Pancreatic islet transplantation: studies on the technique and efficacy of islet isolation and transplantation

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6 Insulinotropic properties of cholecystokinin, gastric inhibitory polypeptide, and glucagon-like peptide-1 during perifusion of short-term cultured canine isolated islets*

Introduction

Insulin secretion from the ß-cells of the islets of Langerhans is regulated by nutrients, hormones and neural factors — the main controller being glucose, which stimulates insulin release and modulates the action of other secretagogues [1,2]. It is well established that oral glucose administration stimulates insulin release more than isoglycemic intravenous infusion [1]. The postprandial release and action of gut hormones, called "incretins", is held responsible for the alimentary augmentation of the insulin response to glucose [1–5]. Recent metabolic studies in dogs suggest that the incretin effect — which, normally accounts for roughly half of postprandial insulin [2,4,6] — may account for most (~80–90%) of the insulin released during mild postprandial hyperglycemia after islet transplantation [7,8]. Insulinotropic effects of physiological levels of the gut hormones cholecystokinin (CCK), gastric inhibitory polypeptide (GIP), and most recently glucagon-like peptide-1 (GLP-1) have been documented in vivo and in the isolated perfused pancreas, in various species — but effects of physiological levels of these peptides on isolated islets are not well established [9].

We, therefore, investigated whether physiological levels of CCK33, a major

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circulating form of duodenal CCK [5], GIP, or GLP-1, stimulate insulin release during perifusion of canine isolated islets at glucose levels as observed postprandially after islet transplantation. Canine islets were employed, because the dog is generally used as a preclinical model for islet transplantation.

Materials and Methods

After a 24-h fast, the splenic pancreas was removed at surgery in 27 adult, female, outbred, beagles (Harlan CPB, Zeist, The Netherlands) for collagenase isolation and density gradient purification of the islets, as described in detail previously [10]. The total volume of isolated islets and purity of the preparations (relative volume of islet tissue) were assessed by morphometry [10], and purity was consistently over 95%. The islets were cultured in bacteriological petri plates for 2 days at 36°C in a humidified ambient atmosphere in RPMI-1640 medium containing 4.2 mM sodium bicarbonate, 20 mM Hepes, 50,000 IU/l penicillin and 50 mg/l streptomycin, and supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratories, Irvine Ayrshire, UK), with a medium change after 24 h.

After culture the islets were pooled and 5% (v/v) aliquots of the islet suspension were transferred to 0.5-ml microchambers equipped with nylon membrane filters (pore size 5 μm; Pall, England) for in parallel perifusion of up to six chambers per experiment in the Acusyst-S system (Endotronics, Coon Rapids, MO, USA). The total volume of islets per chamber, averaged over the experiments, was 0.8 ± 0.2 μl (1 μl islet tissue corresponds to 566 islets with an average diameter of 150 μm). Media were drawn via 3-way disposable stopcocks at 0.25 ml/min, heated to 36°C and gassed with medical grade air. Islet were perifused with 2.5 mM or 7.5 mM glucose during an equilibration period from –90 to 0 min, and next subjected to various protocols as indicated in the figure legends. From –20 min, samples of the effluent perifusate fluid were collected and analysed for insulin content by radioimmunoassay with dog insulin as standard [10]. Data from duplicate chambers were averaged. Basal insulin release was defined as the mean value from –15 to 0 min, and amounted to 178 ± 38 and 49 ± 16 fmol/min per μl islets after equilibration at 7.5 mM and 2.5 mM glucose, respectively. To reduce the variability of insulin release between experiments, the data were expressed in insulin release times basal release. Figures have been corrected for the 1.5 ml dead space in the perifusion apparatus.

The basic perifusion fluid was Krebs-Ringer-Hepes containing 4.2 mM $NaHCO_3$, 10 mM Hepes, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KCl, 1.2 mM

NaH_2PO_4, 118 mM NaCl (at 0 mM glucose), and 0.2% (w/v) bovine albumin fraction V (cell culture tested, Sigma, St. Louis, MO, USA). The sodium chloride content was adjusted to the amount of glucose added to maintain the osmolarity constant. Synthetic porcine CCK33, synthetic porcine GIP, and synthetic human GLP-1 (7–36 amide) were purchased from Peninsula (code 7580, 7192, and 7168 resp., Merseyside, St. Helens, UK). Stock solutions were prepared in a phosphate buffer (4 mM Na_2HPO_4, 1 mM NaH_2PO_4, and 0.5 % (w/v) bovine serum albumin; pH 7.4) aliquoted, and kept below –70°C until use. Net peptide content rather than gross weight was used for calculation of concentrations.

Calculation and statistical analysis

Results are expressed as mean ± SE The (weighed) mean insulin response during individual perifusion experiments was calculated by dividing the integrated insulin response (obtained using the trapezoidal rule) by its time interval. Differences between means were evaluated by analysis of variance with multiple comparisons by Scheffé's test and Fisher's protected least significant difference procedure. Differences were considered NS at $p > .05$.

Results

We first studied the sensitivity of the ß-cells to CCK33 and GIP during sequential perifusion of the islets with 0.1 nM, 1 nM, and 10 nM of the peptides at an ambient glucose level of 7.5 mM. The insulin response curves are shown in Fig. VI.1, and the corresponding mean increment of insulin release over the first 15 min period at each peptide level is listed in Table VI.1. CCK33 elicited

Table VI.1

Insulin response to sequential perifusion of canine islets with 0.1, 1, and 10 nM of either CCK33 or GIP in parallel at 7.5 mM glucose

Peptide levels (nM)	CCK33	GIP
0.1	0.09±0.02*	0.13±0.04†
1	0.18±0.05	0.48±0.16‡
10	0.20±0.05	1.87±0.51

Corresponding response curves are shown in Fig. VI.1. Values are means ± SE ($n = 5$) of the mean insulin response during the first 15 min of perifusion with each of the indicated peptide concentrations, over the basal release rate (defined as the mean value from -15 to 0 min). * $p < .05$ *vs* 1 and 10 nM CCK33. † $p < .01$ *vs* 1 nM GIP; $p < .001$ *vs* 10 nM GIP. ‡ $p < .01$ *vs* 10 nM GIP

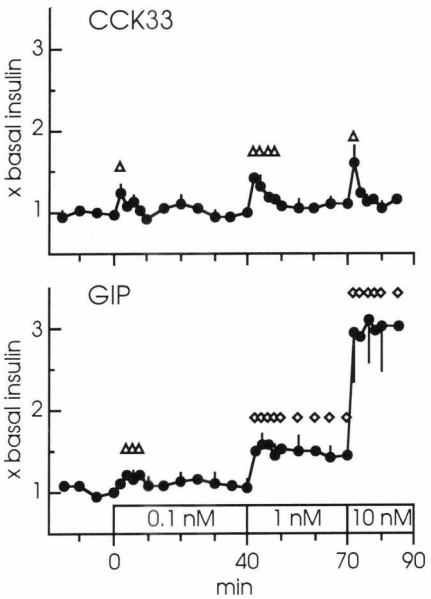

Fig. VI.1

Insulin response to sequential perifusion of canine islets with 0.1 nM (from 0 min), 1 nM (from 40 min), and 10 nM (from 70 min) CCK33, or GIP.
Islets were perifused at a 7.5 mM glucose level, both during the equilibration period (–90 to 0 min), and during the experimental period (0–85 min).
In each experiment both peptides were perifused in duplicate and in parallel. Values are the mean ± SE of 5 experiments. Significance of the difference with baseline level (mean value from –15 to 0 min) is indicated by triangles ($p < .01$) and diamonds ($p < .001$).

an acute, transient, insulin response from the lowest level tested (Fig. VI.1). The incremental insulin output increased from 0.09-times basal insulin at the 0.1 nM CCK33 level, to a maximum of ~0.20-times basal insulin from the 1 nM level (Table VI.1). GIP elicited a small, transient, insulin response at the 0.1 nM level (Fig. VI.1), and sustained dose-dependent insulin increments at higher GIP levels, which averaged 0.48- and 1.87-times basal insulin output in response to 1 and 10 nM GIP, respectively (Table VI.1).

We next studied the potential insulinotropic effects of physiological levels of both CCK33 (20 pM), GIP (500 pM), and GLP-1 (100 pM) by in parallel perifusion at 2.5, 7.5, and 10 mM glucose levels. Prior to perifusion of the peptides a biphasic, sustained, and dose-dependent insulin response to glucose stimulation was observed (Fig. VI.2). No significant insulin response to 20 pM CCK33 was observed at all glucose concentrations. Additional experiments specifically designed to detect possible small insulinotropic effects of CCK33 by in parallel continuous perifusion of the islets with 10 mM glucose in the co-presence or absence of 20 pM CCK33 (graph inserted in the top panel of Fig. VI.2) demonstrated prolonged stable insulin release during the continuous

perifusion with glucose only, and also failed to demonstrate an incretin effect of CCK33. GIP potentiated glucose-stimulated insulin from the 7.5 mM glucose level (Fig. VI.2) and the insulin increment over prestimulus release (i.e., mean insulin output from 30–40 min) doubled from 0.48- to 1.09-times basal insulin output at the 7.5 and 10 mM glucose level, respectively (Table VI.2). GLP-1 transient stimulated insulin release at the 2.5 mM glucose level (Fig. VI.2), and elicited a sustained maximum response amounting to 3– to 4-times basal insulin release, from the 7.5 mM glucose level (Table VI.2). Overall, the mean incretin effect of GLP-1 at both the 7.5 and 10 mM glucose levels (3.9 ± 1.7) was fivefold greater ($p < .05$) than the effect of GIP (0.79 ± 0.15) at these glucose levels.

Discussion

Generally, pharmacological levels (~10–100 nM) of CCK and GIP were required to demonstrate insulinotropic effects in previous studies of isolated islets [11–14]. However, exploration of ß-cell sensitivity in our model during perifusion with graded (0.1–10 nM) doses of these peptides demonstrated insulinotropic effects from the lowest dose used. We, therefore, subsequently focused on the potential insulinotropic effects of physiological levels of CCK33 (20 pM), GIP (500 pM) and GLP-1 (100 pM) — i.e., levels close to the reported circulating peak levels of ~10–20 pM CCK, ~200–400 pM of the biologically active 5-kDa form of GIP, and ~50–100 pM GLP-1 [1,4,6,7,15–17].

Table VI.2

Glucose dependence of the insulin response to physiological GIP (500 pM) and GLP-1 (100 pM) levels

Glucose levels (mM)	GIP	GLP-1
2.5	0.15±0.11*	0.39±0.15†
7.5	0.48±0.10*	3.31±1.82
10.0	1.09±0.22‡	4.46±3.16

Corresponding response curves are shown in Fig. VI.2. Values are means ± SE ($n = 5$) of the mean insulin response during 30 min perifusion with the peptides (from 40-70 min), over the prestimulus release rate (defined as mean value from 30-40 min) at the respective glucose concentrations. * $p < .05$ *vs* effect of GIP at a next higher glucose level. † $p < .01$ *vs* effect of GLP-1 at higher glucose levels. ‡ $p < .001$ *vs* effect of GIP at 2.5 mM glucose

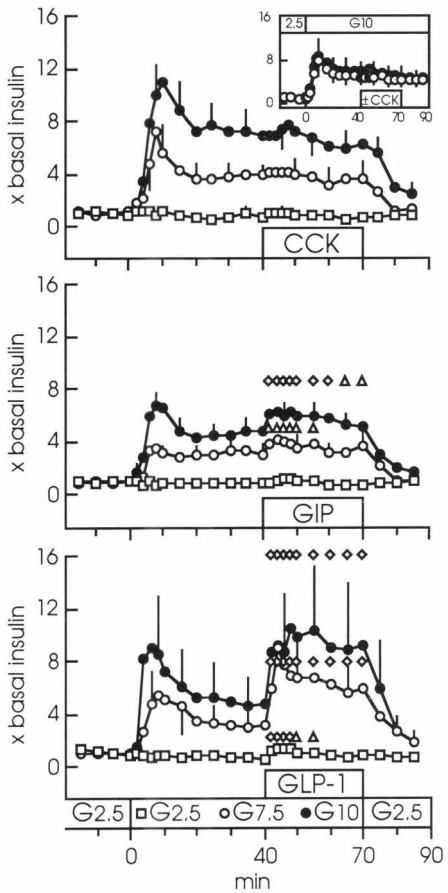

Fig. VI.2

Insulin response to physiological concentrations of CCK33 (20 pM), GIP (500 pM), and GLP-1 (100 pM) during perifusion of canine islets at 2.5, 7.5, and 10 mM glucose.
Islets were perifused with 2.5 mM glucose during the equilibration period (–90 to 0 min), from 0–70 min with either 2.5, 7.5, or 10 mM glucose, and from 70–85 min with 2.5 mM glucose.
In each experiment the peptide, was perifused from 40–70 min at the different glucose levels, in duplicate and in parallel.
Values are the mean ± SE of 5 experiments for each peptide.
Significance of difference with the prestimulus level (mean value from 30 to 40 min) is indicated by triangles ($p < .01$) and diamonds ($p < .001$).

Insert top panel
In 7 additional experiments (graph inserted in the top panel) the insulin response was further examined during in parallel continuous 10 mM glucose perifusion from 0 min, either with (closed symbols) or without (open symbols) the addition of 20 pM CCK33 from 40 to 70 min.

Pharmacological, but not physiological, CCK33 levels stimulated insulin release in our model. Likewise, pharmacological CCK levels evoked a transient insulin response in the perfused dog pancreas [5,18] but no incretin effect has been demonstrated in vivo in dogs and man during low dose CCK infusion, or the postprandial infusion of CCK receptor antagonists [5,17,19,20]. By contrast, in rat studies [2,5,21] incretin effects have been demonstrated during infusion of low dose CCK or CCK receptor antagonists — which, indicates a more predominant role of CCK as an incretin in this species.

Physiological levels of both GIP and GLP-1 potentiated glucose-stimulated insulin release in our model. In concert with these findings, in vivo studies in man [6,15,22] and in vitro studies of the perfused rat pancreas [4,21,23,24] have

demonstrated insulinotropic effects of (near-) physiological doses of exogenous GIP and GLP-1 under mild hyperglycemic conditions. In previous dog studies, pharmacological levels of GIP and GLP-1 have been reported to stimulate insulin secretion in the perfused pancreas [18,24] but in vivo low dose infusion of GLP-1 was ineffective [25]. Our finding that GLP-1, in contrast to GIP, is effective from a 2.5 mM glucose level confirms in vitro rat reports that high dose GLP-1 stimulates insulin secretion from this glucose level [23,26] and further corroborates recent reports that (near-) physiological doses of GLP-1, in contrast to GIP, are effective during basal glycemia in man [3,22,27]. This, and our finding that the maximum effect of GLP-1 was attained at a lower glucose level than observed with GIP, substantiate the contention that GLP-1 is a major incretin during euglycemia. GLP-1 was a considerably more potent incretin than GIP in our model, consistent with previous reports [3,4,6,15,22]. Note, however, that the potency comparison is limited by species differences associated with the use of canine islets and porcine GIP, which differs from canine GIP [28], and human GLP-1, which may be identical to canine GLP-1, because the structure of GLP-1 is identical in all mammals studied so far [3].

In contrast to the in vivo studies and in vitro studies of the perfused pancreas noted above, pharmacological concentrations (~10–100 nM) of CCK, GIP, and GLP-1 were generally required to stimulate insulin release from freshly isolated islets [11–14,26]. The low ß-cell sensitivity to gut hormones in the latter studies has been attributed to the trauma associated with islet isolation, and unknown in vitro conditions. In our study, in contrast, the islets were cultured two days before perifusion, to ensure recovery from isolation-associated trauma. Thus, our results indicate short-term culture of islets of paramount importance in the response to physiological levels of these peptides — consistent with recent reports of the insulinotropic effect of a physiological dose of GIP during static incubation of 24-h cultured rat islets [9] and GLP-1 during static incubation of 4-day cultured rat neonatal dispersed pancreatic cells [29].

In conclusion, short-term cultured isolated islets do respond to physiological ß-cell stimulation with gut peptides. Our findings indicate that CCK is not an incretin in the dog, and corroborate the notion that GLP-1 is a major incretin during euglycemia. The physiological role of GIP during euglycemia is equivocal. However, both GIP and GLP-1 may contribute to a hyperglycemia-enhanced activation of the enteroinsular axis after islet transplantation, and other situations (e.g. type-2 diabetes) characterised by elevated glucose levels.

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