MEK inhibits secretin release and pancreatic secretion: roles of secretin-releasing peptide and somatostatin

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Li, James P., Kae Yol Lee, Ta-Min Chang, and William Y. Chey. MEK inhibits secretin release and pancreatic secretion: roles of secretin-releasing peptide and somatostatin. Am J Physiol Gastrointest Liver Physiol 280: G890–G896, 2001.—We investigated the mechanism of action of methionine enkephalin (MEK) on HCl-stimulated secretin release and pancreatic exocrine secretion. Anesthetized rats with pancreaticobiliary cannulas and isolated upper small intestinal loops were perfused intraduodenally with 0.01 N HCl while bile and pancreatic juice were diverted. The effect of intravenous MEK on acid-stimulated secretin release and pancreatic exocrine secretion was then studied with or without coinfusion of naloxone, an anti-somatostatin (SS) serum, or normal rabbit serum. Duodenal acid perfusate, which contains secretin-releasing peptide (SRP) activity, was collected from donor rats with or without pretreatment with MEK, MEK + naloxone, or MEK + anti-SS serum, concentrated by ultrafiltration, and neutralized. The concentrated acid perfusate (CAP), which contains SRP bioactivity, was infused intraduodenally into recipient rats. MEK increased plasma SS concentration and inhibited secretin release and pancreatic fluid and bicarbonate secretion dose-dependently. The inhibition was partially reversed by naloxone and anti-SS serum but not by normal rabbit serum. In recipient rats, CAP increased plasma secretin level and pancreatic secretion. CAP SRP bioactivity decreased when it was collected from MEK-treated donor rats; this was partially reversed by coinfusion with naloxone or anti-SS serum. These results suggest that in the rat, MEK inhibition of acid-stimulated pancreatic secretion and secretin release involves suppression of SRP activity release. Thus the MEK inhibitory effect appears to be mediated in part by endogenous SS.

METHIONINE ENKEPHALIN (MEK), a pentapeptide with methionine as the COOH-terminal amino acid, was originally isolated from the pituitary and identified in 1975 (11). It has been demonstrated through immunocytochemistry that MEK is widely distributed in the gastrointestinal tract and the pancreas (26). It has a powerful opiate-like effect and participates in the control of a wide variety of motor and secretory functions in the gastrointestinal tract (21). MEK has been shown to exert an inhibitory action on pancreatic exocrine secretion stimulated by duodenal acidification, endogenous secretin, and CCK in the dog (4). The inhibition by MEK appears to be caused, at least in part, by a direct effect on the exocrine pancreas. In addition, the release of secretin, and perhaps CCK, was also reduced by MEK in the dog (4). Konturek et al. (12) reported that MEK inhibited pancreatic secretion elicited by sham feeding or exogenous secretin and CCK in humans that was reversed by naloxone. Gullo et al. (8), however, found that MEK enhanced secretin- and CCK-stimulated exocrine pancreatic secretion in humans. This discrepancy may be due to the different doses of MEK used in the studies (8, 12).

We (18) reported that HCl-stimulated pancreatic exocrine secretion and secretin release are mediated by a secretin-releasing peptide (SRP), which is trypsin sensitive and heat stable. The release of SRP is regulated by a neural mechanism, particularly through mediation by capsaicin-sensitive afferent sensory neurons (17). Both MEK (26) and somatostatin (SS) (23) are found in the upper small intestine. Matsumura et al. (22) observed that duodenal acidification increased plasma endorphin-like immunoreactivity. Lucey et al. (20) reported that duodenal perfusion with acid elevated peripheral plasma levels of SS in humans. Thus it is quite possible that both MEK and SS are involved in the acid-stimulated release of SRP and secretin as well as in pancreatic exocrine secretion. The relationship of MEK and SS in acid-stimulated pancreatic exocrine secretion and release of SRP and secretin has not been studied.

The aim of the present study was to investigate in anesthetized rats the effect of MEK on pancreatic secretion and the release of secretin and SRP bioactivity in response to duodenal acidification and the role of endogenous SS in the inhibitory effect of MEK.

MATERIALS AND METHODS

Materials. The agents used in the present study were from the following sources. MEK was obtained from Bachem (Torrance, CA), and naloxone was from Du Pont Pharmaceuticals.
(Manati, PR). Synthetic porcine secretin was a gift from Dr. David Coy (Tulane University, New Orleans, LA), and goat anti-SS serum was a gift from Dr. A. Arimura (Tulane University). A rabbit anti-secretin serum with a titer of 1:1,000,000 and a rabbit anti-SS serum with a titer of 1:150,000 were both produced in our laboratory.

**Animal preparation.** Adult male Sprague-Dawley rats weighing 230–280 g were fasted for 24 h with free access to drinking water before surgery. Anesthetized rats with pancreaticobiliary canuluses and isolated upper small intestinal loops were prepared as described previously (17, 18).

**Preparation of concentrated acid perfusate in donors.** The upper small intestinal loop of each donor rat was washed with 40 ml of 0.15 M NaCl to remove the remaining pancreatic juice and bile. HCl (0.01 N) was then perfused into the loop at a rate of 0.3 ml/min for 1.5 h while bile and pancreatic juice were continuously diverted (17, 18). The perfusate was collected from the distal end of the loop with an ice-chilled beaker and centrifuged. The supernatant solution was concentrated by ultrafiltration as described previously (17, 18). The concentrated saline perfusate (CSP) was adjusted to pH 7.0 and reinfused into recipient rats via the duodenal cannula of the upper small intestinal loop to test for SRP bioactivity. NaCl (0.15 M) was also infused into the upper small intestinal loop of each donor rat, and the perfusate was collected and treated in the same way as CAP. The concentrated saline perfusate (CSP) was adjusted to pH 7.0 and reinfused into recipient rats as a control group. MEK (40 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\)), MEK plus naloxone (20 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\)), MEK plus rabbit anti-SS serum (1 ml/rat), or MEK plus normal rabbit serum (1 ml/rat) was administered intravenously 30 min before intraduodenal perfusion with 0.01 N HCl. The perfusate was collected and concentrated as described above.

**Experimental procedure.** After the upper small intestine was washed with 40 ml of 0.15 M NaCl administered intraduodenally, each animal was infused intraduodenally with 0.15 M NaCl at 0.3 ml/min for 1 h as the basal period. Various treatments were administered intravenously after the basal period. Pancreatic juice was continuously collected in 30-min intervals through the entire experiment period for determination of fluid volume and bicarbonate output while bile was diverted. In some rats, blood was drawn from the abdominal aorta at the end of the experiments to determine plasma concentrations of secretin and SS.

To study the effect of MEK on acid-stimulated release of secretin and pancreatic secretion, we infused 42 rats with 0.01 N HCl for 2 h. During the second hour of HCl infusion, MEK was administered intraduodenally at 5, 10, 20, 40, or 80 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\) in 30 rats (6 rats for each dosage). In another six rats, naloxone (20 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\)) was administered intravenously 30 min before MEK (40 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\)) was given. In the remaining six rats, naloxone (20 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\)) was infused alone 30 min after infusion of HCl into the loop for 1.5 h.

To study the role of endogenous SS in the MEK inhibition of acid-stimulated release of secretin and pancreatic secretion, we measured plasma SS level before and 2 h after intraduodenal infusion of 0.01 N HCl or intravenous administration of MEK at 10, 20, 40, and 80 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\) in 20 rats (5 rats for each dosage). Two other groups (6 rats/group) received duodenal administration of 0.01 N HCl for 2 h, and then a rabbit anti-SS serum (1 ml/rat) or normal rabbit serum (1 ml/rat) was injected intravenously 30 min before MEK infusion began.

To investigate the effect of MEK on the release of SRP bioactivity, a total of 20 recipient rats were used. In two groups of five rats each, CAP or CSP was infused into the upper small intestinal loop at a rate of 0.3 ml/min for 1.5 h. In another two groups of five rats each, CAP collected from donor rats pretreated with MEK or MEK plus naloxone was infused into the small intestinal loop at the same rate.

To determine whether endogenous SS is involved in MEK inhibition of the release of SRP bioactivity, two groups of five rats each received CAP collected from donor rats pretreated with either the anti-SS serum plus MEK or normal rabbit serum plus MEK under the same conditions as described above.

**Measurement.** Pancreatic juice was collected by inserting the pancreatic cannula into a glass micropette. The volume of pancreatic juice collected in 30-min intervals was determined by measuring the length of pancreatic juice in the micropette. A 10-\( \mu \)l aliquot of pancreatic juice was immediately taken for measurement of bicarbonate concentration by a \( \text{CO}_2 \) analyzer (Beckman Instruments, Fullerton, CA) as described previously (17, 18). Plasma secretin and SS levels were measured by the corresponding specific RIA as described previously (3, 27).

**Statistical analysis.** All data are presented as means ± SE. The percent increase over basal pancreatic secretion was calculated by comparing pancreatic secretion including flow volume and bicarbonate output measured during the 1-h basal period with that during the last hour in the treatment period. The differences in responses among experimental groups were analyzed using ANOVA. The difference between two treatment means was determined by Tukey’s post hoc test. Statistical significance was established at \( P < 0.05 \).

**RESULTS**

**Effect of MEK on pancreatic secretion and plasma secretin in response to duodenal acidification.** Intraduodenal infusion of 0.01 N HCl significantly increased pancreatic juice volume (36.9 ± 4.6 vs. 17.8 ± 1.8 \( \mu \)l/30 min, \( P < 0.01 \)), bicarbonate output (2.02 ± 0.31 vs. 0.85 ± 0.20 \( \mu \)eq/30 min, \( P < 0.01 \)), and plasma secretin level (8.8 ± 1.5 vs. 1.7 ± 0.3 pM, \( P < 0.01 \)) compared with the basal period. MEK, given intravenously at graded doses (5–80 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\)), dose-dependently suppressed the increases in pancreatic exocrine secretion and plasma secretin concentration in response to duodenal acidification (Fig. 1). MEK at 10 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\) was the minimal dose that significantly inhibited the acid-induced pancreatic exocrine secretion. On the other hand, a significant inhibition of plasma secretin level occurred at 40 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\) MEK or above (Fig. 1).

**Plasma concentration of SS in response to acid or MEK administration.** The basal plasma level of SS was 14.6 ± 4.1 pM. After intraduodenal administration of 0.01 N HCl, plasma SS level rose to 29.8 ± 7.3 pM (\( P < 0.05 \)). Intravenous infusion of MEK in graded doses ranging from 10 to 80 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\) also increased plasma concentration of SS in a dose-related manner. However, MEK significantly increased plasma SS concentration over the basal level only when 40 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\) or a larger dose was given (Fig. 2).

**Effect of naloxone on acid-stimulated pancreatic secretion and release of secretin.** Naloxone (20 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\)) alone did not influence basal pancreatic secretion and the release of secretin or SS. Dooley et al. (5) also observed that naloxone had no effect on basal pancre-
atic secretion of fluid and bicarbonate in the dog. However, naloxone partially reversed inhibition by MEK (40 μg·kg⁻¹·h⁻¹) of acid-stimulated pancreatic secretion (53.8% in flow volume and 55.2% in bicarbonate output) and secretin release (7.1 ± 0.6 vs. 5.3 ± 0.5 pM) (Fig. 3).

Effect of anti-SS serum on inhibitory effect of MEK. Intravenous injection of rabbit anti-SS serum (1 ml/rat) did not influence basal pancreatic exocrine secretion and the release of secretin. However, the anti-SS serum significantly reversed the inhibitory effect of MEK on acid-stimulated pancreatic secretion and the release of secretin (7.7 ± 0.8 vs. 5.3 ± 0.5, P < 0.05) (Fig. 4, right). The inhibition by MEK of pancreatic exocrine secretion including flow volume and bicarbonate output was completely reversed by the anti-SS serum (Fig. 4). In contrast, normal rabbit serum did not influence the effect of MEK (Fig. 4).

Effect of MEK on acid-induced release of SRP bioactivity. Infusion of CAP into the small intestinal loop of recipient rats (Fig. 5) markedly increased the volume of pancreatic juice by 79.4 ± 7.3% and bicarbonate output by 146.9 ± 11.1% (P < 0.01). Plasma secretin level also increased from 1.2 ± 0.4 to 4.7 ± 0.7 pM (P < 0.05). These effects were shown to be caused by the release of SRP activity in duodenal acid perfusate (17, 18). In contrast, CSP did not influence the release of secretin or pancreatic exocrine secretion (data not shown). Infusion of CAP from MEK-pretreated donor rats produced 44% less pancreatic fluid and 68.1% less bicarbonate secretion (Fig. 5). The plasma secretin level was also reduced to 2.3 ± 0.4 pM (Fig. 5, right). These results indicated decreased SRP activity in MEK-pretreated CAP. However, the reduction of SRP activity by MEK was partially reversed by pretreatment of donor rats with MEK in the presence of naloxone (51.3% in fluid volume, 79.4% in bicarbonate output, and 4 ± 0.3 pM in plasma concentration of secretin). This result suggested that an opiate receptor or receptors are involved in regulation of the acid-stimulated release of SRP activity.

Effect of anti-SS serum on inhibition of SRP release by MEK. As shown in Fig. 6, CAP obtained from the donor rats pretreated with MEK in the presence of the anti-SS serum produced a greater pancreatic secretion than the MEK-pretreated CAP (68.4 ± 16.2% in volume flow and 74.0 ± 18.1% in bicarbonate output). In addition, plasma secretin concentration (4.8 ± 0.7 pM) was restored to the same level as that produced by control CAP. These results indicated that the anti-SS serum reversed the MEK inhibition of acid-elicited release of SRP activity in donor rats. On the other hand, pretreatment of donor rats with MEK in the
presence of a normal rabbit serum did not affect reduction by MEK of SRP activity in CAP (data not shown).

DISCUSSION

In the present study we have demonstrated that MEK dose-dependently inhibited the release of secretin and pancreatic secretion of fluid and bicarbonate in response to duodenal acidification in anesthetized rats. The inhibitory effect of MEK was significantly reversed by naloxone, indicating mediation by an opiate receptor or receptors. This observation confirmed previous observations on the same effect of MEK in dogs (4, 13) and humans (12). In addition, the present results also indicated that MEK at 40 μg·kg⁻¹·h⁻¹ or higher significantly increased plasma concentration of SS. The role of SS in MEK-evoked inhibition of acid-stimulated release of secretin and pancreatic exocrine secretion therefore was tested. The observation that the anti-SS serum, but not normal rabbit serum, reversed the inhibitory effect of MEK clearly suggested that endogenous SS mediates the actions of MEK on acid-stimulated secretin release and pancreatic exocrine secretion. It should be noted that although plasma concentration of SS is elevated in anesthetized rats

Fig. 3. Effect of MEK with or without naloxone (NL) on acid-stimulated pancreatic secretion and plasma secretin level. HCl was administered at 0.01 N id, MEK at 40–80 μg·kg⁻¹·h⁻¹ iv, and NL at 20–80 μg·kg⁻¹·h⁻¹ iv. ●, HCl alone; ○, HCl + MEK; ▲, HCl + MEK + NL. Values are means ± SE; 6 rats were in each group.*P < 0.05 compared with HCl-stimulated pancreatic secretion or plasma secretin level.

Fig. 4. Effect of rabbit anti-SS serum (anti-SS) and normal rabbit serum (NRS) on pancreatic secretion and plasma secretin level in response to MEK infusion. HCl was administered at 0.01 N id, MEK at 40 μg·kg⁻¹·h⁻¹ iv, anti-SS at 1 ml/rat iv, and NRS at 1 ml/rat iv. ●, HCl alone; ○, HCl + MEK; ●, HCl + MEK + anti-SS; ▲, HCl + MEK + NRS. Values are means ± SE; 6 rats were in each group. *P < 0.05 compared with HCl-stimulated pancreatic secretion or plasma secretin.
(33), it does not affect basal pancreatic exocrine secretion (32). The observation made in the present study, however, confirmed the previous notion that endogenous SS is involved in regulation of stimulated pancreatic exocrine secretion (32).

We reported previously (18) that duodenal acidification-stimulated release of secretin and pancreatic exocrine secretion is mediated through the release of SRP activity in the intestinal lumen. Thus CAP obtained from donor rats was shown to contain a heat-stable and trypsin-sensitive low-molecular-mass (<10 kDa) substance(s) that stimulated secretin release and pancreatic exocrine secretion on infusion into the upper small intestinal lumen of the recipient rats. Administration of a specific anti-secretin serum abolished CAP-stimulated pancreatic exocrine secretion, suggesting that the effect of CAP on the pancreas is mediated through the release of secretin (18). The results of the present study indicated that such a SRP activity in CAP was substantially reduced when CAP was obtained from MEK-pretreated donor rats. Moreover, pretreatment of donor rats with MEK together with naloxone or anti-SS serum partially but significantly reversed the reduction of SRP activity in CAP. Thus it may be concluded that inhibition of acid-stimulated secretin release and pancreatic exocrine secretion by MEK is at least in part mediated through inhibition of the release of SRP activity. This action of MEK involves an opiate receptor-mediated process that is also mediated through the release of endogenous SS.

The mechanism through which SS mediates MEK-elicited inhibition of acid-stimulated release of secretin is not clear at present. Both peptides were shown to inhibit low pH-stimulated secretin secretion from mu-

![Fig. 5. Effect of concentrated acid perfusate (CAP) collected from donor rats pretreated with MEK with or without NL on pancreatic secretion and release of secretin in recipient rats.](image)

![Fig. 6. Effect of CAP collected from donor rats pretreated with MEK with rabbit anti-SS and NRS on pancreatic secretion and release of secretin in recipient rats.](image)
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cosal explants isolated from canine duodenum (24). In addition to its presence in the mucosal endocrine cells (25), SS is also present in the submucosal plexus and nerve fibers projecting to the intestinal mucosa in various mammalian species (1, 7, 9, 19). Similarly, MEK-immunoreactive nerve fibers have been found to innervate the mucosa and submucosal plexus of human duodenum (7). SS is released from human submucosal neurons in vitro (2). Both MEK and SS have been shown to inhibit calcium current in guinea pig submucosal neurons (31) and cholera toxin-induced net fluid secretion and vasoactive intestinal polypeptide release from cat jejunum (6). In addition, SS receptors are expressed in enterocytes and in the submucosal plexus of rat intestine (29). Thus it is possible that exogenous MEK in the present study could act on either mucosal SS-containing nerve fibers or the intestinal D cells to release SS, which in turn inhibit the release of secretin by acting either directly on S cells or through inhibition of SRP release or both. These questions require further study.

The mechanism through which SS mediates MEK-elicted inhibition of acid-stimulated pancreatic exocrine secretion is also unclear. Larsson (14) demonstrated that immunoreactivity of MEK is located in nerve fibers in the endocrine and exocrine pancreas. In addition, pancreatic extract from guinea pigs was shown to contain peptides eluting with similar characteristics to MEK on HPLC (30). In isolated and perfused rat (28) and dog (10) pancreas, MEK was shown to stimulate SS release, suggesting that SS may mediate the action of MEK on the pancreas. The result of the present study has indicated that inhibition of pancreatic exocrine secretion by MEK is completely reversed by cotreatment with the anti-SS serum. In previous studies using isolated and perfused rat (16) and canine (15) pancreas, we showed that endogenous insulin is required for stimulation of pancreatic exocrine secretion by secretin and CCK. Thus infusion of secretin and CCK in the presence of a specific anti-insulin serum abolished the stimulatory effect of the two gut hormones. Coinfusion of the two gut hormones with the anti-insulin serum resulted in an elevation of SS and pancreatic polypeptide in the portal venous effluent. Coinfusion with the anti-insulin serum together with an anti-SS serum, however, restored secretin, and CCK stimulated pancreatic secretion of fluid and bicarbonate (15, 16), thereby suggesting that endogenous SS from the pancreas plays a negative regulatory role in pancreatic exocrine secretion. Thus it is very likely that MEK inhibits pancreatic secretion through suppression of SS release from the pancreatic islet. However, we cannot rule out the possibility that MEK acts to elicit SS release through the enteric nerves, which exert an inhibitory effect on the pancreas via an enteropancreatic reflex mechanism. This matter requires further study to be resolved.

In summary, MEK inhibited acid-stimulated pancreatic exocrine secretion and the release of secretin through suppression of the release of SRP bioactivity. The inhibitory effect of MEK appears to be mediated by endogenous SS in the rat. Further study is required to determine whether this regulatory mechanism plays a significant role under normal physiological conditions.

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