Effects of calcitonin gene-related peptide (CGRP) on islet hormone secretion in the pig

B. Åhrén^{1, 2}, H. Mårtensson¹ and A. Nobin¹

Departments of ¹Surgery and ²Pharmacology, Lund University, Lund, Sweden

Summary. Calcitonin gene-related peptide occurs in intrapancreatic nerves and endocrine cells. The peptide is therefore a candidate for being of physiological importance for pancreatic function. We examined the direct effects of calcitonin gene-related peptide on islet hormone secretion in the pig by infusing the peptide into the superior pancreatic artery. We found that 15 min intrapancreatic infusion of calcitonin gene-related peptide (22 pmol/min) decreased baseline pancreatic insulin output from $48 \pm 10 \mu\text{U}/\text{min}$ to $8 \pm 7 \mu\text{U}/\text{min}$ ($p < 0.01$). Moreover, calcitonin gene-related peptide inhibited glucose-induced insulin secretion by 45% compared to controls ($p < 0.01$), yet left terbutaline (β_2 -adrenoceptor)-stimulated insulin secretion unaffected. Furthermore, while being without effect on baseline glucagon output, cal-

citonin gene-related peptide potentiated terbutaline-induced glucagon secretion more than seven-fold ($p < 0.001$). In contrast, the peptide did not affect baseline or stimulated pancreatic somatostatin output. We conclude that in pigs calcitonin gene-related peptide inhibits insulin secretion and potentiates glucagon secretion by direct effects on the pancreas that are not mediated by primary alterations in pancreatic somatostatin secretion. We suggest that the neuropeptide calcitonin gene-related peptide might be of importance for the intrapancreatic regulation of insulin and glucagon secretion in pigs.

Key words: CGRP, nerve tissue proteins, insulin secretion, glucagon secretion, somatostatin secretion, in vivo, pigs.

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide which is produced by an alternative processing of the calcitonin gene [1, 2]. The peptide is widely distributed within nerves in both the central and peripheral nervous system [2]. It has been shown to exert a variety of effects, including inhibition of gastric acid output and gastrin secretion and induction of vasodilatation [2, 3].

CGRP is one of the newly discovered neuropeptides within the pancreas [4], and the peptide has been reported to occur both in nerves and in endocrine cells within this organ [5–7]. This suggests that CGRP is involved in the regulation of pancreatic function. The peptide has, in fact, been shown to stimulate amylase release from dispersed rat pancreatic acini [8]. CGRP has also been reported to lower basal and glucose-stimulated plasma insulin levels in mice [7], and to inhibit glucose-induced insulin secretion from isolated rat islets [9]. In contrast, basal insulin secretion was not affected by intravenous infusion of the peptide in man [10].

The aim of the present study was to investigate the direct effects of CGRP on islet hormone secretion in

pigs. The peptide was infused directly into the superior pancreatic artery at a low dose rate, and blood samples were taken from the corresponding pancreatic vein and the carotid artery. Since we also measured the pancreatic venous blood flow, we could determine the hormone output from the pig pancreas in situ. We studied the pancreatic output of the three islet hormones, insulin, glucagon and somatostatin, both in the basal state and after intravenous injections of glucose or the β_2 -adrenoceptor agonist terbutaline.

Materials and methods

The experiments were performed in non-fasted pigs weighing 15–20 kg, which were anaesthetized with thiopental (Pentotal® Sodium, Abbot spa, Campoverde, Italy), intubated and ventilated with nitrogen oxide/oxygen.

A carotid arterial catheter was inserted for sampling of peripheral arterial blood, and an internal jugular venous catheter was introduced to slowly infuse saline throughout each experiment and serve as a route for the intravenous injections of glucose and terbutaline.

A midline laparotomy was then performed, and the pancreas exposed. All blood vessels between the duodenum and pancreas were ligated, and an extracorporeal shunt was placed between the superi-

or pancreatic and portal veins. This shunt contained a port for sampling of blood, which after the duodenal exclusion procedure was pancreas-selective venous blood. After inserting the shunt, heparin (Kabi-Vitrum AB, Stockholm, Sweden) was injected intravenously (2500 IU), and a 22-gauge teflon catheter was placed in the superior pancreatic artery for continuous infusion of saline with 1 g/l of gelatine at 0.03 ml/min.

One hour after these surgical procedures, baseline samples were drawn from the carotid artery and from the shunt. CGRP (Peninsula, Belmont, Calif., USA) was then infused at the dose of 22 pmol/min into the pancreatic artery. CGRP was dissolved in saline with addition of gelatine (1 g/l). After 15 min of infusion, glucose (5 g) was injected intravenously; after another 30 min, terbutaline (20 mg; Draco AB, Lund, Sweden) was injected intravenously. Arterial and pancreatic venous samples were drawn at various time intervals during the experiments. Controls were infused with saline/gelatine into the pancreatic artery throughout.

Determinations

Blood samples for insulin determinations were taken in EDTA-tubes and centrifuged immediately after the experiments. Plasma was separated and stored at -20°C . Plasma levels of insulin were determined radioimmunochemically [11]. Blood samples for the glucagon assay were drawn into tubes containing aprotinin and centrifuged immediately after the experiments. Plasma was separated and stored at -20°C . Plasma glucagon levels were determined with a radioimmunoassay on unextracted plasma by the use of an antibody specific to pancreatic glucagon [12, 13]. Plasma levels of somatostatin were also determined with a radioimmunoassay [14]. Blood samples were taken in aprotinin-containing tubes and centrifuged immediately. Plasma was separated and stored at -70°C until assay. Plasma glucose concentrations were determined with a glucose oxidase method.

Regularly during the experiments, blood flow through the extra-corporeal shunt was determined. It was determined by measuring the time necessary for blood to flow between two defined points at the shunt. The blood flow that was determined is thus valid for the area drained by the superior pancreatic vein only, and should not be extrapolated to the entire pancreas. However, it corresponds to the portion of the pancreas that was studied by the infusions in the superior pancreatic artery. The blood flow measurements were calibrated by injection of saline into the shunt regularly during the experiments. Blood flow was recorded simultaneously as blood was sampled from the very shunt, hence (1) the recorded output data is valid for each time point, and (2) flow and concentration are determined from the same portion of pancreas. The two defined points on the shunt were situated close to the entrance of the shunt into the portal vein. The front stream of blood was visualized during each measurement by injecting a small amount of saline into the shunt port. The time needed for the blood to pass from the first point to the second point was divided by the volume in the shunt between those two points; the inversion of this figure is the blood flow. Hematocrit was also measured regularly. The hormone output from the pancreas was then calculated by multiplying the arterio-venous concentration difference times the plasma flow through the shunt ($= (1 - \text{hematocrit}) \times \text{blood flow}$).

Statistical analysis

Data were analysed with a paired Student's *t*-test to compare mean output or concentration during CGRP infusion with the pre-infusion values, and with non-paired Student's *t*-test to compare the responses to glucose and terbutaline between different groups. The integrated hormone outputs under baseline conditions were calculated by subtracting the pre-infusion outputs from the mean of the 5-, 10- and 15-min values. The hormone secretory responses to glucose and terbutaline were calculated by subtracting the output in the pre-injection sample from the mean of the values in the 2-, 4- and 6-min samples.

Fig. 1. Arterial plasma glucose levels in pigs before, during and after intrapancreatic infusion of saline ($\circ$ -- $\circ$, $n=6$) or CGRP ($\bullet$ -- $\bullet$, $n=6$) for 75 min. Glucose (5 g) or terbutaline (20 mg) was injected intravenously as indicated. Means $\pm$ SEM are given

Results

Effects of CGRP on plasma glucose levels (Fig. 1)

Baseline arterial plasma glucose levels were not appreciably affected by the pancreatic CGRP infusion. The terbutaline-induced increase in plasma glucose levels was, however, markedly augmented by CGRP. At 10 min after terbutaline injection, arterial glucose levels were 7.4 ± 0.5 mmol/l in controls and 10.7 ± 0.7 mmol/l in pigs infused with CGRP ($p < 0.01$).

Effects of CGRP on insulin secretion (Fig. 2)

Figure 2 shows that CGRP lowered basal arterial plasma insulin levels and inhibited the basal pancreatic output of insulin. Thus, after 15 min of pancreatic CGRP infusion, basal arterial plasma insulin levels had decreased from 33 ± 4 $\mu\text{U/ml}$ to 27 ± 3 $\mu\text{U/ml}$ ($\Delta = 6 \pm 2$ $\mu\text{U/ml}$; $p < 0.05$), and pancreatic insulin output had decreased from 48 ± 10 $\mu\text{U/min}$ to 8 ± 7 $\mu\text{U/min}$ ($p < 0.01$). The saline infusion did not affect baseline insulin output. Glucose injection (5 g) in controls increased the insulin output to 912 ± 138 $\mu\text{U/min}$ after 4 min. This glucose-induced insulin secretion was impaired to 392 ± 103 $\mu\text{U/min}$ ($p < 0.01$) by CGRP. In contrast, the insulin output at 4 min after terbutaline injection was not affected by CGRP. Figure 2, lower panel, shows the glucose-induced increase in integrated pancreatic insulin output; it was 820 ± 175 $\mu\text{U/min}$ in controls and was reduced to 375 ± 80 $\mu\text{U/min}$ by CGRP ($p < 0.05$).

Effects of CGRP on glucagon secretion (Fig. 3)

Figure 3 shows that CGRP did not affect arterial levels of glucagon or the pancreatic output of glucagon un-

Fig. 2. Arterial plasma insulin levels (upper panel) and pancreatic insulin output (middle panel) in pigs before, during and after intrapancreatic infusion of saline (○--○, $n=6$) or CGRP (●--●, $n=6$) for 75 min. Glucose (5 g) or terbutaline (20 mg) was injected intravenously as indicated. The lower panel shows the integrated insulin output. Saline □, CGRP ■. Means $\pm$ SEM are given. P indicates probability level of random difference between groups. n.s. not significant

der baseline conditions or after injection of glucose. In contrast, the terbutaline-induced glucagon output was potentiated by the peptide; the integrated glucagon response, being 22 ± 4 pg/min in controls, was enhanced to 100 ± 5 pg/min during infusion with CGRP ($p < 0.001$).

Fig. 3. Arterial plasma glucagon levels (upper panel) and pancreatic glucagon output (middle panel) in pigs before, during and after intrapancreatic infusion of saline (○--○, $n=6$) or CGRP (●--●, $n=6$) for 75 min. Glucose (5 g) or terbutaline (20 mg) was injected intravenously as indicated. The lower panel shows the integrated glucagon output. Saline □, CGRP ■. Means $\pm$ SEM are given. P indicates probability level of random difference between groups. n.s. not significant

Effects of CGRP on pancreatic somatostatin secretion (Fig. 4)

Figure 4 shows that CGRP did not affect the pancreatic somatostatin output or arterial somatostatin levels, neither under baseline conditions nor after stimulation

Fig. 4. Arterial plasma somatostatin levels (upper panel) and pancreatic somatostatin output (middle panel) in pigs before, during and after intrapancreatic infusion of saline ($\circ$ — $\circ$, $n=4$) or CGRP ($\bullet$ — $\bullet$, $n=4$). Glucose (5 g) or terbutaline (20 mg) was injected intravenously as indicated. The lower panel shows the integrated pancreatic somatostatin response. Saline $\square$, CGRP $\blacksquare$. Means $\pm$ SEM are given. P indicates probability level of random difference between groups. n.s. not significant

with glucose or terbutaline. In controls, glucose enhanced the integrated pancreatic somatostatin output by 235 ± 60 pg/min ($p < 0.01$), and terbutaline increased the pancreatic somatostatin output by 370 ± 65 pg/min ($p < 0.01$). CGRP did not significantly affect these responses.

Effects of CGRP on pancreatic blood flow (Table 1)

Table 1 shows that CGRP increased the pancreatic blood flow ($p < 0.05$). Injection of glucose or terbutaline did not affect the pancreatic blood flow in these pigs.

Table 1. Blood flow in the superior pancreatic vein in pigs during intrapancreatic infusion of saline or calcitonin gene-related peptide (CGRP) (ml/min)

	Controls	CGRP	
Baseline	2.4 ± 0.3	4.1 ± 0.5	($p < 0.05$)
Glucose	2.7 ± 0.4	4.0 ± 0.5	($p < 0.05$)
Terbutaline	3.7 ± 0.3	4.9 ± 0.4	($p < 0.05$)

The values under baseline conditions or at 6 min after intravenous injection of glucose (5 g) or terbutaline (20 mg) are given (mean $\pm$ SEM). Explanation on the measurement of blood flow is given in the Materials and methods. p indicates probability level of random difference between the groups ($n=6$ in each group)

Discussion

CGRP has previously been found to inhibit glucose-induced insulin secretion in mice when administered intravenously [7]. However, a multitude of effects might be anticipated following the intravenous injection of the peptide [2]. Thus, though suggestable, it could in the previous study not be convincingly established whether CGRP inhibits glucose-induced insulin secretion by a direct pancreatic effect, as is indicated by a recent in vitro study [9], or by indirect effects. For example, CGRP has been shown to markedly stimulate the secretion of gastrointestinal somatostatin [15]. Hypothetically, the released somatostatin could then mediate the inhibitory action on insulin secretion. To study the possible direct effect of CGRP on the pancreas in vivo, we undertook the present investigation. We thereby used a pig model, in which we infused the peptide in the superior pancreatic artery and measured the hormone output in the corresponding vein. The infused CGRP did thus not affect the entire pancreas. This explains why we did not detect any influences of CGRP in the arterial levels of the pancreatic hormones. Since CGRP affected blood flow, we did not base our conclusions on the pancreatic venous levels of the pancreatic hormones, but instead on the calculated pancreatic output of the hormones taking into consideration the blood flow changes. This makes the blood flow determinations crucial. Since the blood flow changes during the different experiments were consistent and reproducible, our conclusions on the hormone output seem valid. We could not, however, demonstrate the finding that glucose increases pancreatic blood flow, as has been shown in other studies [16–18], which might be due to a too small glucose load (5 g) resulting in a modest hyperglycaemia only.

We found that CGRP increased the pancreatic blood flow. Since the pancreatic venous concentrations of insulin dropped markedly, the calculated net output of insulin from the pancreas was shown to be inhibited by CGRP. The glucose-induced insulin secretion was also inhibited by CGRP. In contrast to glucose-induced insulin secretion, the insulin output stimulated by the β_2 -adrenoceptor agonist terbutaline was not in-

hibited by CGRP. It must, however, be considered that the hyperglycaemic response to terbutaline was augmented by CGRP. These findings are interpreted that CGRP either (1) affects certain events in the stimulus-secretion coupling that are of more importance for the effect of glucose than for the effect of terbutaline, or (2) that glucose counteracts any inhibitory action of CGRP on terbutaline-induced insulin secretion. However, more studies are necessary in order to characterize the exact mechanism behind the inhibitory effect of CGRP on insulin secretion.

The direct effects of CGRP on pancreatic glucagon secretion are hitherto unknown. In a previous study in man, basal plasma levels of enteroglucagon did decrease slightly during infusion of CGRP [10]. We found that basal pancreatic glucagon secretion was not affected by CGRP in pigs, and we also found that the peptide could not affect glucagon secretion during hyperglycaemia. In contrast, terbutaline-induced glucagon secretion was markedly potentiated by the peptide. It may then be considered that CGRP induced a potentiated hyperglycaemic response to terbutaline, and despite this marked hyperglycaemia the glucagon secretion was potentiated. This demonstrates that CGRP, while being without effect on glucagon output at low rates of glucagon secretion, potentiates stimulated glucagon secretion in pigs. It is also of interest to note that CGRP potentiated terbutaline-induced glucagon secretion, yet was without effect on terbutaline-induced insulin secretion. It might be speculated that an inhibitory direct action by CGRP on insulin secretion was counteracted by the increased glucagon output. Glucagon is known to stimulate insulin secretion [19].

It has been found that somatostatin released from the pancreas is of marginal importance for the arterial somatostatin levels which instead reflect gastrointestinal somatostatin secretion [20]. Thus, in order to study pancreatic somatostatin secretion, it is necessary to sample pancreatic venous blood as we did in the present investigation. We found that pancreatic somatostatin output was not affected by CGRP in our pigs. This shows that the changes observed in insulin and glucagon secretion are not secondary to any alterations in pancreatic somatostatin secretion.

The observed increase in plasma glucose concentrations after administration of terbutaline was augmented by the pancreatic CGRP infusion. This is consistent with the pattern of the effects of CGRP on insulin and glucagon secretion. Glucagon secretion was potentiated and insulin secretion was unaffected during infusion of the peptide. This exaggerated hyperglycaemic response might in turn influence the glucagon and insulin responses to CGRP.

Since CGRP occurs in intrapancreatic nerves [6, 7], the peptide is one of the many neuropeptides that are candidates for being of importance for islet function [4]. Our present study shows that the peptide affects islet hormone secretion. Though these effects have been

found in this purely pharmacological study, CGRP fulfils two important criteria for establishing the peptide as a physiologically important intrapancreatic neuropeptide: it occurs in intrapancreatic nerves, and it affects islet hormone secretion [4]. We now need (1) determinations of pancreatic venous CGRP levels during pancreatic nerve stimulation to investigate whether the peptide is released from these nerves; and (2) development of specific CGRP antagonists to study whether such antagonists affect neurally-induced changes in islet hormone secretion. It might be mentioned that the inhibition of insulin secretion and the stimulation of glucagon secretion which we observed during CGRP infusion in our pigs are reminiscent of the effects of sympathetic nerve stimulation [21]. Based on several different lines of evidence, it was recently suggested that noradrenaline is not the sole mediator of these sympathetic effects on islet hormone secretion [21, 22], and it might therefore be speculated that these effects are partially mediated by CGRP.

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References

1. Amara SG, Jonas V, Rosenfeld MG, Ong ES, Evans RM (1982) Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature* 298: 240-244
2. Fischer JA, Born W (1985) Novel peptides from the calcitonin gene: Expression, receptors and biological function. *Peptides* 6: 265-271
3. Törnebrandt K, Nobin A, Owman C (1987) Contractile and dilatory action of neuropeptides on isolated human mesenteric blood vessels. *Peptides* (in press)
4. Åhrén B, Taborsky Jr GJ, Porte Jr D (1986) Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. *Diabetologia* 29: 827-836
5. Mulderry PK, Ghatei MA, Bishop AE, Allen YS, Polak JM, Bloom SR (1985) Distribution and chromatographic characterisation of CGRP-like immunoreactivity in the brain and gut of the rat. *Regul Pept* 12: 133-143
6. Sternini C, Brecha N (1986) Immunocytochemical identification of islet cells and nerve fibers containing calcitonin gene-related peptide-like immunoreactivity in the rat pancreas. *Gastroenterology* 90: 1155-1163
7. Pettersson M, Åhrén B, Böttcher G, Sundler F (1986) Calcitonin gene-related peptide: Occurrence in pancreatic islets in the mouse and the rat and inhibition of insulin secretion in the mouse. *Endocrinology* 119: 865-869
8. Seifert H, Sawchenko P, Chesnut J, Rivier J, Vale W, Pandol SJ (1985) Receptor for calcitonin gene-related peptide: binding to exocrine pancreas mediates biological actions. *Am J Physiol* 249: G147-G151
9. Greeley Jr GH, Alwmark A, Cooper CW, Thompson JC (1985) Calcitonin and calcitonin gene-related peptide inhibition of insulin secretion in vitro. *Fed Proc* 44: 1391

10. Kraenzlin ME, Ch'ng JLC, Mulderry PK, Ghatei MA, Bloom SR (1985) Infusion of a novel peptide, calcitonin gene-related peptide (CGRP) in man. Pharmacokinetics and effects on gastric acid secretion and on gastrointestinal hormones. *Regul Pept* 10: 189-197
11. Heding LG (1966) A simplified radioimmunoassay method. In: Donato L, Milhaud G, Sirchis J (eds) *Labelled proteins in tracer studies*. Euratom, Brussels, pp 345-350
12. Heding LG (1971) Radioimmunological determination of pancreatic and gut glucagon in plasma. *Diabetologia* 7: 10-19
13. Ahrén B, Lundquist I (1982) Glucagon immunoreactivity in plasma from normal and dystrophic mice. *Diabetologia* 22: 258-263
14. Ekelund M, Ekman R, Håkanson R, Sundler F (1984) Continuous infusion of somatostatin evokes an escape of gastric acid inhibition in the rat. *Gastroenterology* 86: 861-865
15. Yamatani T, Kadowaki S, Chiba T, Abe H, Chihara K, Fukase M, Fujita T (1986) Calcitonin gene-related peptide stimulates somatostatin release from isolated perfused rat stomach. *Endocrinology* 118: 2144-2145
16. Semb LS, Aune S (1971) The effect of glucose and insulin on pancreatic blood flow in the anesthetized pig. *Scand J Lab Invest* 27: 105-111
17. Fischer U, Hommel H, Schmid E (1975) Dynamics of canine pancreatic blood flow and of insulin secretion during an intravenous glucose load. *Pflügers Arch* 358: 89-100
18. Jansson L, Hellerström C (1983) Stimulation by glucose of the blood flow to the pancreatic islets of the rat. *Diabetologia* 25: 45-50
19. Samols E, Marri G, Marks V (1965) Promotion of insulin secretion by glucagon. *Lancet* 2: 415-416
20. Taborsky Jr GJ, Ensink JW (1984) Contribution of the pancreas to circulating somatostatin-like immunoreactivity in the normal dog. *J Clin Invest* 73: 216-223
21. Ahrén B, Veith RC, Taborsky Jr GJ (1987) Sympathetic nerve stimulation versus pancreatic norepinephrine infusion. 1) Effects on basal release of insulin and glucagon. *Endocrinology* 121: (in press)
22. Dunning BE, Ahrén B, Taborsky Jr GJ (1986) Further evidence that norepinephrine does not mediate neural inhibition of basal insulin secretion. *Proceedings of the 68th Annual Meeting of the Endocrine Society*. pp 313 (Abstract)

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Dr. Bo Ahrén
Department of Pharmacology
Sölvegatan 10
S-223 62 Lund
Sweden