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GASTRIC INHIBITORY PEPTIDE (GIP), PANCREATIC GLUCAGON AND VASOACTIVE INTESTINAL PEPTIDE (VIP) ARE cAMP-INDUCING HORMONES IN THE HUMAN GASTRIC CANCER CELL LINE HGT-1. HOMOLOGOUS DESENSITIZATION OF VIP RECEPTOR ACTIVITY

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Summary. GIP (EC_{50} = 8 x 10^{-9} M, 5-fold stimulation), pancreatic glucagon (EC_{50} = 10^{-8} M, 13-fold) and porcine or chicken VIP (EC_{50} = 2.5 x 10^{-9} M, 10-fold) are shown to activate the cAMP generating system in HGT-1 cells. Combinations of GIP, pancreatic glucagon and VIP indicate the occurence of 3 separate sets of recognitions sites for these 3 peptides. Accordingly, chronic treatment of cultured HGT-1 cells by VIP (10^{-8} M) during 6 days resulted in homologous desensitization of VIP receptor activity. Other peptides structurally related to the secretin-glucagon family, to neurotensin, or to gastrin are either ineffective or very weak agonist (hpGRF). GIP or pancreatic glucagon are inactive on the human colonic cell line HT-29, indicating the gastric specificity of the effect of GIP and glucagon in transformed epithelial cells originating from the human gastrointestinal tract. This implies that GIP and (pancreatic-entero) glucagon peptides may regulate gastric secretions directly, under similar mechanisms that those we evidenced in the rat.

In previous reports, we have established the pharmacological properties of the receptor-cAMP systems that are sensitive to VIP, secretin and (pancreatic-entero) glucagons in gastric glands isolated from the human (1), guinea pig (2, 3) and rat (4-8) stomach. Recently, we have shown that the human gastric cancer cell line HGT-1 (9) possesses both VIP and histamine H_2 receptors similar to those characterized in normal human fundic glands (10-12). We therefore proposed (10-12) that HGT-1 cells can be used as a model to study short-or long-term effects of hormones or drugs on cell surface receptors in the human gastric epithelia.

The purpose of the present work was twofold. First, to investigate short-term effect of GIP and pancreatic glucagon on cAMP production in HGT-1 cells: GIP and pancreatic glucagon are structurally related to the peptides of the secretin family (13-15), both hormones considered as inhibitors of gastric acid secretion *in vivo* (13-21). Results were compared to those obtained with other peptides related to secretin (VIP, somatostatin, growth hormone releasing factor (22)), those related to neurotensin (pancreatic polypeptide and the peptide having N-terminal tyrosine and C-terminal tyrosine: PYY), or to gastrin (cholecystokinin). Second, to determine whether long-term exposure of cultured cells to VIP is associated or not with homologous or heterologous desensitization of HGT-1 cells to VIP, GIP and pancreatic glucagon.

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EXPERIMENTAL

Peptides and drugs: Highly purified natural porcine GIP, porcine and chicken VIP (23) were purchased from Pr. V. Mutt and Dr. A. Nilsson (GIH laboratory Stockholm, Sweden). Crystallized, highly purified porcine glucagon (lot 421306) was from Novo Research Institute (Bagsvaerd, Denmark), Synthetic human pancreatic growth hormone releasing factor (hpGRF) 1-40 was a generous gift from Dr. J. Rivier (Salk Institute, San Diego, Ca, USA). Synthetic cyclic ovine somatostatin - 14, sulphated [Leu^{15}]human gastrin I, the C-terminal fragment of cholecystokinin (CCK 26-33), bovine substance P and neurotensin were from Beckman (Switzerland). Synthetic porcine PYY was purchased from Peninsula Laboratories (Belmont, Ca, USA), crystallized bovine pancreatic peptide (Lot 103411) was from Bachem (Budendorf, Switzerland). Prostaglandins E_2 and $PF_{1\alpha}$, isoproterenol hydrochloride, 3 - isobutyl - 1 - methylxanthine (IBMX), and cylic adenosine 3' - 5' - monophosphate (cAMP) were purchased from Sigma Chemical Co., (St. Louis, Mo. USA). All other chemicals used were of the highest purity available.

HGT-1 cells: The HGT-1 cell line originated from a human gastric cancer localized in fundus (9), was generously provided by Dr. C. Laboisse (INSERM U.239, Faculté de Médecine Xavier Bichat, Paris, France). The cells were routinely cultured in DME (Dulbecco's modified Eagle medium, Gibco) supplemented with 10% heat inactivated foetal bovine serum (Grand Island Biological Co). The culture medium was changed every 3 days and HGT-1 cells were routinely passaged at or just before reaching confluency, about 6-8 days after seeding. To evaluate desensitization of the receptor-cAMP system highly sensitive to VIP in HGT-1 cells, cultured cells were exposed to 10^{-8} M VIP during 6 days. From seeding to confluency, the culture medium was changed every 24 hours. At the end of this period, control or treated HGT-1 cells were washed 3 times at 37°C in 20 ml of PBS (Ca^{2+}-free) buffer and harvested prior cAMP analysis. Adherent cells between passages 66 and 100 were removed from culture flasks by 1-2 min incubation at 37°C with 0.02% EDTA. Then, the cells were washed (3 times, 200 x g, 3 min, 20°C) in Krebs Ringer phosphate buffer (KRP, pH 7.4). Cell viability (trypan blue exclusion test) was about 90-95% and additions of the peptides or agents tested at 10^{-7} - 10^{-6} M did not change this percentage during the standard incubations described below, or after long-term exposure of cultured HGT-1 cells to 10^{-8}M VIP, during 6 days.

HT-29 cells: The HT-29 cell line derived from a human colon carcinoma (24) was provided by Dr. J. Fogh (Sloan Kettering Institute, N.Y. USA). This cell line was cultured by methods described in (24) and adherent cells were removed from Petri dishes by a 2 min incubation with EDTA. Cell viability was about 90% before and after the cell incubations described below.

Cyclic AMP assay: In a standard assay, 150 µl from the HGT-1 or HT-29 cell suspensions (1-2 x 10^6 cells/ml) were preincubated at 20°C in 250 µl of KRP containing 1% BSA and 2 mM IBMX. The reaction was initiated by the addition of appropriate hormones (100µl). The reaction was stopped at the time indicated by adding 50µl 11 N HClO4 and cAMP was determined (10) by the radioimmunoassay method already described (25). None of the hormones tested in the present study interfered with the assay of cAMP.

Processing of the data. Data are given as picomoles of cAMP produced by 10^6 cells. The apparent EC_{50} was the dose required to produce 50% of the maximal stimulation produced by the peptides. Results were analyzed by standard methods using Student's paired t test.

RESULTS

The basal cAMP production in HGT-1 cells incubated at 20°C in the presence of 1 mM IBMX averaged 2.12 ± 0.15 pmol cAMP per 10^6 cells (n = 13) . This basal activity remained constant during the 0-15 min incubation period considered (Figure 1). Significant cAMP generation over basal levels could be observed 3 min after the addition of GIP (5.3 ± 0.3 pmol/10^6 cells, n = 7) and this stimulation became maximal (5-fold) and constant between 10 and

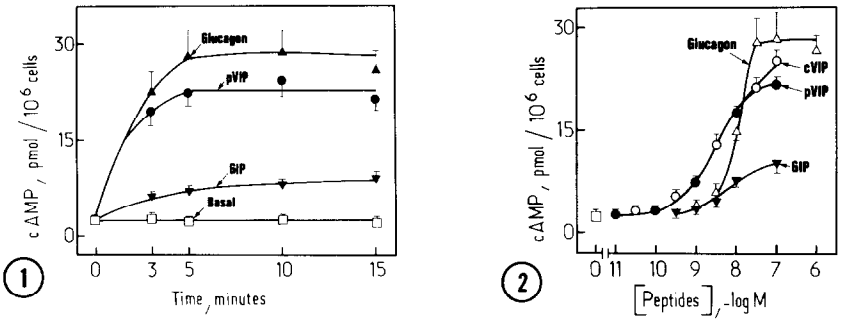

Figure 1: Effect of time on GIP-, pancreatic glucagon - and VIP - induced cAMP production in HGT-1 cells. GIP (10^{-7} M, ▼), pancreatic glucagon (3 X 10^{-7} M, ▲) or porcine VIP (10^{-7} M, ●) were added to HGT-1 cells after a 10 min preincubation at 20°C in Krebs ringer phosphate buffer (pH 7.4) containing 1 mM IBMX as a cAMP-phosphodiesterase inhibitor. The reaction was arrested at the time indicated by adding $HClO_4$ to the mixture. Basal levels in HGT-1 cells (□). Results are mean ± SEM from 3 (basal) to 7 experiments (peptides), each performed in duplicate or triplicate.

Figure 2: Effect of different concentrations of GIP, pancreatic glucagon, porcine or chicken VIP on cAMP production in HGT-1 cells. Cells were preincubated for 10 min at 20°C in the presence of IBMX and incubated for 10 min after the addition of GIP (▼), pancreatic glucagon (▲), porcine (●) or chicken (○) VIP. Basal levels in HGT-1 cells (□). Results are mean ± SEM from 5 to 12 experiments performed in duplicate or triplicate.

15 min. Cyclic AMP levels attained a plateau value within 3-5 min and remained constant for up to 15 min after additions of pancreatic glucagon (3 x 10^{-7} M, 12-fold increase over basal), or porcine VIP (10^{-7} M, 14-fold increase).

The assessment of the concentration dependence of GIP, pancreatic glucagon, porcine and chicken VIP on cAMP production is shown in Figure 2. Significant effects ($p < 0.02$-0.05) on cAMP production could be observed at 10^{-9} M GIP or glucagon, 10^{-10} M porcine VIP or 3.16 x 10^{-10} M chicken VIP. Half-maximal stimulations were elicited by 8 x 10^{-9} M GIP, 10^{-8} M pancreatic glucagon and 2.5 x 10^{-9} M porcine or chicken VIP. At maximally effective doses of peptides, basal cAMP levels were increased 5.4-fold by 10^{-7} M GIP, 13-fold by 3 x 10^{-8}M glucagon and 10-fold by 10^{-7} M chicken or porcine VIP. We have observed that VIP (10^{-8} M) stimulated (30-fold) cAMP production in the human colonic cell line HT-29, as in (26); while GIP or pancreatic glucagon are ineffective under the same experimental conditions (data not shown).

The relationship between the receptor-cAMP systems sensitive to GIP, glucagon and VIP in HGT-1 cells was analyzed by regarding the effect of peptides added simultaneously on isolated cells (Table I) or separately, after chronic treatment of cultured cells by 10^{-8} M VIP (Figure 3). Addition of GIP, at maximally effective concentration (10^{-7} M) produced additive effect (or no inhibition) on cAMP production induced by pancreatic glucagon or VIP, when these two peptides are tested at concentrations giving half-maximal (Exp. 1 in Table I) or maximal stimulation (Exp. 2 in Table I). At maximally

TABLE I: Effect of combinations of cAMP-inducing hormones on HGT-1 cells

Expt.	Additions	cAMP, pmol/10^6 cells
1.	Basal	1.93 ± 0.1
	VIP (3×10^{-9} M)	11.4 ± 0.9
	Glucagon (10^{-8} M)	14 ± 0.9
	GIP (10^{-7} M)	9.7 ± 1.4
	VIP + GIP	18.8 ± 1**
	Glucagon + GIP	22.6 ± 1.7**
2.	Basal	2.2 ± 0.3
	VIP (5×10^{-8} M)	18.4 ± 1.5
	Glucagon (5×10^{-8} M)	26.4 ± 1.3
	GIP (10^{-7} M)	7.4 ± 0.2
	VIP + GIP	23.1 ± 1.7*
	Glucagon + GIP	32 ± 1.7**
	Glucagon + VIP	41.4 ± 2.8

Significantly different at $p < 0.02$* or $p < 0.01$** from the corresponding value. Results are mean ± SEM from 4 experiments performed in duplicate or triplicate.

effective doses, the combined effects of glucagon and VIP were additive (Exp. 2 in Table I). Figure 3 shows that 6 days exposure of cultured HGT-1 cells to VIP resulted in a remarkable desensitization of the VIP receptor activity, since cAMP generation induced by 10^{-8} M VIP in VIP-treated cells is comparable to the effect produced by 10^{-10} M VIP in control cells. In contrast, chronic treatment by VIP do not change the potency and the efficacy of pancreatic glucagon (EC_{50} = 5-10 x 10^{-9} M) or GIP (EC_{50} = 1-1.2 x 10^{-8} M). The receptor specificity of the cAMP generating systems sensitive to GIP, pancreatic glucagon and VIP in HGT-1 cells is further illustrated in Table II: Among the effectors tested, only hpGRF is able to stimulate cAMP production in HGT-1 cells. Other peptides related to the secretin-glucagon family

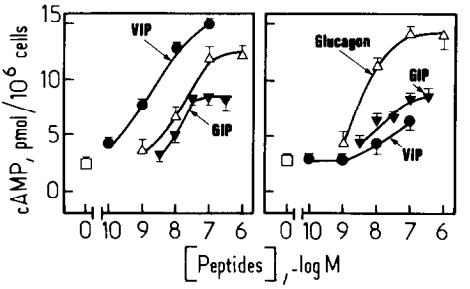

Figure 3: Homologous desensitization of VIP receptor activity in HGT-1 cells after chronic treatment by VIP. HGT-1 cells were cultured during 6 days in the absence (control: left) or in presence of 10^{-8} M VIP (right). Culture medium was changed every day. Control and VIP-treated cells were then compared for their cAMP formation capacity after addition of GIP (▼), pancreatic glucagon (▲) or porcine VIP (●). Basal levels in HGT-1 cells (□). Results are mean ± SEM from 4 experiments performed in duplicate.

(somatostatin-14), to neurotensin (pancreatic polypeptide and PYY), to gastrin (CCK) as well as substance P, the beta adrenergic receptor agonist isoproterenol, or prostaglandins are inactive in the system.

DISCUSSION

Our experiments conducted on the human gastric cancer cell line HGT-1 indicate, for the first time, that GIP is a cAMP-inducing hormone; show the occurence of a receptor-cAMP system sensitive to pancreatic glucagon, previously characterized in rat fundic glands (7, 8) and give the first exemple of homologous desensitization of VIP receptor activity.

It is likely that GIP, pancreatic glucagon and VIP are acting on three separate sets of recognition sites: 1) the maximal cAMP formation capacity is not identical for these three peptides, 2) the effects of glucagon and VIP are additive, 3) GIP does not reduce glucagon-or VIP-induced cAMP formation in HGT-1 cells: If we consider that GIP is a partial agonist for the glucagon or VIP recognition sites in HGT-1 cells, it should also antagonize the effect of glucagon or VIP, as previously shown for the partial histamine receptor agonists impromidine and 2 (2-pyridyl) ethylamine, inhibiting the histamine effect in guinea pig gastric glands (11) or in other systems (27), 4) long-term exposure of HGT-1 cells to VIP resulted in homologous desensitization of the cultured cells to VIP, since the potency and the efficacy of GIP and pancreatic glucagon remained unaffected by this treatment, 5) to our knowledge, there is no evidence in the literature that GIP, glucagon or VIP could share common binding sites, as shown in a variety of tissues containing specific receptors for secretin (5), glucagon (28) or VIP (29). By contrast, it is known that in gastric cells, secretin and PHI could interact with specific VIP/ secretin receptors in guinea pig (2), rat (6) or HGT-1 cells (10), according to the remarkable sequence similarities between PHI, secretin, porcine and chicken VIP (Table III). The human pancreatic GRF (22), which has been also shown to interact with VIP receptors in rat and human intestinal epithelial membranes (30) is a very weak effector in stimulating cAMP production in HGT-1 cells. This peptide, possesses respectively 12, 6, 9, 8 and 5 amino acids in common with PHI, secretin, porcine VIP, glucagon and GIP (Table III), is a partial agonist (10-50% of the maximal VIP effect) with low affinity (700-800 times less potent than VIP) for the receptor-cAMP system specific for VIP in rat and human small intestinal epithelial membranes (30). Other peptides that are related to the secretin - glucagon family (somatostatin 14), to neurotensin (pancreatic polypeptide and PYY), to gastrin (CCK) as well as substance P are ineffective in stimulating cAMP production in HGT-1 cells, indicating the pharmacological specificity of the receptor-cAMP systems sensitive to GIP, glucagon and VIP in HGT-1 cells (Table II).

Table II. Effect of peptides and ubiquitous cAMP-inducing agents on HGT-1 cells

Effectors (10^{-6}M)	cAMP, pmol/10^6 cells
Basal	2.62 ± 0.211
- Somatostatin -14	2.39 ± 0.320*
hp GRF	4.65 ± 0.278
- Neurotensin	2.77 ± 0.649
Pancreatic polypeptide	2.93 ± 0.639
PYY	3.38 ± 0.238
- Gastrin	2.59 ± 0.592
CCK	2.43 ± 0.318
- Substance P	2.97 ± 0.784
- Isoproterenol	2.47 ± 0.233
PGE_2	3.3 ± 0.390
PFI_{α}	2.63 ± 0.290

* Significantly different at $p < 0.001$. Results are mean ± SEM from 3-5 experiments performed in duplicate or triplicate.

The results are also supportive of the gastric cellular specificity of the effects of GIP and glucagon in transformed epithelial cells derived from the human gastrointestinal tract since the two peptides do not activate the cAMP generating system in the human colonic cell line HT-29. Under the conditions tested here, HGT-1 cells are insensitive to the known regulators of the adenylate cyclase, isoproterenol and prostaglandins. This inability contrast with the remarkable efficacy and potency of isoproterenol on the beta 2 receptor that we have characterized in human fundic glands (31), and with the stimulatory effect of prostaglandins in human gastric mucosa (32). It is therefore possible that beta 2 and prostaglandin receptors are not expressed to produce cAMP in HGT-1 cells, due perhaps to an impaired coupling between the receptor and the cyclase. This matter is currently under investigation in our laboratory.

The glucagon-like peptide enteroglucagon, or glucagon - 37(G-37) isolated from the porcine intestine (33) and chemically characterized by Bataille et al (34) is 20 times more potent than pancreatic glucagon (G-29) in stimulating cAMP production in the parietal gland area in rat stomach (7, 8, 35). These two peptides are very weak agonists in antral glands (7, 8, 35) and their order of potencies is inverse in liver plasma membrane, since pancreatic glucagon is about 10 times more potent than enteroglucagon in this system (8, 34-36). We have therefore suggested that the glucagons of pancreatic or intestinal origin could have a physiological regulatory role on the acid-secreting parietal cell and on gastric acid secretion (7, 8, 34, 35). In agreement, Dubrasquet et al (16) have shown that enteroglucagon is also twenty times more potent than pancreatic glucagon in inhibiting pentagastrin-stimulated gastric acid secretion in the rat, in vivo. GIP and (pancreatic-entero) glucagon peptides share a set of remarkable similarities in view of their chemical structure, cellular

Table III: Sequence homologies between the peptides of the secretin-glucagon family that are positive effectors on the cAMP generating systems in the human gastric cancer cell line HGT-1

porcine VIP	HSDAVFTDNYTRLRKQMAVKKYLNSILN
chicken VIP	HSDAVFTDNYSRFRKQMAVKKYLNSVLT
SECRETIN	HSDGTFTSELSRLRDSARLQRLLQGLV
PHI	HADGVFTSDFSRLLGNLSAKKYLQSLI
hp GRF-40	YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGESNQERGA
GLUCAGON	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
GIP	YAEGTFISDYSIAMDKIRQQDFVNWLLAQKGKKSDDWKHNITQ

(porcine, chicken) VIP, secretin, PHI and hpGRF-40 interact with the receptor-cAMP system highly sensitive to VIP in HGT-1 cells (10), while pancreatic glucagon and GIP are acting through distincts recognition sites.

localization and biological action on the stomach. GIP has 15 amino acid identities with pancreatic glucagon in the first 26 positions (Table III); GIP-like peptide is produced in the glucagon cells of the rat pancreas (37); enteroglucagon has been identified in endocrine cells from rat or human intestine (38-40) and both peptides are released after meals (41, 42); GIP, (pancreatic-entero) glucagons and glicentin are potent inhibitors of pentagastrin-stimulated acid secretion in rat or dog stomach but are less potent inhibitors of histamine-stimulated gastric acid secretion (16, 43, 44).

The results presented here indicate that GIP and (pancreatic-entero) glucagons may have a direct effect on human gastric cells. It is suggested that gastric acid secretion in man could be regulated by these two peptides under similar mechanisms that those we have evidenced in the rat (7, 8, 16, 35). It has been also observed that GIP is a potent releasing factor for gastric somatostatin (45), an inhibitor of acid secretion *in vivo* (46) and *in vitro* (2,47,48).It is therefore conceivable that the secretion of somatostatin by the gastric D-cells can be regulated by GIP, *via* a cAMP-dependent process. These properties account for the enterogastrone activity of GIP, originally noticed by Brown and Pederson in the dog (49).

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REFERENCES

1. Dupont, C., Gespach, C., Chenut, B. and Rosselin, G. (1980) FEBS Letters 113, 25-28
2. Gespach, C., Hui Bon Hoa, D. and Rosselin, G. (1983) Endocrinology 112, 1597-1606
3. Mangeat, P., Gespach, C., Marchis-Mouren, G. and Rosselin, G. (1982) Regulatory peptides 3, 155-168

4. Gespach, C., Bataille, D., Dupont, C., Rosselin, G., Wünsch, E. and Jeager, E. (1980) Biochim. Biophys. Acta 630, 433-441
5. Gespach, C., Bataille, D., Vauclin, N., Rosselin, G., Moroder, L. and Wünsch, E. (1981) Peptides 2, 247-251
6. Bataille, D., Gespach, C., Laburthe, M., Amiranoff, B., Tatemoto, K., Vauclin, N., Mutt, V. and Rosselin, G. (1980) FEBS Letters 114, 240-242
7. Gespach, C., Bataille, D., Dutrillaux, M.-C. and Rosselin, G. (1982) Biochim. Biophys. Acta 720, 7-16
8. Bataille, D., Gespach, C., Coudray, A.-M. and Rosselin, G. (1981) Bioscience Reports 1, 151-155
9. Laboisse, C.L., Augeron, C., Couturier-Turpin, M.-H., Gespach, C., Cheret, A.-M. and Potet, F. (1982) Cancer Research 42, 1541-1548
10. Emami, S., Gespach, C., Forgue-Lafitte, M.-E., Broer, Y. and Rosselin, G. (1983) Life Sciences 33, 415-423
11. Gespach, C., Emami, S., Boige, N. and Rosselin, G. (1983) In: Gut Peptides and Ulcer (ed. Miyoshi A.) Biomedical Research Foundation, Tokyo, pp56-63
12. Emami, S., Gespach, C. and Rosselin, G. (1982) Program of the 68th Annual Meeting of the Japanese Society of Gastroenterology on "Gastrointestinal Hormones and Ulcer", Hiroshima, March 29 - April 1
13. Tatemoto, K. and Mutt, V. (1981) Proc. Natl. Acad. Sci. USA 78, 6603-6607
14. Jörnvall, H., Carlqvist, M., Kwank, S., Otte, S.C., McIntosh, C.H.S., Brown, J.C. and Mutt, V. (1981) FEBS Letters 123, 205-210
15. Bataille, D., Tatemoto, K., Gespach, C., Jörnvall, H., Rosselin, G. and Mutt, V. (1982) FEBS Letters 146, 79-86
16. Dubrasquet, M., Bataille, D. and Gespach, C. (1982) Bioscience Reports 2, 391-395
17. Konturek, S.J., Biernat, J., Kwiecien, N. and Oleksy, J. (1975) Gastroenterology 68, 448-454
18. Christiansen, J., Holst, J.J. and Kalaja, E. (1976) Gastroenterology 70, 688-692
19. Brown, J.C. and Dryburgh, J.R. (1971) Can. J. Biochem. 49, 867-872
20. Konturek, S.J., Dembinski, A., Thor, P. and Krol, R. (1976) Pfügers Arch. 361, 175-181
21. Wormsley, K.G. and Grossman, M.I. (1964) Gastroenterology 47, 72-81
22. Vale, W., Vaughar, J., Yamamoto, G., Spiess, J. and Rivier, J. (1983) Endocrinology, 112, 1553-1555
23. Nilsson A. (1975) FEBS Letters 60, 322-326
24. Fogh, J. and Trempe, G. (1975) in Human Tumor cells In vitro, ed. J. Fogh (Plenum, New York) pp 115-159
25. Broer, Y., Fouchereau, M. and Rosselin, G. (1972) C.R. Acad. Sci. Paris 275 (D), 619-622
26. Laburthe, M., Rousset, M., Boissard, C., Chevalier, G., Zweibaum, A. and Rosselin, G. (1978) Proc. Natl. Acad. Sci. USA 75, 2772-2775
27. Gespach, C., Saal, F., Cost, H. and Abita, J.-P. (1982) Molecular Pharmacol. 22, 547-553
28. Holst, J.J. (1983) in Glucagon I, ed P.J. Lefèbvre (Springer-Verlag, Berlin Heidelberg New York Tokyo) pp 245-261
29. Prieto, J.C., Laburthe, M. and Rosselin, G. (1979) Eur. J. Biochem. 96, 229-237
30. Laburthe, M., Amiranoff, B., Boige, N., Rouyer-Fessard, C., Tatemoto, K. and Moroder, L. (1983) FEBS Letters 159, 89-92
31. Boige, N., Dupont, C., Chenut, B., Gespach, C. and Rosselin, G. (1984) Eur. J. Clin. Invest. 14, 42-48
32. Simon, B. and Katler, H. (1980) Eur. J. Clin. Invest. 10, 481-485
33. Bataille, D., Coudray, A.-M., Carlqvist, M., Rosselin, G. and Mutt, V. (1982) FEBS Lett. 146, 73-78
34. Bataille, D., Tatemoto, K., Gespach, C., Jörnvall, H., Rosselin, G. and Mutt, V. (1983) FEBS Lett. 146, 79-86
35. Bataille, D., Gespach, C., Tatemoto, K., Marie, J.-C., Coudray, A.-M., Rosselin, G. and Mutt, V. (1981) Peptides, 2, 41-44
36. Bataille, D., Freychet, P., Kitabgi, P.E. and Rosselin, G. (1973) FEBS Lett. 30, 215-218

37.Sjölund, K., Ekelund, M., Hakanson, R., Moody, A.J. and Sundler, F. (1983) J. Histochem. Cytochem. 31, 811-817
38.Gespach, C., Bataille, D., Jarrousse, C. and Rosselin, G. (1979) Acta Endocr. (Kbh) 90, 307-316
39.Leduque, P., Gespach,C., Brown, J.C., Rosselin, G. and Dubois, P.M. (1982) Acta Endocr. (Kbh) 99, 112-121
40.Leduque, P., Moody, A.J. and Dubois, P.M. (1982) Regulatory Peptides 4, 261-274
41.Christiansen, J., Bech, A., Fahrenkrug, J., Holst, J.J., Lauritsen, K., Moody, A.J. and Schaffalitzky de Muckadell, O. (1979) Scand. J. Gastroenterol. 14, 161-166
42.Brown, J.C., Dryburgh, J.R., Ross, S.A. and Dupré, J. (1975) Rec. Progr. Horm. Res. 31, 487-532
43.Pederson, R.A. and Brown, J.C. (1972) Gastroenterology 62, 393-400
44.Kirkegaard, P., Moody, A.J., Holst, J.J., Loud, F.B., Skov Olsen, P. and Christiansen, J. (1982) Nature 297, 156-157
45.Chiba, T., Taminato, T., Kadowaki, A., Abe, H., Chihara, K., Seino, Y., Matsukura, S. and Fujita, T. (1980) Gastroenterology 79, 67-71
46.Barros, D'Sa, A.A., Bloom, S.R. and Baron, J.H. (1975) Lancet I, 886-887
47.Chew, C.S. Am. J. Physiol. (1983) 245, G221-G229
48.Gespach, C., Dupont, C., Bataille, D. and Rosselin, G. (1980) FEBS Lett. 114, 247-252
49.Brown, J.C. and Pederson, R.A. (1970) Scand. J. Gastroenterol. 5, 537-541