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Prior in vitro exposure to GLP-1 with or without GIP can influence the subsequent beta cell responsiveness

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Abstract

Glucagon-like peptide-1 (7–36) amide (GLP-1) and glucose-dependent insulinotropic peptide (GIP) potentiate glucose-induced insulin release when present at the time of nutrient stimulation. This study examines whether they can also influence rat beta cell responsiveness to subsequent stimulations. When rat beta cells were cultured for 24 h with 1 nM GLP-1, they progressively desensitized to subsequent GLP-1 stimuli, as evidenced by cellular cAMP production. This GLP-1-induced desensitization did not occur when the incretin was only present during three periods of 1 h at 10 mM glucose that alternated with 6–9 h culture at 3 mM glucose. After these 24 h, the beta cells exhibited the same secretory response to glucose (10 mM) and GLP-1 (10 nM at 10 mM glucose), whether GLP-1 was present during the pulses or not. Similarly the presence of 1 nM GIP during these one hour pulses did not influence subsequent secretory responses to glucose and GLP-1. However, when both GLP-1 and GIP, each at 0.5 nM, were added to the one hour pulses, they not only amplified insulin release during the pulses, as was the case with their single addition, but also increased the secretory response to a subsequent stimulation by glucose and GLP-1. These data distinguish between a desensitization effect of a prolonged exposure to GLP-1 and a positive priming effect of a discontinuous exposure to a combination of GLP-1 plus GIP. They may have to be taken into account in drug treatment strategies aiming the mimicking of physiologic patterns in the regulation of insulin release.

Keywords: GLP-1; GIP; Insulin release; Beta cells; Desensitization; Cyclic AMP; Priming

1. Introduction

The acute potentiating effects of the gastrointestinal hormones glucagon-like peptide-1 (7–36) amide (GLP-1) and glucose-dependent insulinotropic peptide (GIP) on nutrient-induced insulin release have been studied extensively [1–3]. Both peptides potentiate the acute effects of glucose on insulin release during a meal via mechanisms involving direct binding to their respective receptors on beta cells and raising the cellular cAMP content [4] and are therefore called incretin hormones [5]. Interestingly, in addition to this well characterized acute response, GLP-1 was recently associated with a whole spectrum of novel chronic effects upon beta cells, including differentiation, growth and protection against apoptosis (reviewed in [6]) and GIP likely has similar effects [7].

As chronic effects of incretin hormones on the beta cell have direct therapeutic application to diabetes and are of general interest for endocrine physiology, we explored in this study the influence of GLP-1, GIP or their combination on secretory function of the mature differentiated rat beta cell in a simplified in vitro model for meal-induced rises in glucose and incretins during a day [2,8]. Our results demonstrate that the combination of GLP-1 and GIP at sub-nanomolar concentrations can effectively contribute to greater secretory responsiveness of beta cells to subsequent stimulations. As such, this is the first study showing that repetitive exposure of primary beta cells to incretins can enhance future secretory function.

Abbreviations: GLP-1, glucagon-like peptide 1; GIP, glucose-dependent insulinotropic peptide; GLP-1-R, GLP-1 receptor; GIP-R, GIP receptor; FACS, fluorescence activated cell sorter; IBMX, 3-isobutyl-1-methylxanthine

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2. Materials and methods

2.1. Beta cell preparation and culture

All studies were carried out according to the Belgium regulation of animal welfare and after approval by the institutions commission for animal experiments. Rat beta cells were more than 95% pure and prepared [9] from male adult Wistar rats by flow cytometry (FACStar plus; Becton-Dickinson, Mountain View, CA, USA). Sorted cells were reaggregated for 2 h (37 °C) at a density of 1×10^5 cells/ml in Ham's F10-medium (Gibco BRL, Grand Island, NY, USA) supplemented with 2 mM L-glutamine, 10 mM glucose, 1% charcoal treated type V bovine serum albumin (Boehringer Mannheim, Germany), 0.075 g/l penicillin (Sigma, St. Louis, MO, USA) and 0.1 g/l streptomycin (Sigma) and precultured statically for 18 h in the same medium. Experimental cell culture was performed for 24 h in Ham's F10-medium at 3 mM glucose (basal) supplemented with either glucose alone (final concentration 10 mM) or 10 mM glucose combined with GLP-1 (Sigma), exendin-4 (synthesized by Dr. J. Vandekerckhove, department of physiological chemistry, University of Ghent, Belgium) or GIP (Sigma) as indicated in the figures. Cell viability was assessed [10] with DNA binding dyes Hoechst 33342 (HO 342; Calbiochem-Novabiochem Corp., La Jolla, CA) and propidium iodide (Sigma Immunochemicals, St. Louis, MO).

2.2. Measurements of insulin release and cellular cAMP content

Insulin accumulation in the media of cultured cells was measured by radioimmunoassay [11]. Perifusion experiments were carried out as described before [12] using 2.5×10^5 beta cells per column. Flow rate was 0.5 ml/min. Samples were collected every minute to measure insulin release [11]. Cellular insulin content was measured at the end of each individual experiment by sonicating the cells from the perifusion column in 2 mM acetic acid with 0.25% bovine serum albumin. In all experiments, the sum of insulin released in the perifusate accounted for 10% or less of the total insulin content in the cells. The cellular cAMP content was measured as described before [4] in cells to which 250 μM of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; Aldrich-Janssen Chimica, Beerse, Belgium) was added during the last 5 min of incubation, using a commercially available ^{125}I cAMP radioimmunoassay (Amersham, Little Chalfont, UK).

2.3. Statistical analysis

Data are presented as means ± S.E.M. of at least three independent experiments. The statistical significance of differences was assessed by two-tailed unpaired Student's *t*-test, accepting *P*-values of 0.05 or less as significant. The data corresponding to Fig. 1B were analyzed by two-tailed paired Student's *t*-test. Bonferroni adjustments were done to the α significance set point, where appropriate, to allow multiple comparisons.

3. Results

3.1. Receptor desensitization occurs with continuous GLP-1 exposure

Since G-protein coupled receptors are expected to desensitize during prolonged agonist exposure [13,14], we optimized the culture conditions for maximal preservation of the incretin hormone sensitivity. Fig. 1A shows the time-dependency of GLP-1 receptor responsiveness in FACS isolated rat beta cells with continuous presence of GLP-1 or exendin-4 [15] as a receptor agonist. The effect of 1 nM GLP-1 to stimulate cAMP accumulation was largely reduced after 1 h of incubation (initial versus 1 h: 53 ± 5 fmol per 10^3 cells per 5 min versus 25 ± 5 fmol per 10^3 cells per 5 min above 10 mM glucose stimuli (basal); $P < 0.05$) and declined progressively with duration of exposure to the peptide (after 24 h: 11 ± 2 fmol per 10^3 cells per 5 min above basal; versus initial: $P < 0.01$). This resulted in almost identical cAMP stimulation after 24 h in GLP-1 cultured cells as compared to cells cultured with glucose alone. This gradually decline of cAMP accumulation is also concentration dependent, as a significant reduction was observed after 1 h exposure with 1 nM exendin-4 concentration ($P = 0.05$), while with a 10-fold lower exendin-4 concentration, reduced cAMP formation was observed after 2 h of exposure ($P < 0.05$).

3.2. Discontinuous agonist exposure allows receptor resensitization

According to current knowledge about receptor desensitization and resensitization, cellular responsiveness should be restored by removal of the ligand and exposure to agonist-free medium for several hours [16]. We therefore cultured FACS-purified beta cells for 6 h without GLP-1 after 1 h agonist exposure (Fig. 1B). During this 1 h period the GLP-1 receptors had desensitized by more than 25% as compared to the initial GLP-1 response (initial versus 1 h: 51 ± 6 fmol cAMP per 10^3 cells per 5 min versus 38 ± 3 fmol cAMP per 10^3 cells per 5 min; $P < 0.05$). When the cells were then incubated for 6 h without GLP-1, their subsequent response to 5 min exposure to GLP-1 had increased to a level that was the same as the initial response (54 ± 6 fmol cAMP per 10^3 cells per 5 min; versus initial: $P > 0.50$), indicating that the cells had resensitized (7 h versus 1 h: $P = 0.06$). Fig. 1B also shows that GIP stimulated cAMP production was lower than with GLP-1 (data at initial 5 min, GIP versus GLP-1: 39 ± 3 fmol per 10^3 cells per 5 min versus 51 ± 6 fmol per

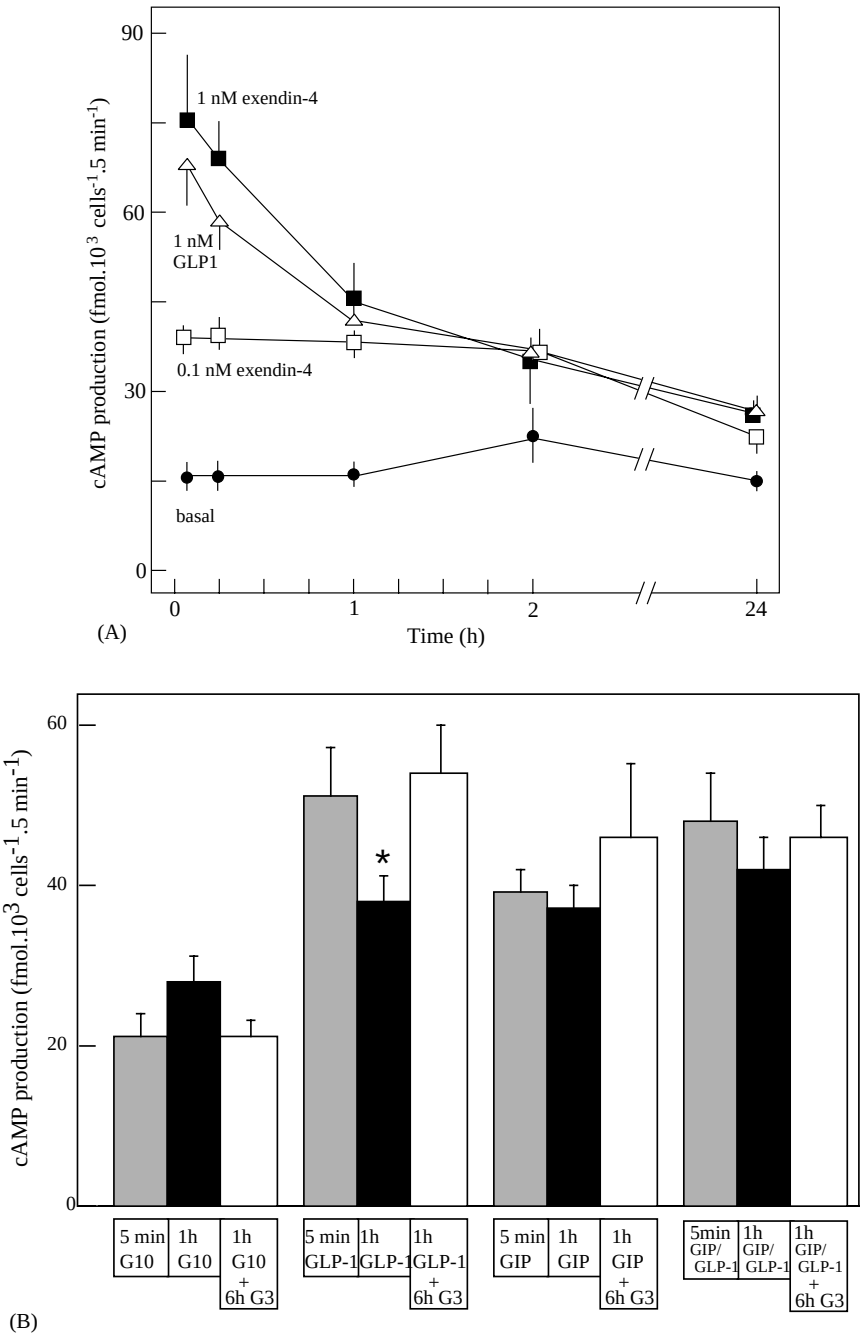

Fig. 1. Desensitization (A) and resensitization (B) of incretin receptors on beta cells. (A) Rat beta cells were exposed for the indicated time-period to culture medium containing 10 mM glucose alone (basal medium; black circles) or 10 mM glucose supplemented with either 1 nM GLP-1 (white triangles) or 1 nM (black squares) versus 0.1 nM (white squares) of the GLP-1 receptor agonist exendin-4. GLP-1 receptor activation was investigated by extracting cellular cAMP 5 min after adding IBMX (250 μM). Data represent mean ± S.E.M. of three to four experiments. (B) Cells were exposed for 1 h to 10 mM glucose alone (G10) or 10 mM glucose combined with either GLP-1 (1 nM), GIP alone (1 nM) or a mixture of both (0.5 nM each). Cellular desensitization was assessed by comparing the cAMP accumulation 5 min following addition of IBMX (250 μM) and fresh peptides (according to the culture), after 5 min versus 1 h of exposure to the peptides; resensitization was investigated by re-exposing the cells to the peptides for 5 min following 6 h of recovery in culture medium (3 mM glucose, G3) without incretins. Data represent mean ± S.E.M. of five experiments. Statistical significance was calculated by two-tailed paired Student's *t*-test. $^*P < 0.05$ versus cAMP production after 5 min of incubation.

10^3 cells per 5 min; $P = 0.05$) whereas the combination of both GLP-1 and GIP (0.5 nM each) gave comparable cAMP production to 1 nM GLP-1 at start of the experiment (48 ± 6 fmol per 10^3 cells per 5 min). In contrast to the GLP-1 condition, the cells were not desensitized significantly after 1 h culture in GIP alone or combined with GLP-1. Exposure of beta cells to a combination of GLP-1 and GIP (0.5 nM each; 1 h) followed by 6 h rest appeared to induce a condition of significant elevated cAMP without desensitizing the cells.

3.3. Priming of beta cells with GLP-1 and GIP affects the subsequent glucose-induced insulin secretion

To address the question whether discontinuous long term exposure of beta cells to incretin hormones can influence the cellular insulin secretory capacity, we designed a protocol that was inspired by the resensitization data and on the in vivo periodicity of meal-induced surges in plasma glucose and incretins [2,8]. The serum-free discontinuous culture protocol (24 h) consisted of three 1 h periods of incretin exposure at 10 mM glucose (S1, S2 and S3 in Fig. 2), resembling periods of food intake, that were separated by recovery periods of at least 6 h having 3 mM glucose medium without incretins. As experimental conditions of incretin action, we tested the conditions used in Fig. 1B, i.e. cells were 24 h discontinuously cultured with either GLP-1 or GIP alone (1 nM) or a combination of both (0.5 nM each). The mean secretory rates during each of the stimulatory periods were potentiated to the same extent for the three incretin conditions (between two- and three-fold versus 10 mM glucose alone; $P < 0.02$). The mean 24 h secretory rate was increased two-fold (control: $0.74 \pm 0.12\%$ of cellular insulin content per hour, GLP-1: 1.52 ± 0.15, GIP: 1.07 ± 0.09 and GLP-1/GIP: 1.69 ± 0.14; GLP-1 with or without GIP: $P < 0.01$ versus control) and was entirely due to the three stimulatory periods, as the basal release rates during the recovery periods varied between 0.37 and 0.56% of cellular insulin content per hour. Because GLP-1 has been proposed to enhance insulin synthesis [17] and protect against beta cell apoptosis [18], we also checked cellular insulin content and viability. The mean insulin content of the cells after the experiment (between 14.4 and 18.9 ng per 10^3 cells) and cellular viability (between 80 and 88%) was not significantly influenced by the experimental condition. Overall, the experiment clearly showed that the incretins were active during culture, by potentiating the glucose-stimulus significantly even after repetitive periods of stimulation and rest.

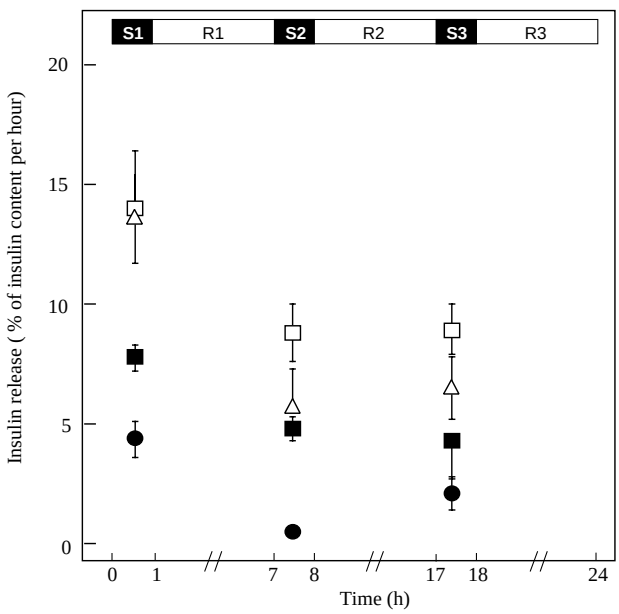

Fig. 2. Incretins potentiate glucose-regulated insulin release in cell culture. Beta cells were cultured for 24 h in HAM's-F10 medium that was basal (3 mM glucose; no incretin hormones) during the recovery phases (R1, R2 and R3 in the scheme) or was stimulatory for insulin release (three periods of 1 h, indicated as S1, S2 and S3). Control cells were stimulated with 10 mM glucose alone (black circles) while experimental conditions consisted of supplementing 10 mM glucose with 1 nM GLP-1 (white triangles), 1 nM GIP (black squares) or GLP-1 and GIP (0.5 nM each, white squares). Data are mean rates of insulin release ± S.E.M. from 3–6 experiments.

To address the possibility that the history of incretin exposure could influence future beta cell insulin secretory capacity, we tested secretory response of beta cells that were discontinuously pre-cultured as in Fig. 2 to elevated glucose alone and glucose plus 10 nM GLP-1 in a dynamic cell perifusion system [12]. After the 24 h pre-culture, all four cultured beta cell preparations were acutely responsive to both 10 mM glucose and 10 nM GLP-1 (Fig. 3), i.e. perifusion with GLP-1 amplified significantly the secretory response to 10 mM glucose alone between 11- and 15-fold ($P < 0.01$ versus glucose alone in all four cell preparations). With regard to the priming effect of incretins, 24 h discontinuously pre-culturing of the cells with either 1 nM GIP (Fig. 3C) or 1 nM GLP-1 (Fig. 3D) had no influence on the subsequent secretory response of the cells when compared to the control culture condition (Fig. 3A). On the contrary, cells that were discontinuously pre-cultured with the mixture of GIP and GLP-1 released twice as much insulin as the control preparation when challenged acutely with either 10 mM glucose alone ($P = 0.01$) or with glucose combined with GLP-1 (Fig. 3A versus 3B; $P < 0.01$).

4. Discussion

In the present study we examined to which extent chronic exposure of rat beta cells to GLP-1 with and without GIP can influence their subsequent responsiveness to glucose and GLP-1. As shown before, acute exposure to GLP-1 stimulates cellular cAMP production and potentiates glucose-stimulated insulin secretion. Prolonged exposure to GLP-1 resulted in a time dependent decrease of receptor stimulated signal transducer cAMP starting after 1 h. This is consistent with the literature on the rapid homologous desensitization of the G-protein coupled receptor in vitro [13,14]. Since IBMX was added as an agent to suppress cAMP breakdown during the last five minutes of stimulation, we interpret this decline as loss of cAMP production in function of time and not as induction of phosphodiesterase activity [19]. As the cells were cultured in serum-free medium, a rapid breakdown of GLP-1 by peptidases [20] is also an unlikely explanation for this phenomenon. Furthermore, we obtained similar data with 1 nM exendin-4 which is

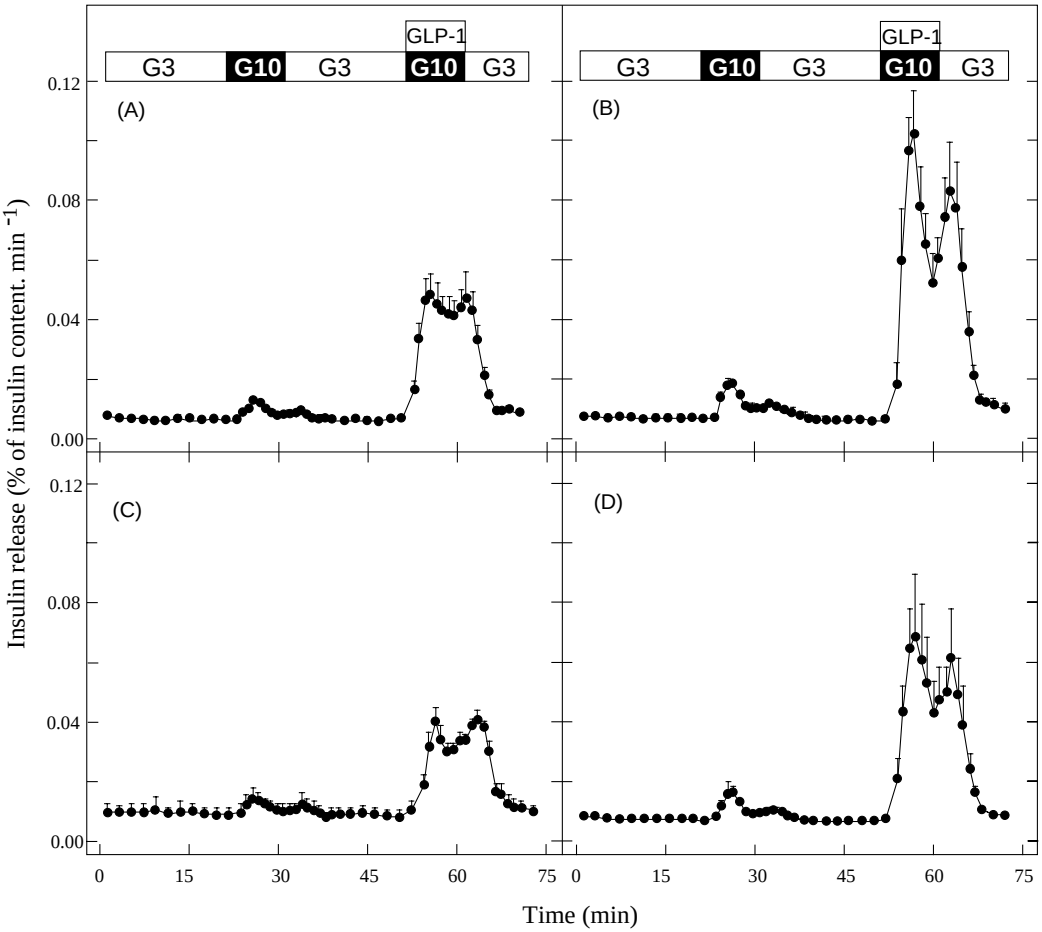

Fig. 3. Discontinuous exposure to GLP-1 and GIP primes secretory function to subsequent stimulation. Acute insulin secretory responsiveness of beta cells was tested after 24 h pre-culture as in Fig. 2, by perifusing the cells with 10 mM glucose alone (G10, 10 min) and 10 mM glucose plus 10 nM GLP-1 (10 min). The four panels differ in the nature of three 1 h periods of stimulation (indicated as S1, S2 and S3 in Fig. 2) during the 24 h preceding the perifusion experiment: 10 mM glucose alone (A, $n = 5$); 10 mM glucose and 0.5 nM GLP-1 plus 0.5 nM GIP (B, $n = 4$); 10 mM glucose and 1 nM GIP (C, $n = 3$), and 10 mM glucose and 1 nM GLP-1 in (D, $n = 6$). Mean rates of insulin release ± S.E.M. are expressed as percent of cellular insulin content per minute.

more resistant than GLP-1 to proteolytic degradation [21]. Along the same line, exposing the beta cells to a 10-time lower exendin-4 concentration slowed down the rate at which functional responsiveness declined. If peptide degradation was responsible for the attenuated cAMP response, the opposite situation would have been expected, i.e. a more rapid loss of function at the lower dose.

A sufficiently high cAMP concentration in purified beta cells is necessary since glucose alone elicits only a partial insulin release [11], whereas the combination of glucose with islet hormone glucagon, which stimulates cAMP accumulation [22] results in a parallel augmentation of insulin release [11]. In vitro observations made by Sjöholm et al. [23] with chronic (7 days) incubations with GLP-1 and GIP on fetal islets, demonstrated that both incretins had no positive effect on glucose responsive insulin secretory rates and islet maturation. But, a short term (10 min–1 h) GLP-1 pre-exposure of glucose unresponsive human fetal beta cells—probably due to insufficient ATP formation and cAMP production—rendered them more responsive for insulin secretion [24].

No receptor desensitization was seen during and following a 24 h culture period which consisted of only three periods of 1 h incretin pulse at 10 mM glucose, interrupted by hormone-free recovery periods at 3 mM glucose (6–9 h). This protocol resembles the in vivo situation more closely, since incretins are released from intestinal cells into the circulation in response to oral glucose load or a mixed meal [2,8] and decline afterwards. It can be hypothesized that shortening of hormone-free recovery periods or consumption of long meals may lead to reduced incretin effect since cAMP accumulation declined during continuous hormone culture in vitro. However, it is not certain whether receptor desensitization does occur in vivo because the circulating bioactive half life of incretins is extremely short as a result of their rapid breakdown by dipeptidyl peptidase IV [20]; regardless, the mechanism of desensitization exists for incretin hormones [13,14].

The observation that repetitive pulse stimulation with 1 nM GLP-1 or GIP individually did not influence the subsequent insulin releasing capacity of the cells while their combination is effective, may be related to the

synergism between the two peptides that was reported before in acute release experiments [25,26]. It is also likely partially due to the fact that significant desensitization of receptor-induced cAMP formation did not occur in that culture condition, while initial cAMP response was as high as seen with 1 nM GLP-1 alone. As GLP-1 and GIP are believed to initiate two partially overlapping signaling cascades [27,28], it is conceivable that under these experimental conditions, the combination of the two peptides will initiate several signaling events that elicit changes that persist when beta cell secretion is initiated at a later point. The responsible molecular mechanism may be related to the expression of cAMP-responsive genes that are involved in exocytosis. These genes can be identified by performing mRNA profiling of beta cells cultured in the presence or absence of incretins [29]. Considering the plasma levels of GLP-1 and GIP, the 0.5 nM concentration used in cell culture are close to the upper limits of the physiological range [30]. Therefore, our data demonstrate for the first time that a mixture of GLP-1 and GIP at physiological concentrations influences the capacity to respond to later secretory stimuli independently of insulin content. Hence, their repeated influence during meals as occurring during a day may not only be relevant in shortening the postprandial phase of elevated glucose but also to prime the beta cell for the next meal. Whether or not this priming effect of GLP-1 plus GIP is relevant for therapeutic schemes in diabetic patients remains to be investigated.

Acknowledgments

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