptide concentration, a situation that probably mimics the physiologic situation more closely than a bolus addition, and the response of a single pancreas can be studied over a defined peptide concentration range. Since inhibitory effects of the enkephalins have been observed,[9,11,12] a high glucose medium was used to elevate the basal levels of somatostatin and insulin. The influence of naloxone on these responses and of naloxone alone has also been studied.

MATERIALS AND METHODS

Overnight-fasted, male Wistar rats (200–300 g) were anesthetized with sodium pentobarbital (60 mg/kg) and the pancreas and associated duodenum isolated according to the method of Grodsky et al.[16] The perfusate consisted of bicarbonate buffer containing 0.2% bovine serum albumin (Sigma

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Chemical Co., St. Louis, Missouri) and 3% Dextran T70 (Pharmacia, Uppsala, Sweden) gassed to achieve pH 7.4. The pancreas was perfused with 80 mg/dl glucose for the first 20 min of equilibration and with 300 mg/dl glucose for a further 10-min equilibration period and throughout the 45-min perfusion. Complete portal vein effluent, after a 30-min period of equilibration, was collected at 1-min intervals. The flow rate was 2 ml/min and fractions were collected into cooled tubes containing aprotinin (Trasylol, Miles Lab., Ontario, Canada) at a concentration of 1000 KIU/ml. The samples were stored at −20°C before assay. Somatostatin and insulin were measured by specific radioimmunoassays as previously described.[17,18] Linear gradients of met-enkephalin (Penninsula Labs., Belmont, California) were generated as described by Chan et al.[18] These have demonstrated linearity for both glucose and bovine serum albumin under the conditions used. Where indicated, naloxone (kindly donated by Endo Labs., New York, New York) was added to both flasks at a concentration of 10^{-6} M.

Secretion rates are expressed as the rate of secretion (mean ± SEM) in picograms (somatostatin) or microunits (insulin) per minute, and the number of perfusions performed under a particular set of conditions (N) is indicated in the legends to the relevant figures in the text. Statistical analysis was performed using Student's *t*-test for paired samples.

RESULTS

THE EFFECT OF MET-ENKEPHALIN ON SOMATOSTATIN AND INSULIN SECRETION

Gradient of met-enkephalin from 0 to 10^{-5} M. Met-enkephalin was perfused as a gradient from 0 to 10^{-5} M in the presence of 300 mg/dl glucose. The somatostatin response depended on the met-enkephalin concentration (Figure 1A). Between 0 and approximately 10^{-6} M, somatostatin secretion was inhibited by 50%. From 10^{-6} to 10^{-5} M, met-enkephalin caused a significant and sustained augmentation of secretion, with an approximate sixfold increase at the end of the gradient. The rate of somatostatin secretion decreased immediately to preperfusion levels on stopping the met-enkephalin gradient.

Insulin secretion increased in response to met-enkephalin with a lag period of approximately 10 min (Figure 2A). There was no significant change during the period in which somatostatin was decreased. Maximal stimulation was approximately five times basal secretion. On stopping the perfusion of met-enkephalin, insulin secretion returned to preinfusion levels.

Gradient of met-enkephalin from 0 to 10^{-6} M. To reproduce the conditions under which a decrease in somatostatin secretion was obtained with low concentrations of met-en-

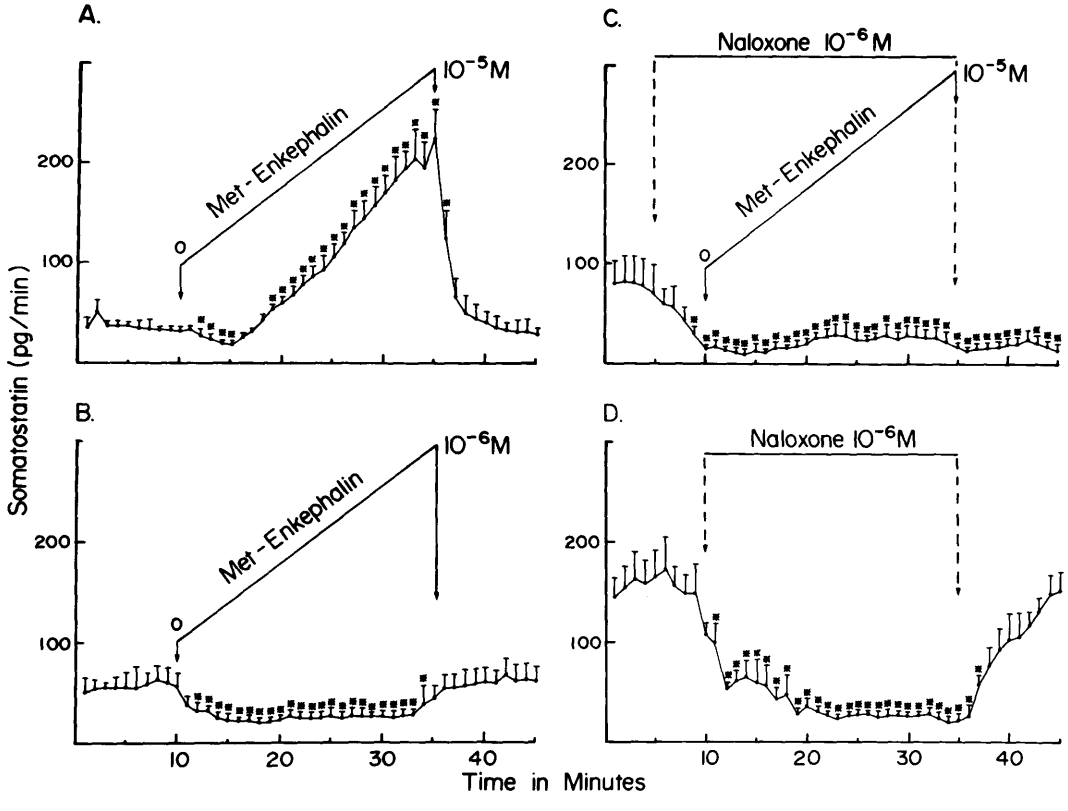

FIGURE 1. The effect of met-enkephalin and naloxone on somatostatin secretion. After perfusion with buffer containing 300 mg/dl glucose alone, met-enkephalin and/or naloxone were introduced: (A) gradient of met-enkephalin (0–10^{-5} M) during periods 10–35, (B) gradient of met-enkephalin (0–10^{-6} M) during periods 10–35, (C) naloxone (10^{-6} M) during periods 5–35 and a gradient of met-enkephalin (0–10^{-5} M) during periods 10–35, and (D) naloxone (10^{-6} M) during periods 10–35. Results are expressed as mean ± SEM; N = 5. Peptide levels were significantly changed from basal to at least the $P < 0.05$ level in the periods indicated (*).

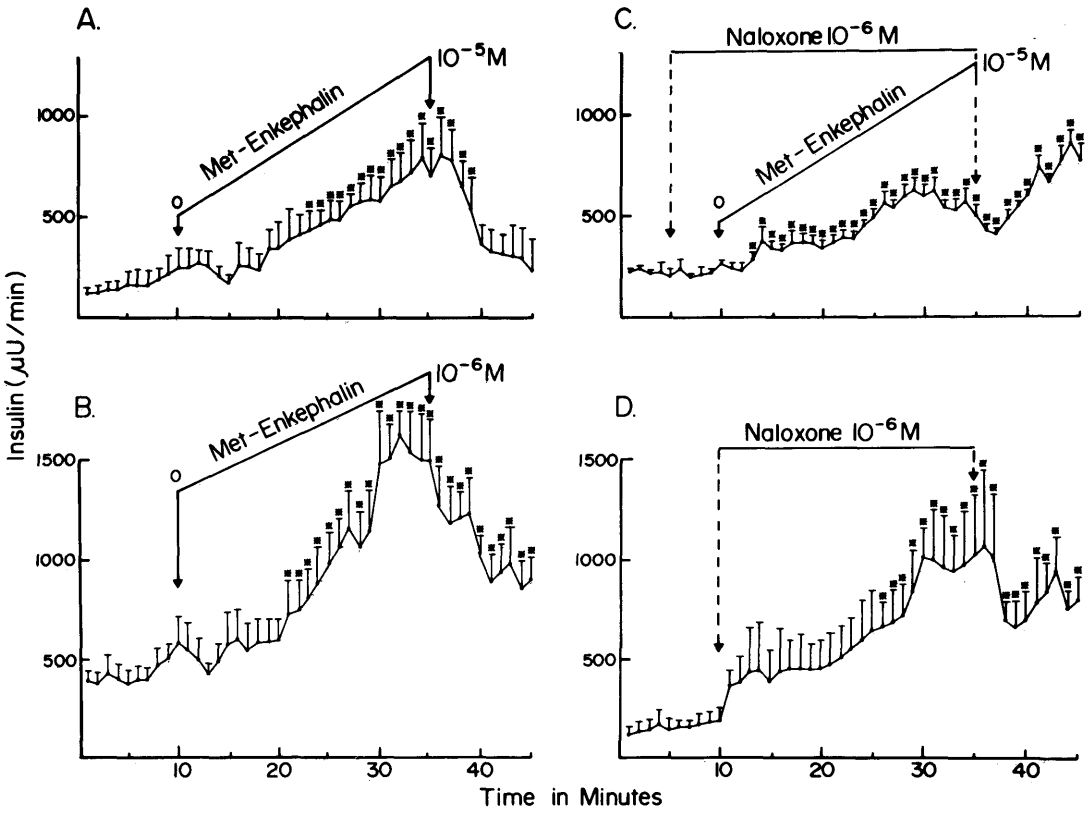

FIGURE 2. The effect of met-enkephalin and naloxone on insulin secretion. After perfusion with buffer containing 300 mg/dl glucose alone, met-enkephalin and/or naloxone were introduced: (A) gradient of met-enkephalin (0–10^{-5} M) during periods 10–35, (B) gradient of met-enkephalin (0–10^{-6} M) during periods 10–35, (C) naloxone (10^{-6} M) during periods 5–35 and a gradient of met-enkephalin (0–10^{-5} M) during periods 10–35, and (D) naloxone (10^{-6} M) during periods 10–35. Results are expressed as mean ± SEM; N = 5. Peptide levels were significantly changed from basal to at least the $P < 0.05$ level in the periods indicated (*).

kephalin, a gradient between 0 and 10^{-6} M was perfused (Figure 1B). The somatostatin response to this stimulus was a decrease of a magnitude similar to that obtained previously. Maximal inhibition was 60%, and at the end of the gradient the rate of somatostatin secretion increased to preperfusion levels.

The 0–10^{-6} M met-enkephalin gradient induced an increase in insulin secretion (Figure 2B) to a maximal stimulation four times basal. At the end of the gradient, the rate of insulin secretion decreased to preperfusion levels.

THE EFFECT OF NALOXONE ON SOMATOSTATIN AND INSULIN RESPONSES TO MET-ENKEPHALIN

Naloxone infusion, at a concentration of 10^{-6} M, was started 5 min before commencing a gradient of met-enkephalin (0–10^{-5} M). Infusion of naloxone during this initial period produced a significant decrease in somatostatin release (Figure 1C) and secretion remained low throughout the perfusion. Maximal inhibition averaged 80%. On stopping the perfusion of both agents, secretion rates remained at basal levels. The met-enkephalin-induced insulin secretion was partially, but not completely, blocked by naloxone (Figure 2C). This effect was significant after 17 min and the release at the end of the gradient was approximately 2.5 times basal release. On stopping perfusion of both agents, the rate of insulin secretion initially decreased slightly and then increased to levels higher than those obtained at the end of the gradient.

THE EFFECT OF NALOXONE ALONE ON SOMATOSTATIN AND INSULIN SECRETION

On perfusion of naloxone (10^{-6} M), somatostatin secretion was significantly decreased within 3 min and reached a maximum inhibition of 80% by 20 min (Figure 1D). Thereafter, it remained inhibited until removal of the naloxone, when levels returned to those obtained before its introduction. Insulin secretion was increased on starting perfusion of naloxone (Figure 2D), and levels reached nine times those obtained under basal conditions. On stopping the naloxone perfusion, secretion decreased but remained above basal.

DISCUSSION

The present results demonstrate that the endogenous opioid peptide, met-enkephalin, at the doses used, can influence endocrine secretion by the isolated perfused rat pancreas. The effect of met-enkephalin was dose dependent at a high glucose concentration (300 mg/dl). An inhibitory effect on somatostatin secretion occurred with concentrations up to 10^{-6} M, whereas concentrations between 10^{-6} and 10^{-5} M caused a stimulation. In a previous study by Hermansen[11] on the perfused dog pancreas, low concentrations of met-enkephalin (10^{-7}–10^{-9} M) were also found to be inhibitory and the effect of met-enkephalin was demonstrated to be more pronounced at a high glucose concentration. Other in vivo[9] and in vitro[12] studies on the dog pancreas have also

shown that leu-enkephalin, morphine, and β-endorphin induced a decrease in somatostatin secretion at low glucose concentrations. Met-enkephalin stimulated insulin release over both concentration ranges and the responses were dose dependent. This is in agreement with findings of a stimulatory action of met-enkephalin on the β-cell in isolated rat islets[13,19] and perfused dog pancreas,[11] as well as in vivo in both the dog[8,20] and rat.[21] Similar responses have also been found with dynorphin[15] and β-endorphin or morphine;[12,22] in the latter study, it was suggested that the decrease in somatostatin secretion may be responsible for the increased release of insulin. Such a mechanism could account for the effect of low concentrations of met-enkephalin in which there was a reciprocal relationship between somatostatin and insulin secretion, but it will probably be necessary to determine the cellular localization of opioid receptors to confirm such a proposition. The reported inhibitory effects of met-enkephalin[10,23] and β-endorphin[24] on insulin secretion may be due to methodologic differences.

The question as to whether or not these responses are physiologic cannot be answered from available data, since the concentration of enkephalin bathing the islet cells is unknown. In the peripheral circulation, enkephalin levels have been reported to be in the picomolar range.[25] However, since immunoreactive enkephalin has been demonstrated in pancreatic nerve elements[3,5] and islet cells,[2] higher concentrations are likely to occur locally in the vicinity of the islet cells. It is more likely that concentrations of 10^{-6} M, rather than 10^{-5} M, are present within a synaptic cleft, and the physiologic action would therefore be inhibitory on somatostatin and stimulatory on insulin.

The similarity of pancreatic hormone responses to the endogenous opioid peptides and morphine suggests an effect via opiate receptors. This suggestion is supported by the fact that naloxone, an opiate receptor antagonist, ablated the stimulatory effect of high concentrations of met-enkephalin on somatostatin release and partially reversed the increase in insulin secretion. The somatostatin results are in conflict with studies on the dog pancreas in which there was no significant effect of naloxone on pancreatic somatostatin secretion either in vivo[8] or in vitro,[11,12] or on the action of β-endorphin on the endocrine pancreas of normal or diabetic man.[26] The in vitro data were, however, obtained under low glucose conditions during which somatostatin secretion was low. Nevertheless, the situation is clearly more complex, since naloxone itself inhibited somatostatin release and produced a marked increase in insulin secretion that was greater than with met-enkephalin itself. Naloxone has a high affinity for μ-type opiate receptors, although it also interacts, with lower affinity, with both σ- and K-receptors. With the high concentration used (1 μM) it would be expected that blockade of all receptor subtypes occurred and that this was near maximal. It is, therefore, unlikely that the responses can be explained on the basis of a differential receptor blockade.

The apparently paradoxical finding, that naloxone had similar actions to opioid peptides, has been previously observed in studies on pancreatic endocrine secretion[10,12,20] and there are a number of possibilities that could account for these results. Opioids in the pancreas may exert a tonic stimulatory action on somatostatin secretion and blockade of their action results in the observed inhibition. This inhibition could then account for the observed increase in insulin release. In addition, at the concentration used, naloxone is known to exhibit agonist activity[27] and to cause induction of opiate receptors,[28] although the latter is unlikely to be involved in the rapid responses observed.

The present results indicate that enkephalinergic pathways are potentially involved in the regulation of pancreatic endocrine secretion similar to those involved in gastric somatostatin release.[29] Whether the endogenous peptides are of neuronal or endocrine origin, and under what conditions they exert this control, remains to be determined.

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REFERENCES

[1] Bruni, J. F., Watkins, W. B., and Yen, S. S. C.: β-Endorphin in the human pancreas. J. Clin. Endocrinol. Metab. 1979; 49:649–51.

[2] Forssmann, W. G., Helmstaedter, V., and Feurle, G.: Relationship of enkephalin and endorphin immunoreactivity with D-cells and G-cells of the stomach. Hepatogastroenterology 1977; 24:488–91.

[3] Larsson, L.: Innervation of the pancreas by substance P, enkephalin, vasoactive intestinal polypeptide and gastrin/CCK immunoreactive nerves. J. Histochem. Cytochem. 1979; 27:1283–84.

[4] Polak, J. M., Bloom, S. R., Sullivan, S. N., Facer, P., and Pearse, A. G. E.: Enkephalin-like immunoreactivity in the human gastrointestinal tract. Lancet 1979; 1:972–74.

[5] Shimosegawa, T., Uchida, T., Kobayashi, S., Ito, S., and Shibata, A.: Met-enkephalin-like immunoreactivity-containing nerve elements in the canine pancreas: a histochemical study. Nippon Naibunpi Gakkai Zasshi 1984; 60:44–53.

[6] Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A., and Morris, H. R.: Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 1975; 258:577–79.

[7] Stern, A. S., Wurzburger, R. J., Barkey, R., and Spector, S.: Opioid polypeptides in guinea pig pancreas. Proc. Natl. Acad. Sci. USA 1982; 79:6703–6706.

[8] Ipp, E., Dhorajiwala, J., Pugh, W., Moossa, A. R., and Rubenstein, A. H.: Effects of an enkephalin analog on pancreatic endocrine function and glucose homeostasis in normal and diabetic dogs. Endocrinology 1982; 111:2110–16.

[9] Schusdziarra, V., Specht, J., Schick, R., de la Fuente, A., Holland, A., and Pfeiffer, E. F.: Effect of morphine, leu-enkephalin and β-casomorphins on basal somatostatin release in dogs. Horm. Metab. Res. 1983; 15:407–408.

[10] Green, I. C., Perrin, D., Pedley, K. C., Leslie, R. D. G., and Pyke, D. A.: Effect of enkephalins and morphine on insulin secretion from isolated rat islets. Diabetologia 1980; 19:158–61.

[11] Hermansen, K.: Enkephalins and the secretion of pancreatic somatostatin and insulin in the dog: studies in vitro. Endocrinology 1983; 113:1149–54.

[12] Ipp, E., Dobbs, R., and Unger, R. H.: Morphine and β-endorphin influence the secretion of the endocrine pancreas. Nature 1978; 276:190–91.

[13] Green, I. C., Ray, K., and Perrin, D.: Opioid peptide effects on insulin release and cAMP in islets of Langerhans. Horm. Metab. Res. 1983; 15:124–28.

[14] Morley, J. E.: The endocrinology of the opiates and opioid peptides. Metabolism 1981; 30:195–209.

[15] Green, I. C., Perrin, D., Penman, E., Yaseen, A., Ray, K., and Howell, S. L.: Effect of dynorphin on insulin and somatostatin secretion, calcium uptake, and cAMP levels in isolated rat islets of Langerhans. Diabetes 1983; 32:685–90.

[16] Grodsky, G. M., Bennett, L. L., Smith, D. R., and Schmid, F. G.: Effect of pulse administration of glucose or glucagon on insulin secretion in vitro. Metabolism 1967; 16:222–23.

[17] McIntosh, C. H. S., Pederson, R. A., Koop, H., and Brown, J. C.: Gastric inhibitory polypeptide stimulated secretion of somatostatin-like immunoreactivity from the stomach: inhibition by acetylcholine or vagal stimulation. Can. J. Physiol. Pharmacol. 1981; 59:468–72.

[18] Chan, C. B., Pederson, R. A., Buchan, A. M. J., Tubesing, K. B., and Brown, J. C.: Gastric inhibitory polypeptide (GIP) and insulin release in the obese Zucker rat. Diabetes 1984; 33:536–42.

[19] Pierluissi, R., Pierluissi, J., and Ashcroft, S. J. H.: Effects of an enkephalin analogue (Damme) on insulin release from cultured rat islets of Langerhans. Diabetologia 1981; 20:642–46.

[20] Werther, G. A., Joffe, S., Artal, R., and Sperling, M. A.: Opiates modulate insulin action in vivo in dogs. Diabetologia 1984; 26:65–69.

[21] Celotti, F., Farina, J. M. S., Motta, M., and Martini, L.: Effect of metenkephalin on portal insulin and glucose in the rat. Horm. Metab. Res. 1980; 12:125–26.

[22] Reid, R. L., and Yen, S. S. C.: β-endorphin stimulates the secretion of insulin and glucagon in humans. J. Clin. Endocrinol. Metab. 1980; 52:592–94.

[23] Kanter, R. A., Ensinck, J. W., and Fujimoto, W. Y.: Disparate effects of enkephalin and morphine upon insulin and glucagon secretion by islet cell cultures. Diabetes 1980; 29:84–86.

[24] Rudman, D., Berry, C. J., Riedeburg, C. H., Hollins, B., M., Kutner, M. H., Lynn, M. J., and Chawla, R. K.: Effects of opioid peptides and opiate alkaloids on insulin secretion in the rabbit. Endocrinology 1983; 112:1702–10.

[25] Clement-Jones, V., Lowry, P. J., Rees, L. H., and Besser, G. M.: Met-enkephalin circulates in human plasma. Nature 1980; 283:295–97.

[26] Feldman, M., Kiser, R. S., Unger, R. H., and Li, C. H.: Beta endorphin and the endocrine pancreas: studies in healthy and diabetic human beings. N. Engl. J. Med. 1983; 308:349–53.

[27] Sawynok, J., Pinsky, C., and LaBella, F. S.: On the specificity of naloxone as an opiate antagonist. Life Sci. 1979; 25:1621–32.

[28] Pert, B. C., Pasternack, G., and Snyder, S. H.: Opiate agonists and antagonists discriminated by receptor binding in brain. Science 1973; 182:1359–61.

[29] McIntosh, C. H. S., Kwok, Y. N., Mordhorst, T., Nishimura, E., Pederson, R. A., and Brown, J. C.: Enkephalinergic control of somatostatin secretion from the perfused rat stomach. Can. J. Physiol. Pharmacol. 1983; 61:657–63.