The Interaction of Vasoactive Intestinal Polypeptide (VIP), Glucose and Arginine on the Secretion of Insulin, Glucagon and Somatostatin in the Perfused Rat Pancreas

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Summary. Vasoactive Intestinal Polypeptide (VIP) increased the release of insulin, glucagon and somatostatin from the perfused rat pancreas. The amount of these hormones released was dependent upon the prevailing glucose concentration. VIP stimulated glucagon release in the absence of glucose, while insulin and somatostatin release were increased by VIP only in the presence of glucose concentrations of 4.4 mmol/l and above. Glucagon secretion stimulated by arginine in the presence of 4.4 mmol/l glucose was potentiated by VIP. In contrast, VIP did not induce any further increase in the secretion of insulin and somatostatin over that stimulated by arginine. At higher concentrations of glucose (6.7, 16.7, and 33.3 mmol/l) VIP continued to stimulate insulin and somatostatin release, this effect being synergistic on early-phase insulin release. The effects of VIP on islet cells thus depend on the levels of modulating nutrients.

Key words: VIP, insulin, glucagon, somatostatin, glucose, arginine, perfused rat pancreas.

Vasoactive Intestinal Polypeptide (VIP) was first isolated from porcine duodenum [22]. Using antibodies against pure porcine VIP in immunoperoxidase and immunofluorescence tests, VIP-immunoreactive cells have been detected in the islets in dog, guinea-pig, and man, but not in rats ([2]; Schultzberg and Hökfelt, personal communication). VIP has also been found in the central nervous system [13, 14] and in peripheral nerves [15, 19, 27, 28]. In the pancreas, VIP containing nerves have been seen in connective

tissues within the exocrine pancreatic parenchyma, and also in association with islets [27].

VIP stimulates insulin and glucagon release from isolated perfused porcine pancreas [16] as well as from dog pancreas perfused *in situ* [12] which, together with the occurrence of VIP in islets, suggests that the peptide may be of importance for the regulation of the secretion of islet hormones.

The present study was designed to characterize the effects of VIP on the release of insulin, glucagon, and somatostatin from the isolated perfused rat pancreas. The study was further extended to investigate the interaction of VIP, glucose, and arginine on pancreatic hormone release.

Material and Methods

Sprague-Dawley rats (Anticimex, Stockholm), weighing 200–250 g, and fed *ad libitum*, were anaesthetized by IP injection of 50 mg/kg of pentobarbitone and the pancreas isolated [17]. The perfusing medium, a red-cell free Krebs-Ringer bicarbonate solution containing 2 g/100 ml bovine serum albumin, was infused into the coeliac artery, and run into the prepared pancreas by an open circuit, a “non-recycling perfusion system” [17]. The rate of perfusion was approximately 3.0 ml/min. The pancreas was equilibrated by perfusion for 20 min before the start of the experiments.

Insulin was measured by radioimmunoassay using a rat insulin standard [10]. The sensitivity of the assay was about 300 ng/l and the coefficient of variation $\pm 10\%$. Glucagon was assayed by the charcoal separation technique with an antibody specific for pancreatic glucagon “30 K” [8]. The sensitivity of this assay was about 50 ng/l and the coefficient of variation $\pm 7\%$. Somatostatin was measured by a radioimmunoassay using locally produced antibodies [4]. Cross-reactivity of this antibody was less than 0.01% with insulin, glucagon, VIP, substance P, LH-RH, vasopressin, and oxytocin. The sensitivity of the somatostatin assay was about 5 ng/l and coefficient of variation $\pm 15\%$.

Pure, natural, porcine VIP was kindly provided by Professor V. Mutt, Karolinska Institute, Stockholm.

The amounts of insulin and somatostatin released were determined by integrating the areas under the respective curves. The initial phase of insulin and somatostatin release corresponds to

Fig 1. Effect of a 15-min infusion of VIP (1 nmol/l) on insulin, glucagon and somatostatin release from the isolated perfused rat pancreas in the absence (upper panel) and presence (lower panel) of 4.4 mmol/l glucose. Results are expressed as mean $\pm$ SEM ($n = 6$). Basal hormone release rates were: At 0 mmol/l glucose: insulin: 0 ng/min – glucagon: 3.04 ± 0.10 ng/min – somatostatin: 2.8 ± 1.0 pg/min. At 4.4 mmol/l glucose: insulin: 2.20 ± 0.26 ng/min – glucagon: 0.37 ± 0.05 ng/min – somatostatin: 12.3 ± 1.6 pg/min

Fig 2. Effect of a 15-min infusion of VIP (0.1, 1.0, 10.0 nmol/l) on insulin and somatostatin release from the isolated perfused rat pancreas. The perfusing medium contained 4.4 mmol/l glucose. Results are given as mean $\pm$ SEM ($n = 6$). Basal hormone release rates were: insulin: 2.20 ± 0.26 ng/min – somatostatin: 12.3 ± 1.6 pg/min

0–7 min after the start of the stimulation, while the second phase comprises the period between 7 and 60 min. Conventional statistical methods were employed. The results are expressed as mean $\pm$ SEM.

Results

Glucose Dependence of the Effect of VIP on the Release of Insulin, Glucagon and Somatostatin (Fig. 1)

In the absence of glucose in the perfusing medium, VIP (1 nmol/l) markedly enhanced glucagon release. The effect was most pronounced during the first five minutes of VIP administration. The peptide alone had no effect on insulin and somatostatin secretion. In contrast, in the presence of 4.4 mmol/l glucose in the perfusing medium, VIP stimulated the release of insulin and somatostatin but not of glucagon (Fig. 1).

Dose Dependence of the Insulin and Somatostatin Response to VIP (Fig. 2)

In these experiments, the pancreas was perfused with a medium containing 4.4 mmol/l glucose. The lowest dose of VIP used (0.1 nmol/l) significantly enhanced insulin as well as somatostatin release. The release of these hormones was maximally stimulated by the presence of 1 nmol/l VIP. Irrespective of the dose of VIP, insulin response was stimulated most during the first five minutes of the administration of the peptide, while somatostatin response to VIP persisted at the same level during the entire experiment (Fig. 2).

Fig 3. Effect of VIP (1 nmol/l) on insulin release stimulated by increasing concentrations of glucose. The isolated perfused rat pancreas was equilibrated for 20 min with 3.3 mmol/l glucose prior to stimulation with higher glucose doses (0–60 min). Broken lines indicate control experiments and solid lines experiments with VIP. VIP was infused from 0–60 min. Results are given as mean $\pm$ SEM. Basal insulin release: 1.70 ± 0.22 ng/min

Table 1. Effect of VIP (1 nmol/l) on glucose induced insulin response of the isolated rat pancreas. Insulin release was calculated by determining incremental area over basal values. NS = not significant

Glucose concentration mmol/l	Insulin release ($\text{ng} \times \text{min} \times \text{ml}^{-1}$)					
	1–7 min			7–60 min		
	glucose	glucose + VIP	P	glucose	glucose + VIP	P
3.3	0	12 ± 3	<0.0025	0	0	0
6.7	33 ± 2	76 ± 4	<0.0025	140 ± 27	240 ± 21	<0.0025
16.7	155 ± 21	268 ± 32	<0.0025	3893 ± 332	4166 ± 707	NS
33.3	225 ± 23	666 ± 99	<0.0025	5468 ± 573	6230 ± 1000	NS

Interaction of Glucose and VIP on the Release of Insulin and Somatostatin (Figs. 3 and 4)

In these experiments, the pancreas was first equilibrated for 20 min with a perfusion medium containing 3.3 mmol/l glucose. In the control experiments, the pancreas was then stimulated by increasing glucose concentrations (6.7, 16.7, and 33.3 mmol/l). In the experiments with VIP, glucose and 1 nmol/l of VIP were perfused simultaneously. As expected, administration of glucose induced a biphasic insulin response. Significant stimulation of insulin release was seen when the glucose concentration was increased from 3.3 mmol/l to 6.7 mmol/l.

Analysis of the two series of results (Fig. 3, Table 1) shows that at all glucose concentrations used, VIP

and glucose exerted synergistic effects on the early phase of insulin release. Late insulin release was significantly enhanced by VIP only in the presence of 6.7 mmol/l glucose. VIP significantly enhanced both the early and the late phase of somatostatin release in the presence of 6.7 mmol/l glucose ($P < 0.01$) and the early phase with 16.7 mmol/l of the hexose (Fig. 4, Table 2).

Effect of VIP on Arginine Induced Release of Insulin, Glucagon and Somatostatin (Fig. 5)

Arginine (20 mmol/l) significantly stimulated the release of insulin, glucagon, and somatostatin from pancreas perfused with a medium containing 4.4 mmol/l glucose. The effect was most pronounced

Fig 4. Effect of VIP (1 nmol/l) on somatostatin release at increasing concentrations of glucose. The isolated perfused rat pancreas was equilibrated for 20 min with 3.3 mmol/l glucose prior to application of higher glucose concentration (0–60 min). Broken lines denote control experiments and solid lines experiments with VIP. VIP was infused from 0–60 min. Results are given as mean $\pm$ SEM. Basal somatostatin release: 4.3 ± 1.2 pg/min

during the first 5 min. VIP significantly enhanced arginine induced glucagon release, whereas there was no such effect on insulin and somatostatin secretion (Fig. 5).

Discussion

VIP containing nerves occur in rat islets ([27]; Schultzberg and Hökfelt, personal communication). The present study demonstrates that in rat VIP stimulates the release of insulin, glucagon and somatostatin from the isolated perfused pancreas, depending on glucose concentration. VIP had a similar effect in the perfused pancreas of other species, in that it stimulated insulin and glucagon release from the porcine pancreas, and insulin and somatostatin from the canine pancreas [12, 16].

In our experimental system VIP stimulated glucagon release in the absence of glucose. In contrast, at 4.4 mmol/l glucose level, the peptide significantly enhanced the secretion of insulin and somatostatin but not of glucagon. Dependence of the effect of VIP on glucose concentration in the perfusing medium also occurs in the isolated porcine pancreas [16]. However, in the porcine pancreas, VIP stimulated glucagon release even in the presence of 5.0 mmol/l glucose, and it was only when the glucose concentration was increased to a hyperglycaemic level (7.5 mmol/l) that VIP enhanced insulin release. Furthermore, under normoglycaemic conditions in the in situ perfused cat pancreas VIP was more

Fig 5. Effect of VIP (1 nmol/l) on arginine (20 mmol/l) induced insulin, glucagon, and somatostatin release from the isolated perfused rat pancreas. Glucose concentration in the perfusate was 4.4 mmol/l. Columns represent insulin, glucagon, somatostatin output during 5 min. Results are given as mean $\pm$ SEM ($n = 6$). Basal hormone release rates were: insulin: 2.70 ± 0.34 ng/min – glucagon: 0.43 ± 0.02 ng/min – somatostatin: 17.2 ± 7.8 pg/min

Table 2. Effect of VIP (1 nmol/l) on glucose induced somatostatin release from the isolated perfused rat pancreas. Somatostatin release was calculated by determining incremental area over basal levels. NS = not significant

Glucose concentration mmol/l	Somatostatin release ($\text{pg} \times \text{min} \times \text{ml}^{-1}$)					
	1–7 min			7–60 min		
	glucose	glucose + VIP	P	glucose	glucose + VIP	P
3.3	-20 ± 11	6 ± 23	NS	-58 ± 28	14 ± 270	NS
6.7	-10 ± 11	50 ± 9	<0.0005	-45 ± 143	161 ± 48	<0.0025
16.7	23 ± 19	72 ± 17	<0.05	544 ± 198	302 ± 62	NS

potent in stimulating glucagon than insulin release. The clear stimulatory effect of VIP on insulin but not glucagon release during normoglycaemia in rats and hyperglycaemia in pigs argues against the suggestion [26] that insulin secretion induced by VIP is secondary to prior stimulation of glucagon release.

An important finding is that during normoglycaemia, VIP also stimulates somatostatin release. The interaction between glucose and substances releasing somatostatin is almost unknown. We have also demonstrated that, in contrast to VIP, a sulphonylurea drug (glibenclamide) markedly stimulates somatostatin release from the perfused rat pancreas in the presence of low glucose concentrations (3.3 mmol/l or less) whereas the effect was less pronounced at 4.4 mmol/l glucose [7].

The significance of glucose for the effects of VIP in the pancreas is further emphasized by studies of its effects on insulin and somatostatin release induced by increasing glucose concentrations. VIP was clearly synergistic with glucose over a broad range of concentrations of this hexose (6.7 to 33.3 mmol/l). A remarkable finding was that the peptide exerted a more prominent effect on early than on late insulin and somatostatin release. This would imply that VIP sensitizes both B and D cells for the action of glucose. On the other hand, VIP seems to sensitize A but not B cells when arginine is used as stimulator.

These marked effects of VIP on the release of islet hormones are in contrast to *in vivo* studies. In rats, a dose as large as 2 $\mu\text{g/kg}$ of VIP induced only a slight increase in serum insulin [29]. When VIP was given as a continuous IV infusion in rats at a dose of 40 ng/kg/min, there was no effect on basal or glucose or arginine induced, insulin or glucagon release (unpublished observations). The comparison between *in vitro* and *in vivo* effects of VIP suggests that, in rats, VIP plays a role in the regulation of the function of A, B, and D cells provided that the peptide released from VIP containing islet nerve terminals exerts a direct local action.

Since in the present experiments we used porcine VIP some caution is warranted when extending our results to a possible physiological significance of VIP in rat islets. Rat VIP is not available at present.

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