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Mechanism of action of glucagon-like peptide-$1^{7\text{-}36NH_2}$ in isolated rat pancreatic islets and abrogation of its effects in long-term incubations

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Glucagon-like peptide-$1^{7\text{-}37/36NH_2}$ (GLP-1) potently stimulates acute glucose-dependent insulin secretion *in vitro* and *in vivo*. Islet cell lines have been used extensively to examine the effects of GLP-$1^{7\text{-}37/36NH_2}$ on adenylyl cyclase as well as the phenomenon of homologous receptor desensitization. However, neither the effects of GLP-$1^{7\text{-}37/36NH_2}$ on the protein kinase A pathway nor its chronic effects on insulin secretion have been examined in normal B cells. The isolated rat pancreatic islet was therefore employed to study these phenomena in a more physiologic model. GLP-$1^{7\text{-}36NH_2}$ (10^{-8} M) increased islet cAMP content to 391 ± 196% of control after 30 min of incubation ($P<0.05$), and stimulated glucose-dependent insulin secretion by 2.0 ± 0.1-fold in acutely perifused islets ($P<0.001$). In chronic 24 h incubations, insulin secretion was stimulated two to fourfold by 10 as compared to 5 mM glucose ($P<0.001$), and three to fourfold by 10 μM forskolin plus 10 μM isobutylmethylxanthine ($P<0.01-0.001$). However, 10^{-8} M GLP-$1^{7\text{-}36NH_2}$ did not stimulate insulin secretion at either 5 or 10 mM glucose, or in the presence of forskolin and IBMX. This specific lack of GLP-$1^{7\text{-}36NH_2}$ effectiveness was not due to peptide degradation or accumulation of somatostatin in the medium. The results of the present study establish for the first time that GLP-$1^{7\text{-}36NH_2}$ induces insulin secretion in normal B cells through a protein kinase A-dependent mechanism. The loss of insulinotropic effect of GLP-$1^{7\text{-}36NH_2}$ in long-term incubations, despite the ability of glucose and cAMP to increase insulin secretion, suggests that the GLP-$1^{7\text{-}37/36NH_2}$ receptor in normal islets may undergo homologous desensitization during chronic exposure to GLP-$1^{7\text{-}36NH_2}$.

Keywords: glucagon-like peptide-1; insulin; cyclic AMP; rat islets; homologous desensitization; receptor

Introduction

Incretins are endocrine factors, secreted by the intestine in response to nutrient ingestion, that stimulate insulin secretion from the pancreas in a glucose-dependent fashion (Creutzfeldt & Ebert, 1985). Glucagon-like peptide-$1^{7\text{-}37}$ and -$1^{7\text{-}36NH_2}$ (GLP-1) are intestinal products of proglucagon processing (Orskov *et al.*, 1986; Mojsov *et al.*, 1986). These peptides were first demonstrated to exert insulinotropic effects in 1987 (Drucker *et al.*, 1987; Holst *et al.*, 1987; Kreymann *et al.*, 1987; Mojsov *et al.*, 1987) and were subsequently shown to be equipotent (Suzuki *et al.*, 1989; Weir *et al.*, 1989). Indeed, GLP-$1^{7\text{-}37/36NH_2}$ increases glucose-dependent insulin secretion in a variety of experimental systems and *in vivo* (Orskov *et al.*, 1988; D'Alessio *et al.*, 1989; Kawai *et al.*, 1989; Suzuki *et al.*, 1989; Weir *et al.*, 1989; Fehmann & Habener, 1991b; Fridolf & Ahren, 1991; Fehmann & Habener, 1992; Gutniak *et al.*, 1992; Nathan *et al.*, 1992; Siegel *et al.*, 1992; Hendrick *et al.*, 1993; Nauck *et al.*, 1993b). Furthermore, increases in the plasma levels of GLP-$1^{7\text{-}36NH_2}$ have been observed following an oral glucose load in man (Kreymann *et al.*, 1987; Orskov *et al.*, 1991; Gutniak *et al.*, 1992). Thus, GLP-$1^{7\text{-}37/36NH_2}$ fulfils all the criteria for classification as an incretin. In addition, due to its glucose-dependent insulinotropic effects, GLP-$1^{7\text{-}37/36NH_2}$ has recently been proposed as a treatment for Type II diabetes (Nathan *et al.*, 1992; Gutniak *et al.*, 1992).

A specific receptor for GLP-$1^{7\text{-}37/36NH_2}$ has been identified in various tissues, including the pancreas, as well as in several islet-derived cell lines (Goke & Conlon, 1988; Orskov & Nielsen, 1988; Fehmann & Habener, 1991a,b; Orskov & Poulsen, 1991; Goke *et al.*, 1992). The sequence of the receptor is homologous to that of other G_s protein-linked receptors (Thorens, 1992), and consistent with this finding is the fact that GLP-$1^{7\text{-}37/36NH_2}$ increases cAMP biosynthesis in a number of different insulin-producing cell lines (Drucker *et al.*, 1987; Goke & Conlon, 1988; Gefel *et al.*, 1990; Fehmann & Habener, 1991a,b; Fehmann & Habener, 1992; Thorens, 1992; Van Eyll *et al.*, 1994). GLP-$1^{7\text{-}37/36NH_2}$ also increases cAMP levels when its receptor is expressed in non-insulin producing COS and CHO cells (Wheeler *et al.*, 1993; Fehmann *et al.*, 1994). While no studies to date have examined whether GLP-$1^{7\text{-}37/36NH_2}$ exerts any effects through the protein kinase A pathway in islets, several investigators have found evidence for an involvement of calcium and the protein kinase C pathway in GLP-$1^{7\text{-}37/36NH_2}$ signal transduction in normal islets (Fridolf & Ahren, 1991; Yada *et al.*, 1993; Cullinan *et al.*, 1994). Similarly, it has been reported that the GLP-$1^{7\text{-}37/36NH_2}$ receptor undergoes homologous desensitization in the HIT T15 islet cell line (Fehmann & Habener, 1991b), however, this has not yet been examined in the normal B cell. Thus, the goal of the present study was to examine the acute and chronic effects of GLP-$1^{7\text{-}36NH_2}$ on the protein kinase-A pathway and insulin secretion by the normal B cell, using the isolated rat pancreatic islet model.

Results

To establish whether GLP-$1^{7\text{-}36NH_2}$ activates adenylyl cyclase in normal islets, the cAMP content of the islets was measured after incubation under static conditions for 30 min (Figure 1). Control islets contained 10.5 ± 4.4 pmoles of cAMP, and incubation of the islets with forskolin and IBMX increased the cAMP content to 690 ± 292% of control levels ($P<0.01$), indicating that the adenylyl cyclase system was intact and functional. Islets incubated with 10^{-8} M GLP-$1^{7\text{-}36NH_2}$ and IBMX also exhibited increased cAMP production, to 391 ± 196% of control ($P<0.05$), indicating that GLP-$1^{7\text{-}36NH_2}$ exerts its actions on normal islet cells via the cAMP second messenger system. cAMP levels had returned to basal after 24 h of incubation with either forskolin or GLP-$1^{7\text{-}36NH_2}$ (data not shown).

To verify that the isolated islet preparation exhibited the expected effects of GLP-$1^{7\text{-}36NH_2}$ on acute insulin secretion, an islet perifusion system was employed. Islets that were preperifused for 10 min with 10 mM glucose secreted twofold greater amounts of insulin than did islets that were preperifused with 2.5 mM glucose (0.9 ± 0.2 *vs* 0.4 ± 0.2 ng/2 min, respectively, $P<0.001$), indicating the glucose-responsiveness of the system. Addition of 10^{-8} M GLP-

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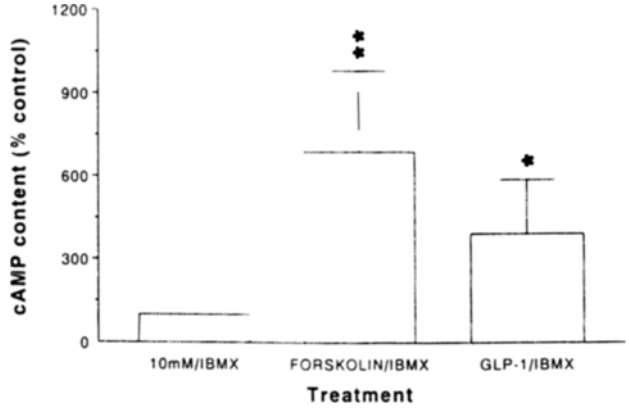

Figure 1 cAMP biosynthesis by groups of 100 islets incubated for 30 min with 10 mM glucose plus 100 μM IBMX alone (control), or with 100 μM forskolin or 10^{-8} M GLP-1^{7-36NH_2} ($n = 4$). *, $P<0.05$, **$P<0.01$ *vs* control

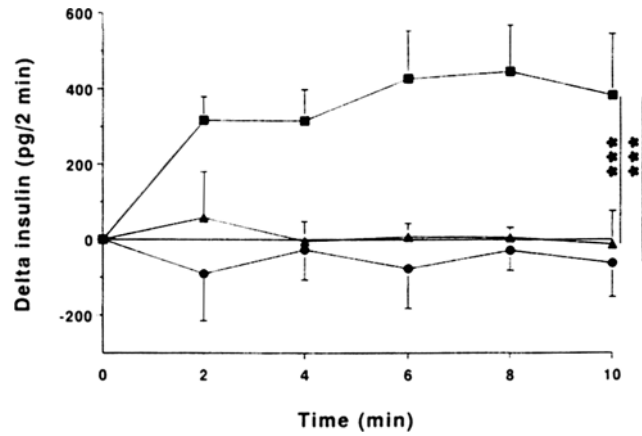

Figure 2 Insulin secretion by groups of 100 islets perifused for 10 min with 10 mM glucose alone (●), 10 mM glucose plus 10^{-8} M GLP-1^{7-36NH_2} (■), or 2.5 mM glucose plus 10^{-8} M GLP-1^{7-36NH_2} (▲) ($n = 4-8$). ***, $P<0.001$

1^{7-36NH_2} in the presence of 10 mM glucose increased insulin secretion by twofold, to 1.7 ± 0.4 ng/2 min ($P<0.001$, Figure 2). In contrast, addition of GLP-1^{7-36NH_2} to the 2.5 mM glucose perfusate did not alter the rate of insulin secretion (0.4 ± 0.2 ng/2 min) from basal values. Thus, GLP-1^{7-36NH_2} increased acute insulin secretion in a glucose-dependent fashion in the islet system utilized.

To examine the long-term effects of GLP-1^{7-36NH_2} on insulin secretion, static incubations were performed for 24 h. Islets incubated in 5 mM glucose alone secreted the same amount of insulin as islets incubated in 5 mM glucose plus 10^{-8} M GLP-1^{7-36NH_2} (Figure 3). Unexpectedly, the islets incubated with 10 mM glucose plus GLP-1^{7-36NH_2} also failed to secrete more insulin than the islets incubated with 10 mM glucose alone. Glucose-dependent insulin secretion was observed, however, in both control and GLP-1^{7-36NH_2}-treated islets: 10 mM glucose alone stimulated insulin secretion fourfold as compared to 5 mM glucose alone ($P<0.001$), and 10 mM glucose plus GLP-1^{7-36NH_2} stimulated insulin secretion twofold as compared to 5 mM glocose plus GLP-1^{7-36NH_2} ($P<0.001$). To ensure that the GLP-1^{7-36NH_2} was not being degraded during the static incubation, ^{125}I-GLP-1^{7-36NH_2} was added to 100 islets for 24 h. The recovery of iodinated peptide after 24 h of incubation with islets was 96 ± 16% of that recovered from medium incubated in the absence of islets, and analysis by reversed-phase HPLC demonstrated that the recovered peptide was identical to intact ^{125}I-GLP-1^{7-36NH_2} (data not shown).

To establish that the lack of stimulation of insulin secretion by GLP-1^{7-36NH_2} in long-term incubations was not due to either B cell exhaustion or to uncoupling of adenylyl cyclase from the insulin secretory machinery, islets were treated for 24 h with 10 mM glucose alone (control), or with 10^{-8} M GLP-1^{7-36NH_2}, with forskolin and IBMX, or with GLP-1^{7-36NH_2} plus forskolin and IBMX (Figure 4). Although GLP-1^{7-36NH_2} was unable to stimulate insulin secretion by islets incubated with 10 mM glucose, islets treated with forskolin and IBMX secreted 3.1 ± 0.9-fold more insulin than islets incubated with 10 mM glucose alone ($P<0.01$). However, incubation with GLP-1^{7-36NH_2} plus forskolin and IBMX did not further augment insulin secretion (4.1 ± 1.2-fold increase *vs* 10 mM glucose alone, $P<0.001$). Thus, although the islets incubated for 24 h with 10 mM glucose had an intact protein kinase A system and were capable of secreting insulin in response to activation of this pathway, GLP-1^{7-36NH_2} did not stimulate insulin secretion under these conditions.

As GLP-1^{7-36NH_2} stimulates acute release of somatostatin (D'Alessio *et al.*, 1989), a known inhibitor of insulin secretion (Howell & Montague, 1973; Koch *et al.*, 1985; Schuit *et al.*, 1989), it was possible that the abrogation of the GLP-1^{7-36NH_2}-induced stimulation of insulin over the 24 h incubation period was due to accumulation of somatostatin in the culture medium. Accordingly, somatostatin secretion was investigated in islets treated for 24 h with 10 mM glucose alone (control), or with 10^{-8} M GLP-1^{7-36NH_2}, with forskolin and IBMX, or with GLP-1^{7-36NH_2} plus forskolin and IBMX. Islets treated with forskolin and IBMX secreted 2.2 ± 0.6-fold more somatostatin than islets incubated with 10 mM glucose alone ($P<0.05$) (Figure 5). Somatostatin secretion was not stimulated by incubation with GLP-1^{7-36NH_2} alone, while incubation with GLP-1^{7-36NH_2} plus forskolin and IBMX did not increase somatostatin secretion beyond that seen with forskolin and IBMX alone. Thus, the lack of stimulation of insulin secretion by GLP-1^{7-36NH_2} was not due to accumulation of somatostatin in the culture medium.

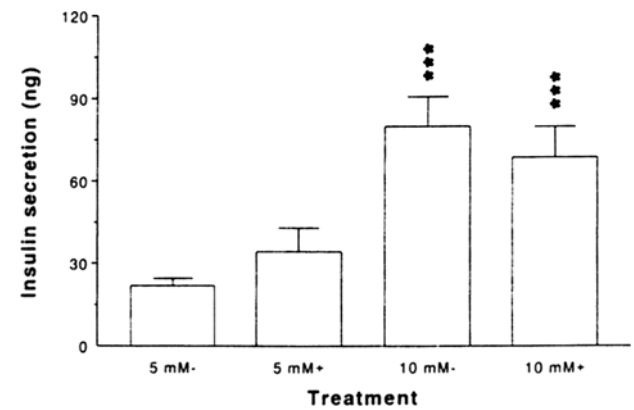

Figure 3 Insulin secretion by groups of 20 islets incubated for 24 h with 5 or 10 mM glucose in the absence (−) or presence (+) of 10^{-8} M GLP-1^{7-36NH_2}. Secretion is defined as the total immunoreactive insulin contained in the medium at the end of the incubation period ($n = 5-8$). ***, $P<0.001$ *vs* the same treatment at 5 mM glucose

Discussion

Although GLP-$1^{7-37\ 36NH_2}$ is undergoing extensive testing as an insulinotropic agent, controversy remains as to the mechanism by which it stimulates insulin secretion by normal, non-transformed B cells. Studies utilizing several islet models have yielded contradictory results, suggesting that GLP-$1^{7-37/36NH_2}$ induces insulin secretion through a protein kinase A, protein kinase C and/or a calcium-dependent pathway (Drucker *et al.*, 1987; Goke & Conlon, 1988; Fehmann

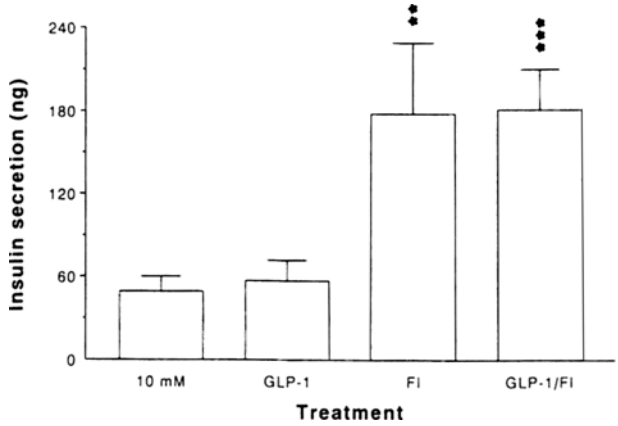

Figure 4 Insulin secretion by groups of 20 islets incubated for 24 h with 10 mM glucose alone (control), 10 mM glucose plus 10^{-8} M GLP-1^{7-36NH_2}, 10 mM glucose plus 10 μM forskolin plus 10 μM IBMX, or 10 mM glucose plus GLP-1^{7-36NH_2}, forskolin and IBMX. Secretion is defined as the total immunoreactive insulin contained in the medium at the end of the incubation period ($n = 5$). **, $P<0.01$; **, $P<0.001$ *vs* control

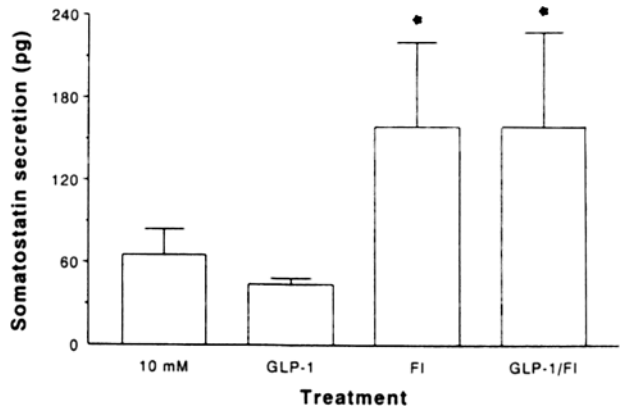

Figure 5 Somatostatin secretion by groups of 20 islets incubated for 24 h with 10 mM glucose alone (control), 10 mM glucose plus 10^{-8} M GLP-1^{7-36NH_2}, 10 mM glucose plus 10 μM forskolin plus 10 μM IBMX, or 10 mM glucose plus GLP-1^{7-36NH_2}, forskolin and IBMX. Secretion is defined as the total immunoreactive somatostatin contained in the medium at the end of the incubation period ($n = 5$). *, $P<0.05$ *vs* control

& Habener, 1991a,b; Fridolf & Ahren, 1991; Fehmann & Habener, 1992; Thorens, 1992; Yada *et al.*, 1993; Cullinan *et al.*, 1994; Van Eyll *et al.*, 1994). In the present study, GLP-1^{7-36NH_2} increased cAMP biosynthesis in isolated normal rat islets by fourfold, demonstrating a common mechanism of action of this peptide in normal islets and immortalized islet cells (Drucker *et al.*, 1987; Goke & Conlon, 1988; Fehmann & Habener, 1991a,b; Fehmann & Habener, 1992; Thorens, 1992), and validating the use of these cell lines for further studies on GLP-$1^{7-37/36NH_2}$. These findings are consistent with the observation that the GLP-$1^{7-37/36NH_2}$ receptor shares structural homology with a number of other adenylyl cyclase-linked receptors (Thorens, 1992), and demonstrates this linkage in normal islets for the first time.

The insulinotropic effects of GLP-$1^{7-37/36NH_2}$ have been well documented in the isolated perfused pancreas (Holst *et al.*, 1987; Mojsov *et al.*, 1987; Orskov *et al.*, 1988; Kawai *et al.*, 1989; Suzuki *et al.*, 1989; Weir *et al.*, 1989) and *in vivo* (Kreymann *et al.*, 1987; Gutniak *et al.*, 1992; Nathan *et al.*, 1992; Nauck *et al.*, 1993a). However, relatively few studies have employed the isolated pancreatic islet system. In these studies, GLP-$1^{7-37/36NH_2}$ increased insulin release at glucose concentrations of 2.8 mM (Goke *et al.*, 1993) and 3.3 mM (Fridolf & Ahren, 1991) or greater, but not at 2.75 mM (Zawalich *et al.*, 1993). Consistent with these findings, GLP-1^{7-36NH_2} stimulated insulin secretion by perifused islets at 10 but not 2.5 mM glucose in the present study. When taken together, these findings suggest a glucose threshold of approximately 2.8 mM for the insulinotropic effects of GLP-$1^{7-37/36NH_2}$.

Although a few studies have examined the acute effects of GLP-$1^{7-37/36NH_2}$ using perifusion systems or short-term (30–60 min) static incubations of intact or dispersed cultured islets (D'Alessio *et al.*, 1989; Fridolf & Ahren, 1991; Zawalich *et al.*, 1993; Siegel *et al.*, 1992), none of these systems have been used to determine the chronic effects of GLP-$1^{7-37/36NH_2}$ on insulin secretion by normal islets. In the present study, GLP-1^{7-36NH_2} stimulated glucose-dependent insulin secretion acutely in the perifused rat islets, in agreement with previously published studies (Fridolf & Ahren, 1991; Zawalich *et al.*, 1993). Unexpectedly, however, GLP-1^{7-36NH_2} had no effect on insulin secretion in chronic, 24 h incubations of islets, regardless of whether glucose levels were elevated. This was not due to degradation of the peptide, as GLP-1^{7-36NH_2} was recovered intact after long-term incubation with islets. Furthermore, insulin secretion was increased by 10 mM glucose alone in these islets, demonstrating that the islets exhibited normal glucose-dependent insulin secretion (Giddings *et al.*, 1982; Nielsen *et al.*, 1985; Weir *et al.*, 1986; German *et al.*, 1990; Purrello *et al.*, 1993). These responses to glucose were augmented by concomitant activation of the protein kinase A pathway with forskolin, as expected since this pathway is known to exert stimulatory effects on insulin secretion (Pipeleers *et al.*, 1985a; Pipeleers *et al.*, 1985b; Drucker *et al.*, 1991). A similar pattern of somatostatin secretion was also observed, such that forskolin, but not GLP-1^{7-36NH_2}, stimulated somatostatin secretion in 24 h incubations. These findings indicate that GLP-1^{7-36NH_2}-induced accumulation of somatostatin was not responsible for the lack of stimulation of insulin secretion observed during chronic incubations. Thus, abrogation of the effects of GLP-1^{7-36NH_2} in the long-term incubations, despite the ability of the islets to respond to both forskolin and glucose, suggests a dissociation between the GLP-$1^{7-37/36NH_2}$ receptor and adenylyl cyclase in the B cell. This uncoupling of the receptor appears to have occurred in a time-dependent fashion, as the peptide stimulated both cAMP biosynthesis and insulin secretion in acute incubations of 30 min or less.

A likely explanation for the lack of response of the islets to GLP-1^{7-36NH_2} in the 24 h incubations is that of homologous receptor desensitization. The GLP-$1^{7-37/36NH_2}$ receptor on HIT-T15 insulinoma cells is homologously desensitized after 10 min of exposure to GLP-1^{7-37} (Fehmann & Habener, 1991b). Although the mechanism underlying this phenomenon has not been elucidated, the fact that the desensitization is reversible indicates that neither receptor degradation nor internalization are involved. Interestingly, our findings suggest that desensitization of the GLP-$1^{7-37/36NH_2}$ receptor also occurs on the somatostatin-producing D cell, an observation that has not previously been reported. Homologous receptor desensitization has been observed in a number of other G_s-linked receptors, including the β-adrenergic, GIP and glucagon receptors (Murphy *et al.*, 1987; Benovic *et al.*, 1988; Fehmann & Habener, 1991b). In the case of the β-adrenergic receptor, homologous desensitization requires the presence of a specific receptor kinase and the co-factor β-arrestin, resulting in functional uncoupling of the receptor from adenylyl cyclase (Benovic *et al.*, 1988; Lohse *et al.*, 1990). In the present study, forskolin increased both insulin and somatostatin secretion in the absence of a GLP-1^{7-36NH_2} effect. Thus, the specific loss of GLP-1^{7-36NH_2} bioactivity in 24 h incubations of normal islets appears to be related to a dissociation of the GLP-$1^{7-37/36NH_2}$ receptor from adenylyl cyclase, and not to a dissociation of cAMP from stimulation of hormone secretion. Physiologically, such desensitization of the GLP-$1^{7-37\,36NH_2}$ receptor likely serves to

protect the organism from hypoglycemia due to excessive GLP-$1^{7-37/36NH_2}$-induced insulin secretion.

In conclusion, GLP-1^{7-36NH_2} acutely stimulates both cAMP biosynthesis and insulin secretion in normal rat pancreatic islets. The insulinotropic effects of GLP-1^{7-36NH_2} are lost in long-term incubations, possibly due to GLP-$1^{7-37/36NH_2}$ receptor desensitization.

Materials and methods

Islet isolation

Pancreatic islets were isolated from male Wistar rats weighing 250–400 *g* (Charles River Canada Inc., St. Constant, Quebec) as described previously (Drucker *et al.*, 1991). Briefly, rats were lightly anaesthetized by halothane inhalation before ip injection of sodium pentobarbital (65 mg/kg of body weight). The pancreas was filled via the bile duct with 12 ml of type XI collagenase (0.4 mg/ml, Sigma Chemical Co., St. Louis, MO) in modified Hank's balanced salt solution (MHBSS), and then removed and shaken gently at 37°C for 18–22 min. After vigorous shaking to break up the tissue and several washings with MHBSS, islets were purified by density-gradient centrifugation, using Ficoll 400 (ICN Biochemicals, Cleveland, OH) at concentrations from 11–25% (w/v). Islets were individually collected by suction using a plastic catheter under a microscope into Dulbecco's minimal essential medium (Medical Sciences Media Service, University of Toronto, Toronto, Ontario) with 5 mM glucose and 5% (v/v) fetal calf serum (GIBCO, Life Technologies Inc., Grand Island, NY) and left to recover for 48 h in a tissue culture incubator with 95% (v/v) air and 5% (v/v) CO_2 at 37°C. The average yield per pancreas was 325 islets.

cAMP bioassay

After the recovery period, islets were picked into groups of 100 and 1 ml of experimental media was then added. The experimental medium contained 10 mM glucose plus 100 μM isobutylmethylxanthine (IBMX, Sigma) alone (control) or with either 100 μM forskolin (Sigma) or 10^{-8} M GLP-1^{7-36NH_2} (Bachem, Torrance, CA). After a 30 min incubation period, the medium was discarded and the cells were homogenized in ice-cold absolute ethanol and assayed for cAMP content.

Perifusion experiments

After the recovery period, islets were picked into groups of 100. Potential differences in peptide secretion and content were compensated for by picking a consistent number of small, medium and large islets into each experimental group. The perifusion apparatus (kindly supplied by Dr Anthony Sun, Dept. of Physiology, University of Toronto) consisted of a 1 ml hollow chamber with an inflow and an outflow tube. The inflow tube supplied the experimental medium, which was stored in the incubator to ensure that the correct temperature and gas composition were maintained. The outflow tube passed from the chamber to a fraction collector. All experimental groups were pre-perifused at 1 ml/min for 25 min under basal conditions, and then for 10 min with medium containing either 10 or 2.5 mM glucose, as appropriate for the subsequent perifusion period. Control islets were then perifused for 10 min with 10 mM glucose, while the other two groups were perifused as follows: 10 min with 10 mM glucose plus 10^{-8} M GLP-1^{7-36NH_2}, or 10 min with 2.5 mM glucose plus 10^{-8} M GLP-1^{7-36NH_2}. Fractions were collected every two min and acidified with 1 ml 0.1% (v/v) trifluoroacetic acid (BDH, Toronto, Ontario). Peptides were isolated from the medium by adsorption to C18 silica (Sep-Pak cartridge, Waters Assoc., Bedford, MA) as previously described (Brubaker & Vranic, 1987; Brubaker, 1988; Drucker *et al.*, 1991) and the samples were then assayed for insulin content.

Static incubations

Two series of experiments were performed on islets after the 48 h recovery period. In both cases, islets were picked into groups of 20. Again, the potential differences in peptide secretion were compensated for by picking a consistent number of small, medium and large islets into each experimental groups. For each experiment, the results from 2–8 different groups of 20 islets were averaged to make $n = 1$. In the first series of experiments, the media contained 5 or 10 mM glucose, alone (controls) or with 10^{-8} M GLP-1^{7-36NH_2}. In the second set of experiments, the medium contained 10 mM glucose alone (control), or with 10^{-8} M GLP-1^{7-36NH_2}, with 10 μM forskolin and 10 μM IBMX or with GLP-1^{7-36NH_2} plus forskolin and IBMX. After a 24 h incubation period, cells and media were separated. Islets were homogenized in extraction medium as previously described (Brubaker & Vranic, 1987; Brubaker, 1988; Drucker *et al.*, 1991) and peptides were isolated from both medium and cells by adsorption to C18 silica. The samples were then assayed for insulin, somatostatin and cAMP content.

Assays

RIAs for immunoreactive insulin and somatostatin-like immunoreactivity in medium and cells were performed as previously described (Brubaker *et al.*, 1990; Drucker *et al.*, 1991). cAMP was assayed using an RIA kit (Biomedical Technologies Inc., Stoughton, MA).

GLP-1^{7-36NH_2} degradation studies

After the 48 h recovery period, 100 islets were placed into medium containing 10 mM glucose and 10^5 c.p.m. of ^{125}I-GLP-1^{7-36NH_2} (prepared using the chloramine T method and purified by adsorption to C_{18} silica). Two identical samples were also prepared without islets (controls). Peptides were immediately isolated from one of the controls by adsorption to C18 silica. The other two samples were incubated for 24 h, after which peptides were extracted. All samples were then subjected to high pressure liquid chromatography to determine the extent of ^{125}I-GLP-1^{7-36NH_2} degradation over the incubation period. Samples were run through a Waters Liquid Chromatograph (Waters Associates, Milford MA) on a Waters C18 μBondapak column using a linear gradient of 10–80% (v/v) Solvent B [Solvent A: 0.1% (v/v) TFA; Solvent B: 80% (v/v) acetonitrile containing 0.1% (v/v) TFA], followed by a 10 min purge with 99% (v/v) Solvent B. Samples were collected at 1 min intervals and subsequently placed in a gamma counter.

Data analysis

The total culture content (media and cells) of immunoreactive insulin (209 ± 28 ng/20 islets) and somatostatin (224 ± 44 pg/20 islets) was not altered by any of the experimental treatments. Therefore, the amount of hormone in the medium alone was taken to reflect hormone secretion. Data is shown as the mean of 3–8 experiments ± SEM. Statistical significance was determined, as appropriate, by paired Student's *t*-test or ANOVA. In some cases data were $\log_{10}$-transformed to normalize variances.

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