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Different effects of bombesin on glucose- and tolbutamide-induced insulin release in man

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1 The effect of bombesin, a neurograstrointestinal peptide, on basal and stimulated insulin release was studied in man.

2 Two different stimuli were used, hyperglycaemic (20 g glucose) and hypoglycaemic (1 g tolbutamide). They were injected intravenously to two groups of male healthy volunteers during saline or bombesin (5 ng kg^{-1} min^{-1} for 60 min) infusion.

3 The peptide had no significant effect on basal levels of glucose and insulin. However, the insulin response to intravenous glucose was strongly potentiated by bombesin, the integrated insulin response being 2.23 ± 0.59 mu $ml^{-1} \cdot 90$ min and 0.98 ± 0.19 mu $ml^{-1} \cdot 90$ min during infusion of bombesin and saline, respectively ($P < 0.05$). The behaviour of plasma glucose was not significantly modified by the peptide. Indeed, the glucose disappearance rate (K of Conard, mg $min\ 10^{-2}$) changed from 2.5 ± 0.3 during saline to 2.4 ± 0.4 during bombesin infusion.

4 When the hypoglycaemic stimulus (i.e. tolbutamide) was used, no effect of the peptide on insulin release could be detected. Here again, the drop in plasma glucose (expressed as Marigo's coefficient) was not affected by the peptide, with a value of 92.8 ± 12.6 and 84.0 ± 10.9 during bombesin and saline administration.

5 These data therefore show that, at normal or low blood glucose levels, the dose of bombesin used is unable to modify insulin release and suggest that this peptide might be regarded as a glucose-dependent insulinotropic peptide.

Introduction

Bombesin is a frog skin tetradecapeptide which has been found to have similar immunologically characterized counterparts in mammalian gut (Anastasi *et al.*, 1971; Brown & Vale, 1979; Ersparmer & Melchiorri, 1980). A heptacosapeptide has been isolated from porcine non-antral tissue, the so-called Gastrin Releasing Peptide (GRP) (McDonald *et al.*, 1979). Evidence has been presented indicating that GRP must be regarded as mammalian bombesin (Brown *et al.*, 1980).

Bombesin-like immunoreactivity was found to be present throughout the alimentary tract (Walsh *et al.*, 1979; Yanaihara *et al.*, 1980) and pancreas of several animal species, including man (Holst *et al.*, 1983; Ghatei *et al.*, 1984). This broad localization in the gut raises questions about the possible physiological significance of bombesin-like peptides.

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Both bombesin and GRP are potent and polyvalent releasers of several gastrointestinal hormones (Ghatei *et al.*, 1982; Knigge *et al.*, 1984) and have a similar effect on many biological functions (McDonald *et al.*, 1981; Fletcher *et al.*, 1983; Lambert *et al.*, 1984). This overlapping spectrum of biological activity is the consequence of their structural homology. The carboxy-terminal decapeptides of bombesin and GRP are, indeed, identical except for a Gln/His interchange at position 8 from the carboxyl terminus (McDonald *et al.*, 1979) (Figure 1).

Although their main physiological action seems to be the release of gastrin with consequent increase in acid secretion (Knuhtsen *et al.*, 1984), their effect on insulin secretion was recently emphasised. A stimulation of insulin release by intravenous bombesin or GRP was reported in both animals (Kaneto *et al.*, 1978; McDonald *et al.*, 1981; Vaysse *et al.*, 1981; Vagne *et al.*, 1982; Greeley & Thompson, 1984) and man (Ghatei *et al.*, 1982; Bruzzone *et al.*, 1983;

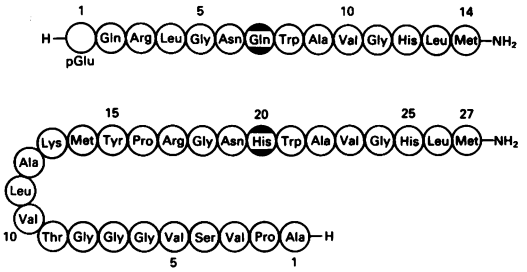

Figure 1 Primary structure of bombesin and porcine Gastrin Related Peptide (GRP).

Wood *et al.*, 1983; Knigge *et al.*, 1984). The direct stimulatory effect of bombesin on insulin release was confirmed in the isolated and perfused rat (Martindale *et al.*, 1982) and dog (Ipp & Unger, 1979) pancreas.

In previous papers, we have shown that bombesin affects the insulin response to mixed meal (Scarpignato & Micali, 1986) and to oral as well as intravenous glucose load (Scarpignato *et al.*, 1987b). However, no effect on *basal* insulin levels was observed. Furthermore, it was recently reported that GRP is able to stimulate insulin secretion in fed (serum glucose: $167 \pm 4\,mg\,dl^{-1}$) but not in fasted (serum glucose: $83 \pm 3\,mg\,dl^{-1}$) rats (Greeley & Thompson, 1984). Finally, the release of insulin in response to bombesin was found to be enhanced by raising the concentration of glucose in the isolated and perfused rat pancreas (Martindale *et al.*, 1982). All these data, taken together, suggest that the effect of bombesin-like peptides on insulin release may be dependent on blood sugar levels.

The aim of the present investigation was, therefore, to test this hypothesis. We evaluated the effect of bombesin on insulin response to two different stimuli (i.e. intravenous glucose and tolbutamide), hyper- and hypo-glycaemic respectively, in healthy male volunteers. This paper was presented at the First Joint Meeting of the Italian and Dutch Pharmacological Societies (Florence, May 10–13, 1987) and appeared in abstract form (Scarpignato *et al.*, 1987a).

Methods

Subjects

Two groups of 6 male healthy volunteers (average age 25 years) participated in the study; written informed consent was obtained from all of them. They were medical students or soldiers without any gastrointestinal, endocrine or metabolic diseases. When compared to average weights based on heights and age (tables Geigy), all subjects were within 10% of their predicted (ideal) value.

Experimental design

After an overnight fast and abstinence from smoking (Janzon *et al.*, 1983), all the subjects came to the laboratory at 08 h 30 min. They remained in the supine position during the entire period of the study. An indwelling intravenous catheter (Abbocath) was inserted in each forearm: one for the infusion, the other for blood sampling. Patency was preserved by a slow infusion of physiological saline.

All the volunteers underwent the test twice, once with an intravenous infusion of bombesin, the second time with a control saline infusion in a single blind randomised order. Infusion of bombesin in saline ($5\,ng\,kg^{-1}\,min^{-1}$) began 15 min before glucose (20 g intravenously over 5 min) [first group] or tolbutamide (1 g intravenously over 5 min) [second group of volunteers] and continued for 60 min. The doses of both compounds represent a submaximal stimulus for insulin release. Blood for insulin and glucose assay was sampled at various time intervals throughout the experiments (see figures).

Evaluation of side effects

Respiratory rate was monitored through impedance variation between two electrodes placed on the chest and heart rate (HR) was obtained from a cardiotachometer driven by R waves from a chest wall electrocardiogram. In addition, axillary skin temperature was measured with a thermocouple taped to the skin. All these parameters were continuously displayed throughout the experiments on the screen of a SENTINEL 2 monitor (Gambro-Soxil SpA, Milan, Italy) and recorded at 15 min intervals. Systolic and diastolic blood pressure (BP) were recorded by means of an automatic device (BP-103N, Nippon-Colin Co., Japan) and an inflatable cuff around the non dominant arm.

Side effects as reported spontaneously by the subjects were recorded together with the experimenter's observations.

Laboratory analyses

Blood was collected in prechilled tubes containing 1.2 mg of disodium edetate per ml of blood. Specimens were centrifuged within 1 h and plasma stored frozen at −20°C until the time of assay.

Protein-free filtrates for plasma glucose were made immediately after the blood samples had been obtained. Plasma glucose was then determined on the supernatant by a semimicro glucose-oxidase method (Trinder, 1969).

Plasma immunoreactive insulin (IRI) was assayed by a modification (Cornale *et al.*, 1981) of the double antibody method of Hales & Randle (1963), by using a Liso-phase Insulin System (Lepetit Diagnostic Products, Milan, Italy). In this assay the separation of insulin bound to antibody from unbound insulin was carried out by affinity chromatography (Sepharose gel to which the second antibody has been covalently linked according to Cornale *et al.*, 1981). Human insulin was used as a standard and diluted in insulin-free human plasma. In our hands, the method has an intra-assay coefficient of variation of 3%; the minimal sensitivity of this method is $2\,\mu u\,ml^{-1}$. All samples from a single subject were assayed in duplicate and in the same run in order to avoid inter-assay variations. Bombesin did not interfere in the insulin radioimmunoassay up to a concentration of $1\,ng\,ml^{-1}$.

Evaluation of data

The fitting of the standard curve of radioimmunoassay was performed by a LKB-WALLAC computer on line with the gamma counter (Rack-Gamma, LKB, Sweden) using the spline approximation (Marschner *et al.*, 1980). Quantitative evaluation of hormone production (Integrated Insulin Response, IIR) was made by an integration of areas under the curve of immunoreactivity in plasma, after subtraction of the basal values.

After the intravenous glucose load, the glucose disappearance rate (K) according to Conard *et al.* (1953) was calculated by the method of least squares taking the natural logs of the glucose values from 10 to 60 min and expressed in $mg\,min\ 10^{-2}$.

After the intravenous tolbutamide load, the fall in plasma glucose was quantitated through the calculation of the D coefficient according to the following formula (Marigo, 1967):

$$D = \frac{3r + 2s + t}{3}$$

where r is the percentage fall of glycaemia at 20, s at 30 and t at 40 min after intravenous tolbutamide. This formula was employed to emphasise the speed of the glycaemic fall resulting from the tolbutamide stimulus (Marigo, 1967).

All values are presented as mean ± s.e.mean. Two-way analysis of variance (ANOVA) was used for statistical evaluation of the data (Snedecor & Cochran, 1967). According to Elashoff (1981), Student's *t* test for paired data was employed to check differences between the means of summarised responses (i.e. IHR values).

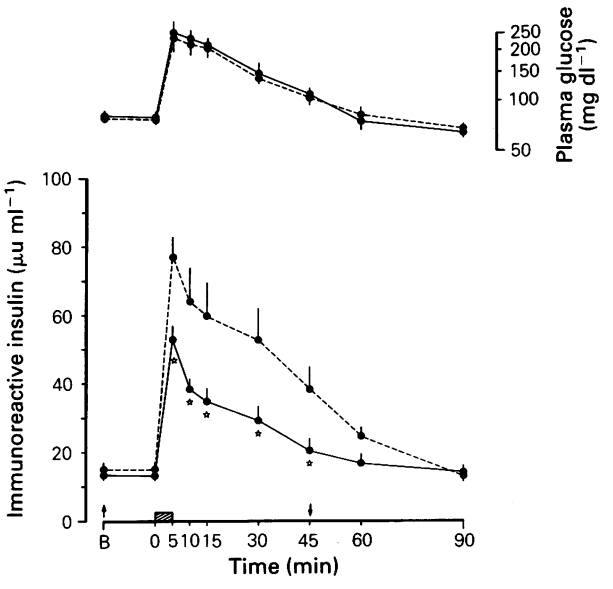

Figure 2 Plasma insulin and glucose after an intravenous glucose load (20 g) during saline (continuous line) or bombesin (broken line) infusion in healthy volunteers. Each point refers to the mean of the values obtained from 6 subjects. Vertical bars are standard errors. ↑ = start of the infusion; ↓ = end of the infusion. B = basal and refers to the mean of two consecutive blood samples (−30 and −15 min) obtained before bombesin or saline infusion. The hatched horizontal bar represents the time taken to inject glucose. $*P < 0.05$.

All the calculations were performed using a computer programme running on an Apple II (Barlow, 1983).

Drugs

Tolbutamide (as Rastinon test ampoules) was kindly supplied by Hoechst Italia SpA (Scoppito, Italy). Synthetic bombesin was a generous gift of Dr Chiara De Paolis (Farmitalia-Carlo Erba Research Labs, Milan, Italy).

Results

Intravenous glucose load

Plasma glucose and insulin after the intravenous glucose load (20 g) during bombesin or saline infusion are depicted in Figure 2. As previously described (Scarpignato & Micali, 1986; Scarpignato *et al.*, 1987b), bombesin did not affect basal levels of both parameters. It was, however, able to increase

significantly the insulinogenic power of glucose. Indeed, during the infusion of the peptide the insulin response to intravenous glucose was greater than that observed after saline (Figure 4). The Integrated Insulin Response (IIR) was 0.98 ± 0.19 mu $ml^{-1} \cdot 90$ min during saline and 2.23 ± 0.59 mu $ml^{-1} \cdot 90$ min during bombesin infusion, the difference being statistically significant ($P < 0.05$).

The behaviour of plasma glucose was not significantly modified by bombesin. As a consequence, the glucose disappearance rate (K of Conard) was virtually identical during either bombesin or saline infusion (2.4 ± 0.4 mg min $\cdot 10^{-2}$ *versus* 2.5 ± 0.3 mg min $\cdot 10^{-2}$).

Intravenous tolbutamide load

When tolbutamide (1 g) was injected intravenously to stimulate insulin release, no effect of bombesin on insulin levels was evident (Figure 3.) The administration of the sulphonylurea was followed, as expected, by a strong release of insulin with a consequent drop in plasma glucose (Figure 3). The behaviour of both parameters was virtually identical with or without bombesin. As a consequence, neither the IIR (Figure 4) nor the D coefficient were significantly modified by the peptide. The coefficient of Marigo was indeed 84 ± 10.9 during saline and 92.8 ± 12.6 during bombesin infusion.

Side effects

No significant changes in respiratory rate, HR, BP or body temperature were observed with bombesin at the dose employed in the present investigation. During the infusion of the peptide, nausea and a slight abdominal discomfort were reported by 2 out of 12 volunteers. These effects disappeared spontaneously after stopping the infusion.

All the six volunteers became symptomatically hypoglycaemic after intravenous tolbutamide and their subjective feeling was not modified by the administration of the peptide.

Discussion

Bombesin belongs to a series of structurally related peptides isolated from amphibian skin. In 1971 Erspamer first reported that intravenous infusion of one of these, alytesin, causes a threefold increase of immunoreactive insulin levels in dogs. Some years later Brown & Fisher (1980) showed that, like other peptides, bombesin can influence glucose homeostasis and affect insulin secretion. Afterwards, insulin release was found to be stimulated by the peptide in

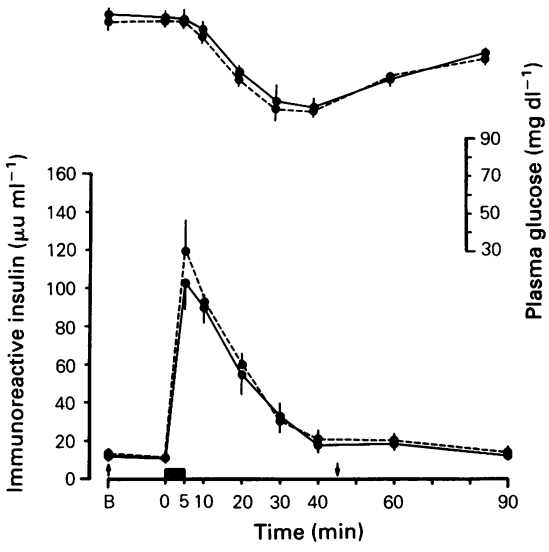

Figure 3 Plasma insulin and glucose after an intravenous tolbutamide load (1 g) during saline (continuous line) or bombesin (broken line) infusion in healthy volunteers. Each point refers to the mean of the values obtained from 6 subjects. Vertical bars are standard errors. ↑ = start of the infusion; ↓ = end of the infusion. B = basal and refers to the mean of two consecutive blood samples (−30 and −15 min) obtained before bombesin or saline infusion. The hatched horizontal bar represents the time taken to inject tolbutamide. *$P < 0.05$.

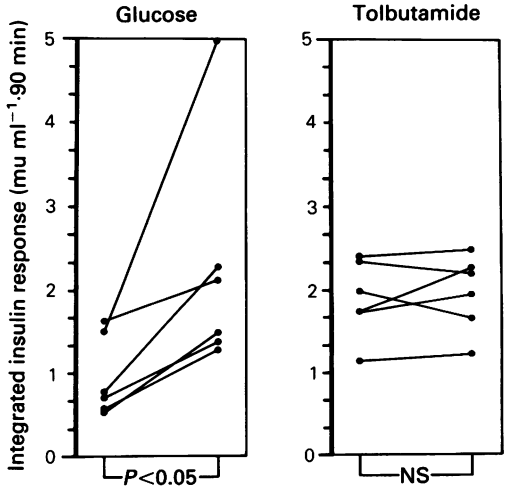

Figure 4 Integrated insulin responses during saline or bombesin infusion after glucose or tolbutamide stimulation in 2 groups of 6 male healthy volunteers. The lines join the integrated hormone responses observed with saline and bombesin for each subject.

dogs (Kaneto *et al.*, 1978; Vaysse *et al.*, 1981), cats (Vagne *et al.*, 1982) and man (Gathei *et al.*, 1982; Bruzzone *et al.*, 1983).

Recently a significant amount of GRP-like immunoreactivity was detected in porcine pancreatic tissue (Holst *et al.*, 1982). In addition, GRP was found to be present in the venous effluent of the isolated and perfused porcine pancreas, its output being considerably increased after electrical vagal stimulation (Holst *et al.*, 1982). In this preparation, exogenous GRP affects somatostatin and pancreatic polypeptide (PP) secretion as well as exocrine pancreatic secretion (Holst *et al.*, 1982). Therefore, it is quite reasonable to expect that GRP may also affect endocrine pancreatic function. In fact, this peptide was found to modify insulin release in rats (Greeley & Thompson, 1984), dogs (McDonald *et al.*, 1981) and man (Wood *et al.*, 1983; Knigge *et al.*, 1984).

In our experimental conditions, a 15 min bombesin infusion had no effect on basal levels of insulin and glucose, in agreement with previous reports (Scarpignato & Micali, 1986; Scarpignato *et al.*, 1987b).

Although no effect on basal insulin levels was observed after bombesin administration, a clear-cut enhancement of insulin release was evident when glucose was injected intravenously. These results are in line with the elegant work of Wood *et al.* (1983) in which GRP was found to potentiate glucose-dependent insulin secretion. On the contrary, when the hypoglycaemic sulphonylurea tolbutamide was used as a stimulus, no effect of bombesin was observed. These data could suggest that at normal or low blood glucose concentrations (when insulin levels after tolbutamide are still elevated) bombesin is unable to affect insulin release.

In both (glucose and tolbutamide) studies, plasma glucose levels were not significantly modified by the peptide. The lack of effect of bombesin may be the consequence of a simultaneous release of pancreatic and intestinal glucagon induced by the peptide (Ghatei *et al.*, 1982; Bruzzone *et al.*, 1983). It is worth mentioning that, when plasma levels of insulin are high, a further increase of hormone concentrations are not followed by a significant and proportional increase in glucose oxidation (Felber *et al.*, 1981). Furthermore, a diminished tissue sensitivity to insulin, induced by bombesin administration, cannot be excluded on the basis of our experiments.

In man, intravenous administration of bombesin releases many gastrointestinal peptides (Ghatei *et al.*, 1982), some of which (like gastrin, CCK, GIP and neurotensin) have an insulinotropic action. Although results from experiments on isolated and perfused dog (Ipp & Unger, 1979) and rat (Martindale *et al.*, 1982) pancreas suggest a direct stimulatory effect on insulin release, the participation of one or more peptides released by bombesin in its *in vivo* beta-cytotropic action cannot be excluded.

Results of the present investigation show that, at normal or low plasma glucose levels, bombesin is unable to modify insulin release and suggest that this peptide might be regarded as a glucose-dependent (Andersen *et al.*, 1978). Experiments, in which the glucose clamp technique is used, are now in progress to confirm this hypothesis and to establish the physiological significance of these observations.

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References

ANASTASI, A., ERSPAMER, V. & BUCCI, M. (1971). Isolation and structure of bombesin and alytesin, two analogous active peptides from the skin of the European amphibians bombina and alytes. *Experientia*, **27**, 166–167.

ANDERSEN, D.K., ELAHI, D., BROWN, J.C., TOBIN, J.D. & ANDRES, R. (1978). Oral glucose augmentation of insulin secretion. Interactions of gastric inhibitory polypeptide with ambient glucose and insulin levels. *J. Clin. Invest.*, **62**, 152–161.

BARLOW, R.B. (1983). *Biodata Handling with Microcomputers*. Cambridge: Elsevier Biosoft.

BROWN, M.R. & FISHER, D.A. (1980). Glucoregulation and the sympathetic nervous system: CNS control by brain peptides. In *Peptides – Integrators of Cells and Tissue Function*. ed. Bloom, F.E. pp. 81–97. New York: Raven Press.

BROWN, M., MARKI, W. & RIVIER, J. (1980). Is gastrin releasing peptide mammalian bombesin? *Life Sci.*, **27**, 125–129.

BROWN, M.R. & VALE, W. (1979). Bombesin: a putative mammalian neurogastrointestinal peptide. *Trends Neurosci.*, **2**, 95–97.

BRUZZONE, R., TAMBURRANO, G., LALA, A., MAUCERI, M., ANNIBALE, B., SEVERI, C., DE MAGISTRIS, L., LEONETTI, F. & DELLE FAVE G.F. (1983). Effect of bombesin on plasma insulin, pancreatic glucagon and gut glucagon in man. *J. Clin. Endocrinol. Metab.*, **56**, 643–647.

CONARD, V., FRANCKSON, J.R.M., BASTENIE, P.A., KESTENS, J. & KOVACS, L. (1953). Etude critique du triangle d'hyperglycémie intraveineux chez l'homme normal et détermination d'un 'coefficient d'assimilation glucidique'. *Arch. Int. Pharmacodyn. Thér.*, **93**, 277–292.

CORNALE, P., BONAZZI, M., MULTINU, C., ROMELLI, P., VANCHERI, L. & PENNISI, F. (1981). Column affinity chromatography for bound/free separation in ligand assays. I. Radio-immunoassay of choriomammotropin (human placental lactogen). *Clin. Chem.*, **27**, 896–900.

ELASHOFF, J.D. (1981). Down with multiple t tests! *Gastroenterology*, **80**, 615–620.

ERSPAMER, V. (1971). Biogenic amines and active polypeptides of the amphibian skin. *Ann. Rev. Pharmacol.*, **11**, 327–350.

ERSPAMER, V. & MELCHIORRI, P. (1980). Active polypeptides: from amphibian skin to gastrointestinal tract and brain of mammals. *Trends Pharmacol. Sci.*, **1**, 391–395.

FELBER, J.P., JACOT, E., THIEBAUD, D., MAEDER, E. & JEQUIER, E. (1981). The effect of different levels of insulinaemia on carbohydrate (CHO) metabolism. *Diabetologia*, **21**, 269–270.

FLETCHER, D.R., SHULKES, A., BLADIN, P.H.D. & HARDY, K.J. (1983). The effect of atropine on bombesin and gastrin releasing peptide stimulated gastrin, pancreatic polypeptide and neurotensin release in man. *Regul. Peptides*, **7**, 31–40.

GHATEI, M.A., GEIRGE, S.K., MAJOR, J.H., CARLEI, F., POLAK, J.M. & BLOOM, S.R. (1984). Bombesin-like immunoreactivity in the pancreas of man and other mammalian species. *Experientia*, **40**, 884–886.

GHATEI, M.A., JUNG, R.T., STEVENSON, J.C., HILLYARD, C.J., LEE, Y.C., CHRISTOFIDES, N.D., SARSON, D.L., MASHITER, K., MACINTYRE, I. & BLOOM, S.R. (1982). Bombesin: action on gut hormones and calcium in man. *J. Clin. Endocrinol. Metab.*, **54**, 980–985.

GREELEY, G.H. & THOMPSON, J.C. (1984). Insulinotropic and gastrin-releasing action of gastrin-releasing peptide (GRP). *Regul. Peptides*, **8**, 97–103.

HALES, C.N. & RANDLE, P.J. (1963). Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.*, **88**, 137–146.

HOLST, J.J., KNUHTSEN, S., FAHRENKRUG, J., JENSEN, S.L., SCHWARTZ, T.W., NIELSEN, O.V. & REHFELD, J.F. (1982). Regulatory peptides in the control of the pancreas. In *Systemic Role of Regulatory Peptides*. ed. Bloom, S.R., Polak, J.M. & Lindenlaub, E. pp. 171–190. Stuttgart: Schattauer Verlag.

HOLST, J.J., KNUHTSEN, S., JENSEN, S.L., NIELSEN, O.V. & SCHWARTZ, T.W. (1983). GRP-nerves in the control of pancreatic endocrine secretion. *Diabetologia*, **25**, 165–169.

IPP, E. & UNGER, R.H. (1979). Bombesin stimulates the release of insulin and glucagon, but not pancreatic somatostatin, from the isolated perfused dog pancreas. *Endocr. Res. Commun.*, **6**, 37–42.

JANZON, L., BERNTORP, K., HANSON, M., LINDELL, S-E. & TRELL, E. (1983). Glucose tolerance and smoking: a population study of oral and intravenous glucose tolerance tests in middle-aged man. *Diabetologia*, **25**, 86–88.

KANETO, A., KANEKO, T., NAKAYA, S., KAJINUMA, H. & KOSAKA, K. (1978). Effect of bombesin infused intrapancreatically on glucagon and insulin secretion. *Metabolism*, **27**, 549–553.

KNIGGE, U., HOLST, J.J., KNUHTSEN, S.T., PETERSEN, B., KRARUP, T., HOLST-PETERSEN, J. & CHRISTIANSEN, P.M. (1984). Gastrin-releasing peptide: pharmacokinetics and effects on gastro-entero-pancreatic hormones and gastric secretion in normal man. *J. Clin. Endrocrinol. Metab.*, **59**, 310–315.

KNUHTSEN, S., HOLST, J.J., KNIGGE, U., OLESEN, M. & NIELSEN, O.V. (1984). Radioimmunoassay, pharmacokinetics and neuronal release of gastrin-releasing peptide in anesthetized pigs. *Gastroenterology*, **87**, 372-378.

LAMBERT, J.R., HANSKY, J., SOVENY, C. & HUNT, P. (1984). Comparative effects of bombesin and porcine gastrin-releasing peptide in the dog. *Dig. Dis. Sci.*, **29**, 1036–1040.

MARIGO, S. (1967). The evaluation of the intravenous tolbutamide test by means of the coefficient 'D'. *Acta Diabetol. Latina*, **4** (Suppl. 1), 63–74.

MARSCHNER, I., HERNDL, R. & SCRIBA, P.C. (1980). Comparison of four different algorithms for the calculation of radioimmunoassay standard curves. *J. Clin. Chem. Clin. Biochem.*, **18**, 105–109.

MARTINDALE, R., LEVIN, S. & ALFIN-SLATER, R. (1982). Effects of caerulein and bombesin on insulin and glucagon secretion from the isolated, perfused rat pancreas. *Regul. Peptides*, **3**, 313–324.

McDONALD, T.J., GHATEI, M.A., BLOOM, S.R., TRACK, N.S., RADZIUK, J., DUPRÉ, J. & MUTT, V. (1981). A qualitative comparison of canine plasma gastroenteropancreatic hormone responses to bombesin and porcine gastrin-releasing peptide (GRP). *Regul. Peptides*, **2**, 293–304.

McDONALD, T.J., JORNVALL, H., NILSSON, G., VAGNE, M., GHATEI, M., BLOOM, S.R. & MUTT, V. (1979). Characterization of a gastrin releasing peptide from porcine non antral gastric tissue. *Biochem. Biophys. Res. Comm.*, **90**, 227–233.

SCARPIGNATO, C. & MICALI, B. (1986). Opposite effects of bombesin on insulin and gastrin response to food in humans. *Gut*, **27**, 499–504.

SCARPIGNATO, C., GIOFFRÉ, M., GULINO, F.M. & MICALI, B. (1987a). Bombesin: another Glucose-dependent Insulinotropic Peptide? *Abstracts of First Joint Meeting of Italian and Dutch Pharmacological Societies*, (Florence, May 10–13), p. 34.

SCARPIGNATO, C., GIOFFRÉ, M. & MICALI, B. (1987b). Effect of bombesin on glucose-induced insulin release in humans. *Digestion*, **37**, 114–124.

SNEDECOR, G.W. & COCHRAN, W.G. (1967). *Statistical Methods*, 6th ed. Ames, Iowa: Iowa State University Press.

TRINDER, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxigen acceptor. *Ann. Clin. Biochem.*, **6**, 24–27.

VAGNE, M., GELIN, M.L., McDONALD, T.J., CHAYVIALLE, J.A. & MINAIRE, Y. (1982). Effect of bombesin on gastric secretion and motility in the cat. *Digestion*, **24**, 5–13.

VAYSSE, N., PRADAYROL, L., CHAYVIALLE, J.A., PIGNAL, F., ESTEVE, J.P., SUSINI, C., DESCOS, F. & RIBET, A. (1981). Effects of somatostatin-14 and somatostatin-28 on bombesin-stimulated release of gastrin, insulin and glucagon in the dog. *Endocrinology*, **108**, 1843–1847.

WALSH, J.H., WONG, H.C. & DOCRAY, G.J. (1979). Bombesin-like peptides in mammals. *Fed. Proc.*, **38**, 2315–2319.

WOOD, S.M., JUNG, R.T., WEBSTER, J.D., GHATEI, M.A., ADRIAN, T.E., YANAIHARA, N. & BLOOM, S.R. (1983). The effect of the mammalian neuropeptide, gastrin releasing peptide (GRP), on gastrointestinal and pancreatic hormone secretion in man. *Clin. Sci.*, **65**, 365–371.

YANAIHARA, C., INOUE, A., MOCHIZUKI, T., OZAKI, J., SATO, H. & YANAIHARA, N. (1980). Bombesin-like immunoreactivity in mammalian tissues. *Biomed. Res.*, **1**, 96–100.

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