Release of Cholecystokinin by Amino Acids (37308)

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Intestinal perfusion with essential amino acids results in the liberation of cholecystokinin (CCK) and subsequent stimulation of pancreatic enzyme secretion (1–4). It has been demonstrated that only L-isomers of neutral amino acids are effective and that the amount of releasable CCK is related to the load of amino acid and to the length of the intestine exposed to amino acid (2, 4). However, no systematic study has been performed to compare the relative effectiveness of various amino acids in the release of CCK from the intestinal musoca.

In this report the activity of individual amino acids, both essential and nonessential, perfused through intestinal Thiry-Vella loops in releasing CCK has been tested in conscious dogs with gastric and pancreatic fistulas using the stimulation of pancreatic protein secretion and the potentiation of secretin-induced pancreatic bicarbonate secretion as the criteria of CCK release.

Methods. Four mongrel dogs, weighing 18–20 kg, were prepared surgically with gastric fistulas by inserting a Thomas cannula in the stomach and pancreatic fistulas made according to a modification of the method of Herrera *et al.* (5). In addition, the animals were provided with Thiry-Vella loops fashioned from the distal portion of the duodenum just below the entrance of the main pancreatic duct and the upper jejunum. Each intestinal loop was about 50 cm long and cannulated at both ends by Gregory cannulas. Gastrointestinal continuity was restored by end to end duodenojejunostomy.

Secretory studies were started about 4 wk after surgery. The animals were deprived of food for at least 18 hr before each test. The stomach was rinsed by irrigation through the gastric fistula, which was kept open throughout the experiment to prevent the acid from the main stomach from entering the duodenum and releasing endogenous hormones. Pancreatic secretion was examined first under basal conditions during two 15 min periods. No tests were started unless there were at least two consecutive 15 min periods with basal secretion less than 2.0 ml. In each test, two infusions of 0.15 *M* NaCl were given at a constant rate of 100 ml/hr by a peristaltic pump (Unipan, Poland), one through a polyethylene tube (PE 50) inserted into the leg vein and the other into the Thiry-Vella loop through the cannula placed at the proximal end of the loop.

Secretion from the pancreatic fistula was collected continuously and separated into 15 min samples. The volume of each sample was recorded to the nearest 0.1 ml. Bicarbonate concentration was measured by adding 0.5 ml of pancreatic juice to 1.0 ml of 0.1 *N* HCl, boiling the solution briefly, cooling and backtitrating the residual HCl with 0.1 *N* NaOH. Bicarbonate output was expressed as milliequivalents per 15 or 30 min period. Protein concentration was determined with a Perkin-Elmer spectrophotometer as absorption at 280 nm and compared to a standard solution of human serum albumin. Protein output was expressed as milligrams per 15 or 30 min period.

The activity of amino acids in releasing CCK from the intestinal mucosa was examined by perfusion of the duodenojejunal loop with equimolar (50 m*M*) solution of L-isomers of essential and nonessential amino acids. Sufficient dilute NaOH was added to this solution to bring the pH to 7.0. Mannitol was added as required to achieve an osmolarity of about 300 mOsm/liter. Osmolarity was measured by freezing de-

pression with a Fiske osmometer. In all tests with amino acids a background dose (0.2 units/kg/hr) of synthetic secretin (gift from M. A. Ondetti of Squibb Institute, New Brunswick, NJ) was given intravenously throughout the experiment to enhance the sensitivity of the assay. Sixty minutes after beginning intravenous infusion of secretin, when the rate of pancreatic secretion had become relatively constant, amino acid solution instead of saline was infused into the intestinal loop by a peristaltic pump at a constant rate of 100 ml/hr for a 1 hr period. The animals then continued to receive saline intraduodenally for one additional hour. The CCK-releasing activity of each amino acid was determined by comparing pancreatic outputs in the two last 15 min periods before amino acid instillation with those occurring during the two last 15 min periods of amino acid instillation. The increase of the volume protein and bicarbonate outputs during amino acid instillation above the mean level before infusion is referred to as the CCK-releasing activity of the amino acid. Control experiments also were conducted during which the animals received only secretin intravenously and saline intraduodenally for the duration of the test.

The relationship between the amount of releasable CCK and the rate of amino acid perfusion was determined by instillation into the intestinal loop of constant volume (100 ml/hr) of the solution of tryptophan in increasing concentration ranging from 3.5 to 100 m*M*. A background dose of 0.2 units/kg/hr of secretin was also given in these tests.

A possible release of secretin by amino acids has been tested by establishing a plateau of secretion in response to a supramaximal dose (25 units/kg/hr) of CCK (gift of Professor E. Jorpes of GIH Research Unit, Karolinska Institutet, Stockholm, Sweden) and then infusing amino acid into the intestinal loop against this background of exogenous stimulation. An increase in bicarbonate secretion was considered as evidence of secretin release by amino acid and was compared with exogenous secretin given intravenously in the dose producing an equal rate of pancreatic bicarbonate secretion.

Only one individual amino acid and only one rate of administration was used on a given day and experiments were conducted on alternate days no more than three times a week.

The significance of differences between the protein and bicarbonate outputs from the pancreatic fistula before and during perfusion of the intestinal loops with individual amino acids was determined by the Mann-Whitney (6) U test. As used in the text, significance indicates $p < 0.05$.

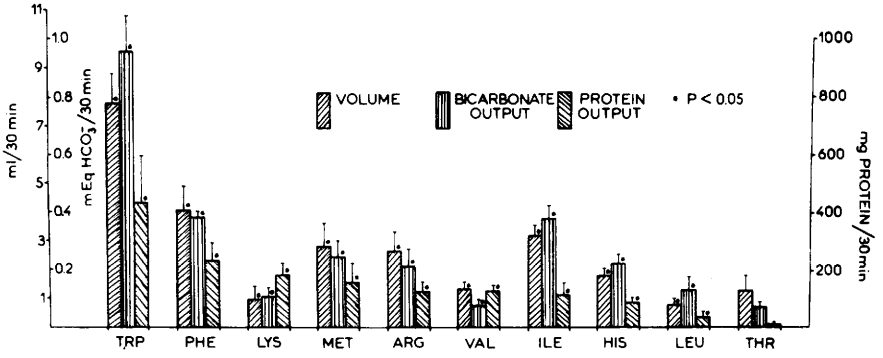

FIG. 1. The increase in pancreatic volume, bicarbonate and protein outputs over control levels in response to individual essential amino acids perfused through the intestinal Thiry-Vella loop. In this and subsequent figures each line is a mean of 3 tests on each of 4 dogs. Vertical bars indicate standard error of the mean (SEM). In all tests in Figs. 1–3 a threshold dose (0.2 units/kg hr) of secretin was given throughout the test. In control period secretin background was administered intravenously and saline was perfused through the intestinal loop.

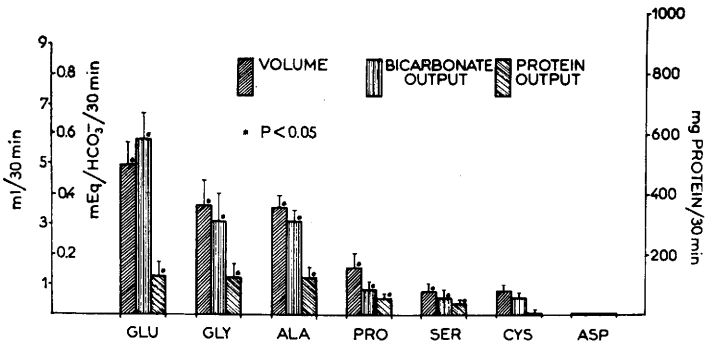

FIG. 2. The increase in pancreatic volume, bicarbonate and protein outputs over control level in response to individual nonessential amino acids perfused through the intestinal Thiry-Vella loop.

Results. Figures 1 and 2 demonstrate the relative activities of individual amino acids in releasing CCK. Using the stimulation of pancreatic protein and an increase of bicarbonate secretion above the control level as the criteria for the release of CCK, it was found that all tested essential amino acids except threonine and all nonessential amino acids except cysteine and aspartic acid were effective. Among essential amino acids, the most potent releasers of CCK were tryptophan and phenylalanine and among nonessential amino acids the most potent were glutamic acid, glycine and alanine.

Tryptophan instilled into the intestinal loop at various concentrations produced a dose-related increase in pancreatic secretion reaching the maximum at the concentration of 50 m*M* (Fig. 3). Further increase in tryptophan concentration did not result in further increase of pancreatic secretion. The highest pancreatic response to tryptophan in this study was equal to about 80% of that evoked by a dose of 25 units/kg/hr of CCK.

The results of the study on the ability of phenylalanine and tryptophan to release secretin are shown on Fig. 4. A large load of each amino acid (16 mmoles/hr) produced a significant increase of bicarbonate output about that obtained with CCK alone. This increase was equivalent to that obtained with exogenous secretin at a dose of 0.01 units/kg/hr.

Discussion. This study provides evidence that almost all L-isomers of essential and nonessential amino acids release CCK from the intestinal mucosa and that the most effective appear to be tryptophan and phenylalanine.

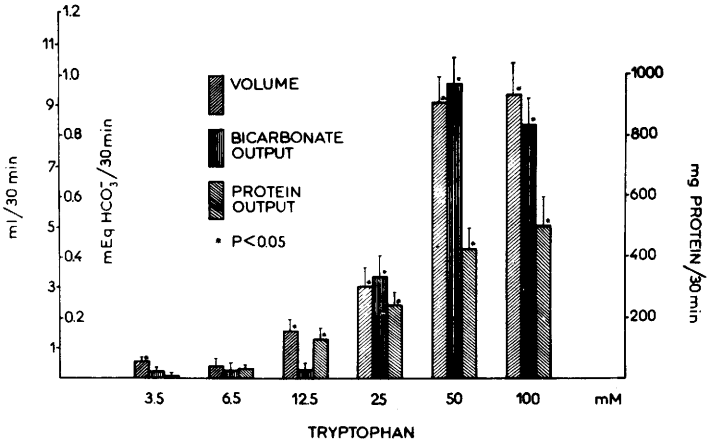

FIG. 3. The effect of intestinal perfusion with a constant volume of tryptophan in concentration varying from 6.5 to 100 m*M*.

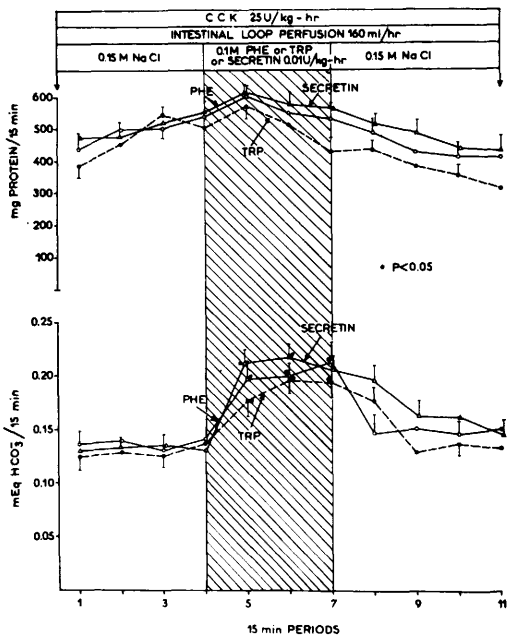

FIG. 4. Protein and bicarbonate outputs in response to phenylalanine and tryptophan instilled into the intestinal loop during a plateau of secretion to supramaximal dose of exogenous CCK (25 units/kg hr).

The amount of CCK released by tryptophan was related to the concentration of this amino acid in the perfusion solution and achieved the peak at the 50 m*M* concentration. Both potent releasers of CCK, tryptophan and phenylalanine, also stimulate the release of small amounts of secretin from the intestinal mucosa.

Similar comparison of the potencies of some amino acids has been reported recently by Meyer and Grossman (2), who found that only L-isomers of neutral amino acids were active releasers of CCK and that among the neutral amino acids phenylalanine and tryptophan were most effective. Phenylalanine infused into the duodenum did not augment bicarbonate response to a high background dose of exogenous CCK, indicating that all of its effects on pancreatic secretion could be fully accounted for by the release of CCK. Such amino acids as methionine, arginine, histidine, lysine, glycine, aspartic acid and glutamic acid were not effective in the release of CCK. Several amino acids such isoleucine, proline, serine, cysteine and threonine were not tested.

The reason for the discrepancy between our present results and those reported by Meyer and Grossman could be attributed at least partly to the difference in the technique employed to release CCK. Although in both studies the same parts of the small bowel were perfused with amino acids, namely duodenum and upper jejunum, in our preparation the intestinal loop was excluded from gastrointestinal continuity as a Thiry-Vella loop and therefore it was permanently deprived of contact with bile and pancreatic juice, whereas in Meyer and Grossman's study the intact intestinal segments were perfused with amino acids which had access to the entire small intestine. The infused test solution was diverted to the outside of the animal. Since it was shown that the presence of bile salts (7, 8) and pancreatic enzymes (9) in the intestine may modify the secretory activity of the pancreas, it is not excluded that the use of the isolated intestinal loop for CCK release could increase the sensivity of our assay.

In man (3) an intestinal perfusion with essential amino acids caused an increase in pancreatic enzyme output comparable to that induced by perfusion with original amino acid mixture, whereas perfusion with nonessential amino acids had no effect. Among essential amino acids tested individually only phenylalanine, methionine and valine caused significant stimulation of pancreatic enzyme secretion, whereas tryptophan was less effective. The difference in the ability of individual amino acids to release CCK between man and dog may reflect a species difference or may be due to the different techniques used. In this study in man exogenous secretin was given to increase the sensitivity of the assay and only a short segment of distal duodenum was perfused with amino acids resulting in a relatively small release of CCK as the amount of releasable hormone is a function of the length of the gut exposed to amino acids.

The physiological significance of the fact that the majority of amino acid stimulate pancreatic secretion is obvious. The stimula-

tion of CCK release may begin already in the duodenum and continue through the major part of the jejunum (10) as free amino acids are split from peptides and brush border exopeptidases. In this way a large amount of CCK is released along the gut providing a strong stimulus for pancreatic enzyme secretion, which is additionally potentiated by secretin released simultaneously from the upper duodenum by gastric acid.

Summary. The ability of essential and nonessential amino acids to stimulate the release of CCK was investigated in dogs using a bioassay procedure based on the perfusion of duodenojejunal Thiry-Vella loops and determining the increase of pancreatic protein response as well as the potentiation of pancreatic bicarbonate secretion induced by a background dose of secretin (0.2 units/kg/hr). All essential amino acids except threonine and all nonessential amino acids except cysteine and aspartic acid were effective in CCK release when perfused in equimolar concentration (50 m*M*) through the intestinal loop. The highest activity for release of CCK was exhibited by tryptophan and phenylalanine, which were also shown to release small amounts of secretin. The peak pancreatic protein response to tryptophan was 80% of the maximal response to exogenous CCK (25 units/kg/hr).

The authors are deeply grateful to Dr. Morton I. Grossman, Dr. Eugene D. Jacobson and Dr. James H. Meyer for their critical advice in the preparation of the manuscript.

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Received Jan. 2, 1973. P.S.E.B.M., 1973, Vol. 143.