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Stimulation of insulin release in isolated rat islets by GIP in physiological concentrations and its relation to islet cyclic AMP content

E. G. Siegel and W. Creutzfeldt

Department of Medicine, Division of Gastroenterology and Metabolism, University of Göttingen, Göttingen, FRG

Summary. Several insulinotropic hormones have been shown to increase the level of cyclic AMP in isolated islets. This study was performed to investigate whether gastric inhibitory polypeptide (glucose-dependent insulin-releasing polypeptide) has a similar effect, in particular at concentrations close to the physiological level in blood. Collagenase isolated rat islets were maintained for 24 h in tissue culture (medium 199) and then incubated for 30 min for measurement of insulin release and cyclic AMP content. Glucose-induced (16.7 mmol/l) insulin release was enhanced by gastric inhibitory polypeptide 1–100 ng/ml (0.196–19.6 nmol/l) in a dose-related fashion. The cyclic AMP content was enhanced only by 100 ng/ml. However, when 0.1 mmol/l of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine was present, even 1 ng/ml of gastric inhibitory polypeptide increased both cyclic AMP content and insulin release. Such a concentration of the hormone can be measured in human blood after a meal. In contrast, in freshly isolated islets no effect of the hormone on glucose-induced insulin release or cyclic AMP content could be detected for concentrations ranging from 1 to 100 ng/ml. These findings demonstrate that the hormone sensitivity of isolated islets is markedly enhanced by short-term maintenance in tissue culture. The results suggest that an increase in cyclic AMP is seen in response to gastric inhibitory polypeptide and may be causally related to the insulinotropic effect of the hormone.

Key words: Isolated islets, tissue culture, insulin release, cyclic AMP, gastric inhibitory polypeptide, perifusion.

Glucose ingestion evokes a greater insulin response than its intravenous application [1]. This appears to be due largely to the release of intestinal peptides, but neural factors are also involved. The significance of intact innervation for normal glucose tolerance has been documented for transplanted islets [2] and normal rats [3]. Among the intestinal peptides which are of possible importance, gastric inhibitory polypeptide (GIP) fulfils several criteria of such an 'incretin'. It is released after a meal and increases in the blood [1, 4–6]; when infused in vivo to achieve levels seen after a meal, insulin is released [7], provided that the blood glucose concentration is elevated [1, 7]. In vitro, GIP also causes insulin release at stimulatory glucose concentrations. In the perfused pancreas, this has been demonstrated for GIP concentrations close to the physiological level [8], whereas in isolated islets GIP levels several-fold higher are needed to release insulin [9–11].

When investigating intracellular mechanisms one is obliged to use isolated islets or cells. The effects of hormones on cell activation appear to be largely mediated by the adenylate cyclase-cyclic AMP system [12, 13], the 'classical' hormone stimulator of insulin release being glucagon. The present study was designed to investigate whether in isolated islets insulin release and increased cyclic AMP levels could be observed by GIP concentrations as close to the physiological level as possible. For this purpose islets were used after a short time (1 day) in tissue culture.

Material and methods

Preparation of islets and maintenance in tissue culture

Pancreatic islets were isolated by the collagenase digestion technique [14] from male Wistar rats weighing 180–250 g (Collagenase from Worthington Biochemical Corp.). The islets were separated from the remaining exocrine tissue by handpicking. Batches of 250–400 islets were maintained for 24 h in plastic Petri dishes, 6 cm diameter (Greiner, Nürtingen, FRG) containing 4 ml tissue culture medium 199 [15] supplemented with 10% heat-inactivated newborn calf serum (Grand Island Biochemical Corp.), 14 mmol/l $NaHCO_3$, 400 IU/ml sodium penicillin G, 200 µg/ml streptomycin sulfate, 1 mmol/l N-2-hydroxyethylpiperazine-N′-ethane sulfonic acid (Hepes) and a glucose concentration of 8.3 mmol/l. The islets were maintained at 37 °C, pH 7.4 in an atmosphere saturated with water and gassed with an air-CO_2 mixture.

Static incubation for measurement of insulin release and cyclic AMP content

After the maintenance period, the islets were washed twice with a modified Krebs-Ringer bicarbonate buffer (KRB-Hepes) containing 2.8 mmol/l glucose by centrifugation (150 g) at room temperature, as described in detail previously [16]. This buffer was also used for the static incubation and contained 5 mmol/l $NaHCO_3$, 1 mmol/l $CaCl_2$, 0.2% bovine serum albumin and, in addition, 10 mmol/l Hepes. For the experiment, batches of 10 islets were then incubated for 30 min at 37° in 2 ml KRB-Hepes containing the respective stimulus. Flat bottom glass vials were used. Usually, 250 islets were used for one experiment allowing an $n = 5$ for each of the test conditions. The experiment was stopped by picking out the islets under the microscope and transferring them immediately into 200 µl of boiling acetate buffer (0.05 mol/l pH 6.2). The incubation buffer was chilled immediately.

Insulin release was determined by radioimmunoassay [17] using a rat standard and a guinea pig antiserum raised against beef insulin. The sensitivity of the assay was 0.25 ng/ml, the interassay variance 7.8% and the intraassay variance 5.6%. When freshly isolated islets were used, experiments were performed within 1 h after collagenase isolation. ^{125}I-Insulin and rat insulin standard were purchased from Behringwerke, Marburg, FRG.

Measurement of islet cyclic AMP content

After the experiment the islets were boiled for at least 20 min in 0.05 mol/l acetate buffer. Blanks for each of the incubation buffers were treated in the same way. Islet cyclic AMP levels were then determined by radioimmunoassay using a commercially available kit (Becton Dickinson and Co., Heidelberg, FRG) after succinylation of samples and standards according to the method of Cailla et al. [18]. This procedure considerably increases the sensitivity, allowing for the determination of less than 0.88 fmol/vial. This lowest standard was always clearly distinct from the zero binding. Samples were assayed in duplicate; the blanks were always less than 4% of the measured cyclic AMP content of the islets and not different between the respective incubation buffers; they were therefore not subtracted. Insulin release and cyclic AMP content were always determined for the same batch of islets.

Measurement of gastric inhibitory polypeptide (GIP)

GIP was measured using a radioimmunoassay described in detail previously [19]. A rabbit antiserum was used raised against porcine GIP. The sensitivity of the assay was 31 pg/ml and the intra assay variance was 7.9%. The molecular weight of GIP is 5105, i.e. 1 ng/ml = 0.196 nmol/l.

Perifusion for measurement of dynamic insulin release

The islets were perifused using a perifusion system described in detail previously [15, 20]. In brief, 40 islets were placed in a microchamber (volume 70 µl) and perifused with a flow rate of 1 ml/min resulting in a turnover of approximately 14 chamber volumes/min. The perifusate consisted of a KRB buffer containing 1.0 mmol/l Ca, 0.2% dialysed bovine serum albumin and 14 mmol/l $NaHCO_3$. The buffer was gassed with 95% O_2 and 5% CO_2 which allowed for a constant pH of 7.4 over the time of perifusion. The dead space of the whole system was 1.5 ml, and was always taken into consideration. Four chambers were always used in parallel. Usually, after 30 min of perifusion with a buffer containing glucose 2.8 mmol/l the islets were exposed to 16.7 mmol/l glucose for another 40 min. The buffer was then switched back to the buffer containing low glucose. Samples of the effluate were collected every minute, from minute 25 to min 80. These 1-min samples were assayed for insulin every minute, from min 25 to min 40, and then every 5th min until min 70, and, thereafter, again min 71, 72, 73, 75, 77, and 80.

Statistics

Statistical analysis was performed by Student's two-tailed t-test for unpaired data unless stated differently.

Results

Comparison of fresh and cultured islets in perifusion and static incubation

As both fresh and 24-h cultured islets were used, dynamic insulin release from the two types of islets was compared in perifusion. Both types of islets exhibited a rather sharp rise in the rate of insulin release starting 1 min after the exposure to glucose 16.7 mmol/l (Fig. 1). A first peak was reached at 3 min. For freshly isolated islets a slight nadir at 4–5 min was followed by a rapidly increasing second phase. For 24 h cultured islets, a pronounced nadir at 5–7 min was followed by a gradually increasing second phase allowing for the clear distinction of the two phases. Both types of islets returned rapidly to basal release values upon reexposure to the non-stimulating buffer containing 2.8 mmol/l glucose.

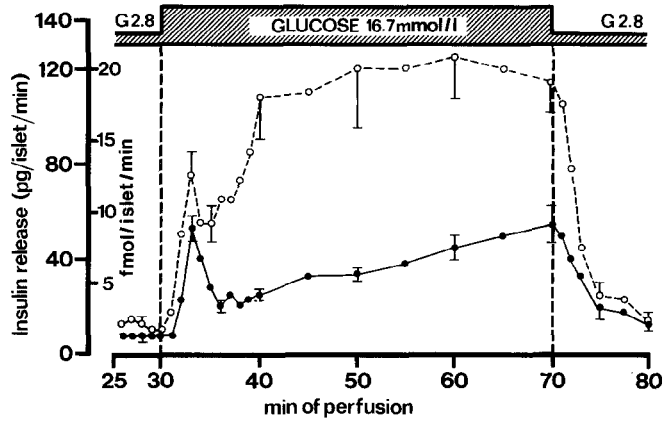

Fig. 1. Dynamic insulin release from freshly isolated and 24 h cultured islets. Both types of islets were perifused in microchambers for 30 min with a KRB buffer containing 2.8 mmol/l glucose and then stimulated for 40 min with glucose 16.7 mmol/l. They were then exposed to glucose 2.8 mmol/l. Values are means ± SEM, numbers of observations in parentheses; G, glucose; ○-----○ freshly isolated islets ($n=7$); ●—● 24 h cultured islets ($n=10$)

Since in subsequent experiments static incubation was used, the effect of glucose was also compared in fresh and cultured islets in 30-min static incubation. This was performed by pooling the results of a large series of experiments carried out in our laboratory. Basal release (2.8 mmol/l glucose) was not different for fresh (0.41 ± 0.06 ng/islet/30 min, $n=53$) and 24 h cultured islets (0.40 ± 0.04, $n=52$). Glucose 16.7 mmol/l increased the release of insulin in freshly isolated islets to 1.59 ± 0.13 ng/islet/30 min, $n=53$, and to 1.17 ± 0.05, $n=52$, in 24 h cultured islets. Thus, glucose-induced insulin release is slightly less in cultured islets ($p<0.005$), as in perifusion. However, there is less variance in the results of cultured islets per the SEMs. The insulin content decreased slightly during 24-h tissue culture from 55.8 ± 6.3 ng/islet, $n=13$ in freshly isolated islets, to 39.5 ± 1.3, $n=4$ after 24 h culture, the difference being not significant ($p>0.10$).

Effect of GIP on glucose-induced insulin release and cyclic AMP content in cultured islets

Insulin release was clearly stimulated by glucose 16.7 mmol/l, from 0.52 to 1.26 ng/islet, $p<0.001$ (Fig. 2). The addition of GIP resulted in a marked potentiation of insulin release. Even a concentration as low as 1 ng/ml caused a twofold increase ($p<0.02$). The effect of 16.7 mmol/l glucose on islet cyclic AMP content was only marginal ($0.05<p<0.10$). GIP 100 ng/ml enhanced the cyclic AMP content from 10.8 ± 0.9 to 14.1 ± 1.4 fmol/islet, $p<0.05$. No significant effect on cyclic AMP levels was detected in response to GIP 10 and 1 ng/ml.

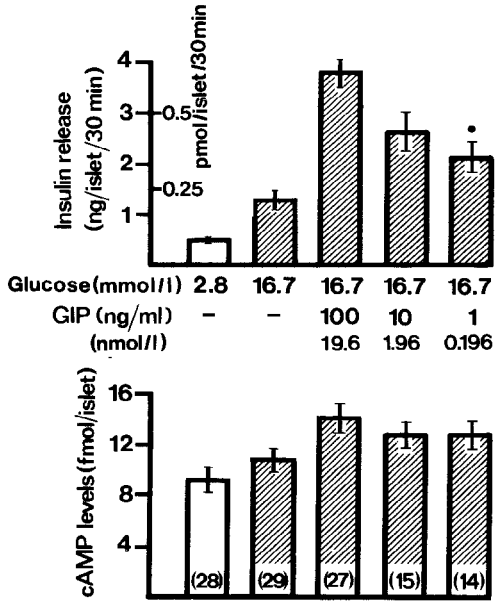

Fig. 2. Effect of GIP on insulin release and cyclic AMP levels in 24 h cultured islets. Islets were used after 24-h maintenance in tissue culture. They were then incubated in KRB Hepes buffer for 20 min. Insulin release and cyclic AMP content were always measured on the same batch of 10 islets per vial. Values are means ± SEM, numbers of observations in parentheses; GIP, gastric inhibitory polypeptide. 1 ng/ml GIP is equivalent to 0.196 mmol/l. ● denotes $p < 0.02$ vs glucose 16.7 mmol/l alone

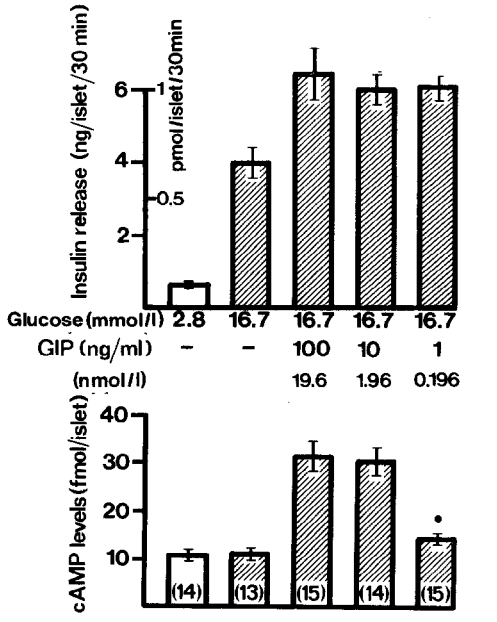

Fig. 3. Effect of GIP on insulin release and cyclic AMP levels in 24 h cultured islets (0.1 mmol/l IBMX present). Experiments were performed like in Figure 2, except that 0.1 mmol/l of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) was present during the 30-min incubation for all conditions. 1 ng/ml GIP is equivalent to 0.196 nmol/l. ● denotes $p < 0.05$ vs glucose 16.7 mmol/l

As glucose 16.7 mmol/l had only a marginal influence on islet cyclic AMP content, and a tendency to increase could be seen in most of the experiments, the effect of glucose was investigated in a large series of experiments carried out in our laboratory in part for other purposes. Cyclic AMP content was 7.9 ± 0.6 ($n = 43$) for glucose 2.8 and 9.7 ± 0.8 fmol/islet ($n = 43$) for glucose 16.7 mmol/l, which was again marginal ($0.05 < p < 0.10$). Since in almost every experiment (usually $n = 5$ for each of the conditions) there was a slight increase, the effect of glucose was also compared on a paired basis always using the mean of one experiment obtained at either 2.8 or 16.7 mmol/l glucose. For this way of analysis, the incremental effect of 16.7 mmol/l glucose was 1.85 ± 0.75 ($n = 9$, $p < 0.05$), which was significant. At 2.8 mmol/l glucose, no effect of GIP on insulin release was seen up to 400 ng/ml (data not shown).

Effect of GIP on glucose-induced insulin release and cyclic AMP content in the presence of IBMX

As cyclic AMP is rapidly disintegrated by phosphodiesterases, the same protocol as for Figure 2 was employed in the presence of 0.1 mmol/l of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX). Results are shown in Figure 3. Consciously, not a high concentration of IBMX was used. Glucose-induced insulin release was clearly enhanced again by all the concentrations of GIP employed ($p < 0.001$ for each of the conditions). GIP also augmented the cyclic AMP content of the islets, with the effect achieving significance even for the lowest concentration employed: GIP 1 ng/ml increased the cyclic AMP levels from 11.4 ± 1.0 to 14.6 ± 1.0 fmol/islet, $p < 0.05$.

Effect of IBMX on insulin release and cyclic AMP content

Comparing Figures 2 and 3, it might seem as if 0.1 mmol/l IBMX had no effect on cyclic AMP levels at 2.8 and 16.7 mmol/l glucose, although a twofold increase is observed when GIP 10 or 100 ng/ml is present. However, the islets originated from different pools and can, therefore, not be compared. Subsequently, the effects of IBMX were investigated at both low and high glucose in islets originating from the same pool(s) (Table 1). Under such controlled conditions, 0.1 mmol/l IBMX caused an increase of insulin release and cyclic AMP content both at low and high glucose ($p < 0.02$ or less). When in another series of experiments a high concentration of IBMX was tested, these effects were even more pronounced (Table 1 B).

Effect of GIP in freshly isolated islets on insulin release and islet cyclic AMP content

The experiments shown in Table 2 were carried out on freshly isolated islets in the same fashion as in Figure 2. In freshly isolated islets, 16.7 mmol/l glucose caused a threefold increase in insulin release and cyclic AMP content after 30 min. No significant effect of GIP could be detected on insulin release or cyclic AMP content. This was also the case when, in a small series, 0.1 mmol/l IBMX was present in the incubation medium (data not shown). Since this could be due to breakdown of the hormone GIP, its concentration was mea-

Table 1. Effect of IBMX (3-isobutyl-1-methylxanthine) on insulin release and cyclic AMP levels in 24-h cultured islets

		Insulin release (ng/islet/30 min)	Cyclic AMP level (fmol/islet)
A	G 2.8 mmol/l	0.34 ± 0.06 (13)	8.8 ± 1.0
	G 2.8 mmol/l + IBMX 0.1 mmol/l	0.76 ± 0.12 (14)	15.0 ± 2.0
	G 16.7 mmol/l	1.50 ± 0.12 (13)	9.5 ± 1.1
	G 16.7 mmol/l + IBMX 0.1 mmol/l	4.70 ± 0.46 (14)	21.1 ± 2.9
B	G 2.8 mmol/l	0.28 ± 0.06 (12)	10.2 ± 1.1
	G 2.8 mmol/l + IBMX 1 mmol/l	0.92 ± 0.10 (12)	47.2 ± 6.2
	G 16.7 mmol/l	0.80 ± 0.12 (12)	17.0 ± 3.1
	G 16.7 mmol/l + IBMX 1 mmol/l	7.10 ± 0.42 (12)	55.0 ± 6.6

Islets were incubated after culture in KRB-Hepes buffer for 30 min. Values are presented as mean ± SEM, number of experiments in parentheses. G, glucose

Table 2. Effect of GIP on insulin release and cyclic AMP levels in freshly isolated islets after 30-min incubation

	Insulin release (ng/islet/30 min)	cyclic AMP level (fmol/islet)
G 2.8 mmol/l	0.46 ± 0.03 (15)	9.6 ± 2.2
G 16.7 mmol/l	1.45 ± 0.30 (15)	27.5 ± 2.5
G 16.7 mmol/l + GIP 100 ng/ml	1.55 ± 0.36 (14)	25.9 ± 2.5
G 16.7 mmol/l + GIP 10 ng/ml	1.84 ± 0.39 (14)	29.9 ± 3.0
G 16.7 mmol/l + GIP 1 ng/ml	1.67 ± 0.26 (14)	21.3 ± 2.0[a]

[a] $p > 0.05$ vs G 16.7 mmol/l

Islet cAMP content was measured as described in Methods. 1 ng/ml GIP is equivalent to 0.196 mmol/l. 1 ng/ml insulin = 0.17 nmol/l. Values are presented as mean ± SEM, numbers of experiments in parentheses. G = glucose; GIP = gastric inhibitory polypeptide.

sured before and after an additional experiment. For the buffer containing the addition of 10 ng/ml, the concentration determined by radioimmunoassay was 14.0 ± 0.4 ($n = 3$) before the experiment, 12.1 ± 0.8 ($n = 3$) after the experiment and 13.7 ± 0.9 ($n = 5$) for incubation vials treated in the same way but without islets. The measured concentration of GIP after the experiment was 104.5 ± 4.2 ($n = 4$) when islets were incubated at GIP 100 ng/ml and 1.02 ± 0.03 ($n = 5$) when islets were incubated at 1 ng/ml.

Discussion

The in vitro maintenance of isolated islets in tissue culture offers several advantages compared to the use of freshly isolated islets. Insulin release is clearly biphasic with a clear nadir separating the two phases (Fig. 1), a pattern very close to the release pattern seen in humans under hyperglycaemic glucose clamp conditions [7] and in the perfused pancreas [8]. Furthermore, such islets can be loaded with isotopes to isotopic equilibrium, e.g. ^{45}Ca [21], facilitating the interpretation of flux studies. Third, the hormone sensitivity after culture has been demonstrated to be increased compared to freshly isolated islets with respect to glucagon [22] and somatostatin [23]. This has been attributed to a possible regeneration of membrane receptors which probably are damaged by the collagenase digestion. With respect to GIP, a striking increase in the hormone sensitivity is seen in the results presented in this study.

Results shown in Figure 2 demonstrate a dose-related increase in glucose-induced insulin release for GIP concentrations between 100 and 1 ng/ml. The lowest concentration is clearly in the physiological range since values of 2 ng/ml are observed after an oral glucose tolerance [1, 4, 7] or a mixed meal [1, 5]. Thus, the hormone sensitivity of these cultured islets is comparable to the in vivo situation. This is in marked contrast to the finding that in freshly isolated islets even the highest GIP concentration had no effect on insulin release. In two previous studies concentrations greater than 1 μg/ml had to be employed [9, 11]; 1 μg/ml just caused a 5% increase [9]. In two recent studies, 10 and 15 ng/ml had stimulatory effects [10, 24]. Since apparently the GIP concentration surrounding the islets is not altered during the 30-min incubation, the lack of a stimulatory effect is not due to major breakdown of the hormone. One may, therefore, assume that receptor function is impaired during islet isolation and that the hormone sensitivity can be completely restored by the 24 h maintenance in tissue culture.

A significant increase in islet cyclic AMP content could only be observed in response to the highest GIP concentration used (Fig. 2). Nevertheless one might expect that also the low GIP concentration should be associated with changes in the adenylate-cyclase cyclic AMP system, since hormones have been shown to act through activation of adenylate cyclase [12, 13]. This has been reported also for GIP [25]. An effect on cyclic AMP content could have been missed either due to the time course (highest cyclic AMP contents are seen early [13, 22]), compartmentalization of cyclic AMP and/or activation of phosphodiesterase [12, 13]. Therefore, experiments were also carried out in the presence of the phosphodiesterase inhibitor IBMX. An intermediate concentration of 0.1 mmol/l was used which under controlled conditions augmented both insulin release and cAMP content (Table 1). As expected, glucose-induced insulin release was clearly enhanced. Under these conditions the lowest concentration of GIP also increased cAMP content apart from stimulating insulin release. In freshly isolated islets, a stimulatory effect on ^{3}H-cyclic AMP accumulation and insulin release could only be seen in response to a concentration of approximately 15 ng/ml, whereas 1.5 ng/ml were ineffective [10].

The lack of a clear stimulatory effect of glucose on cyclic AMP content in these islets contrasts with its marked effect in freshly isolated islets (Table 2). This has been found before [16] and also when ^{3}H-cyclic AMP accumulation was measured in the two types of islets in paired conditions [22]. In the present study there was a marginal effect in cultured islets when a large series of experiments was evaluated, the value of

significance being $p < 0.05$ under paired and $0.05 < p < 0.10$ under unpaired conditions. Therefore, an effect of glucose on cAMP content cannot be excluded by the present study; on the other hand, clarification of this point was not the purpose of the study.

The further mechanism of cyclic AMP action on intracellular activation is still a matter of investigation, in particular whether increased cAMP is a primary or secondary event. Calcium ions play an important role and an increase of ionized calcium in the B-cell cytosol is generally thought to be one important step [12, 13, 20, 26]. Agents that increase cAMP like IBMX via phosphodiesterase inhibition or glucagon via activation of adenylate cyclase have both been demonstrated to increase $^{45}Ca^{++}$ efflux [16, 27] which may reflect an increase in cytosol Ca^{++}. On the other hand, Ca^{++} with the need of calmodulin activates adenylate cyclase [28, 29]. Certainly, the calcium-calmodulin-cAMP interactions are still a matter of investigation. With respect to this the present study demonstrates that, in vitro, a physiological hormonal stimulus is able to stimulate insulin release at physiological concentrations. The content of one important second messenger, cyclic AMP, is increased.

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Dr. E. G. Siegel
Zentrum Innere Medizin, Georg-August-Universität
Robert-Koch-Str. 40
D-3400 Göttingen, FRG

Note added in proof. Using this system of cultured islets, it has been shown recently [Schmidt WE, Siegel EG, Creutzfeldt W (1985) Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. Diabetologia 28: 704-707] that glucagon-like peptide-1 (GLP-1) exerted a GIP-like effect on glucose-induced insulin release.