Pergamon

Life Sciences, Vol. 55, No. 4, pp. 317-325, 1994

0024-3205/94 $6.00 + .00

0024-3205(94)00121-9

SERUM GASTRIN LEVEL AND GASTRIC SOMATOSTATIN CONTENT AND BINDING IN LONG-TERM PYLOROMYOTOMIZED CHILDREN

V. Barrios (a), M.J.M. Urrutia (b), M. Hernández (a), R. Lama (b), M.D. García-Novo (a), A. Hernanz (b) and E. Arilla (c)*.

(a) Hospital Niño Jesús, Autonomous University, Madrid,
(b) Hospital La Paz, Autonomous University, Madrid, and
(c) Department of Biochemistry and Molecular Biology, University of Alcalá de Henares, Madrid, Spain.

(Received in final form May 16, 1994)

Summary

Since somatostatin inhibits basal and stimulated gastric acid secretion and gastrin release, it is conceivable that decreased gastric somatostatin concentration may be one of the factors responsible for gastric hypersecretion found in patients who have undergone long-term pylorotomy for hypertrophic pyloric stenosis. To investigate this proposal the somatostatin-like immunoreactivity concentration was determined in antral and fundic mucosa samples from control and long-term pyloromyotomized children. In addition, somatostatin binding to cytosol from gastric (fundus and antrum) mucosa and fasting serum gastrin levels and serum gastrin response to a standard breakfast were also studied. The mean fundic and antral somatostatin-like immunoreactivity concentrations were significantly lower in long-term pyloromyotomized children than in control children. The depletion of fundic and antral somatostatin-like immunoreactivity content was associated with an increase in the number of gastric somatostatin binding sites. The fasting serum gastrin levels and serum gastrin response to a standard breakfast (after 60 min) in long-term pyloromyotomized children was significantly higher than those in control children. Since, together with the increase of somatostatin binding to gastric mucosa, there is an increase in the gastrin serum levels, despite the inhibitory effect of somatostatin on gastrin release, the binding capacity cannot be the main factor determining the response to somatostatin in long-term pyloromyotomized children. The present results suggest that both somatostatin and gastrin have some pathophysiologic importance in long-term pyloromyotomized children.

Key Words: gastrin, gastric somatostatin receptors, hypertrophic pyloric stenosis, pylorotomy

Hypertrophic pyloric stenosis is a relatively common condition and there is recent evidence that the incidence is increasing (1). Long-term follow-up of patients pyloromyotomized for hypertrophic pyloric stenosis has shown an increased incidence of ulcer symptoms, and many patients had increased basal gastric acidity (2). To date, the mechanism underlying these findings is unknown. However, it is possible that somatostatin and gastrin may be implicated. Somatostatin and gastrin, two potent regulatory peptides in the human digestive system, play an important role in gastrointestinal physiology. Both hormones influence the secretion of gastric acid, which is stimulated by gastrin (3) and inhibited by somatostatin (4, 5), depending on the pH value in the stomach and duodenum. Somatostatin seems to act directly on parietal cells (6) and can also inhibit gastric acid secretion by suppressing the release of gastrin from the antrum (7). These inhibitory actions of somatostatin in the stomach suggest that this peptide may play a role in states in which there is an abnormal gastric secretion.

The present study investigates the concentration of gastric mucosal somatostatin-like immunoreactivity in fundic and antral mucosa samples from controls and from children who had been pyloromyotomized for hypertrophic pyloric stenosis (between 4 and 15 years after Fredet-Ramstedt pylorotomy). In addition, somatostatin binding to cytosol from gastric (fundus and antrum) mucosa and the fasting serum gastrin levels and the serum gastrin response to a standard breakfast were also studied.

Patients and Methods

Chemicals

Synthetic [Tyr^{11}]-somatostatin and somatostatin tetradecapeptide were purchased from Universal Biological Ltd (Cambridge, U.K.), aprotinin (Trasylol) from Bayer (Leverkusen, F.R.G.), trypsin inhibitor and bovine serum albumin from Sigma (St. Louis, MO, U.S.A.) and carrier-free Na^{125}I (IMS 30; 100 mCi/mL) from the Radiochemical Centre (Amersham, Bucks, U.K.). The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham, Bucks, U.K.). All other chemicals were reagent grade.

Patients

Endoscopic samples were taken from 59 patients (Table I) who were divided into two groups: a) control group and b) patients who underwent long-term pylorotomy for hypertrophic pyloric stenosis. These children were examined between 4 and 15 years after Fredet-Ramsted pylorotomy. All of the parents were informed about the risks and aims of the study and gave their consent before sample collection. The study was also approved by the local ethical commitee.

The control group included 36 patients who underwent endoscopic exploration for different reasons: dyspepsia in 7 patients, hiatal hernia in 15 patients and epigastric pain in 14 patients. None of them had visible changes in the stomach during the exploration or showed histopathological changes of the gastric mucosa.

TABLE I

Groups of children studied

Groups	n	Sex		Age (years)	
		Boys	Girls	Mean	Range
Control	36	28	8	10.4	4-15
Pyloromyotomized	23	17	6	9.8	4-15

n: number of individuals studied.

Radioimmunoassay of somatostatin and gastrin

a) Somatostatin: The somatostatin antiserum was raised in rabbits against somatostatin-14 conjugated to bovine serum albumin and is specific for somatostatin but, since somatostatin-14 constitutes the C-terminal portions of both somatostatin-25 and somatostatin-28, the antiserum does not distinguish between these three forms.

Endoscopic biopsies were obtained from antral and fundic mucosa. The samples were divided in three parts for radioimmunoanalysis, binding somatostatin experiments and histological analysis. One sample from every child was stained by the hematoxiline-eosine method in order to verify the absence of histological alterations before further processing. Some gastric mucosa samples were immediately frozen; the frozen tissue was homogenized with a pestle, boiled for 5 min in acetic acid (1 mol/l) in order to destroy the proteolytic enzymes and coagulate the bulk of the proteins and then homogenized briefly (1 min) a second time with a motor-driven teflon pestle. This

procedure does not destroy somatostatin detectability, as Gerich et al. (8) have already shown. The homogenate was centrifuged at 15000 x g for 15 min at 4°C, and the resulting supernatant was stored at -70°C until assay. Just before assay, extracts were neutralized with NaOH (1 mol/l). Somatostatin concentration was determined by a radioimmunoassay method (10) with a sensitivity limit of 10 pg/mL, using a rabbit anti-SS serum (final dilution usually 1:20000). A phosphate buffer (0.01 mmol/l, pH 7.4) containing NaCl (0.15 mol/l), EDTA (0.05 mol/L), 0.1% (wt/vol) bovine serum albumin and 100 kallikrein inhibitor units (KIU) aprotinin (Trasylol)/ml was used in the assay system. Incubation tubes were prepared in triplicate and the following substances were added to the assay tubes in sequence: 200 µl sample or standard containing 0-320 pg cyclic somatostatin; 200 µl ^{125}I-labelled [Tyr^{11}]- somatostatin (5000 cpm; equivalent to 5-10 pg) and 400 µl appropriately diluted antibody (final dilution usually 1:20000). After brief agitation with a Vortex mixer, the tubes were incubated at 4°C for 48 h; separation of bound and free hormone was accomplished by addition of 500 µl dextran-coated charcoal (dextran T-70, 0.025% wt/vol; Pharmacia, Uppsala, Sweden. Charcoal, Norit A, 0.25% wt/vol; Serva, Feinbiochemica, Heidelberg, F.R.G.). Dilution curves for mucosa gastric extracts were parallel to the standard curve. The percent recovery of ^{125}I-labelled [Tyr^{11}]-somatostatin added to tissue extracts was 89 ± 5 (SEM) %. The intra- and interassay coefficients of variation were 7.2 and 9.5% respectively.

b) Gastrin: Blood serum immunoreactive gastrin level was determined in each child after four-hour fasting period. Additionally, immunoreactive gastrin curves were determined by taking 3 blood samples during a 1-hour period after feeding, the first being taken 15 min after a standard breakfast. This breakfast contained 380 Kcal made up of 16% proteins, 30% fat and 54% carbohydrates. The fasting level obtained immediately before the meal was taken as a reference value.

Blood serum immunoreactive gastrin level was determined by the radioimmunoassay technique using a Clinical Assays Gamma Dab [^{125}I] Gastrin radioimmunoassay kit from Baxter (USA). The gastrin antibody used in this study could be completely absorbed by gastrin-34 and gastrin-17, but not by somatostatin. Neither antibody cross-reacted with cholecystokinin and secretin. The precipitating antiserum reagent, containing the second antibody in the polymer solution, was used to separate the antibody-bound tracer from unbound tracer by immunoprecipitation. The assay tubes were centrifuged and supernatants decanted. The antibody-bound tracer, which is in the precipitate, was counted in a gamma counter.

Binding assay

Synthetic [Tyr^{11}]-somatostatin was radioiodinated by the method described by Greenwood et al. (9) and purified by chromatography on a Sephadex G-25 column (100 x 1 cm) preequilibrated with acetic acid (0.1 mol/l) containing 0.1% (wt/vol) bovine serum albumin. The specific activity of radioiodinated somatostatin was approximately 400 Ci/g.

The cytosolic fraction of antral and fundic mucosa was isolated according to the method of Reyl-Desmars & Lewin (10). Briefly, homogenates were prepared from antral and fundic mucosa using a motor-driven Potter-Elvejhem teflon glass homogenizer (1 min at 1800 rpm) in a 10% suspension (wt/wt) in ice-cooled Tris (10 mmol/l)/sucrose (250 mmol/l) buffer (pH 7.4), containing 0.1 mg trypsin inhibitor/ml. Cytosolic extracts were prepared by centrifuging the homogenates for 1 hour at 105000 x g. Protein concentration was estimated by the method of Lowry et al. (11) using bovine serum albumin as a standard.

Binding studies were performed in optimal conditions according to a previously reported technique (12, 13). The interaction of ^{125}I-labelled [Tyr^{11}]-somatostatin with cytosol from fundic and antral mucosa is rapidly reversible, specific, saturable, and temperature-dependent. Briefly, the cytosolic fraction of fundic and antral mucosa (0.2 mg protein/ml) was incubated in 0.5 ml medium (pH 7.4) with the following composition: NaH_2PO_4 (0.5 mmol/l), Na_2HPO_4 (1mmol/l), NaCl (80 mmol/l), KCl (5 mmol/l), $CaCl_2$ (1 mmol/l), $MgCl_2$ (1.5 mmol/l), HEPES (50 mmol/l), glucose (11 mmol/l), 0.1% bovine serum albumin, trypsin inhibitor (0.1 mg/ml), and ^{125}I-labelled [Tyr^{11}]-somatostatin (50 pmol/l) either alone or together with increasing concentrations of unlabelled somatostatin. Unless otherwise indicated, incubations were performed at 25°C for 60 min. The amount of ^{125}I-labelled [Tyr^{11}]-somatostatin associated with cytosolic proteins was determined

after removal of unbound tracer by the addition of 0.25% charcoal, 0.5% bovine serum albumin and 0.025% dextran T-70 (10). "Specific" binding was estimated as the difference between "total" binding (i.e. in the presence of tracer alone) and "non-specific" binding as measured in the presence of unlabelled somatostatin (4 mmol/l). This non-specific component represented about 30% of the binding observed in the absence of unlabelled somatostatin.

The degradation of ^{125}I-labelled [Tyr^{11}]-somatostatin was assessed by determining the ability of the label remaining in the incubation medium supernatants to bind to talc (14). Samples of the supernatants (200 µl) were added to chilled HEPES (50 mmol/l) buffer, pH 7.5, containing 0.25% human serum albumin (1 ml). After agitation using a Vortex mixer, the samples were centrifuged for 10 min at 4°C and 1700 x g and the supernatants removed by aspiration. The radioactivity in the pellet was compared to that in an aliquot of the original sample (200 µl).

Data analysis

Binding parameters for somatostatin binding were determined by least squares analysis of Scatchard plots (15). Statistical analysis was performed using the Student's t-test for unpaired samples to determine significance. Differences with p values lower than 0.05 were considered significant. Each individual experiment was performed in triplicate. All results are expressed as means ± SEM.

Results

Since no differences existed between male and female gastric somatostatin content and binding and gastrin values, all values in each group were pooled for the calculations.

The mean antral and fundic somatostatin-like immunoreactivity concentrations in long-term pyloromyotomized children was significantly lower than those in control children (Fig. 1).

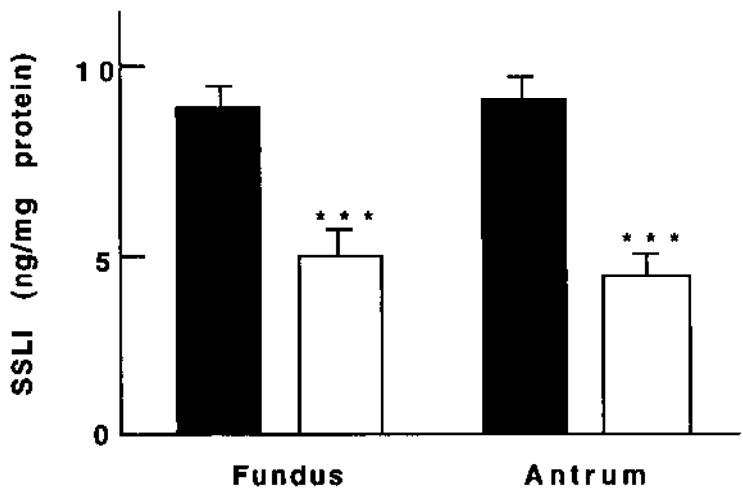

FIG. 1

Somatostatin-like immunoreactivity (SSLI) concentration in the antral and fundic mucosa from control (n=36, full bars) and long-term pyloromyotomized children (n=23, open bars). Values are the mean ± SEM. Units for SSLI are ng of somatostatin per mg of protein. *** $p < 0.001$ compared with control (Student's t-test).

The specific binding of ^{125}I-labelled-[Tyr^{11}]-somatostatin to cytosol from gastric (antral and fundic) mucosa from either control or long-term pyloromyotomized children was time-dependent becoming maximal at 60 min and then remaining stable for at least another 2 hours in agreement with previous studies (13, 16). All subsequent studies were performed for 60 min at 25°C. To rule

out the possibility of different somatostatin-degrading activities in the cytosolic preparations that might affect the interpretation of the results, the degradation of the tracer was determined. The percentage of labelled somatostatin degraded by the cytosolic preparation during the binding experiments was similar in both control and long-term pyloromyotomized children, being 21.2 and 23.5% respectively.

Increasing concentrations of unlabelled somatostatin competitively inhibited the specific binding of ^{125}I-labelled-[Tyr^{11}]-somatostatin to gastric (fundus and antrum) cytosolic preparations from both the control and long-term pyloromyotomized children (Fig. 2, left panel). However, the specific binding of the tracer in gastric cytosolic preparations from long-term pyloromyotomized children was significantly higher than that in the control group. Scatchard analysis of these results indicates that there was an increase in the number of somatostatin binding sites in the cytosolic fraction of fundic and antral mucosa from long-term pyloromyotomized children (without affecting the dissociation constant) as compared with control children (Fig. 2, right panel; and Fig. 3).

Sixty minutes after a standard breakfast, the fasting serum gastrin levels and serum gastrin response were significantly higher in previously pyloromyotomized children than in control children (Table II). The area under the curve of controls was 2276.4 ± 88.7 pg of gastrin, whereas that of long-term pyloromyotomized children was 2933.4 ± 234.6 pg of gastrin, this value being significantly different.

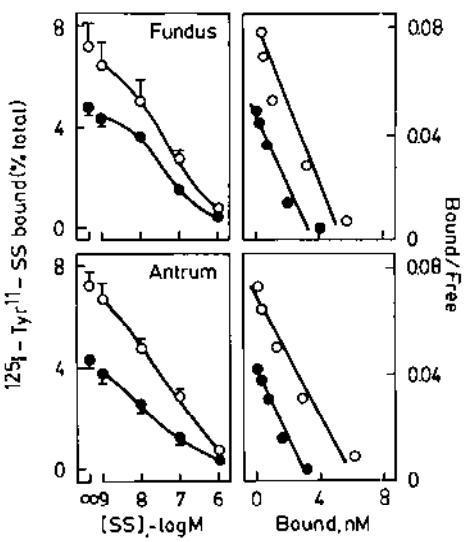

FIG.2

Left panel: Competitive inhibition of specific ^{125}I-labelled [Tyr^{11}]-somatostatin (^{125}I-Tyr^{11}-SS; 50 pmol/l) binding to cytosol (0.2 mg protein/ml) from gastric (fundus and antrum) mucosa by unlabelled somatostatin. Points correspond to control children (●) and long-term pyloromyotomized children (O). Mean values ± SEM are given. Right panel: Scatchard analysis of the same data. The kinetic constants calculated by Scatchard analysis are given in Fig. 3.

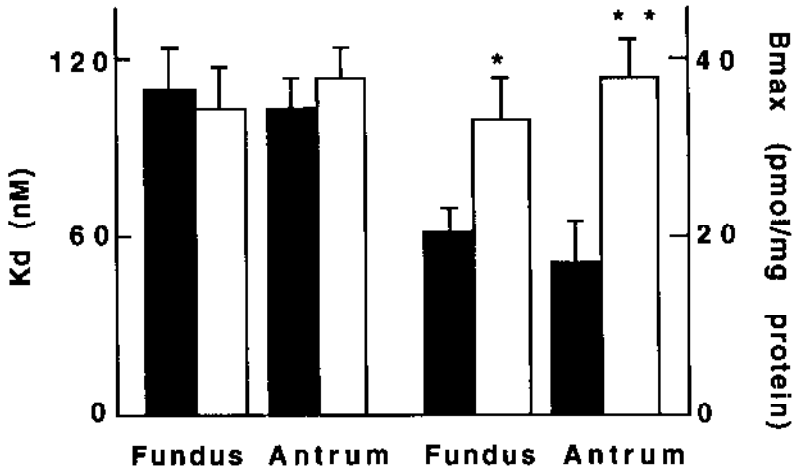

FIG.3

Equilibrium parameters of somatostatin binding to cytosol of gastric (fundus and antrum) mucosa from control (n=36, full bars) and long-term pyloromyotomized children (n=23, open bars). Values are the mean ± SEM. Binding parameters were calculated from Scatchard plots by linear regression. Units for dissociation constant (Kd) are nmol/l and units for binding capacity (Bmax) are pmol of somatostatin bound per mg of protein. * $p < 0.05$, ** $p < 0.01$ compared with control (Student's t-test).

TABLE II

Serum gastrin levels (pg/ml) in controls (n=36) and long-term pyloromyotomized children (n=23) before and after standard breakfast.

Groups	Time (min)			
	0	15	30	60
Control	56.0 ± 2.5	131.5 ± 2.5	121.3 ± 1.4	49.5 ± 2.6
Pyloromyotomized	72.7 ± 4.2*	141.7 ± 15.1	129.5 ± 9.1	108.8 ± 8.6**

The groups are further explained in the Patients and Methods section. *p<0.05; **p<0.001 compared with control (Student's t-test).

Discussion

The fasting serum gastrin levels and the serum gastrin response to a standard breakfast (after 60 min) in long-term pyloromyotomized children were significantly higher than in control children. A marked rise in mean fasting gastrin levels was also noted 4 days to 4 weeks after pylorotomy by some authors (17, 18) but not by others (19, 20).

Conlon et al. (21) have demonstrated that the rate of degradation of ^{125}I-Tyr^{11}-somatostatin, as measured as the loss of ability to bind to talc, is similar to that of somatostatin as measured by radioimmunoassay. The extent of somatostatin degradation in this study was relatively important but it reached similar values in all the experimental groups, so it was possible to compare the type values. The values calculated for Kd and binding can be modified by ^{125}I-Tyr^{11}-somatostatin degradation and so they must be considered as apparent.

The coexistence of somatostatin and nitric oxidase synthase in myenteric neurons has been shown (22). Recently, Vanderwinden et al. (23) suggest that a lack of nitric oxidase synthase in

pyloric tissue is responsable for pylorospasm in infantile hypertrophic pyloric stenosis. At the present moment, the relationship between these findings and the decrease in somatostatin content found in our study is unknown.

The rise in serum immunoreactive gastrin is not due to age differences between the two groups of children studied since both groups have a similar age range.

The mechanism of hypergastrinaemia has not been elucidated in the present study. Somatostatin is thought to inhibit gastrin release in the antrum and acid secretion in the fundic mucosa by both hormonal and paracrine pathways (7). A significant reduction in antral somatostatin may be the pathogenetic basis of hypergastrinaemia, although the possibility that other factors may also be involved cannot be precluded (7).

As previously shown, the hormonal content of routine biopsies can be analyzed accurately (24). In the present study, the somatostatin-like immunoreactivity levels as well as the binding parameters of somatostatin binding sites in the gastric (fundus and antrum) mucosa in the control children were similar to those previously reported by others (25, 26). The mean fundic and antral somatostatin-like immunoreactivity concentrations in long-term pyloromyotomized children were significantly lower than those of control children. This finding is consistent with the results of Chen et al. (27) who showed that hypergastrinemic rats had a reduced number and density of somatostatin cells.

We have been unable to demonstrate specific somatostatin binding in isolated cell membranes from gastric mucosa in the variety of experimental conditions tested here. Intracellular somatostatin binding sites were first described by Ogawa et al. (28). They suggested that the somatostatin binding protein is an easily soluble membrane protein. Recently, it has been demonstrated that somatostatin binding sites exist in secretion vesicles in anterior pituitary cells and pancreatic islets in rats (29), in plasma membranes from pig gut (30) and rat stomach (31) and in canine gastric parietal cells (6). Evidence that somatostatin can penetrate the plasma membrane in vitro is supported by previous binding studies on isolated rat gastric mucosal cells (32). The intracellular binding sites for somatostatin in a number of tissues, including the gastric mucosa, may behave as a phosphoprotein phosphatase regulatory subunit and therefore, somatostatin could lead to the corresponding physiological effects (10).

The affinity of the somatostatin binding site is comparable to that reported in previous studies on somatostatin binding to cytosol from rat small intestinal ephithelium (33) and human placental membranes (34). However, somatostatin bound with a higher affinity to the cytosolic fraction of both rat pancreas and gastric mucosa (35) and pig gut membranes (30). The present affinity values are not compatible with the low circulating levels of the peptide. However, the presence of somatostatin in both paracrine cells and nerve endings in the gastric mucosa may make local concentration of somatostatin high enough to interact with the reported binding sites.

The depletion of gastric (antrum and fundus) somatostatin-like immunoreactivity was associated with an increase in the number of gastric somatostatin receptors. These results suggest that a decrease in endogenous somatostatin-like immunoreactivity leads to sensitization or up-regulation of somatostatin receptors in the gastric mucosa. This is consistent with reports showing that cysteamine-induced depletion of brain (36) and gastroduodenal mucosal somatostatin has been associated with an increase of the number of the somatostatin receptors in both tissues (12, 36, 37).

The relationship between somatostatin binding and somatostatin action has not been studied in this work. Other factors subsequent to binding may significantly modify somatostatin action in certain circumstances.Thus, in rats with cysteamine-induced duodenal ulcer, somatostatin action on gastrin output is impaired (38) whereas somatostatin duodenal mucosa binding is enhanced (12). Rate-limiting intracellular events probably exist in long-term pyloromyotomized children since, although their somatostatin binding is increased, their somatostatin action is impaired.

In conclusion, the present results suggest that both somatostatin and gastrin may be of pathophysiologic importance in long-term pyloromyotomized children.

Acknowledgements

This study was supported by grants from the Dirección General de Investigación Científica y Técnica (PM 92-0049) and from the Fondo de Investigaciones Sanitarias de la Seguridad Social of Spain (94/401). The authors thank Carol F. Warren from the Alcalá de Henares University Institute of Education Sciences and Lillian Puebla for their assitance in the stylistic revision of the manuscript.

References

1. P.F. MASON, J. Roy. Coll. Surg. Edinburgh. 36 293-294 (1991).
2. B. WANSCHER and H.E. JENSEN, Scand. J. Gastroenterol. 6 597-599 (1971).
3. J.H. WALSH and M.I. GROSSMAN, N. Engl. J. Med. 292 1324-34 (1975).
4. A. GOMEZ-PAN, J.D. REED, M. ALBINUS, B. SHAW, R. HALL and G.M. BESSER, Lancet 1 888-890 (1975).
5. A.A.J. BARROS D'SA, S.R. BLOOM and J.H. BARON, Lancet 1 886-887 (1975).
6. J. PARK, T. CHIBA and T. YAMADA, J. Biol. Chem. 262 14190-14196 (1987).
7. L.I. LARSSON, N. GOLTERMANN, L. DE MAGISTRIS, J.F. REHFELD and T.W. SCHWARTZ, Science 205 1393-1395 (1979).
8. J. GERICH, K. GREENE, M. HARA, R. RIZZA and G. PATTON, J. Lab. Clin. Med. 93 1009-1017 (1979).
9. F.C. GREENWOOD, W.M. HUNTER and J.S. GLOVER, Biochem. J. 89 114-123 (1963).
10. F. REYL and M.J.M. LEWIN, Proc. Natl. Acad. Sci. USA 76 978-982 (1982).
11. O.H. LOWRY, N.J. ROSEBROUGH, A.L. FARR and R.J. RANDALL, J. Biol. Chem. 193 265-275 (1951).
12. L.G. GUIJARRO, M.P. LOPEZ-RUIZ, J.C. PRIETO and E. ARILLA, Gen. Pharmacol. 6 637-639 (1986).
13. V. BARRIOS, J. MARTINEZ-GOMEZ, M.D. GARCIA-NOVO, E. ARILLA and M. HERNANDEZ, [Abstract]. Proceedings of the XVII Spanish Congress of Paediatrics, pp. 53 (1988).
14. J.M. CONLON, J. WHITTAKER, V. HAMMOND and K.G.M.M. ALBERTI, Biochim. Biophys. Acta. 677 234-242 (1981).
15. G. SCATCHARD, Ann. NY. Acad. Sci. 51 660-672 (1949).
16. L.G. GUIJARRO, E. ARILLA, M.P. LOPEZ-RUIZ, J.C. PRIETO, C. WHITFORD and B.H. HIRST, Regul. Peptides. 10 207-215 (1985).
17. J.M. CASASA, J.M. LAFUENTE, J. BOIX-OCHOA, R. TORMO, D. INFANTE and J. CAROL, Ann. Chir. Infant. (Paris) 18 363-369 (1977).
18. M.A. HAMBOURG, M. MIGNON, R. RICOUR, J. ACCARY and D. PELLERIN, Arch. Dis. Child. 54 208-212 (1979).
19. I.M. ROGERS, I.K. DRAINER, M.R. MOORE and K.D. BUCHANAN, Arch. Dis. Child. 50 467-71 (1975).
20. M.A. BLEICHER, B. SHANDLING, W. ZINGG, H.W.A. KARL and N.S. TRACK, Gut 19 794-797 (1978).
21. J.M. CONLON, J. WHITTAKER, V. HAMMOND and K.G.M.M. ALBERTI, Biochim. Biophys. Acta 677 234-242 (1981)
22. S.R. VINCENT and B.T. HOPE, Trends Neurosci. 15 108-113 (1992)
23. J.M. VANDERWINDEN, P. MAILLEUX, S.N. SCHIFFMANN, J.J. VANDEHAEGHEN and M.H. DE LAET, N. Engl. J. Med. 327 511-515 (1992)
24. M.G. BRYANT, S.R. BLOOM, J.M. POLAK, S. HOBBS, W. DOMSCHKE and S. DOMSCHKE, Gut 24 114-119 (1983).
25. M. BRYANT, A. BUCHAN and M. GREGOR, Gastroenterology 83 47-51 (1982).
26. E. PENMAN, J.A.H. WASS, M.G. BUTLER, E.S. PENNY, J. PRICE and P. WU, Regul. Peptides 7 53-65 (1983).
27. D. CHEN, A. URIBE, R. HAKANSON and F. SUNDLER, Scand. J. Gastroenterol. 27 479-482 (1992).
28. N. OGAWA, T. THOMPSON, H.G. FRIESEN, J.B. MARTIN and P. BRAZEAU, Biochem. J. 165 269-277 (1977).
29. B. DRAZNIN, P. MEHLER, J.W. LEITNER, K.E. SUSSMAN, R. DAHL and A. VATTER, J. Recept. Res. 5 83-103 (1985).

30. M. WEBER, T. COLE and J.M. CONLON, Am. J. Physiol. 250 G679-685 (1986).
31. W.J. ROSSOWSKI, A. OZDEN, A. ERTAN, M. MAUMUS and A. ARIMURA, Scand. J. Gastroenterol. 23 717-725 (1988).
32. F. REYL, C. SILVE and M.J.M. LEWIN, Hormone Receptors in Digestion and Nutrition, pp. 391-400, Elsevier/North-Holland, Amsterdam (1979).
33. E. ARILLA, M.P. LOPEZ-RUIZ, L. GONZALEZ-GUIJARRO, J.C. PRIETO, A. GOMEZ-PAN and B.H. HIRST BH, Biochim. Biophys. Acta. 802 203-208 (1984).
34. E. TSALIKIAN, T.P. FOLEY and D.J. BECKER, Pediatr. Res. 18 953-957 (1984).
35. F. REYL and M.J.M. LEWIN, Biochem. Biophys. Res. Commun. 109 1324-1331 (1982).
36. C.B. SRIKANT and Y.C. PATEL, Endocrinology 115 990-995 (1984).
37. S. SZABO and S. RIECHLIN, Endocrinology 109 2255-2257 (1981).
38. U. SCHWEDES, P. ALTHOFF, S. SZABO and K. SCHÖFFLING, Acta. Endocrinol. 87 65-66 (1978)