Diabetologia 14, 413–417 (1978)

Diabetologia

Pancreatic Polypeptide, Glucagon and Insulin Secretion from the Isolated Perfused Canine Pancreas

T. E. Adrian, S. R. Bloom, K. Hermansen[1] and J. Iversen[1, 2]

Department of Medicine, Royal Postgraduate Medical School, London, England and [1]Second University Clinic of Internal Medicine, Kommunehospitalet, Aarhus, Denmark

Summary. The release of pancreatic polypeptide (PP) by gut hormones, acetyl choline and adrenaline was investigated in an isolated perfused pancreas preparation. PP was potently released by 1 nmol/l caerulein (186 ± 12%, $p < 0.001$) and gastric inhibitory peptide (GIP) (211 ± 31%, $p < 0.005$) as well as by 1 µmol/l acetyl choline (1097 ± 59%, $p < 0.001$). A significant two-fold release of PP was also evoked by 1 nmol/l vasoactive intestinal peptide (VIP) (129 ± 38%, $p < 0.02$ and gastrin (108 ± 25% $p < 0.01$). Insulin release, induced by high glucose concentration was enhanced by both GIP (210 ± 38%, $p < (0.01)$ and VIP (48 ± 5%, $p < 0.001$). In addition GIP enhanced the release of glucagon by 179 ± 18% ($p < 0.001$) at 1.4 mmol/l glucose and by 127 ± 24% ($p < 0.005$) at 8.3 mmol/l glucose. Thus no simple inter-relationship appears to exist between the control of the three circulating islet hormones.

Key words: Isolated perfused canine pancreas, VIP, GIP, caerulein, gastrin, secretin, glucagon, bombesin, acetyl choline, adrenaline, release of insulin, release of glucagon, release of PP.

It is now clear that the pancreatic islets form a complex endocrine unit. They contain three types of cell, A, B and PP cell, which undoubtedly secrete their product into the circulation and other less numerous cell types producing, for example, somatostatin which are thought to act mainly locally. In addition there is a complex innervation which includes the three neural elements, sympathetic, parasympathetic and peptidergic (eg VIPergic). The release of the individual hormones, insulin and glucagon, from the islets has been extensively studied but there has been little attempt so far to investigate the degree of integration of control, in particular between the three pancreatic hormones whose main actions appear to be via the circulation.

Gastrointestinal hormones have been shown to be potent releasers of pancreatic polypeptide (PP) in man and probably play a major role in the rise of this hormone after a meal [1, 2]. The aim of this study was to establish whether the observed effect of gut hormones on the PP cell was direct or indirect, and also to investigate the relative potencies of the various gut hormones in the release of PP.

We have used an isolated perfused canine pancreas preparation which has previously been useful in the investigation of the direct release of glucagon and insulin by some gastrointestinal hormones [3] and also other substances [4, 5]. Thus, in addition to new information on PP release, the direct effects of the new gut hormones, gastric inhibitory peptide (GIP), VIP, bombesin and PP on the release of insulin and glucagon has been compared with that of gastrin, secretin and cholecystokinin, which have already been reported [3].

Methods

The pancreas donors were overnight fasted male mongrel dogs weighing 18–25 kg. The operative procedure for isolation of the pancreas and the perfusion system have been described in detail elsewhere [6]. The coeliac artery, the splenic artery and the portal vein were catheterised and the pancreas perfused, without recirculation, with an oxygenated synthetic medium which consisted of Krebs Ringer buffer pH 7.4 adjusted to the electrolyte concentrations of dog plasma with 40 g/l dextran, bovine serum albumin at

[2] Present Address: Department of Medicine, State University Hospital, Rigshospitalet, DK-2100 Aarhus, Denmark

0012-186X/78/0014/0413/$01.00

Table 1. The mean basal and peak PP concentrations in pmol/l in the efflux perfusate of the isolated dog pancreas during perfusion with hormones and neurotransmitters

Perfused substance	Number of preparations	Mean PP concentration pmol/l		
		Zero	Peak	P
Caerulein	5	22.8±3.9	64.4± 2.7	<0.001
Gastrin	6	17.7±4.4	35.5± 4.3	<0.05
Glucagon	4	18.3±2.1	18.3± 1.3	NS
Secretin	5	16.2±2.2	23.0± 4.0	NS
GIP	6	13.3±4.1	43.5± 4.4	<0.005
VIP	6	20.5±4.1	44.8± 7.4	<0.05
Bombesin	2	10.0	12.0	
Acetyl choline	6	18.5±4.6	223 ±11	<0.001
Adrenaline	6	20.8±4.4	27.7± 5.2	NS

Table 2. Mean efflux insulin and glucagon concentration both at low and high glucose concentrations in the isolated perfused dog pancreas

Glucose mmol/l	Insulin pmol/l	Glucagon pmol/l
1.4	38.2± 11.5	154±35
8.3	4846 ±907	52±12

Table 3. Percentage change in glucagon and insulin secretion from the isolated perfused dog pancreas. As no increase in insulin secretion occurred at low glucose concentrations with any stimulus this column has been omitted. (NT = not tested)

	Percentage change		
	Glucagon	Insulin	Glucagon
Perfusate	Low glucose (1.4 mmol/l)	High glucose (8.3 mmol/l)	High glucose (8.3 mmol/l)
Caerulein	662±100 p<0.01 (n=4)	154±14 p<0.005 (n=4)	115±21 p<0.02 (n=4)
Gastrin	193±28 p<0.005 (n=5)	49±6 p<0.005 (n=5)	145±15 p<0.001 (n=5)
Secretin	−59±5 p<0.001 (n=4)	229±72 p<0.05 (n=4)	−62±3 p<0.001 (n=4)
GIP	179±18 p<0.001 (n=4)	210±38 p<0.01 (n=5)	127±24 p<0.005 (n=5)
VIP	90±43 NS (n=4)	48±5 p<0.001 (n=5)	6±10 NS (n=5)
Acetyl choline	NT	130±20 p<0.01 (n=4)	431±19 p<0.001 (n=4)

0.35 mmol/l and glutamate, fumarate and pyruvate, each at a concentration of 0.5 mmol/l. The perfusion flow rate was 18–20 ml/min (approximately 0.25 ml/min/g of pancreas wet weight) and the perfusion pressure was 30–40 mmHg. Both parameters were constant during the perfusion experiment. The oxygen uptake of the isolated pancreas was 0.005 ml/min/g [5]. Each perfusion experiment was carried out with hourly alternations with glucose concentrations of 1.4 and 8.3 mmol/l respectively.

The pure peptide hormones, human gastrin (synthetic human gastrin I, ICI Ltd), caerulein – an analogue of cholecystokinin (synthetic "Caeruletide", Farmitalia Ltd), porcine pancreatic glucagon, (Novo Ltd), bovine pancreatic polypeptide (BPP) (Eli Lilly Ltd) porcine GIP (Prof. J. Brown, Vancouver University), porcine VIP (Prof. S. I. Said, Dallas University) and bombesin (synthetic, Farmitalia Ltd) were added to the perfusate in random order at concentration of 1 nmol/l. Acetyl choline was perfused at a concentration of 1 μmol/l and adrenaline at 11 nmol/l. When investigating the effect of inhibitors on the release of the pancreatic hormones synthetic cyclic ovine somatostatin was added to the perfusate at 60 nmol/l and atropine at 25 μmol/l. The pancreas preparations were perfused with each substance for ten minutes with a recovery period of at least 20 min before the next test substance. Samples were taken every minute from the influx and the efflux perfusate. Even though there is no evidence that proteolytic enzymes escape into the venous drainage from the perfused pancreas [4] a proteolytic enzyme inhibitor, aprotinin (Trasylol, Bayer Ltd) was added to the tubes in which the efflux samples were collected to give a final concentration of 400 KI units/ml. PP, glucagon and insulin were all measured in the perfusate by specific and sensitive radioimmunoassays using antisera raised to the pure peptides [3, 7, 8, 9]. The assays were capable of detecting changes of PP concentration of 5 pmol/l, glucagon 3 pmol/l and insulin 12 pmol/l with 95% confidence. For each system the intra-assay and interassay variation were less than 10% and 20% respectively.

Results

The release of PP by the gut peptide hormones can be seen in Figure 1 and the mean zero and peak of each stimulus is summarised in Table 1. The basal and stimulated PP concentrations were not altered by the different glucose concentrations used; for example at 8.3 mmol/l glucose concentration basal PP was 23.0 ± 5.8 pmol/l, and the peak after caerulein 66.0 ± 2.3, while at 1.4 mmol/l glucose concentration basal PP was 22.5 ± 5.5 and caerulein peak 62.0 ± 4.0 (both measured in the same 4 preparations). Results for PP stimulation therefore represent the concentrations after the first application of the stimulus in each pancreas preparation regardless of glucose concentration. Caerulein and GIP caused a highly significant release of PP from all preparations and the release elicited by gastrin or VIP was also significant. Secretin and glucagon, however, did not release PP at the dose used in this in vitro preparation. Acetyl choline is extremely potent in its capacity to release PP (Fig. 2); the data shown represent experiments carried out with a dose of 1 μmol/l acetyl choline. However, in a single experiment, a dose of 10 pmol/l gave a response which was 50% of

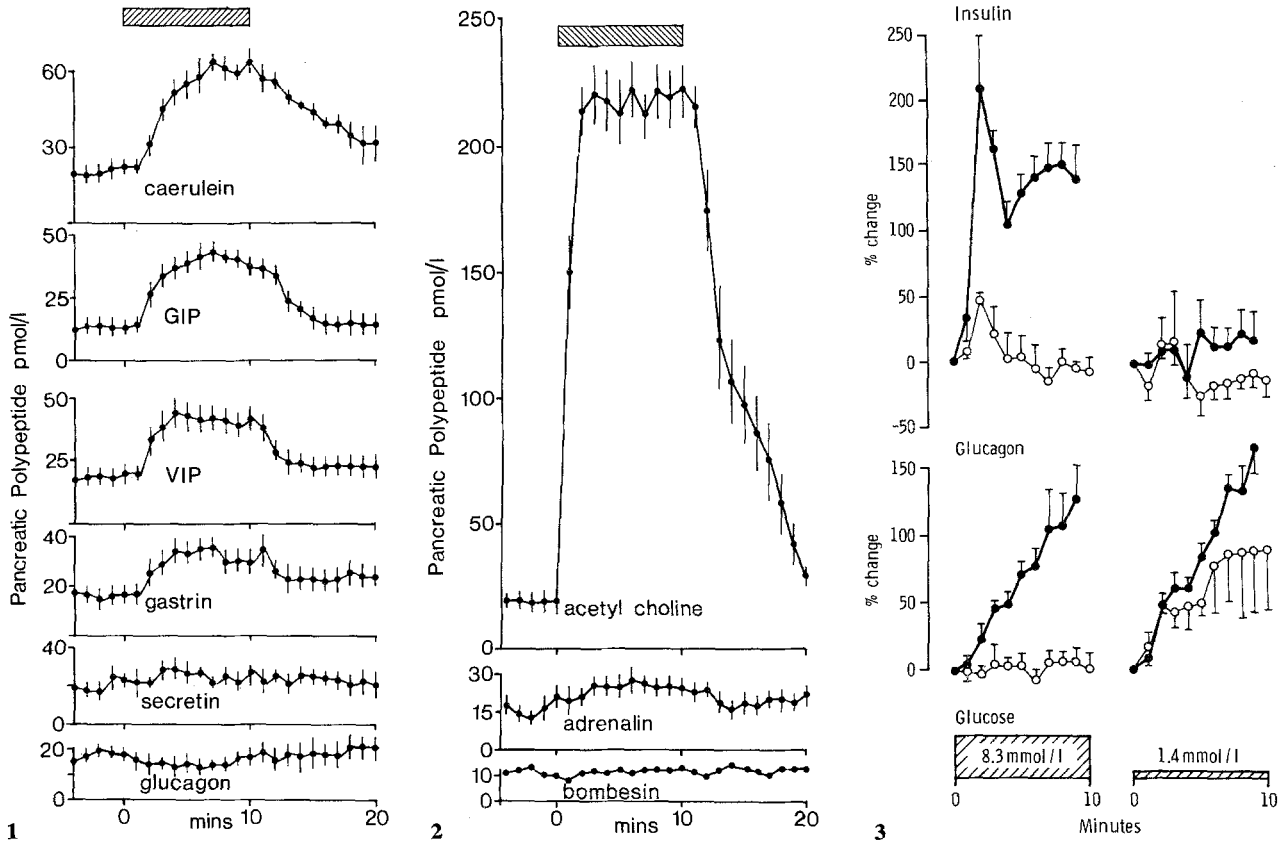

Fig. 1. The effect of 10 min perfusion of gut peptide hormones (1 nmol/l) on efflux pancreatic polypeptide concentrations from the isolated perfused canine pancreas. The number of experiments for each hormone is given in Table 1

Fig. 2. The effect of 10 min perfusion of acetyl choline (1 μmol/l), adrenaline (11 nmol/l) and bombesin (1 nmol/l) on efflux pancreatic polypeptide concentrations from the isolated perfused canine pancreas. The number of experiments for each stimulus is given in Table 1

Fig. 3. The effect of GIP and VIP on the secretion of insulin and glucagon during perfusion with a glucose concentration of 1.4 and 8.3 mmol/l. The effect is depicted as change in percent from zero. GIP ●—●, VIP ○—○. Glucose concentration 1.4 nmol/l n = 4. Glucose concentration 8.3 nmol/l n = 5

maximum. No significant rise in PP concentration occurred when adrenaline was perfused (Fig. 2). Bombesin was perfused in only two preparations and no effect on PP was observed (Fig. 2). Atropine completely abolished the release of PP elicited by acetyl choline by (% inhibition 97.3 ± 1.7, n = 4), but had no effect on caerulein-induced PP release in two preparations. Somatostatin inhibited the release of PP evoked by acetyl choline by 61.3 ± 9.8% (n = 4) and completely blocked the release of PP induced by caerulein in two preparations.

Throughout the experiments changes in the infusate glucose concentration induced the expected changes [3] in insulin and glucagon, as seen in Table 2. The high glucose concentration stimulated insulin secretion whilst suppressing that of glucagon. The low glucose concentration had the opposite effects. The effect of acetyl choline, gastrin, secretin, caerulein, GIP and VIP is summarised in Table 3. Acetyl choline is an extremely potent stimulator of insulin and glucagon [10] as well as PP secretion in this vitro preparation. It has previously been reported [3] that insulin secretion is enhanced by gastrin, secretin and cholecystokinin. In this study similar effects were noted at high glucose concentrations, but not at low, with gastrin, secretin and caerulein. Similarly glucagon was released, as expected, by gastrin and caerulein, the effects being greater at low glucose concentration than at high.

The effect of GIP and VIP on insulin and glucagon secretion is summarised in Figure 3. GIP releases insulin at high glucose concentration. It also produced a release of glucagon, but this effect was more significant at low glucose concentration. VIP has a stimulatory effect on glucagon at low glucose concentration and slightly enhanced insulin release at

high glucose concentrations. Bombesin and BPP were each perfused in only two preparations and in these no effect on either insulin or glucagon release was seen.

Discussion

It is now well established that PP circulates in plasma and there is a rapid and substantial rise after a meal [2, 9, 11]. As PP is found almost exclusively in the pancreas, it has been proposed that an entero-PP axis [1], analogous to the well-established entero-insular axis, is responsible for release of this hormone following intestinal stimulation by food. The entero-PP axis appears to be mediated partly through the vagal innervation and partly through circulating gut hormones. Circulating nutrients appear to have little effect and this is confirmed in this study by the absence of any glucose effect on PP release from the isolated pancreas. The experiments reported here have demonstrated at the tissue level that circulating hormones and acetyl choline can release PP directly and thus are possible physiological components of the entero-PP axis. The release by acetyl choline is in agreement with preliminary findings reported by other workers [12], as is the minor effect of catecholamines on PP release [13]. As expected, the PP release by acetyl choline is totally blocked by atropine. Somatostatin reduced the PP response to the high dose of acetyl choline used in the experiments and effectively blocked the PP release induced by caerulein; this is expected from previous work in man [14]. In one series of experiments (unpublished observations) VIP (1 nmol/l) was given 20 min after acetyl choline (1 μmol/l) and the PP release was tenfold higher than with VIP alone. The apparent synergism observed between acetyl choline and VIP is of great interest and may be presumed to reflect the in vivo situation where some degree of continuous vagal tone is probable.

In spite of the anatomical grouping of the A, B and PP cells in the islets of Langerhans the release of PP does not appear to be integrated with the release of insulin and glucagon. This is perhaps not surprising as in some species, eg dog, many of the PP cells lie outside the islets, eg between the acinar cells and in duct walls [15]. PP is mainly influenced by circulating hormones, acetyl choline and peptidergic neurotransmitters whilst insulin and glucagon release is, in addition, powerfully affected by metabolites and catecholamines. These experiments demonstrate directly how the observed influence of the gut on the hormones of the endocrine pancreas can be accounted for by an integrated combination of metabolite, hormonal and nervous control.

It has recently been established that, in addition to the well recognised adrenergic and cholinergic nerve fibres, a third component of the autonomic nervous system exists [16]. This is the peptidergic system, which may be anatomically identical, in part, with the previously described system to which the term "purinergic" has been applied. Thus the islets of Langerhans are richly innervated with, for example, VIP-containing nerve fibres [17, 18]. It is impossible at present to evaluate the possible concentration of peptide neurotransmitter which may be present at the target cell. Our finding of the effectiveness of low concentrations of VIP in releasing PP, glucagon and also insulin, however, adds considerable emphasis to its possible role in the physiological control mechanisms of the endocrine pancreas.

Acknowledgements. We are grateful for the skillful technical assistance of Mrs. Karen Just. The pure peptide hormones used in this study were generous gifts from Dr. R. E. Chance (Lilly Research Laboratories, Indianapolis), Professor V. Mutt, (Karolinska Institute, Stockholm), Professor J. Brown (Vancouver University), Professor S. I. Said (Dallas University) and Professor V. Erspamer and Professor R. Castiglione (Farmitalia, Milan). Antibody specific for pancreatic polypeptide was provided by Dr. R. E. Chance. This study was supported by grants from the Danish Medical Research Council, Novo S. A., and also the British Diabetic Association.

References

1. Adrian, T. E., Bloom, S. R., Besterman, H. S., Barnes, A. J., Cooke, T. J. C., Russell, R. C. G., Faber, R. C.: Mechanism of pancreatic polypeptide release in man. Lancet **1977 I**, 161–163
2. Floyd, J. C., Fajans, S. S., Pek, S.: Regulation in healthy subjects of the secretion of human pancreatic polypeptide, a newly recognised pancreatic islet polypeptide. Recent Prog. Horm. Res. **33**, 519–570 (1977)
3. Iversen, J.: Secretion of glucagon from the isolated perfused canine pancreas. J. Clin. Invest. **50**, 2123–2136 (1971)
4. Iversen, J.: Adrenergic receptors and the secretion of glucagon and insulin from the isolated perfused canine pancreas. J. Clin. Invest. **52**, 2102–2116 (1973)
5. Iversen, J.: Inhibition of pancreatic glucagon release by somatostatin: in vitro. Scand. J. Clin. Invest. **33**, 125–129 (1974)
6. Iversen, J., Miles, D. W.: Evidence for a feedback inhibition of insulin on insulin secretion in the isolated, perfused canine pancreas. Diabetes **20**, 1–9 (1971)
7. Ørskov, H.: Wick chromatography for the immunoassay of insulin. Scand. J. Clin. Lab. Invest. **20**, 297–304 (1967)
8. Ørskov, H., Thomsen, H. G., Yde, H.: Wick chromatography for the rapid and reliable immunoassay of insulin, glucagon and growth hormone. Nature **219**, 193–195 (1968)
9. Adrian, T. E., Bloom, S. R., Bryant, M. G., Polak, J. M., Heitz, Ph., Barnes, A. J.: Distribution and release of human pancreatic polypeptide. Gut **17**, 940–944 (1976)
10. Iversen, J.: Effect of acetyl choline on the secretion of glucagon and insulin from the isolated perfused canine pancreas. Diabetes **22**, 381–387 (1973)
11. Schwartz, T. W., Stadil, F., Chance, R. E., Rehfeld, J. E., Larsson, L. I., Moon, N.: Pancreatic polypeptide response to food

in duodenal ulcer patients before and after vagotomy. Lancet **1976 I**, 1102–1105
12. Schwartz, T. W., Rehfeld, J. J.: Mechanism of pancreatic-polypeptide release. Lancet **1977 I**, 697–698
13. Samols, E., Weir, G. C., Patel, Y. C., Loo, S. W., Gabbay, K. H.: Autonomic control of somatostatin and pancreatic peptide secretion by the isolated perfused canine pancreas. Clin. Res. **25**, 499 A (1977)
14. Adrian, T. E., Besterman, H. S., Mallinson, C. N., Czaykowska, W. M., Bloom, S. R.: Studies on the release of pancreatic polypeptide and its relationship to pancreatic exocrine function. J. Endocrinol. **75**, 35 P (1977)
15. Larson, L. I., Sundler, F., Hakanson, R.: Pancreatic polypeptide – a postulated new hormone: identification of its cellular storage site by light and electron microscopic immunocytochemistry. Diabetologia **12**, 211–226 (1976)
16. Bloom, S. R., Polak, J. M.: Peptidergic versus purinergic. Lancet **1978 I**, 93
17. Bryant, M. G., Bloom, S. R., Polak, J. M., Albuquerque, R. H., Modlin, I., Pearse, A. G. E.: Possible dual role for vasoactive intestinal peptide as gastrointestinal hormone and neurotransmitter substance. Lancet **1976 I**, 991–993
18. Sundler, F., Alumets, J., Hakanson, R.: Peptides in the gut with a dual distribution in nerves and endocrine cells. In: S. R. Bloom (Ed): Gut Hormones, pp. 406–413. Edinburgh: Churchill Livingstone 1978

Received: October 26, 1977,
and in revised form: February 20, 1978

Dr. S. R. Bloom
Department of Medicine
Royal Postgraduate Medical School
Hammersmith Hospital
Du Cane Road
London W12 0HS
England