Upsala J Med Sci 94: 111–122, 1989

Cholecystokinin and Gastrin Inhibit Histamine Stimulated Aminopyrine Uptake in Isolated Rabbit Gastric Glands

Per Bengtsson and Göran Nilsson

From the Department of Physiology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden and the Department of Surgery and the Research Center, Karolinska Institute, Huddinge University Hospital, Sweden

ABSTRACT

In the present study we have analyzed if cholecystokinin (CCK) or gastrin (G) can inhibit acid production in isolated rabbit gastric glands as revealed by the aminopyrine technique.

The results show that G 17 I, CCK 8 NS, CCK 8 S, ceruletide and CCK 39 significantly inhibit histamine induced aminopyrine accumulation. No significant inhibition was noted for G 4, G 34 and NT G 1-13. As a group the CCK peptides were more effective than the gastrin peptides in inhibiting the aminopyrine uptake. CCK 8 S and ceruletide, the most potent inhibitors, reduced histamine induced aminopyrine accumulation with an ED_{50} of 10^{-9} and 10^{-10} M respectively. These potencies are similar to those by which CCK peptides stimulate isolated pancreatic acini to secrete amylase. Inhibition evoked by CCK 8 S was most effective following 20-40 min of incubation time, possibly indicating that the effect is mediated by the release of an intermediate substance.

The results may therefore indicate a role for cholecystokinin as a physiological inhibitor of acid secretion in the rabbit. The results may also contribute to explain why the potent gastric secretagogue gastrin per se fails to stimulate acid formation in gastric glands isolated from the rabbit.

INTRODUCTION

From in vivo studies in several species it is known that gastrin and the structurally related cholecystokinin peptides in certain doses may evoke inhibition of gastric

acid secretion. Such inhibition has been demonstrated in the cat (18), dog (8,10), bullfrog (13), cod (11) and pig (1) by gastrin and in the dog (9,16), man (6), cod (11) and pig (1) by cholecystokinin peptides. In general, relatively high doses of gastrin and cholecystokinin have been used in these experiments to evoke secretory inhibition. The results have therefore been considered as pharmacological rather than physiological .

About ten years ago an in vitro method was developed that made it possible to study the formation of acid in isolated gastric glands (2,3). This technique has become a useful tool in the study of parietal cell function. Surprisingly, gastrin per se does not stimulate acid formation in such isolated gastric glands (3,4,7,5). An explanation for this phenomenon could be that gastrin stimulates parietal cells by the release of histamine which fails to activate the glands since it becomes too diluted in the incubation medium (5). There may, however, be other explanations as to why gastrin does not stimulate acid production in isolated gastric glands.

In this study we therefore investigated whether peptides that are structurally related to gastrin and cholecystokinin may inhibit isolated gastric glands. If such a mechanism exists it may offer an alternate explanation as to why gastrin does not stimulate isolated gastric glands. In the present experiments rabbit gastric glands were stimulated by histamine with or without the presence of the mentioned peptides. The uptake of isotope labelled aminopyrine in the glands was taken as evidence for stimulation of parietal cells.

METHODS

Preparation of gastric glands

The isolation and preparation of gastric glands from male New Zealand white rabbits (1.5 - 2 kg body weight) were essentially carried out as described by Berglindh & Öbrink (2). This involved perfusion of the gastric artery at high pressure with oxygenized phosphate buffered saline at 37°C under Nembutal anaesthesia (Mebumal® 30 - 60 mg/kg i.v). After removal of the stomach the fundic mucosa was separated from the muscular layer and minced with a pair of scissors before it was subjected to collagenase digestion. The selection of collagenase batches as well as

the titration of a suitable concentration of collagenase for digestion had to be performed with care. If not, no inhibition at all or very poor inhibition was detected. The collagenase digestion was carried out for 40 - 65 min, i.e. until the suspension appeared to be homogeneous as judged by optical examination. The appearance of the digested glands was also frequently controlled by microscopic examination. The digestion was interrupted by dilution with 200 ml of incubation medium and the suspension was then filtered through coarse nylon mesh. The glands were allowed to settle in 10 ml conical tubes and were then washed three times in incubation medium. The volume was adjusted to give a final dilution (three times) at the experiments. All solutions used in these procedures were kept at room temperature.

Measurement of aminopyrine accumulation

^{14}C labelled aminopyrine was added to the gland suspension to give a final concentration of 0.33 μM. Since aminopyrine is a weak base it will accumulate in acid compartments and thereby reflect the amount of trapped acid (3).

Samples of 1.5 ml of the gland suspension (on average 3.4 mg dry wt/ml) were incubated together with the agents to be tested and placed in a gyratory shaking bath at 37°C and at a speed of 120 rpm. At the end of the incubation period the samples were transferred to plastic vials and centrifuged (10000 g) for 8 sec. The estimation of ^{14}C radioactivity and the treatment of the supernatant and pellet were performed according to Berglindh et al (3). The accumulation of aminopyrine was expressed as the ratio (R_{AP}) of ^{14}C radioactivity in intracellular water to extracellular water as described elsewhere (3).

Chemicals

Histamine dihydrochloride for stimulation of glands and rabbit albumin were purchased from Sigma Chemicals, S:t Louis, Mo., USA. The unsulphated heptadecapeptide of gastrin (G 17 I), tetragastrin (G 4), the N-terminal tridecapeptide of gastrin (NT-G 1-13), the unsulphated (CCK 8 NS) and sulphated (CCK 8 S) octapeptide of cholecystokinin were all purchased from CRB, Cambridge, England. Natural gastrin 34 (G 34) and cholecystokinin 39 (CCK 39) were kindly donated by professors G. Wünsch, Munich, West Germany, and V. Mutt, Stockholm, Sweden, respectively. The synthetic decapeptide ceruletide, which belongs to a group of amphibian peptides having close chemical and functional similarities to the gastrointestinal hormones cholecystokinin and gastrin, was generously supplied by

professor G. Bertaccini, Farmitalia, Milan, Italy. The molecular structure of ceruletide is identical to CCK 10 S except for having threonin instead of methionin in the sixth position. All peptides except for G 4 were solubilized in incubation medium devoid of glucose. G 4 was solubilized in 0.42 mM Tris buffer containing 30 % methanol and was further diluted 45 times with incubation medium until use at 10^{-5} M. Separate controls for 10^{-5} M and 10^{-6} M G 4 were prepared with the same concentration of the solubilization buffer as those containing the peptide. Collagenase type I for digestion of glands was obtained from Worthington Diagnostic System Inc., Freehold, N.J, USA. and ^{14}C aminopyrine from New England Nuclear, Boston, Ma., USA. Salts, phenol red and glucose were purchased from Kebo, Stockholm, Sweden.

Solutions: Phosphate buffered saline contained (mM): NaCl 149.6; K_2HPO_4 3; NaH_2PO_4 0.64; pH 7.4. Collagenase medium consisted of (mM if not specified): NaCl 130; $NaHCO_3$ 12; NaH_2PO_4 3; $MgSO_4$ 2; $CaCl_2$ 1; phenol red 10 mg/ml, collagenase 0.2-1.0 mg/ml, rabbit serum albumin 1 mg/ml and glucose 2 mg/ml. The incubation medium consisted of (mM if not specified): NaCl 132.4; KCl 5.4; Na_2HPO_4 5; NaH_2PO_4 1; $MgSO_4$ 1.2; CaCl 1; phenol red 10 mg/ml, pH 7.4. Before use 2 mg/ml rabbit albumin and 2 mg/ml glucose were added to the incubation medium.

Treatment and expression of results

In experiments where several different doses of histamine or various incubation periods were used for stimulation results are expressed as per cent of maximal histamine stimulation (Fig. 1). In this case Student's t-test for paired data was used when comparing results from control stimulation by histamine with results from samples also including the inhibitor (*=$p<0.05$, ** $p=<0.01$). When only one dose (Table 1) or two doses (Fig. 2) of histamine were used for stimulation and several doses of the inhibitor were tested results are expressed as per cent of control stimulation (Table 1) or per cent inhibition (Fig. 2). In order to avoid the problem of mass significance, analysis of variance in combination with the studentized range test of means (17) was applied to find the level at which inhibition becomes significant ($p<0.05$ depicted as *). When the inhibitory capacity of two different peptides was compared at the same dose level Student's t-test for unpaired data was employed. In the special case when two dose response curves prepared in the same experiment were compared (Fig. 2) the Student's t-test for paired samples

was used for comparing the means of the inhibitory response (see Results).

RESULTS

Effects of CCK 8 S on unstimulated glands

CCK 8 S (10^{-13} - 10^{-6} M) did not change the basal aminopyrine uptake as studied following 40 min incubation of unstimulated glands. Neither did 10^{-6} M CCK 8 S significantly change the basal aminopyrine uptake at incubation periods varying from 0 - 60 min (data not shown).

Effects of CCK and gastrin peptides on stimulated glands

Time course of CCK 8 S induced inhibition. Preliminary experiments showed that CCK 8 S inhibited histamine stimulated aminopyrine uptake. To elucidate the time course of CCK 8 S induced inhibition glands were incubated for 0 - 60 min with 10^{-6} M CCK 8 S and 5 x 10^{-5} M histamine . The results shown in Fig. 1 illustrate that CCK 8 S inhibits the glands with some latency. A small inhibitory effect is evident at 10 min, whereas maximal inhibition is established at 20 - 40 min of incubation. During this period the control stimulation decreases while the fraction that is inhibited

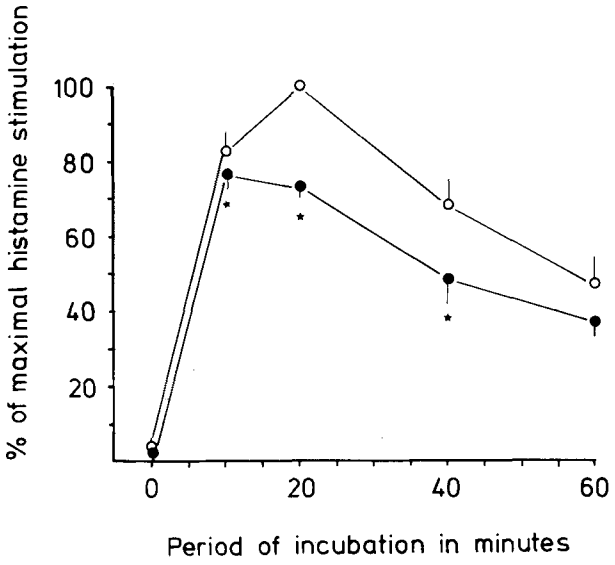

Fig.1. Effect of CCK 8 S (10^{-6} M) on aminopyrine accumulation in per cent of the maximal response in R_{AP} units (48 ±14) on stimulation with histamine (5x10^{-5} M) following incubation periods varying from 0 - 60 min. Each symbol represents the mean of 4 experiments and vertical bars the standard error of mean. Open ( ) symbols represents controls and closed symbols ( ) experiments with CCK 8 S.

Table 1 Aminopyrine accumulation in per cent (mean±SE) of control stimulation with $5x10^{-5}$ M Histamine

Peptide	Log conc M; -13	-12	-11	-10	-9	-8	-7	-6	-5	n
G4		101±6	105±5	103±3	105±5	102±4	100±2	96±2	83±6	5
G 17 I	104±4	103±4	103±4	98±2	94±3	101±3	91±2	84±4	76±4 *	6
G 34		102±2	105±5	114±4	100±6	99±5	95±1	82±6		5
NT G 1-13		105±1	102±2	102±3	95±5	92±5	96±1	102±4	86±6	5
CCK 8 NS	98±3	95±2	98±5	96±2	94±1	93±2	84±2	73±6 *		6
CCK 8 S	100±4	96±3	94±4	93±5	83±8 •	76±6 **	62±4 *	68±3 *		5
Ceruletide	98±2	89±3	89±4 •	83±3 •	67±3 *	63±2 **	66±6 *	64±4 *		6
CCK 39	98±3	104±4	103±3	98±2	98±1	93±1	86±3	76±5 *		5

remains constant. In subsequent series of experiments a more detailed study of the dose response relationship between histamine and the most potent of the mammalian peptides CCK 8 S was undertaken.

Effects of various doses of CCK and gastrin peptides on stimulation induced by a maximal dose of histamine. A number of CCK and gastrin peptides were tested on their ability to inhibit glands stimulated with a maximally effective dose of histamine (5×10^{-5} M) (Table 1). No significant inhibition was noted when G 4, G 34, or NT-G 1-13 were tested . On the other hand significant inhibition was observed when using G 17 I, CCK 39, CCK 8 NS, CCK 8 S, and the amphibian peptide ceruletide. These peptides inhibited the aminopyrine accumulation at different molar concentrations and this effect in general became increasingly evident as concentrations were raised. The most potent inhibitor was ceruletide reducing the maximal histamine stimulation by 35% with an ED_{50} of 10^{-10} M. Also CCK 8 S was an effective inhibitor of the histamine induced stimulation showing an ED_{50} of 10^{-9} M. Of the two CCK peptides the sulfated form was a more potent inhibitor (100-fold) than the unsulphated. At molar concentrations of 10^{-8} and 10^{-7} the difference between the two peptides was significant. When compared with CCK 39 the inhibitory potency was also 100-fold greater. As a group the gastrin peptides were less effective in inhibiting the aminopyrine uptake. Of the gastrin peptides G 17 I and G 34 seemed equally capable of causing inhibition of histamine induced stimulation although this inhibition was not significant at 10^{-6} M. Due to the limited amount available of G 34 doses higher than 10^{-6} M were not tested.

Dose response relationship of CCK 8 S induced inhibition. In the first series of experiments gastric glands were stimulated with two doses of histamine (2×10^{-6} M or 5×10^{-5} M), and inhibition by CCK 8 S was studied at concentrations varying between 10^{-12} - 10^{-7} M. For each concentration of CCK 8 S inhibition was greater on stimulation with the moderate dose of histamine than with the maximal dose (Fig. 2). A moderate degree of histamine stimulation thus significantly reduced the concentration needed to obtain an inhibitory effect of CCK 8. The second series of experiments was performed with doses of histamine that varied between threshold (10^{-7} M) and supramaximal (10^{-3} M). Results are illustrated in Fig. 3. Inhibition was produced by CCK 8 S at molar concentration of 10^{-9} or 10^{-7}. Generally, inhibition with the higher dose of CCK 8 S was greater than that evoked by the lower CCK 8

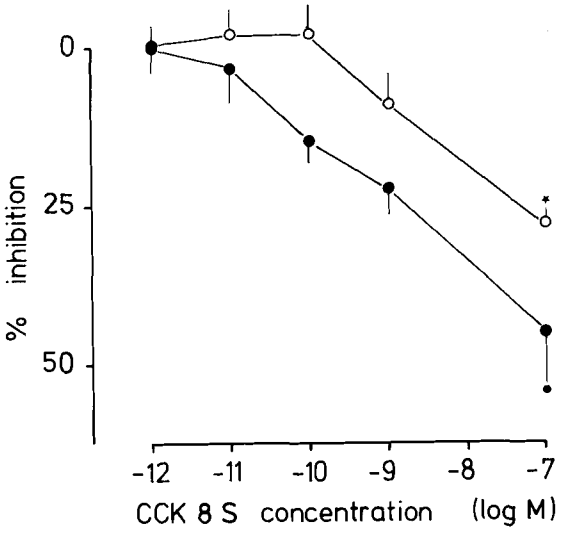

Fig. 2. Effect of CCK 8 S (10^{-12}-10^{-7} M) on aminopyrine accumulation induced by histamine at 2 x 10^{-6} M ( ) and 5 x 10^{-5} M ( ). Symbols indicate the mean of 5 experiments and vertical bars the standard errors. Control R_{AP} values were 34 ± 1 (2 x 10^{-6} M) and 51 ± 3 (5 x 10^{-5} M) (mean ± SE of mean). The difference between the two dose response curves was significant in the region where CCK 8 S produced inhibition (10^{-9} and 10^{-7} M).

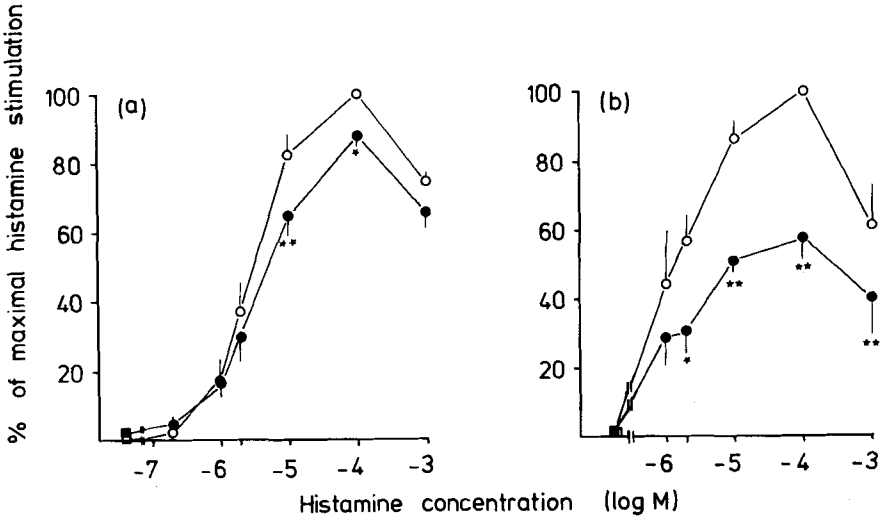

Fig. 3 Effect of 10^{-9} M (a) and 10^{-7} M (b) of CCK 8 S on aminopyrine accumulation induced by different doses of histamine (10^{-7} -10^{-3} M). Open symbols represent control experiments with ( ) or without ( ) histamine stimulation. Closed symbols represent experiments with CCK 8 S alone ( ) or CCK 8 S in combination with histamine ( ) in the incubation medium. The maximal R_{AP} (mean ± SE) responses to control stimulation with histamine were 48 ± 7 (a) and 50 ± 10 (b), n=4.

dose. In the presence of the inhibitor the histamine response did not reach the maximal level of the control experiments despite the fact that the histamine dose was raised supramaximally indicating a non competitive mode of inhibition. Also, the ED_{50} for histamine stimulation was essentially unchanged under the influence of CCK 8 S.

DISCUSSION

The aminopyrine technique measures in vitro the uptake of labelled aminopyrine into the acid secreting glands. This uptake is considered to reflect the acid production of parietal cells and has been used in studies of mechanisms activating these glands. The present study utilizes this technique to study inhibition of acid production induced by the structurally related peptides CCK and gastrin. The results demonstrate that CCK and to some extent gastrin reduce the uptake of aminopyrine into the gastric glands.

In general the CCK peptides were more effective inhibitors of the glands than were the gastrin peptides. The most efficient inhibitor as expressed on a molar basis was the amphibian peptide ceruletide. Also the octapeptides of CCK were strong inhibitors of the gland stimulation. Of these peptides the naturally occurring sulphated CCK 8 was the most potent.

In a recent study (15) it was reported that the N-terminal fragment (NT-G 1-13) supresses gastric secretion in man. No certain inhibition of parietal cell stimulation could be detected in the present experiments despite the fact that the peptide was tested over a concentration range varying between 10^{-12}-10^{-5} M. Nor have we been able to detect inhibition of gastric secretion in in vivo experiments in pigs by this gastrin peptide (1). The general impression of the results is that inhibition of aminopyrine accumulation is dependent on the molecular length of the structurally interrelated peptides used, optimal inhibition probably being accomplished at an approximate molecular length of 10 amino acids. Also, the existence of a sulphate group seems to be of importance for the inhibiting effect.

Of the peptides tested, CCK 8 S was found to be the most potent mammalian peptide. Therefore, it was chosen for further studies of the inhibitory mechanism. In

such experiments it was demonstrated that the inhibition of histamine stimulation was established with some latency. This observation may suggest that CCK 8 S does not act directly on the parietal cells but possibly by some intermediate substance the concentration of which has to be built up in this in vitro system before its effect becomes fully evident.

In experiments performed to elucidate the character of inhibition a dose dependent relation between the amount of inhibitory peptide present in the incubation medium and the degree of inhibition produced was demonstrated. This was found whether the stimulation by histamine was kept constant and the dose was varied or vice versa. The mode of inhibition seemed to be non competitive since the ED_{50} of histamine stimulation was essentially unchanged and the response at maximal histamine stimulation in the control experiment was not attained in the presence of CCK 8 S. It is however difficult to conclude from experiments in this system whether the inhibitory mechanism is in fact competitive or not competitive. Thus, the substances involved in the experiments may exert dual effects on the acid secreting glands and more than one receptor system may take part in the reactions. For example, it cannot be excluded that CCK and gastrin in addition to their inhibitory effect on the acid secreting glands also may induce a stimulation of these glands. A week stimulation of gastrin on acid secreting glands has previously been indicated (4,7). Also histamine may exert a dual action on acid secreting glands and cause both stimulation and inhibition (12). When acting together gastrin and histamine have been shown to promote each others stimulatory effects on gastric acid secretion in vivo (14) and in vitro gastrin has been shown to cause stimulation of isolated gastric glands in the presence of histamine (7).

In the present experiments it was not possible to completely inhibit the histamine induced aminopyrine accumulation, although the maximal inhibition observed (45%), must be regarded as considerable. It is possible that greater inhibition can be demonstrated as the experimental conditions become further improved. Even though the inhibition was not complete significant inhibition was accomplished by low concentrations of peptides. Thus, ED_{50} for partial inhibition by ceruletide and the CCK 8 S were 10^{-10} M and 10^{-9} M. These figures are similar to the ED_{50} values that have been observed in in vitro studies of amylase secretion from pancreatic acini with CCK 10 S and CCK 8 S (19).

Previous investigators have reported the unexpected finding that gastrin per se does not stimulate isolated rabbit gastric glands (3,4,7,5). A small stimulatory response has however been obtained with the aid of reducing (7) or facilitating (4) agents. The failure of gastrin per se to activate glands may be due to dilution of the gastrin released histamine by the incubation medium (5). However, also other mechanisms may be involved. The results in this paper , demonstrating CCK and gastrin induced inhibition of histamine stimulated rabbit gastric glands, may for example also contribute to the explanation of the poor stimulating effect of gastrin in this system. Thus, the CCK and gastrin activated inhibitory mechanism counteracts the stimulatory response of gastrin that otherwise would be evident. Whether the inhibitory mechanism demonstrated in this paper is of physiological significant importance or not cannot at present be determined. As mentioned, high doses of gastrin and CCK are needed to evoke inhibition of gastric acid secretion in vivo. The observation, in this paper, that CCK 8 S and ceruletide inhibits acid production in gastric glands with similar potencies as CCK 8 S and CCK 10 S stimulate amylase secretion from dispersed pancreatic acini, would however favour a physiological relevance of the mechanism at least in the rabbit.

REFERENCES

1. Bengtsson, P. & Nilsson, G.: Effects of gastrin and CCK like peptides on gastric acid secretion in pigs. (To be published).
2. Berglindh, T., & Öbrink, K.J.: A method for preparing isolated glands from the rabbit gastric mucosa. Acta Physiol Scand 96: 150-159, 1976.
3. Berglindh, T., Helander, H,F. & Öbrink, K.J.: Effects of secretagogues on oxygen consumption, aminopyrine accumulation and morphology on isolated gastric glands from the rabbit. Acta Physiol Scand 97: 401-414, 1976.
4. Berglindh, T., Sachs, G. & Takeguchi, N.: Ca^{2+}-dependent secretagogue stimulation in isolated rabbit gastric glands. Am J Physiol 239: G90-94, 1980.
5. Bergqvist, E. & Öbrink, K.J.: Gastrin-Histamine as a normal sequence in gastric acid stimulation in the rabbit. Ups J Med Sci 84, 145-154, 1979.
6. Brooks, A.M., Agosti, A., Bertaccini, G. & Grossman, M.I.: Inhibition of gastric acid secretion in man by peptide analogues of cholecystokinin. N Engl J Med 282: 535-538, 1970.

7. Chew, C.S., & Hersey, S.J.: Gastrin stimulation of isolated gastric glands. Am J Physiol 242: G504-512, 1982.
8. Gillespie, I.E. & Grossman, M.I.: Inhibition of gastric acid secretion by extracts containing gastrin. Gastroenterology 44, 301-310, 1963.
9. Gillespie, I.E. & Grossman, M.I.: Inhibitory effect of secretin and cholecystokinin on Heidenhein pouch responses to gastrin extract and histamine. Gut 5: 342-345, 1964.
10. Gregory, R. A. & Tracy, H.: The constitution and properties of two gastrins extracted from hog antral mucosa. Gut 5: 103-117, 1964.
11. Holstein, B.: Inhibition of gastric acid secretion in the Atlantic cod, Gadus morhua, by sulphated and desulphated gastrin,caerulein, and CCK-octapeptide. Acta Physiol Scand 114: 453-459, 1982.
12. Impicciatore, M., Bertaccini, G., Mossini, F., Hansen, D. & Grossman, M.I.: N-methyl, 5-methyl histamine evokes a higher maximal rate of gastric acid secretion than histamine. Proc Soc Exp Biol Med 156: 296-298, 1977.
13. Morrisey, S.M. & So, Y.C.: The effect of gastrin on gastric secretion in Rana Catesbeiana (American Bullfrog). Comparative Biochemistry and Physiology 34: 521-533, 1970.
14. Passaro, E.P., Gillespie, I.E., & Grossman, M.I.: Potentiation between gastrin and histamine in stimulation of gastric secretion. Proc Soc Exp Biol Med 156: 296-298, 1977.
15. Petersen, B., Christiansen, J .& Rehfeld, J.F.: The N-terminal trideca-peptide fragment of gastrin-17 inhibits gastric acid secretion. Regul Pept 7: 323-334, 1983.
16. Sjödin, L.: Influence of secretin and cholecystokinin on canine gastric secretion elicited by food and by exogenous gastrin. Acta Physiol Scand 85: 110-117, 1972.
17. Snedecor, G.W. & Cochran, W.G.: In: Statistical Methods, Chapter 10. Ames: The Iowa State University Press, Iowa, 1967.
18. Uvnäs, B.: The gastric secretory excitant from the pyloric mucosa. Acta Physiol Scand 6: 97-107, 1943.
19. Villaneuva, M.L., Collins, S.M., Jensen, R.T. & Gardner, J.D.: Structural requirements for action of cholecystokinin on enzyme secretion from pancreatic acini. Am J Physiol 242: G416-422, 1982.