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Methionine-Enkephalin Stimulates Gastric Secretion and Gastric Mucosal Blood Flow (40161)

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The naturally occurring enkephalins and endorphins and synthetic opiate substances exert their biological effects by complexing reversibly with specific opiate receptor sites identified in the brain and other tissues (1–3). Enkephalins, which probably serve as neurotransmitters of specific neuronal systems, have been recently localized by immunofluorescence in brain tissues (4–5).

The most striking observation, however, was the detection of enkephalins outside the brain, namely, in the gastrointestinal tract (6). The role of these substances in the regulation of the digestive system so far has not been determined, but several other hormonal peptides such as somatostatin, neurotensin and substance P, first isolated from the brain, were later shown to have immunoreactive counterparts in the alimentary canal (7). Similarly, hormonal peptides originally detected in the gut such as gastrin and vasoactive intestinal peptides act as neurotransmitters in the brain. The fact that the distribution of these peptides is largely limited to the brain and the gut may be related to the common embryologic origin of the nervous and digestive system from the neuroectoderm (8).

The present study was designed to explore the effects of methionine–enkephalin (9) (Met-enkephalin) on gastric secretion and gastric mucosal blood flow of an *in vivo* canine stomach preparation, and to compare these findings with those obtained with morphine itself before and after blockade of opiate receptors with naloxone.

Materials and methods. Thirty fasted mongrel dogs of either sex weighing from 8 to 17 kg were anesthetized by intravenous injection (1 ml/kg) of a solution containing 9.25 g chloralose and 95.2 g ethyl carbamate in 150 ml normal saline and endotracheal intubation was then carried out. A femoral artery was cannulated and connected to a pressure transducer for continuous monitoring of systemic blood pressure. Both femoral veins were cannulated for infusion of drugs and supplemental anesthetic.

An *in vivo* stomach chamber preparation of the oxyntic gland area with intact blood supply was made as previously described (10–11). A polyethylene catheter was introduced into a branch of the splenic artery, so that normal saline, Met-enkephalin or morphine could be administered into the gastroepiploic artery and directly to the stomach tissue. The effluent from the chambers was collected every 15 min and titrated to pH 7 to determine the rate of gastric acid secretion. Pepsin outputs were also measured in the effluents by the modified Anson hemoglobin method (12). Gastric mucosal blood flow was determined by aminopyrine clearance adapted to this preparation (13). Animals with a mean systolic blood pressure below 80 mm Hg were discarded.

Basal gastric secretion was collected for two 15 min periods and then submaximal stimulation was obtained by intravenous infusion of histamine (80 μg/kg-hr) through a femoral vein. After 60 min of intravenous histamine infusion, either normal saline (control tests), Met-enkephalin, or morphine hydrochloride was infused intra-arterially in graded doses. The dose was doubled every 60 min. In separate series of experiments, Met-enkephalin or morphine was given intra-arterially in graded doses without intravenous infusion of histamine. Since both opiate compounds were poor stimulants of acid secretion, the aminopyrine clearance was measured only in one half of the Lucite chamber by placing isotonic HCl solution (0.15 *N*) and recovering it at 15-min intervals (14). Secretory volume was calculated as the change in weight between the instilled and recovered solution. The other half of the chamber was

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designed for determining gastric secretion.

In additional experiments, Met-enkephalin or morphine was given intra-arterially in a constant dose for a two hour period during constant histamine stimulation (80 μg/kg-hr). When the morphine or Met-enkephalin had been going for 60 min, naloxone (60 μg/kg-hr) was given by intra-arterial infusion for 15 min. After cessation of Met-enkephalin or morphine administration, histamine alone or histamine combined with Met-enkephalin or morphine without naloxone was administered for the period of experiment.

Results. Intravenous administration of histamine alone resulted in an increase of gastric acid and pepsin secretion reaching a peak within the first hour and then followed by a well-sustained plateau for the entire period of drug infusion. Met-enkephalin or morphine infused intra-arterially in equimolar doses (10 nmol/kg-hr) together with histamine resulted in a significant increase of acid and pepsin outputs accompanied by a rise in gastric mucosal blood flow. After withdrawal of opiate infusion, gastric secretion as well as gastric mucosal blood flow tended to return towards control level (Fig. 1). Met-enkephalin infused intra-arterially in doses ranging from 4 to 64 nmol/kg-hr caused a dose-dependent augmentation of histamine-induced gastric acid and pepsin secretion and gastric mucosal blood flow. Acid outputs reached a peak at a dose of 32 nmol/kg-hr whereas pepsin outputs and mucosal blood flow showed a tendency for further increase. Morphine given intra-arterially at an equimolar dose caused a similar increase in pepsin outputs and mucosal blood flow whereas acid output reached a peak at a dose of 128 nmol/kg-hr and it was about twice as high as that obtained with Met-enkephalin (Fig. 2). Met-enkephalin or morphine given intra-arterially in graded doses during basal secretion (without histamine) did not affect gastric acid or pepsin secretion but caused a dose-dependent increase in gastric mucosal blood flow (Fig. 3).

The augmentation of histamine induced gastric acid and pepsin secretion and mucosal blood flow was also demonstrated when a constant dose of Met-enkephalin or morphine was given intravenously into the femoral vein but about ten times larger doses were required than in tests with the intra-arterial

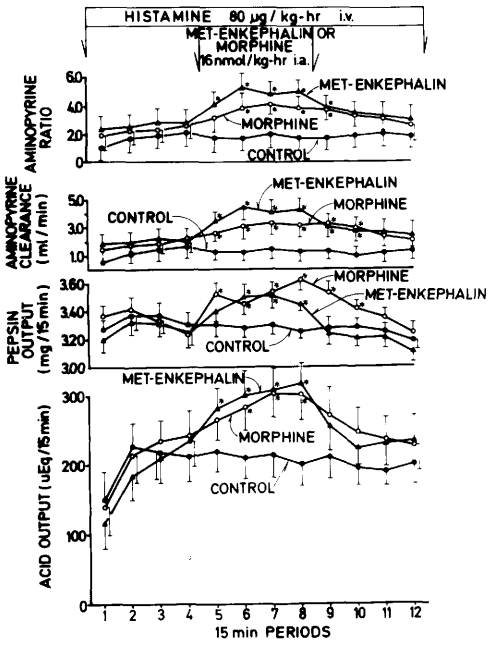

FIG. 1. Effect of intra arterial infusion of Met-enkephalin or morphine on histamine-induced gastric acid and pepsin outputs and gastric mucosal blood flow. In control experiments histamine alone was given intravenously for the period of experiment. Mean values for six dogs ± SEM. In this and subsequent figures asterisks indicate statistically significant (P 0.05) increase above control value with histamine alone after the same elapsed time from the start of the experiment.

administration. These data are omitted for the clarity of presentation.

Naloxone added to intra-arterial infusion in a dose of 60 μg/kg-hr for 15-min period resulted in a significant suppression of gastric secretion and mucosal blood flow induced by a combination of histamine and Met-enkephalin (Fig. 4) or morphine (Fig. 5). The decrease of gastric secretion and gastric circulation resulting from the administration of naloxone was much stronger and more prolonged in tests with enkephalin than with morphine. Naloxone given during histamine infusion, without opiate compounds, did not affect significantly gastric secretion or mucosal blood flow. These data are omitted from the presentation.

Discussion. This study provides evidence that both morphine and Met-enkephalin, a natural ligand for opiate receptor, produce dose-dependent augmentation of histamine-

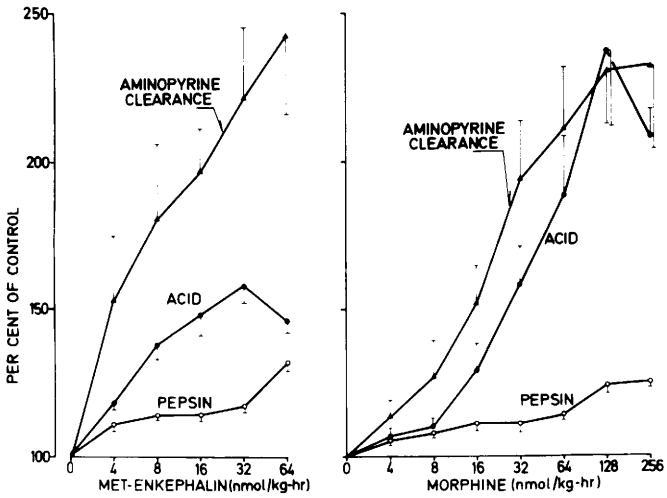

FIG. 2. Effects of intra-arterial infusion of Met-enkephalin or morphine on histamine-induced gastric acid and pepsin outputs and gastric mucosal blood flow expressed as percent of the control values (in test with histamine alone). Mean values for 12 dogs ± SEM.

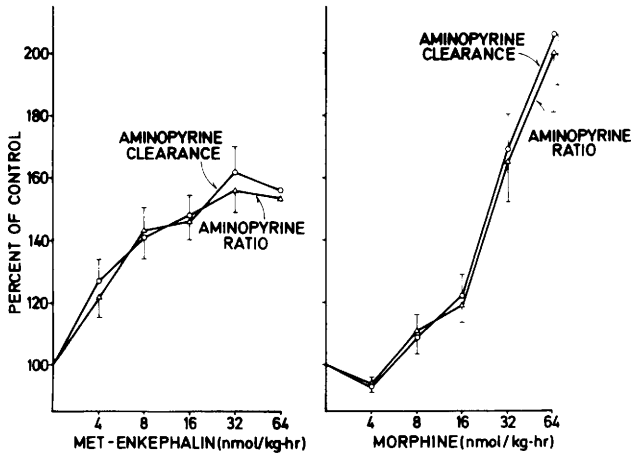

FIG. 3. Effect of intra-arterial infusion of Met-enkephalin or morphine on aminopyrine clearance and ratio expressed as percent of the control values. Mean values for six dogs ± SEM.

induced gastric acid and pepsin secretion and strongly increase gastric mucosal blood flow. Whether opiate peptides present in the digestive tract are involved in the physiological stimulation of gastric secretion remains to be determined. The finding that naloxone, a potent opiate antagonist, diminished or abolished the stimulatory effects of morphine and Met-enkephalin on gastric secretion indicates that the oxyntic cells may possess high affinity binding sites which are saturable and stereospecific for opiates. The observation of this study, however, that naloxone does not affect basal or histamine-induced gastric secretion suggests that endogenous opiate peptides present in the gastric wall may not be directly involved in the activation of oxyntic cells.

It is well established that gastric secretion depends directly upon gastric tissue perfusion (15). It follows that changes in the ratio of gastric mucosal blood flow to gastric secretion may demonstrate the primary site of action of drugs that affect gastric secretion. Both morphine and Met-enkephalin given either alone or in a combination with a histamine background were found in this study to cause

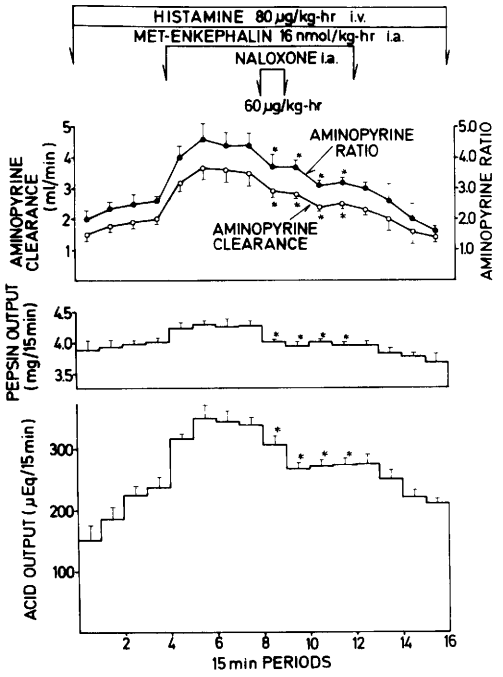

FIG. 4. Effects on intra-arterial infusion of naloxone on aminopyrine clearance and acid and pepsin outputs stimulated by the combination of Met-enkephalin given intra-arterially and histamine administered intravenously. Mean values for six dogs ± SEM. Asterisks indicate significant decreases from histamine and Met-enkephalin.

a marked and dose-dependent increase in gastric mucosal blood flow and a rise in the ratio of aminopyrine to the rate of secretion. Such a relationship between gastric mucosal circulation and secretion indicates that opiates may be classified as potent vasoactive substances. The augmentation of histamine-induced gastric secretion by these compounds may be due at least in part to a primary increase in gastric microcirculation and increased delivery of histamine to the oxyntic glands. This is supported by our finding that opiates alone without a histamine background did not affect unstimulated gastric acid secretion but caused significant and dose-dependent rise in gastric mucosal blood flow. It is of interest that the only substances known to be capable of augmenting histamine-induced gastric secretion but unable to initiate gastric secretion from the unstimulated stomach, are the gastric inhibitors of phosphodiesterase such as methylxanthines and papaverine or cyclic AMP itself (10, 16). It remains to be established whether opiate compounds increase cyclic AMP content in the gastric mucosa and whether opiate-stimulated gastric mucosal blood flow is mediated by this cyclic nucleotide as in the case of phosphodiesterase inhibitors (10, 16).

It is also likely that both secretory and circulatory effects of opiate compounds could be mediated by endogenous histamine as it was reported previously that morphine may increase endogenous release of histamine (17). It has also been suggested that the release of gastrin and cholinergic stimulation are involved in the opiate-induced gastric secretion (18, 19, 29) but neither of these factors seem to be responsible for this secretion as the gastric flap preparation used in our study was deprived of endogenous hormonal and vagal influences.

Summary. Intra-arterial infusion of methionine–enkaphalin or morphine caused a dose-dependent increase in histamine-in-

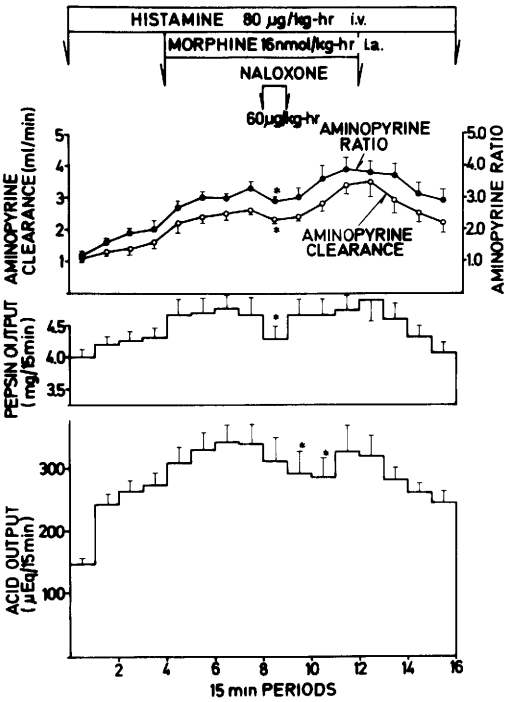

FIG. 5. Effects of intra-arterial infusion of naloxone on aminopyrine clearance and acid and pepsin outputs stimulated by the combination of morphine given intra-arterially and histamine administered intravenously. Mean values for 6 dogs ± SEM. Asterisks indicate significant decreases from morphine and histamine control.

duced gastric secretion and gastric mucosal blood flow. Administration of naloxone resulted in the reduction of both gastric secretion and mucosal circulation indicating that oxyntic cells possess specific opiate receptors and that opiate peptides may be involved in the stimulation of gastric secretion.

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