Bombesin promotes pancreatic growth in suckling rats

M. Papp, I. Dobronyi, G. Varga and C. Scarpignato*

*Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest (Hungary), and *Laboratory of Clinical Pharmacology, Institute of Pharmacology, University of Parma, Parma (Italy), 16 April 1986*

Summary. The pancreatic growth promoting effect of long term administration of bombesin was investigated in suckling rats. The authors showed that bombesin given in 10 μg/kg b.wt doses s.c. every 8 h for 10 days from the day of parturition stimulated pancreatic growth: it increased pancreatic weight, protein and DNA content, trypsin and amylase activity and trypsin/DNA ratio. Conclusion: Bombesin is an effective stimulator of pancreatic growth in suckling rats.
Key words. Bombesin; pancreatic growth; stimulation; suckling rats.

Bombesin is a tetradecapeptide of amphibian origin[1,2]. Bombesin-like immunoreactivity was shown in the mammalian gastrointestinal tract[3-5], respiratory tract[6], and the central nervous system[4] and bombesin-like peptides have been extracted from mammalian and avian tissues[7]. This peptide is an effective stimulator of some gastrointestinal functions. Among others it increases gastric acid output in dogs and rats [2,8,9], pancreatic juice volume and protein output in man[10], and in dogs in vivo[9,11,12]. In rats and other rodents, bombesin increases pancreatic amylase output in vitro[13-17], acting on single class receptors[14]; atropine did not alter this effect[15]. It has been reported that chronic administration of bombesin induces antral hypertrophy[18] and promotes pancreatic growth[19] in adult rats.

In the present study, we investigated the effect of bombesin treatment on pancreatic growth and tissue composition in suckling rats.

Material and methods. CFY female suckling rats from the third litter were selected and divided randomly. In each cage 7 suckling rats were nourished by one dam. They were kept in light controlled and air conditioned rooms. The day of parturition was called day 1. Two groups of suckling rats with about equal average body weight were used: all the rats in a given litter received saline (28 rats) or bombesin (28 rats). Saline or bombesin (UCB, Bioproducts Peptide Department, Belgium), 10 μg/kg b.wt, were injected s.c. every 8 h for 10 days. Three suckling rats from the bombesin treated group and two from the saline treated newborns died on the first or second day of treatment. The body weight of the suckling rats was checked every day in the evening. In the morning of day 11 the animals were decapitated and the pancreas was removed, weighed and analyzed.

The pancreas was homogenized in a buffer consisting of 100 mM Tris-HCl, 100 mM KCl and 20 mM $CaCl_2$, pH 8.0. Its protein-[20] and DNA[21,22] content, trypsin[23] and amylase[24] activity were determined. Trypsinogen was activated by enterokinase[25].

Mathematical-statistical analyses were performed by analysis of variance (ANOVA). Means ± SEM are demonstrated in the table.

Results. It is shown in Table 1 that bombesin treatment increased pancreatic weight, total pancreatic protein, DNA content, trypsin and amylase activity. Bombesin did not affect body weight. Related to g body weight the corresponding parameters showed similar trends. Related to mg pancreatic DNA a significant increase in total pancreatic trypsin activity was observed. It was 87.8 ± 5.2 U/mg DNA in the bombesin-treated group vs 62.4 ± 3.8 U/mg DNA in the saline-treated group; $p. < 0.01$. On the other hand, pancreatic protein content and amylase activity related to mg pancreatic DNA did not change after bombesin treatment (data not shown).

Discussion. The basic conclusion of the present study is that bombesin treatment stimulates pancreatic growth in 11-day-old suckling rats. Using an increase in pancreatic DNA content as an index of hyperplasia and increase in total pancreatic protein content, enzyme activity and trypsin/DNA ratio as indices of hypertrophy[26-28] it is suggested that the growth promoting effect of bombesin is due to both pancreatic hyperplasia and hypertrophy biochemically. Our results are in good agreement with the previous findings[19] that bombesin promotes pancreatic growth in adult rats.

The mean percentual increase in pancreatic proteolytic enzyme activity (185% of control) exceeded the increase in amylase activity (136% of control) induced by bombesin.

The number of acinar cells and cellular content of trypsin significantly increased while cellular protein and amylase did not change as a result of treatment with this peptide. This clearly shows that the effect of bombesin is independent of glucocorticoid release which preferentially increases pancreatic amylase activity in suckling rats[29].

The growth-promoting effect of bombesin is rather similar to the effect of caerulein under the same condition. Previously, we found that caerulein treatment lasting for 5, 10 or 20 days had a trophic effect on the pancreatic growth of suckling rats (Papp et al., unpublished results). A characteristic feature of this growth-promoting effect of caerulein was the increase in pancreatic tryptic activity. In the present study bombesin also enhanced pancreatic growth by increasing pancreatic tryptic enzyme activity. CCK receptors of acinar cells have a high affinity for caerulein[30] and bombesin also acts directly on pancreatic acinar cells binding to bombesin receptors[14-17]. Both CCK and bombesin receptors are present within the same acinar cell unit[17] and both CCK and bombesin act on the plasma membrane of acini the same way[16]. Besides the existence of bombesin receptors, bombesin-like immunoreactivity has been demonstrated in pancreatic nerves, particularly in the acinar parenchyma[5]. On the basis of these observations, it is possible that bombesin acts directly on the rat exocrine pancreas. We cannot exclude, however, the possibility that bombesin releases CCK from the duodenum and intestines in rats, likewise in dogs[11,12]. To clarify the detailed mechanism of bombesin action stimulating pancreatic growth in suckling rats we have to investigate more precisely the hormonal background of the trophic action of this peptide on the rat pancreas.

1 Anastasi, A., Erspamer, V., and Bucci, M., Experientia *27* (1971) 166.

Effect of bombesin treatment, 10 μg/kg s.c. every 8 h for 10 days, on pancreatic growth and composition in 11 day old suckling rats

Groups	Body weight (g)	Pancreatic weight (mg)	Total pancreatic Protein (mg)	DNA (μg)	Trypsin (U)	Amylase (U)
Saline (26)	27.5* ± 0.5	57.7 ± 1.7	8.85 ± 0.26	339 ± 19	19.5 ± 0.8	313 ± 16
Bombesin (25)	28.4 ± 0.6	64.8 ± 1.9	10.84 ± 0.48	439 ± 28	36.0 ± 1.8	427 ± 24
ANOVA p <	N.S.	0.01	0.01	0.01	0.01	0.01

* mean ± SEM; ( ), number of rats; N.S., not significant.

2 Erspamer, M., Peptides of amphibian skin active on the gut. II. Bombesin-like peptides: isolation, structure and basic functions, in: Gastrointestinal Hormones, p. 343. Ed. J.G.B. Glass. Raven Press, New York 1980.
3 Polak, J.M., Bloom, S.R., Hobbs, S., and Solcia, E., Lancet *1* (1976) 1109.
4 Walsh, J.H., and Dockray, G.J., Gastroenterology *74* (1978) 1108.
5 Ghatei, M.A., George, S.K., Major, J.H., Carlei, F., Polak, J.M., and Bloom, S.R., Experientia *40* (1984) 884.
6 Wharton, J., Polak, J.M., Bloom, S.R., Ghatei, M.A., Solcia, E., Brown, M.R., and Pearse, A.G.E., Nature *273* (1978) 769.
7 Melchiorri, P., Bombesin-like peptides activity in the gastrointestinal tract of mammals and birds, in: Gastrointestinal Hormones. p. 717. Ed. J.G.B. Glass. Raven Press, New York 1980.
8 Bertaccini, G., Erspamer, V., and Impiciatore, M., Br. J. Pharmac. *49* (1973) 437.
9 Fender, H.R., Curtis, P.J., Rayford, P.L., and Thompson, J.C., Surg. Forum *27* (1976) 414.
10 Basso, N., Giri, S., Improta, G., Lezoche, E., Melchiorri, P., Percoco, M., and Speranza, V., Gut *16* (1975) 994.
11 Erspamer, V., Importa, G., Melchiorri, P., and Sopranzi, N., Br. J. Pharmac. *52* (1974) 227.
12 Konturek, S.J., Krol, R., and Tasler, J., J. Physiol. *257* (1976) 663.
13 Deschodt-Lanckman, M., Robberecht, P., De Need, P., Lammens, M., and Christophe, J., J. clin. Invest. *58* (1976) 891.
14 Jensen, R.T., Moody, T., Rivier, J.E., and Gardner, J.D., Proc. natn. Acad. Sci. USA *75* (1978) 6139.
15 May, R.J., Conlon, T.P., Erspamer, V., and Gardner, J.D., Am. J. Physiol. *235* (1978) E 112.
16 Iwatsuki, N., and Petersen, O.H., J. clin. Invest *61* (1978) 41.
17 Philipott, H.G., and Petersen, O.H., Pflügers Arch. *382* (1979) 63.
18 Lehy, T., Accary, J.P., Labeille, D., and Dubrasquet, M., Gastroenterology *84* (1983) 914.
19 Solomon, T.E., Petersen, H., Elashoff, J., and Grossman, M.I., Effects of chemical messenger peptides on pancreatic growth in rats, in: Gut Peptides, p. 213. Ed. A. Miyoshi. Kondasho, Tokyo 1979.
20 Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
21 Burton, K., Biochem. J. *62* (1956) 315.
22 Munro, H.N., and Fleck, A., Analyst *91* (1966) 78.
23 Hummel, B.C., Can J. Biochem. Physiol. *37* (1959) 1393.
24 Bernfeld, P., Amylase alpha and beta, in: Methods of Enzymology, p. 149. Eds S.P. Colowic and N.O. Kaplan. Academic Press, New York 1955.
25 Solomon, T.E., Petersen, H., Elashoff, J., and Grossman, M.I., Am. J. Physiol. *235* (1978) E 714.
26 Morisset, J., Jolicœur, L., Genik, P., and Lord, A., Am. J. Physiol. 241 (1981) G 37.
27 Solomon, T.E., Regulation of exocrine pancreatic cell proliferation and enzyme synthesis, in: Physiology of the Gastrointestinal Tract, vol. 2, p. 857. Ed. L.R. Johnson. Raven Press, New York 1981.
28 Fölsch, U.R., Clinics Gastroent. *13* (1984) 679.
29 Morisset, J., and Jolicœur, L., Am. J. Physiol. *239* (1980) G 95.
30 Meyer, J.H., Control of pancreatic secretion (Receptors for CCK and structurally related peptides), in: Physiology of the Gastrointestinal Tract, Vol. 2, p. 857. Ed. L.R. Johnson. Raven Press, New York 1981.

0014-4754/87/020201-02$1.50 + 0.20/0

Serological and genetical studies on the evolution of substrate specificity of flavone glycosyltransferase genes in *Silene*

J. van Brederode, R. Kamps-Heinsbroek and J. Steyns

Department of Population and Evolutionary Biology, University of Utrecht, Padualaan 8, NL-3584 CH Utrecht (The Netherlands), 28 March 1986

Summary. The variation in flavone glycosylation patterns in *Silene* is the result of the expression of six genetic loci, which control either the presence of allozymes differing in substrate specificity or isozymes regulated differently during development. Serological studies showed that at least three of these six loci are evolutionarily related. The genetic mechanisms leading to these complicated variation patterns and the role of this polymorphism for the plant in its interaction with the environment are discussed.
Key words. Evolution; substrate specificity; serological homologies; flavone biosynthesis; *Silene;* glycosyltransferases.

The flavones present in *Silene pratensis* and *S. dioica,* the white and red campion respectively, belong to the class of the C-glycosylflavones. The main flavone skeleton found is isovitexin (fig. 1). The 7-OH of the A ring and the 2″-OH of the C–C bound glucose may be glycosylated[1].

Until now 11 dominant genes, spread over 6 loci, which control the glycosylation of isovitexin have been identified in *Silene*. The 2″-O-glycosylation is controlled by the loci gl, f and D6a; the 7-O-glycosylation by the loci g, 07g and Xgal. Three alleles have been identified both for the locus gl (gl, glA, glR) and f (f, fG, fX). The allozymes encoded by glA and glR transfer arabinose and rhamnose respectively[2,3], whereas those encoded by fG and fX transfer glucose and xylose[4]. Five alleles have been identified for the g locus (g, gX, gX′, gGm and gGd). The transfer of xylose to the 7-OH group of isovitexin or its 2″-O-glycosides is accomplished by the allozymes encoded by gX and gX′, which differ in maximal reaction velocity[5,6]. The allozyme encoded by gGm transfers glucose to isovitexin but not to isovitexin 2″-O-rhamnoside, whereas the enzyme encoded by gGd preferentially recognizes the 2″-O-rhamnoside and not isovitexin[7]. The loci gl, f and g segregate independently. Plants homozygous for the recessive alleles of these loci (genotype g/g, gl/gl, f/f) are unable to glycosylate isovitexin in the petals[8]. Yet, in the cotyledons and the rosette leaves of these plants glycosides of isovitexin are present[9]. This is due to the expression of the genes 07g and D6a, which control the 7-O-galactosylation and the 2″-O-arabinosylation respectively[10]. Biosynthesis of isovitexin 7-O-galactoside beyond the rosette leaf stage, controlled by Xgal, was found to be dependent on the presence of gene gX[11]. Hitherto no recessives or other alleles have been identified for the loci D6a, 07g and Xgal. The dominant flavone glycosylation genes identified until now in *Silene* plants have been summarized in figure 1.

So far, the study of the 7-O- and 2″-O- glycosylation of isovitexin has revealed the existence of allozymes differing in substrate specificity for the sugar donor as well as for the flavone acceptor, and the existence of differently regulated genes encoding the same product (glA/D6a and Xgal/07g). Variation in expression of isovitexin glycosylation genes is found not only within the plant, but also between geographical races and between the various *Silene* species within section Elisanthe[12]. The complicated pattern of variation in the expression of isovitexin glycosylation genes suggests an adaptive component in this variation, which can easily be tailored by the plant. This leaves us with the question of how this is accomplished by the plant, i.e. what are the evolutionary relationships between these various alleles and loci.