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Synergistic stimulatory effect of glucagon-like peptide-1 (7–36) amide and glucose-dependent insulin-releasing polypeptide on the endocrine rat pancreas

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The interaction of glucagon-like peptide-1 (7–36)amide (GLP-1) and glucose-dependent insulin-releasing polypeptide (GIP) on insulin release from the perfused rat pancreas was studied. The GLP-1 stimulated (0.5 nmol/l), glucose-induced (6.7 mmol/l) insulin secretory answer was enhanced by GIP (0.1, 1.0 and 10.0 nmol/l) to the arterial perfusate. This effect was maximal at 1 nmol/l GIP and smaller but still significant at 0.1 nmol/l GIP. The high GIP concentration of 10 nmol/l GIP did not further increase insulin secretion compared to the stimulation by 1 nmol/l GIP. Our data demonstrate an additive synergistic effect of GLP-1 and GIP on the glucose-induced insulin release. This supports the concept of an action 'in concert' of gastrointestinal incretin hormones postprandially released on the endocrine pancreas to guarantee adequate insulin answers after meals.

Incretin; Glucagon-like peptide-1 (7–36)amide; Glucose-dependent insulin-releasing polypeptide; Insulin secretion

1. INTRODUCTION

It is believed that hormones released from the gastrointestinal tract exert an influence upon the secretion of pancreatic islet cell hormones [1]. The physiological function of such an 'entero-insular axis' would be to augment or modify the islet cell hormone responses to ingested substrates [2]. Incretin hormones, one part of this system, refer to the endocrine factors liberated from the gut which potentiate the insulin response to oral ingestion of food [1]. Previously, since it acts as potent insulin secretagogue GIP was the leading candidate for a physiological relevant incretin [3]. However, strong evidence from various studies suggested that additional factors are of importance to fully explain the incretin effect [4,5]. Recent studies claim the proglucagon derived glucagon-like peptide-1 (7–36)amide (GLP-1) to represent an important, powerful incretin hormone in animals and humans [6–9,24]. The current study was undertaken to examine the possible interaction of GIP and GLP-1 on insulin secretion. This was of interest since previous studies showed that functional interactions of different gut hormones at the pancreatic B-cell are of significance in regulating the insulin response to achieve adequate postprandial insulin answer [10,11].

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2. MATERIALS AND METHODS

2.1. *Substances*

Synthetic GLP-1 and GIP were purchased from Peninsula (St. Helens, Merseyside). Bovine serum albumin (fraction V) was from Serva (Heidelberg, FRG) and aprotinin (Trasylol) from Bayer (Leverkusen, FRG). All other chemicals used were of analytical grade and were purchased from Merck (Darmstadt, FRG).

2.2. *Animals*

Male albino Wistar rats (180–240 g) kept in a light and temperature controlled room were fed a standard diet (Altromin, Lage, FRG) and had free access to water ad libitum.

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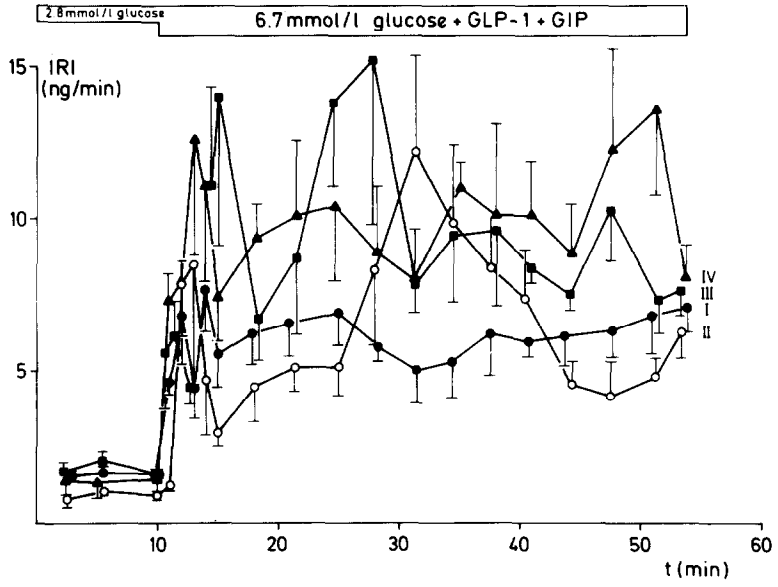

Fig.1. Effect of GIP (0.1, 1, 10 nmol/l) on glucose (6.7 mmol/l)-induced and GLP-1 (0.5 nmol/l)-stimulated insulin secretion from the isolated perfused rat pancreas. (I) Glucose + GLP-1; (II) glucose + GLP-1 + GIP (0.1 nmol/l); (III) glucose + GLP-1 + GIP (1 nmol/l); (IV) glucose + GLP-1 + GIP (10 nmol/l).

2.3. *Perfusion experiments*

Rats were anesthetized by an intraperitoneal injection of pentobarbitone sodium (45 mg/kg body wt). The pancreas, spleen, stomach, and proximal part of the duodenum were perfused through the cannulated abdominal aorta as described previously [12]. The entire preparation was removed from the cadaver and placed into a perfusion chamber. The perfusion media consisted of a Krebs-Henseleit bicarbonate buffer (pH 7.4 when gassed with 95% O and 5% CO) as was detailed before [20]. The venous effluent was collected on aprotinin (1000 U/fraction) in intervals by a cannula inserted into the portal vein. The perfusion pumps were adjusted to generate a constant flow rate

Table 1

Effect of glucagon-like peptide-1 (7–36)amide and GIP on insulin secretion from the isolated perfused rat pancreas

Glucose (mM)	GLP-1 (nM)	GIP (nM)	*n*	First phase (0–8 min)	Second phase (9–44 min)
6.7	–	–	7	25.7 ± 10.1	100.9 ± 43.8
6.7	0.5	–	6	68.1 ± 11.8[a]	248.6 ± 164.5[a]
6.7	0.5	0.1	6	51.2 ± 24.8	385.6 ± 37.0[bc]
6.7	0.5	1	6	77.0 ± 20.4[c]	439.6 ± 43.7[bc]
6.7	0.5	10	6	77.6 ± 10.5[c]	444.9 ± 67.5[bc]
6.7	–	0.1	5	30.7 ± 10.0	137.2 ± 37.7
6.7	–	1	6	30.6 ± 6.7	157.7 ± 20.6
6.7	–	10	6	46.5 ± 9.2[a]	208.0 ± 29.9[a]

[a] Significantly different integrated insulin secretion rate compared to controls (sole glucose, $p < 0.05$)

[b] Significantly different integrated insulin secretion rate compared to experiments with glucose and GLP-1 ($p < 0.05$)

[c] Significantly different integrated insulin secretion rate compared to experiments with glucose and GIP ($p < 0.05$)

Given are means ± SE of (n) experiments

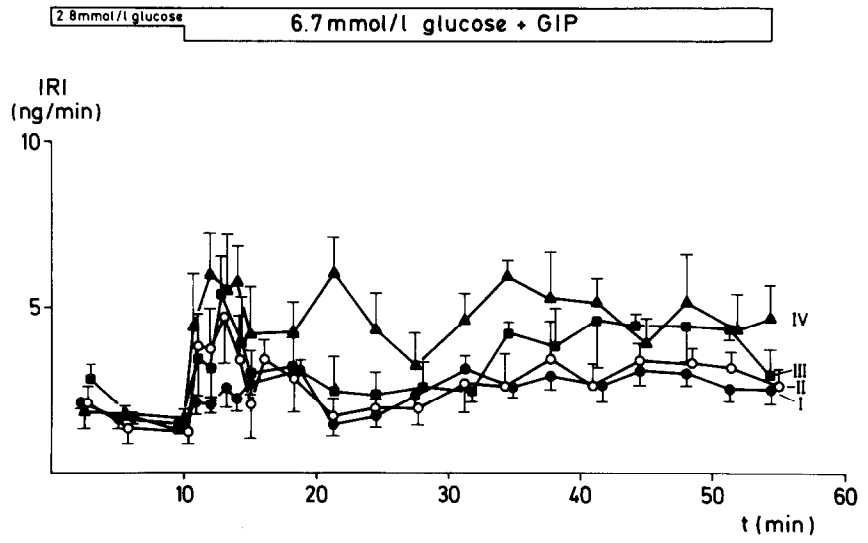

Fig.2. Effect of 0.1, 1 and 10 nmol/l GIP on glucose (6.7 mmol/l)-induced insulin secretion from the isolated perfused rat pancreas. (I) Glucose; (II) glucose + 0.1 nmol/l GIP; (III) glucose + 1 nmol/l GIP; (IV) glucose + 10 nmol/l GIP.

of 4 ml/min. One perfusion experiment lasted for 54 min. After a basal period of 10 min for equilibration (2.8 mmol/l glucose) the glucose concentration was raised to 6.7 mmol/l glucose. Under the latter condition the effect of GIP (0.1, 1.0 and 10 nmol/l) on the glucose-induced (6.7 mmol/l) and GLP-1 (0.5 nmol/l) stimulated insulin was investigated. Insulin was measured by radioimmunoassay [13]. The standard was a mixture of rat insulin I and II and was obtained from Novo (Mainz, FRG). Integrated insulin secretion was determined as secretion rate of the first (ng insulin/0–8 min) and the second phase (ng insulin/9–44 min) of the biphasic insulin answer from the perfused rat pancreas.

2.4. *Statistics*

Statistical analysis was performed by the U-test. Statistical significance was set at the 5% level.

3. RESULTS

After perfusion of the pancreata by media containing glucose (6.7 mmol/l) or glucose plus GLP-1 (0.5 nmol/l) a typical biphasic pattern of the insulin answer was observed (fig.1). The GLP-1 concentration considered in the present experiments exerted a significant but weak stimulatory effect on insulin secretion (fig.1). This finding corroborates recent reports describing the dose-response relationship between GLP-1 stimulation and insulin release from the perfused pancreas [7,14,15]. 0.5 nmol/l GLP-1 increased the glucose-induced insulin release in both the first and the second phase of the secretory insulin response compared to glucose stimulation of secretion alone (fig.2, table 1). GIP in our hands showed a glucose- and concentration-dependent insulinotropic action as was described before [15,16]. The combination of GLP-1 (0.5 nmol/l) and GIP revealed an additive, synergistic effect on insulin release (fig.1, table 1). This effect was dependent on the GIP concentration used. In the presence of 0.5 nmol/l GLP-1 this effect was maximal at 1 nmol/l GIP and smaller but still significant at 0.1 nmol/l GIP (table 1). The effect was not further enhanced at the higher GIP concentration of 10 nmol/l GIP. The synergistic interaction of both hormones on insulin release was more pronounced on the second phase of the secretory answer (table 1).

4. DISCUSSION

It is believed that the nutrient-dependent release of gut hormones is of significance in the regulation of endocrine pancreatic function [2]. Strong evidence exists now that this regulation is facilitated by interactions of various incretin factors [10,11]. It was of special interest to study the interaction of GIP and GLP-1 on insulin release. GIP until recently, played a unique role as incretin

candidate, and GLP-1 is now a promising new candidate peptide for the modulation of insulin secretion in response to carbohydrates [6–9,14].

In a recent study Zawalich reported that the combination of GIP plus cholecystokinin (CCK) together with 7 mmol/l glucose induced a markedly amplified insulin response from rat perifused islets [11]. Ahren et al. [10] demonstrated previously that GIP and CCK-8 in low doses possess strong insulinotropic activity when injected together in mice. It is known that GIP facilitates the glucose-induced insulin output by elevating islet cAMP [17]. Furthermore, CCK, via the phosphoinositides, generates at least several second messengers in islets [11]. Therefore, it was suggested that CCK and GIP modulate the levels of separate second messengers and that their synergistic impact on insulin secretion might be explained by these separate actions on those second messenger systems [11].

Recently, we have shown that the GLP-1 stimulated, glucose-induced insulin release is strongly potentiated by addition of CCK-8 [19,20]. Since the signal transmission after binding of GLP-1 to B-cells is adenylate-cyclase linked [21–23], we have explained the CCK potentiation of the GLP-1 effect on insulin by a synergistic interaction of the different second messenger systems as was suggested by Zawalich in the case of GIP and CCK. Our present results indicate an additive effect of GLP-1 and GIP on insulin secretion. This effect was maximal at 1 nmol/l GIP and was not further enhanced at the higher GIP concentration. However, when the glucose-induced insulin secretion was stimulated by GIP alone (fig.2) the resulting insulin release was stronger in response to 10 nmol/l GIP compared to the lower concentration of 1 nmol/l. Since both hormones, GIP and GLP-1, act via the adenylate cyclase system the combination of both seems to result in only an additive effect. It seems likely, that the interaction of lower, i.e. respectively submaximal, effective concentrations of GIP and GLP-1 could result in the full cAMP answer in the B-cell which is not further increased by higher amounts of the individual hormones. In conclusion, our data suggest an additive effect of GLP-1 and GIP on the glucose-induced insulin release from the perfused pancreas. This supports the concept of an existence of several incretin factors in the enteroinsulinar axis with functional interactions on the pancreatic B-cell level.

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