UC Davis

UC Davis Previously Published Works

Title

Effects of cholecystokinin and glucagon-like peptide 1 on the secretion of pancreatic polypeptide in mice.

Permalink

<https://escholarship.org/uc/item/40h2m84k>

Journal

Regulatory peptides, 59(3)

ISSN

0167-0115

Authors

Ahrén, B
Gingerich, RL
Havel, PJ

Publication Date

1995-11-01

DOI

10.1016/0167-0115(95)00081-I

Peer reviewed

Effects of cholecystokinin and glucagon-like peptide 1 on the secretion of pancreatic polypeptide in mice

Bo Åhrén^{a,*}, Ronald L. Gingerich^b, Peter J. Havel^c

^a Department of Medicine, Lund University, S-205 02 Malmö, Sweden

^b Washington University, School of Medicine and Linco Research, St. Louis, MO, USA

^c Departments of Nutrition and Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, University of California, Davis, CA, USA

Received 1 March 1995; revised 5 June 1995; accepted 6 June 1995

Abstract

The gut hormones, cholecystokinin (CCK) and truncated glucagon-like peptide 1 (GLP-1(7–36)amide or GLP-1) both stimulate insulin secretion and affect glucagon secretion in mice, but their effects on the secretion of other islet hormones have not been established in rodents. In the present study, we have examined the influence of the C-terminal octapeptide of CCK, CCK-8, and GLP-1 on the secretion of pancreatic polypeptide (PP) in the mouse by the use of a radioimmunoassay for rodent PP. Mice were injected intravenously with CCK-8 (doses in the range of 0.053–5.3 nmol/kg) or with GLP-1 (doses in the range of 1–32 nmol/kg) and blood was sampled at 2, 6 or 10 min after the injection. Controls were injected with saline. It was found that CCK-8 at 5.3 nmol/kg increases plasma levels of both PP and insulin when the sample was taken at 2 min, but not at 6 or 10 min, after injection. These effects were blocked by the selective CCK_A-receptor antagonist, L-364,718 (2.4 µmol/kg). GLP-1 increased plasma insulin levels at 32 nmol/kg at 2 and 6 min after the injection, but plasma PP levels were unaltered. In conclusion, this study, using a newly developed radioimmunoassay for PP in rodents, shows that CCK-8 but not GLP-1 stimulates PP secretion in mice at dose levels where both peptides stimulate insulin secretion. Furthermore, PP secretion in response to CCK-8 showed a similarity with that of insulin in terms of dose- and time-response characteristics as well as sensitivity to CCK_A-receptor antagonism.

Keywords: Cholecystokinin; Glucagon-like peptide 1; Pancreatic polypeptide secretion; Pancreas; Mouse

1. Introduction

Pancreatic polypeptide (PP) is synthesized in and secreted from endocrine cells located in the periphery of the pancreatic islets [1]. The physiological function of the peptide is poorly understood, although it has been demonstrated that under experimental conditions, the peptide inhibits the secretion of insulin in rats, dogs and mice [2–4] and inhibits

exocrine pancreatic secretion in humans and in dogs [5,6].

The secretion of PP is predominantly regulated by the parasympathetic nervous system [7,8]. Thus, PP secretion is stimulated both by electrical stimulation of the vagus and administration of cholinergic agonists [9,10]. Also glucopenic stress induced by hypoglycemia or administration of 2-deoxy-D-glucose (2-DG) stimulates PP secretion predominantly by cholinergic muscarinic receptor activation [7,8,11,12]. PP secretion is also increased during

* Corresponding author. Fax: +46 40 337041.

food intake [13–15]. The mechanism responsible for this increase is not known, but meal-stimulated PP secretion could be mediated by the pancreatic autonomic nerves or by the gastrointestinal hormones which are released during feeding analogous to the incretin factors that stimulate insulin secretion [16].

The peptidergic regulation of PP secretion has not been investigated in rodents. Studies undertaken in other species have shown that the C-terminal octapeptide of cholecystokinin (CCK-8) stimulates PP secretion in dogs [17] and humans [18–20], and that the amphibian peptide bombesin, which in mammals corresponds to gastrin releasing peptide (GRP), stimulates PP secretion when infused into humans [21].

In the present study, a newly developed radioimmunoassay for the determination of rodent PP [22] was used to examine the regulation of PP secretion in mice. Previously, we have presented evidence that in this species, PP secretion is increased during hypoglycemia or neuroglycopenia induced by 2-DG by nicotinic and muscarinic mechanisms [12]. Furthermore, in rats, combined muscarinic and adrenergic blockade do not abolish the PP response to hypoglycemia, which suggests involvement of neuropeptidergic mediation [11]. In the present study, we examined the influence of intravenous administration of the C-terminal octapeptide of CCK (CCK-8) and the truncated amidated form of glucagon-like peptide-1, i.e., GLP-1(7–36)amide or, as it will be called here, GLP-1, on plasma PP levels. It is known that both CCK and GLP-1 are released from the gut during meal intake [23,24], and GLP-1 is currently thought to be a major incretin factor [25]. In addition, there is evidence that CCK functions as an intrapancreatic neuropeptide [26]. We have previously shown that both these peptides stimulate insulin secretion in the mouse [27–29], and that CCK-8 stimulates insulin secretion by a CCK_A-receptor-mediated mechanism [30]. In contrast, the effects of CCK-8 and GLP-1 on PP secretion in rodents have not been investigated.

2. Materials and methods

2.1. Animals

Female mice of the NMRI strain (Bomholtgård Breeding and Research Centre Ltd, Ry, Denmark),

weighing 25–30 g, were used. The animals were fed a standard pellet diet (Astra-Ewos) and tap water ad libitum. All experiments were conducted on conscious animals. The study was approved by the Ethic Committee at Lund University.

2.2. Experiments

Synthetic CCK-8 (Sigma Chemical Co., St Louis, MO, USA) or synthetic human GLP-1 (Peninsula Laboratories Europe, St Helene, Merseyside, UK) was dissolved in saline with the addition of 1% gelatin. CCK-8 was injected intravenously at various dose levels in the range from 53 pmol/kg to 5.3 nmol/kg and GLP-1 was injected at 1, 8, or 32 nmol/kg. All injections were performed in a tail vein in the unanesthetized mice. In one series of experiments, the selective CCK_A-receptor antagonist, L-364,718 (3*S*(–)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-yl)-1*H*-indole-2-carboxamide; a kind gift of Merck Sharp and Dohme Research Laboratories, West Point, PA, USA) was injected intravenously at a dose of 2.4 μmol/kg together with CCK-8. L-364,718 has previously been demonstrated to be a selective CCK_A-receptor antagonist [31] which inhibits CCK-8-stimulated insulin secretion in mice at this dose [28,30]. L-364,718 was dissolved in methylcellulose (0.5%). Controls were injected with saline–gelatin except in the experimental series involving L-364,718, in which controls were injected with methylcellulose. The volume load was 10 μl/g. Blood was sampled from the retrobulbar venous plexus at 2, 6 or 10 min after the injection.

2.3. Assays

Plasma samples for PP determinations were lyophilized and shipped from Lund, Sweden, to St Louis, MO. The samples were stored at –20°C until they were reconstituted to the original volume with distilled water. PP concentrations were determined with a new radioimmunoassay using a guinea pig anti-rat antibody, ¹²⁵I-labeled rat as PP as tracer and rat PP as standard (Linco Research, St Louis, MO). The radioimmunoassay is able to detect rodent PP, but does not crossreact with neuropeptide Y or peptide YY [22]. The intra- and interassay variations of

this assay are 6.4 and 5.9%, respectively, and the detection limit is 19 pg/ml. Plasma levels of immunoreactive insulin were determined by radioimmunoassay using a guinea pig anti-rat insulin antibody (Linco Res), ^{125} I-labeled porcine insulin (Novo Nordic, Bagsvaerd, Denmark) as tracer and rat insulin (Linco Res) as standard. The bound antigen-antibody complex was precipitated by the use of an anti-IgG (goat anti-guinea pig) antibody. In this assay, the detection limit is 18 pmol/l, and the intra-

Fig. 1. Plasma levels of insulin, PP and glucose in unanesthetized mice at 2 min after intravenous injection of saline or CCK-8 at various dose levels. Means $\pm$ S.E.M. are shown. There were 8 animals in each group. Asterisks indicate the probability level of random difference versus the saline-injected control group: *** P < 0.001; * P < 0.05.

Fig. 2. Plasma levels of insulin and PP in unanesthetized mice at 2 min after an intravenous injection of saline or CCK-8 at 5.3 nmol/kg alone or together with the CCK_A-receptor antagonist, L-364,718 at 2.4 μ mol/kg. Means $\pm$ S.E.M. are shown. There were 8–10 animals in each group. Asterisks indicate the probability level of random difference versus the control group: *** P < 0.001; * P < 0.05.

and interassay variations are 6.6 and 7.2%, respectively. Plasma glucose levels were determined by the glucose oxidase method [32].

2.4. Statistics

Means $\pm$ S.E.M. are shown. Student's *t*-test with Bonferroni correction for multiple comparisons was used for the statistical comparisons.

3. Results

3.1. Effects of CCK-8 on plasma PP and insulin levels in mice

In the first experimental series, CCK-8 was injected intravenously at increasing doses and plasma PP and insulin levels were determined in samples

taken at 2 min after the injection. It was found that at 5.3 nmol/kg, CCK-8 increased the plasma levels of both PP (116 ± 12 vs. 78 ± 11 pg/ml in saline-injected controls; $P < 0.05$) and insulin (231 ± 24 vs. 77 ± 18 pmol/l in controls; $P < 0.001$). In contrast, at lower doses, CCK-8 was without effect, and CCK-8 did not affect plasma glucose levels during the 2-min study period (Fig. 1). In the second series of experiments, CCK-8 was injected at 5.3 nmol/kg and samples were taken at 2, 6 or 10 min after the

injection. It was found that, again, plasma PP and insulin levels were increased in CCK-8-injected animals versus in saline-injected controls in samples taken at 2 min after the injection ($P < 0.05$ and $P < 0.001$, respectively). However, at 6 or 10 min after the injection, there were no significant differences in PP or insulin levels in CCK-8-injected animals versus controls (data not shown). Finally, in the third experimental series, CCK-8 was injected at 5.3 nmol/kg alone or together with the selective CCK_A-receptor antagonist, L-364,718 (2.4 μ mol/kg), and a sample was taken after 2 min. It was found that the CCK_A-receptor antagonist inhibited the increase in plasma levels of both PP ($P < 0.05$) and insulin ($P < 0.001$) induced by CCK-8 (Fig. 2).

3.2. Effects of GLP-1 on plasma PP and insulin levels in mice

The intravenous injection of GLP-1 at 1 or 8 nmol/kg did not affect plasma levels of insulin or glucose (data not shown). In contrast, at 32 nmol/kg, GLP-1 increased plasma insulin levels ($P < 0.01$), but did not affect plasma PP levels in samples taken at 2 and 6 min after the injection (Fig. 3). When the samples were taken at 10 min after the injection of GLP-1 at 32 nmol/kg, plasma levels of PP and insulin were not significantly different from those in saline-injected controls. However, plasma glucose levels were significantly reduced at 10 min after injection of GLP-1 ($P < 0.001$; Fig. 3).

4. Discussion

We have previously demonstrated that CCK-8 markedly increases plasma insulin levels in mice when injected intravenously at doses of 5.3 nmol/kg and higher [27,28,30]. We confirm in this report that CCK-8 at 5.3 nmol/kg potently stimulates insulin secretion. CCK-8 was without effect at 1.5 nmol/kg, which is a threshold dose for stimulating insulin secretion [27]. We also confirm our previous results that the peak level in plasma insulin after an intravenous injection of CCK-8 in mice occurs at 2 min and that the CCK-8-induced insulin secretion is markedly suppressed by the CCK_A-receptor antago-

Fig. 3. Plasma levels of insulin, PP and glucose in unanesthetized mice at 2, 6 or 10 min after an intravenous injection of saline or GLP-1 at 32 nmol/kg. Means $\pm$ S.E.M. are shown. There were 8–10 animals in each group. Asterisks indicate the probability level of random difference versus the saline-injected control group: *** $P < 0.001$; ** $P < 0.01$.

nist, L-364,718 [27,28,30,33]. In this study, we demonstrate that CCK-8 increases plasma levels of PP in mice, as previously has been demonstrated in dogs *in vitro* [17] and *in vivo* [33], and in humans [18–20]. We also found that the PP response to CCK-8 shows similarities to the CCK-8-induced increase in plasma insulin. Thus, also for PP secretion, CCK-8 was stimulatory at 5.3 nmol/kg, the time pattern showed a peak level after 2 min and CCK_A-receptor antagonism abolished the PP response.

In contrast, we found that GLP-1 stimulated insulin secretion without affecting PP secretion in mice. We and others have demonstrated that GLP-1 stimulates insulin secretion and inhibits glucagon secretion [25,29,34–37]. In contrast, the issue whether the peptide affects the secretion of the other islet hormones, PP and somatostatin, has not been previously investigated. In the present study, plasma PP levels were not altered by the intravenous injection of GLP-1 at a dose that stimulates insulin secretion. As we previously observed, GLP-1 stimulated insulin secretion with a peak level of plasma insulin seen after two min [29]. GLP-1 also reduced plasma glucose levels at 10 min after the intravenous injection of the peptide. This hypoglycemic action might be mediated by the fast insulinotropic action, a glucagonostatic action or a peripheral action of GLP-1, since it is known that the peptide stimulates insulin secretion, inhibits glucagon secretion, inhibits the hepatic glucose production as well as stimulates the peripheral uptake of glucose [38,39].

In conclusion, CCK-8, but not GLP-1, stimulates PP secretion in mice at doses where both peptides stimulate insulin secretion. Furthermore, PP secretion in response to CCK-8 showed similarity to that of insulin in terms of dose- and time-response characteristics as well as sensitivity to CCK_A-receptor antagonism. Additional studies are now required to further characterize the *in vivo* PP secretion in relation to that of other islet hormones. The newly developed radioimmunoassay for PP in rodents makes such comparisons also possible in small laboratory animals.

Acknowledgements

The technical assistance of Lilian Bengtsson and Lena Kvist is gratefully acknowledged. The study

was supported by the Swedish Medical Research Council (14X-6834 and 12X-712), Albert Pålsson, Ernhold Lundström, Crafoord and Novo Nordic Foundations, The Swedish Diabetes Association, and by the Faculty of Medicine, Lund University.

References

- [1] Larsson, L.I., Sundler, F. and Håkanson, R., Pancreatic polypeptide – a postulated new hormone. Identification of its cellular storage site by light and electron microscopic immunocytochemistry, *Diabetologia*, 12 (1976) 211–226.
- [2] Lundquist, I., Sundler, F., Åhrén, B., Alumets, J. and Håkanson, R., Somatostatin, pancreatic polypeptide, substance P, and neurotensin: cellular distribution and effects on stimulated insulin secretion in the mouse, *Endocrinology*, 104 (1979) 832–838.
- [3] Williams, R.H. and Champagne, J., Effects of cholecystokinin, secretin and pancreatic polypeptide on the secretion of gastric inhibitory polypeptide, insulin and glucagon, *Life Sci.*, 25 (1979) 947–956.
- [4] Murphy, W.A., Fries, J.L., Meyers, C.A. and Coy, D.H., Human pancreatic polypeptide inhibits insulin release in the rat, *Biochem. Biophys. Res. Commun.*, 101 (1981) 189–193.
- [5] Greenberg, G.R., McLoy, R.F., Chadwicks, V.S., Adrian, T.E., Baron, J.H. and Bloom, S.R., Effect of bovine pancreatic polypeptide on basal pancreatic and biliary outputs in man, *Dig. Dis. Sci.*, 24 (1979) 11–14.
- [6] Taylor, I.L., Solomon, T.E., Walsh, J.H. and Grossman, M.I., Pancreatic polypeptide: metabolism and effect on pancreatic secretion in dogs, *Gastroenterology*, 76 (1979) 524–528.
- [7] Schwartz, T.W., Pancreatic polypeptide: a hormone under vagal control, *Gastroenterology*, 85 (1983) 1411–1425.
- [8] Havel, P.J. and Taborsky, G.J., Jr., The contribution of autonomic nervous system to changes of glucagon and insulin secretion during hypoglycemic stress, *Endocrinol. Rev.*, 10 (1989) 322–350.
- [9] Åhrén, B., Paquette, T.L. and Taborsky, G.J., Jr., Effect and mechanism of vagal nerve stimulation on somatostatin secretion in dogs, *Am. J. Physiol.*, 250 (1986) E212–E217.
- [10] Taborsky, G.J., Jr., Paquette, T.L., Pfeifer, M.A. and Gingerich, R.L., Pentobarbital suppresses basal and reflexive pancreatic polypeptide release in dogs, *Am. J. Physiol.*, 249 (1985) E577–E583.
- [11] Havel, P.J., Parry, S.J., Curry, D.L., Stern, J.S., Akpan, J.O. and Gingerich, R.L., Autonomic nervous system mediation of the pancreatic polypeptide response to insulin-induced hypoglycemia in conscious rats, *Endocrinology*, 130 (1992) 2225–2229.
- [12] Havel, P.J., Akpan, J.O., Curry, D.L., Stern, J.S., Gingerich, R.L. and Åhrén, B., Autonomic control of pancreatic polypeptide and glucagon secretion during neuroglycopenia and hypoglycemia in mice, *Am. J. Physiol.*, 265 (1993) R246–R254.

- [13] Floyd, J.C., Jr., Fajans, S.S., Pek, S. and Chance, R.E., A newly recognized pancreatic polypeptide: plasma levels in health and disease, *Rec. Progr. Horm. Res.*, 33 (1977) 519–570.
- [14] Villanueva, M.L., Hedó, J.A. and Marco, J., Fluctuations of human pancreatic polypeptide in plasma: effect of normal food ingestion and fasting, *Proc. Soc. Exp. Biol. Med.*, 159 (1978) 245–248.
- [15] Jorde, R. and Burhol, P.G., Fasting and diurnal plasma PP, and the effect of fasting and somatostatin on postprandial plasma PP, *Acta Physiol. Scand.*, 115 (1982) 421–425.
- [16] Dupré, J., Influences of the gut on the endocrine pancreas. An overview of established and potential physiological mechanisms. In: Samols (Ed.), *The Endocrine Pancreas*, Raven Press, New York, 1991, pp. 253–281.
- [17] Adrian, T.E., Bloom, S.R., Hermansen, K. and Iversen, J., Pancreatic polypeptide, glucagon and insulin secretion from the isolated perfused canine pancreas, *Diabetologia*, 14 (1978) 413–417.
- [18] Schmid, R., Schusdziarra, V., Schulte-Frohline, E., Mahler, V. and Classen, M., Effect of CCK on insulin, glucagon and pancreatic polypeptide level in humans, *Pancreas*, 4 (1989) 653–661.
- [19] Lonovics, J., Guzman, S., Devitt, P., Hejtmancik, K.E., Suddith, R.L., Rayford, P.L. and Thompson, J.C., Release of pancreatic polypeptide in humans by infusion of cholecystokinin, *Gastroenterology*, 79 (1980) 817–822.
- [20] Åhrén, B., Pettersson, M., Uvnäs-Moberg, K., Gutniak, M. and Efendic, S., Effects of cholecystokinin (CCK)-8, CCK-33, and gastric inhibitory polypeptide (GIP) on basal and meal-stimulated pancreatic hormone secretion in man, *Diabet. Res. Clin. Pract.*, 13 (1991) 153–162.
- [21] De Magistris, L., Delle Fave, G., Kohn, A. and Schwartz, T.W., Differential stimulation of pancreatic-polypeptide and gastrin secretion by bombesin in man, *Life Sci.*, 28 (1979) 2617–2621.
- [22] Akpan, J.O., Havel, P.J., Parry, S.J., Shalwitz, R.A. and Gingerich, R.L., The characterization of a radioimmunoassay for rat pancreatic polypeptide in serum, *Regul. Pept.*, 37 (1992) 59–69.
- [23] Liddle, R.A., Goldfine, I.D., Rosen, M.S., Taplitz, R.A. and Williams, J.A., Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction, *J. Clin. Invest.*, 75 (1985) 1144–1152.
- [24] Kreyman, B., Williams, G., Ghatei, M.A. and Bloom, S.R., Glucagon-like peptide 1 7–36: a physiological incretin in man, *Lancet*, 2 (1987) 1300–1304.
- [25] Göke, R., Fehmann, H.C. and Göke, B., Glucagon-like peptide-1 (7–36)amide is a new incretin/enterogastrone candidate, *Eur. J. Clin. Inv.*, 21 (1991) 135–144.
- [26] Rehfeld, J.F., Larsson, L.I., Goltermann, N.R., Schwartz, T.W., Holst, J.J., Jensen, S.L. and Morley, J.S., Neural regulation of pancreatic hormone secretion by the C-terminal tetrapeptide of CCK, *Nature*, 284 (1980) 33–38.
- [27] Åhrén, B. and Lundquist, I., Effects of two cholecystokinin variants, CCK-39 and CCK-8, on basal and stimulated insulin secretion, *Acta Diabetol.*, 18 (1981) 345–356.
- [28] Karlsson, S. and Åhrén, B., Effects of three different cholecystokinin receptor antagonists on basal and stimulated insulin and glucagon secretion in mice, *Acta Physiol. Scand.*, 135 (1989) 271–278.
- [29] Fridolf, T., Böttcher, G., Sundler, F. and Åhrén, B., GLP-1 and GLP-1(7–36)amide: influences on basal and stimulated insulin and glucagon secretion in the mouse, *Pancreas*, 6 (1991) 208–215.
- [30] Karlsson, S. and Åhrén, B., CCK-8-stimulated insulin secretion in vivo is mediated by CCK_A receptors, *Eur. J. Pharmacol.*, 213 (1992) 145–146.
- [31] Chang, R.S.L. and Lotti, V.J., Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist, *Proc. Natl. Acad. Sci. USA*, 83 (1986) 4923–4926.
- [32] Bruss, M.L. and Black, A.L., Enzymatic microdetermination of glycogen, *Analyt. Biochem.*, 84 (1978) 309–312.
- [33] Dunning, B.E., Havel, P.J. and Taborsky, G.J., Jr., The CCK antagonist, L-364,718, blocks the endocrine pancreatic response to exogenous CCK but not that to vagal nerve stimulation in dogs. *Proc. 71st Annual Meeting Endocrine Society*, abstract 1360, (1989).
- [34] Holst, J.J., Ørskov, C., Nielsen, O.V. and Schwartz, T.W., Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut, *FEBS Lett.*, 211 (1987) 169–174.
- [35] Ørskov, C., Glucagon-like peptide-I, a new hormone of the entero-insular axis, *Diabetologia*, 35 (1992) 701–711.
- [36] Gutniak, M., Ørskov, C., Holst, J.J., Åhrén, B. and Efendic, S., Antidiabetogenic effect of glucagon-like peptide-1(7–36)amide in normal subjects and patients with diabetes mellitus, *New Engl. J. Med.*, 326 (1992) 1316–1322.
- [37] Åhrén, B., Insulinotropic action of truncated glucagon-like peptide-1 in mice, *Acta Physiol. Scand.*, 153 (1995) 205–206.
- [38] D'Alessio, D.A., Kahn, S.E., Leusner, C.R. and Ensink, J.W., Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal, *J. Clin. Invest.*, 93 (1994) 2263–2266.
- [39] Hvidberg, A., Nielsen, M.T., Hilsted, J., Ørskov, C. and Holst, J.J., Effect of glucagon-like peptide-1 (proglucagon 78–107amide) on hepatic glucose production in healthy man, *Metabolism*, 43 (1994) 104–108.