Exendin-(9–39) Is an Inverse Agonist of the Murine Glucagon-Like Peptide-1 Receptor: Implications for Basal Intracellular Cyclic Adenosine 3′,5′-Monophosphate Levels and β-Cell Glucose Competence

Serre Beinier, Véronique; Dolci, Wanda; Schaerer, Elizabeth; Scrocchi, Louise; Drucker, Daniel; Efrat, Shimon; Thorens, Bernard

0013-7227/98/$03.00/0
Endocrinology

Vol. 139, No. 11
Printed in U.S.A.

Exendin-(9–39) Is an Inverse Agonist of the Murine Glucagon-Like Peptide-1 Receptor: Implications for Basal Intracellular Cyclic Adenosine 3′,5′-Monophosphate Levels and β-Cell Glucose Competence*

VÉRONIQUE SERRE, WANDA DOLCI, ELIZABETH SCHAERER, LOUISE SCROCCHI, DANIEL DRUCKER, SHIMON EFRAT, AND BERNARD THORENS

Institute of Pharmacology and Toxicology, University of Lausanne (V.S., W.D., E.S., B.T.), CH-1005 Lausanne, Switzerland; the Department of Molecular Pharmacology, Albert Einstein College of Medicine (S.E.), Bronx, New York 10461; and the Endocrine Division, The Toronto Hospital (L.S., D.D.), Toronto, Ontario, Canada M5G 2C4

ABSTRACT

The effect of exendin-(9–39), a described antagonist of the glucagon-like peptide-1 (GLP-1) receptor, was evaluated on the formation of cAMP- and glucose-stimulated insulin secretion (GSIS) by the conditionally immortalized murine βTC-Tet cells. These cells have a basal intracellular cAMP level that can be increased by GLP-1 with an EC_{50} of approximately 1 nM and can be decreased dose dependently by exendin-(9–39). This latter effect was receptor dependent, as a β-cell line not expressing the GLP-1 receptor was not affected by exendin-(9–39). It was also not due to the endogenous production of GLP-1, because this effect was observed in the absence of detectable preproglucagon messenger RNA levels and radioimmunoassayable GLP-1. Importantly, GSIS was shown to be sensitive to this basal level of cAMP, as perifusion of βTC-Tet cells in the presence of exendin-(9–39) strongly reduced insulin secretion. This reduction of GSIS, however, was observed only with growth-arrested, not proliferating, βTC-Tet cells; it was also seen with nontransformed mouse β-cells perifused in similar conditions. These data therefore demonstrated that 1) exendin-(9–39) is an inverse agonist of the murine GLP-1 receptor; 2) the decreased basal cAMP levels induced by this peptide inhibit the secretory response of βTC-Tet cells and mouse pancreatic islets to glucose; 3) as this effect was observed only with growth-arrested cells, this indicates that the mechanism by which cAMP leads to potentiation of insulin secretion is different in proliferating and growth-arrested cells; and 4) the presence of the GLP-1 receptor, even in the absence of bound peptide, is important for maintaining elevated intracellular cAMP levels and, therefore, the glucose competence of the β-cells. (*Endocrinology* **139:** 4448–4454, 1998)

THE SECRETION of insulin by pancreatic β-cells is mainly under the control of blood glucose. However, this glucose-dependent secretory activity can be modulated in a positive or negative manner by several hormones and neurotransmitters. In the postprandial state, two peptidic hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are secreted by gut endocrine cells in response mainly to fat and carbohydrates (1, 2). Their insulinotropic action is mediated by binding to specific β-cell plasma membrane receptors of the G protein-coupled receptor superfamily that are linked to activation of the cAMP pathway (3–5). Activation of protein kinase A and phosphorylation of different proteins participating in the glucose signaling pathway, such as the glucose transporter GLUT2 and the L-type Ca^{2+} channel, have been hypothesized to be key events in the stimulatory action of these hormones (6, 7). A quantitatively very important effect of cAMP at the level of insulin granules exocytosis has also been demonstrated (8). Beside these effects, which require an acute elevation of intracellular cAMP, a basal level of cAMP is required to maintain pancreatic β-cells in a glucose-competent state. Indeed, as reported many years ago (9), purified rat islet β-cells have low intracellular cAMP levels and respond poorly to glucose. Only after elevation of the intracellular cAMP levels by hormones or pharmacological agents is the glucose-dependent secretory activity restored. It is not clear to date how the *in vivo* control of basal cAMP levels in pancreatic β-cells is maintained. Rat pancreatic β-cells express several receptors that are coupled to the adenylyl cyclase pathway, such as the GLP-1 (3, 4), GIP (5, 10), and glucagon receptors (11, 12). Basal circulating levels of GLP-1, GIP, and glucagon may be sufficient to maintain a minimal activity of these receptors and therefore a minimal level of cAMP (6).

In the present report, we demonstrate that exendin-(9–39) is an inverse agonist of the murine β-cell GLP-1 receptor and that decreasing the basal level of cAMP with this peptide strongly decreases the glucose-dependent insulin secretory activity. These data suggest, therefore, that the GLP-1 receptor is constitutively active in the ligand-free state and that this activity is important for maintaining the glucose competence of the β-cells.

Received February 23, 1998.
Address all correspondence and requests for reprints to: Dr. Bernard Thorens, Institute of Pharmacology and Toxicology, 27 rue du Bugnon, CH-1005 Lausanne, Switzerland. E-mail: bernard.thorens@ipharm.unil.ch.
* This work was supported by Grant 31-46958.96 from the Swiss National Science Foundation (to B.T.).

Materials and Methods

Chemicals

GLP-1 was purchased from Bachem (Switzerland). Exendin-(9–39) was a gift from Dr. J. Eng (Bronx, NY). Insulin RIAs were obtained from Linco Research, Inc. (St. Louis, MO).

Cells and culture

The βTC-Tet cell line (13) was cultured in DMEM containing 2.5% FCS, 15% horse serum, 2 mM glutamine, and 50 U/ml streptomycin/penicillin. They were used between passages 17–25. For the perifusion studies, clusters of βTC-Tet cells of about 100 μm in diameter were formed by culturing the cells in 10-cm petri dishes maintained for 2–4 days on a rotating (70 rpm) platform placed in the cell culture incubator. The βTC-Tet cells were growth arrested in the presence of 1 μg/ml tetracycline. The βTCR^{-} cell line was established from β-cell tumors that developed in GLP-1-$R^{-/-}$ mice (14) crossed with RIP-Tag mice (15) (our manuscript in preparation). The cells were cultured in RPMI 1640 medium containing 10% FCS, 2 mM glutamine, and 50 U/ml streptomycin/penicillin.

cAMP assays

cAMP assays were performed as described previously (16). Briefly, cells were grown for 2 days in 12-well plates and loaded with 2 μCi tritiated adenine (TRK311, Amersham, Aylesbury, UK) for 5 h at 37 C. Cells were then washed twice with 130 mM NaCl, 20 mM HEPES (pH 7.4), 0.9 mM NaH_2PO_4, 0.8 mM $MgSO_4$, 5.4 mM KCl, 1.8 mM $CaCl_2$, 25 mM glucose, and 25 μM phenol red and exposed to peptides at different concentrations in the presence of 0.1 mM forskolin for 8 min at 37 C. After removal of the medium, cells were lysed with 1 ml 5% trichloroacetic acid containing 0.1 mM cAMP and 0.1 mM ATP. Tritiated cAMP was sequentially separated on Dowex cation exchange resin and aluminum oxide columns (17). For study of the effect of exendin-(9–39) on basal cAMP levels, the cells were prelabeled with 10 μCi tritiated adenine for 5 h at 37 C.

RNA extraction and Northern blot analysis

RNA was isolated by the guanidinium isothiocyanate method (18). Total RNA was size-fractionated on 1 × 3-[*N*-morpholino] propanesulfonic acid-1.2% agarose gels containing formaldehyde. Gels were transferred overnight by diffusion [10 × SSC (standard saline citrate)] to a GeneScreen membrane (DuPont, Wilmington, DE). Membranes were UV cross-linked and prehybridized for 4 h at 42 C. After prehybridization, the blots were hybridized with random primed (Boehringer Mannheim, Mannheim, Germany) rat GLP-1 receptor (4) and proglucagon (19) probes in 5 × SSC, 100 mM $NaPO_4$ (pH 6.5), 5 × Denhardt's solution, 50% formamide, 10 mM EDTA, 1% SDS, and 100 μg/ml yeast transfer RNA overnight at 42 C. The blots were washed in 2 × SSC at room temperature, followed, for the proglucagon blots, by 2 × SSC-0.1 SDS at 42 C and 0.2 × SSC-0.1% SDS at 55 C. Blots were then exposed to Hyperfilm-MP (Amersham) at −70 C.

Perifusion

Batches of 200–300 βTC-Tet clusters were placed in a perifusion chamber. The perifusion buffer was a Krebs-Ringer solution containing 0.5% BSA, and the flow rate was adjusted to 1 ml/min. Perifusion experiments consisted of a 30-min equilibration period in the presence of 2.8 mM glucose, a 15-min perifusion period in the presence of 2.8 mM glucose and 0.1 mM isobutylmethylxanthine (IBMX), a 20-min stimulation period in the presence of 11.1 mM glucose, 0.1 mM IBMX, and the presence or absence of peptides, and a 20-min period in the presence of 2.8 mM glucose and 0.1 mM IBMX. The perifusion chamber was maintained at 37 C, and the gas phase was 95% O_2-5% CO_2. Fractions were collected every minute. Insulin was quantitated by RIA, using rat insulin as standard.

Quantitative analysis

Results are presented as the mean ± SEM. Statistical differences were analyzed by Student's *t* test.

Results

In the present study we used the conditionally immortalized βTC-Tet cell line. These cells can be maintained in a growth-arrested state in the presence of tetracycline and resume their proliferation in the absence of the antibiotic due to the regulated expression of the simian virus 40 (SV-40) T antigen by a tetracycline operator/tetracycline transactivator system (13).

First, we determined by Northern blot analysis that these cells express the GLP-1 receptor in the proliferating and growth-arrested state (not shown). The dose-dependent production of intracellular cAMP in response to increasing concentrations of GLP-1 was then evaluated. Figure 1 shows that both growth-arrested and proliferating cells respond to the addition of GLP-1 with EC_{50} values that were not significantly different (2.05 ± 0.31 *vs.* 0.96 ± 0.20 nM for growth-arrested and proliferating cells, respectively). Next, we determined that the basal level of cAMP observed in the absence of added GLP-1 could be decreased by increasing concentrations of exendin-(9–39) (Fig. 2). This effect was observed with growth-arrested as well as proliferating cells. It was, however, not observed with $\beta TC\text{-}R^{-}$ cells, which do not express the GLP-1 receptor. These cells have been established in culture from pancreatic β-cells of GLP-1 receptor knockout mice expressing the SV-40 Tag under the control of the rat insulin promoter. To ensure that the decrease in cAMP induced by exendin-(9–39) was not due to displacement of GLP-1 ectopically expressed by the βTC-Tet cells and bound to their own receptors, we analyzed by Northern blot analysis the expression of the preproglucagon messenger RNA (mRNA). As shown in Fig. 3, proliferating βTC-Tet cells express some preproglucagon mRNA, but at a considerably lower level compared with INR1-G9 glucagonoma cells. Importantly, when the cells were growth arrested in the presence of tetracycline, preproglucagon mRNA was no longer detectable. Also, no GLP-1 could be detected by RIA (detection limit, 1 fmol) in the 24-h conditioned medium of 3×10^6 proliferating or growth-arrested cells. This indicates that a

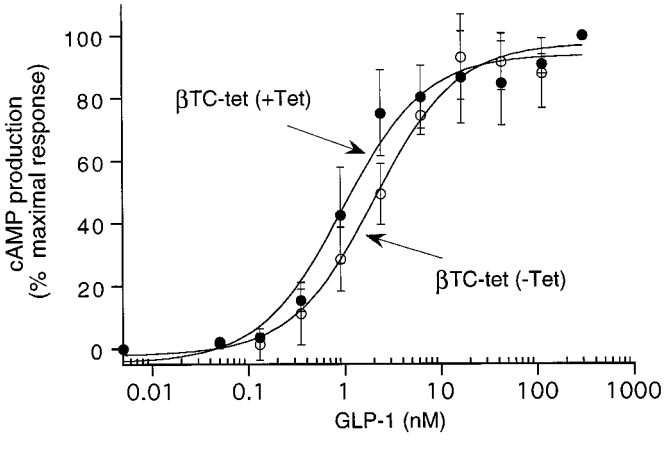

FIG. 1. GLP-1-induced cAMP accumulation in βTC-Tet cells. βTC-Tet cells were growth arrested in the presence of tetracycline (+Tet) or were not arrested (-Tet) and were exposed to increasing concentrations of GLP-1 for 8 min in the presence of IBMX. GLP-1 induced accumulation of intracellular cAMP with the same EC_{50} in both growth-arrested and proliferating cells (2.05 ± 0.31 *vs.* 0.96 ± 0.20 nM).

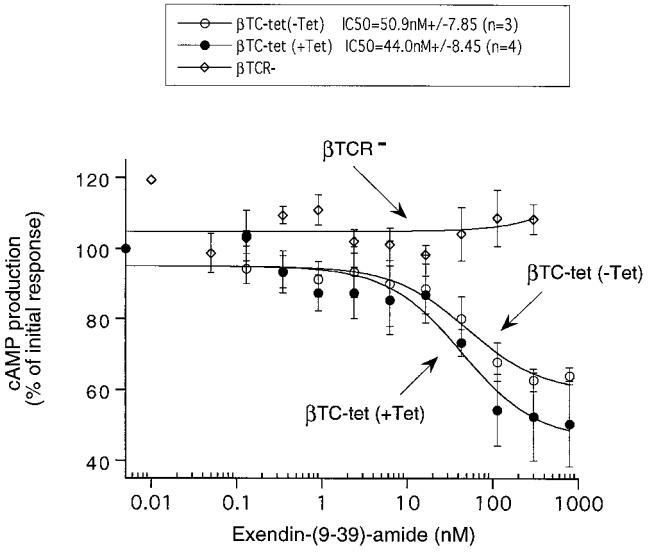

FIG. 2. Exendin-(9–39) dose dependently decreased the basal level of cAMP in βTC-Tet cells. Proliferating (-Tet) or growth-arrested (+Tet) βTC-Tet cells were exposed to increasing concentrations of exendin-(9–39), and intracellular cAMP was measured. A dose-dependent decrease in basal cAMP levels was observed with the same IC_{50} values for both proliferating and growth-arrested cells. The basal level of cAMP in a βTC cell line (βTC-R^-) derived from GLP-1-receptor$^{-/-}$ mice was not modified by exendin-(9–39). The basal levels of cAMP were 1166 ± 78 cpm (n = 4) for βTC-Tet (+Tet), 806 ± 225 cpm (n = 3) for βTC-Tet (−Tet), and 807 ± 177 cpm (n = 3) for βTC-R^-.

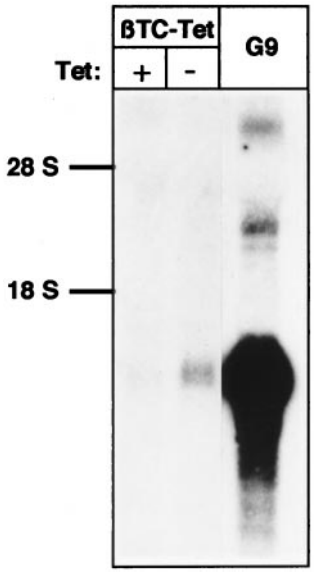

FIG. 3. Expression of the preproglucagon mRNA in βTC-Tet cells. Total RNAs extracted from growth-arrested (+Tet) or proliferating (−Tet) βTC-Tet cells and from the glucagonoma cells INR1-G9 were separated by electrophoresis on agarose gel, and the preproglucagon mRNA was detected by Northern blot analysis. In the growth-arrested state, βTC-Tet cells no longer express detectable levels of preproglucagon mRNA.

simple antagonistic effect of exendin-(9–39) on preventing endogenously produced GLP-1 from binding to the GLP-1 receptor was unlikely to be the cause of the decreased production of cAMP.

We next assessed the secretory activity of βTC-Tet cells in the proliferating and growth-arrested states and the potentiation effect of GLP-1 on insulin secretion. For these experiments, βTC-Tet cells were grown as aggregates about 100 μm in diameter in rotating suspension cultures. The secretory activity of these clusters was evaluated in perifusion experiments in the presence of 2.8 and 11.1 mM glucose. As previously described (13), these cells respond to elevations in extracellular glucose with a normal dose-response curve. Figure 4, A and B, shows that in both proliferating and growth-arrested states, the addition of 1 nM GLP-1 strongly stimulated the secretion of insulin induced by 11.1 mM glucose. Quantitation of the stimulatory effect on first and second phase secretion is presented in Fig. 4C. As there is some variability in the size of the clusters that also affects the efficiency of glucose-induced insulin secretion, the results are presented as a percentage of the insulin content for individual perifusion experiments, and quantitation of the area under the curve is presented as a percentage of the control AUC. We then determined whether exendin-(9–39), which decreases the basal intracellular level of cAMP, as described above, would cause an inhibition of glucose-induced insulin secretion. Figure 5A shows that the secretory activity of growth-arrested cells perifused at 11.1 mM glucose was strongly inhibited when 100 nM exendin-(9–39) was added to the 11.1-mM glucose solution. Interestingly, however, this inhibitory effect was much lower when using cells in the proliferating state (Fig. 5B). Figure 5C shows the quantification of these results for first and second phases.

To determine whether this inhibitory effect was also detectable with nontransformed β-cells, the glucose-stimulated insulin secretion of mouse pancreatic islets was assessed in the presence and absence of exendin-(9–39). Figure 6A shows that a strong inhibition of insulin secretion was observed that mostly affected the second phase of secretion without modifying the initial rate of secretion or the height of the first phase. This is quantitated in Fig. 6B.

Discussion

In the present study, we showed that exendin-(9–39) reduces the basal intracellular cAMP content of βTC-Tet cells and that this was correlated with an inhibition of glucose-induced insulin secretion in growth-arrested βTC-Tet cells and isolated pancreatic islets, but not in proliferating βTC-Tet cells. Our results suggest that the GLP-1 receptor is constitutively active and thus maintains a basal production of cAMP even in the absence of bound ligand. This may be required to maintain the glucose competence of β-cells. Interestingly, as the decreased basal cAMP content leads to a reduced secretory activity only in growth-arrested cells, this suggests that different mechanisms may be involved in the control by cAMP of insulin granule exocytosis in proliferating and growth-arrested cells.

The βTC-Tet cells are conditionally immortalized cells whose proliferation can be stopped by tetracycline, as expression of the transforming SV-40 T antigen is under the control of a tetracycline-regulated promoter (13). These cells display normal glucose dose-dependent insulin secretion and an insulin content close to that of normal β-cells. Here we further showed by Northern blot analysis that βTC-Tet

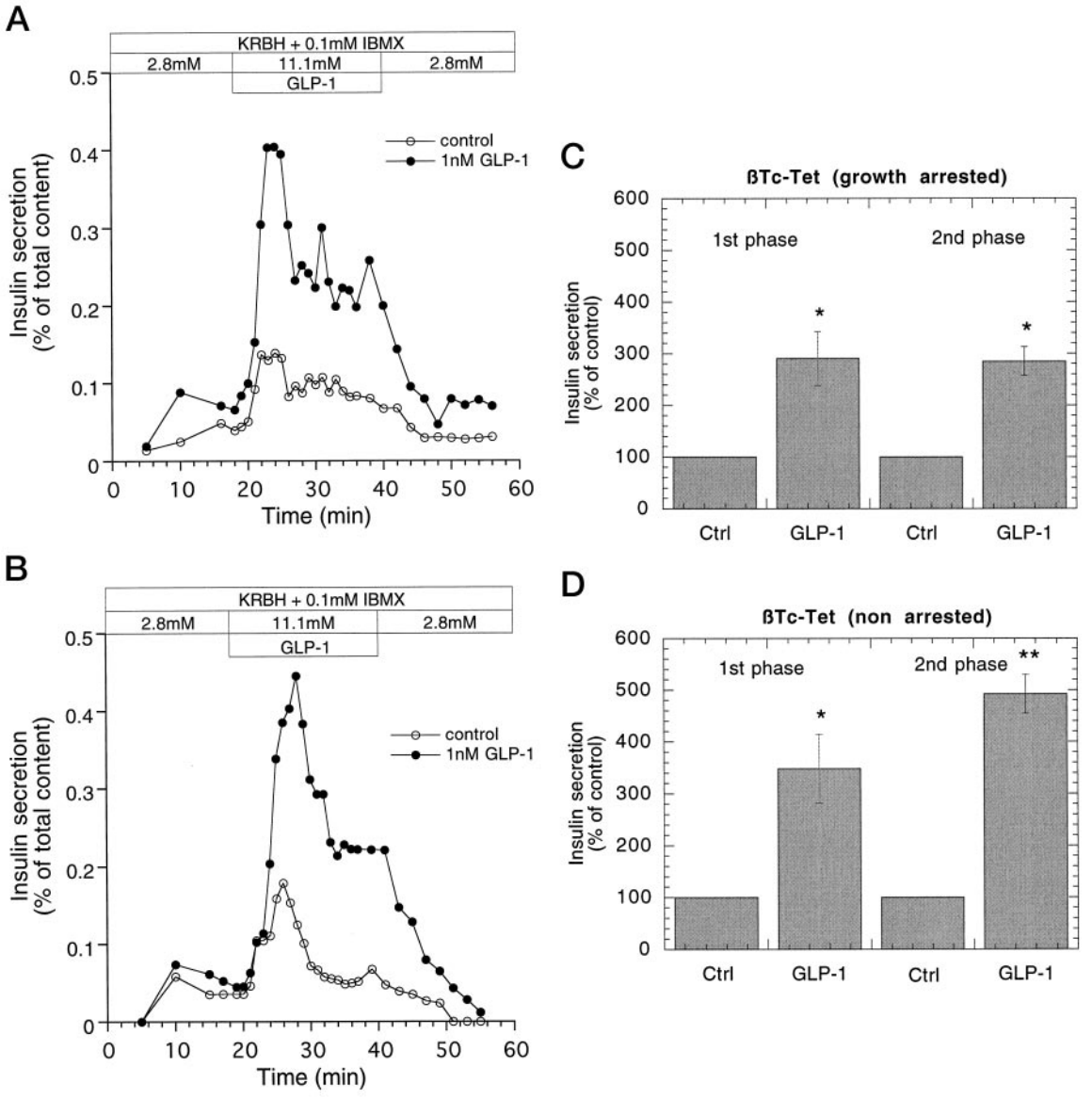

FIG. 4. GLP-1 stimulation of insulin secretion by βTC-Tet cells. The secretory activity of βTC-Tet cells in response to glucose and 0.1 mM IBMX and in the presence or absence of 1 nM GLP-1 was assessed in perifusion experiments. A, The cells were grown in clusters and growth arrested in the presence of tetracycline. The secretion of insulin stimulated by 11.1 mM glucose was strongly amplified when 1 nM GLP-1 was added to the perifusion solution. B, Same as in A, but the cells were not growth arrested. C and D, Quantitation of the area under the curve (AUC) of the 11.1 mM glucose-stimulated insulin secretion curve in the presence and absence of GLP-1. Data are presented for first and second phase secretions. The first phase is taken as the first 10-min period and second phase as the second 10-min period of high glucose stimulation. Data are for growth-arrested (C) and nonarrested (D) cells. *, $P < 0.05$; **, $P < 0.01$ (n = 3).

cells express the GLP-1 receptor, that they increase their intracellular cAMP content with the normal GLP-1 dose dependence, and that the glucose-dependent secretory activity can be amplified by GLP-1 similarly in growth-arrested and proliferating cells.

Exendin-(9–39) is a truncated form of exendin-4, a peptide isolated from the venom of *Heloderma suspectum* (20). Exendin-(9–39) is an antagonist of the rat and human GLP-1 receptors (21, 22). It has been used in *in vivo* experiments to evaluate the importance of GLP-1 insulinotropic activity on the control of postprandial insulin secretion and blood glucose levels (23–25). These experiments, performed in both rats and baboons, demonstrated that GLP-1 has an important role in the postprandial control of glycemia.

Here we demonstrated that exendin-(9–39) not only had an antagonistic activity on the GLP-1 receptor, but it dose dependently decreased the basal intracellular levels of cAMP in growth-arrested or proliferating βTC-Tet cells. This effect was receptor specific, as it could not be observed in a β-cell line lacking the GLP-1 receptor. Furthermore, the inhibitory effect could not be explained by a simple displacement by exendin-(9–39) of endogenously produced GLP-1 bound to the receptor, because the preproglucagon mRNA was no longer detectable in growth-arrested cells, and no radioim-

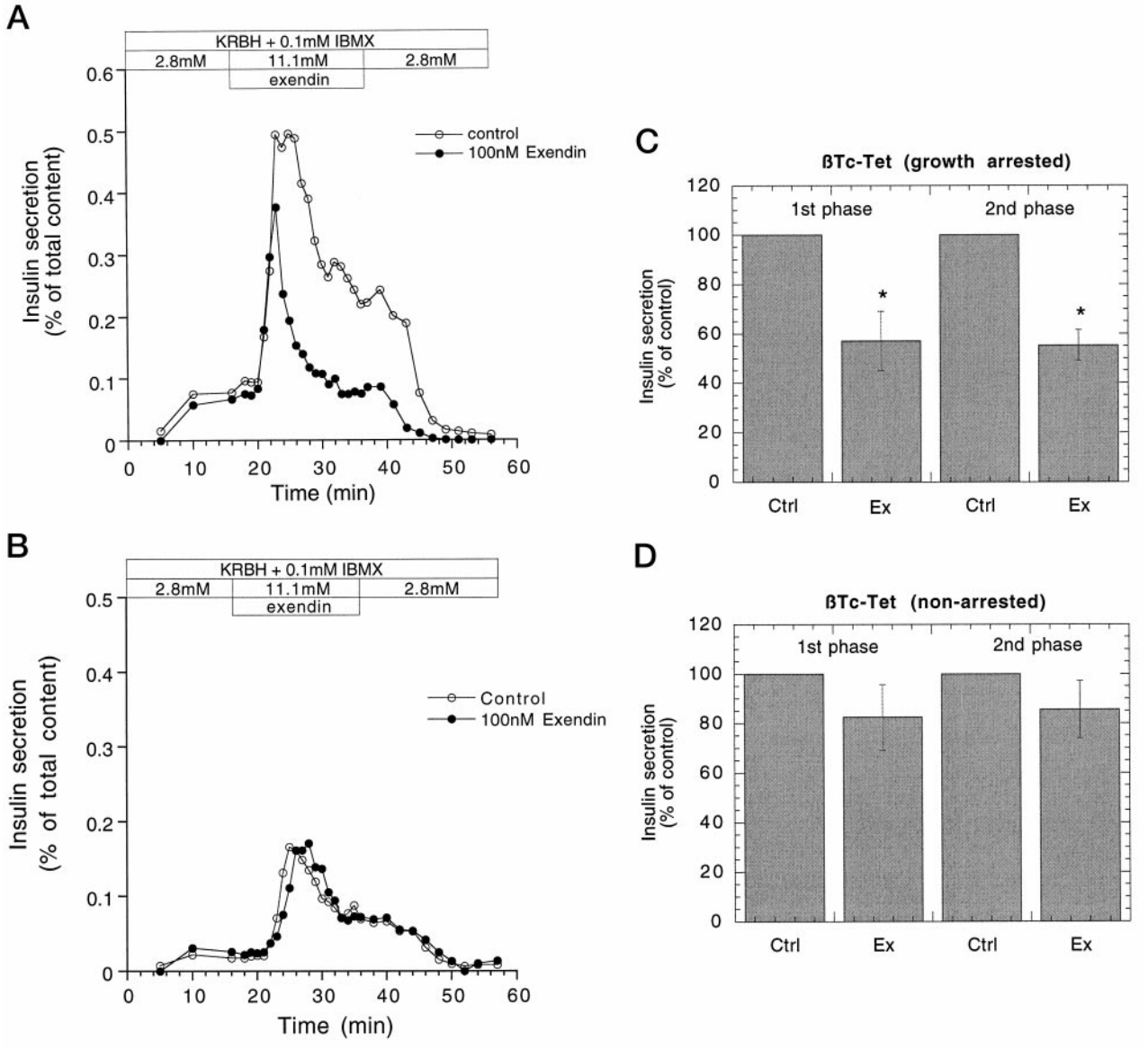

FIG. 5. Exendin-(9–39) inhibits glucose-induced insulin secretion in growth-arrested cells. βTC-Tet cells grown as clusters were either growth arrested in the presence of tetracycline (A) or kept in the proliferating state (B). Insulin secretory activity was then assessed in perifusion experiments in response to 11.1 mM glucose and 0.1 mM IBMX. The presence of exendin-(9–39) (100 nM; Ex) led to a strong reduction in the glucose-stimulated secretory activity of growth-arrested, but not proliferating, cells. C and D, Quantitation of the area under the curve (AUC) of the 11.1 mM glucose-stimulated insulin secretion curve in the presence and absence of exendin-(9–39). Data are presented for first and second phase secretion. The first phase is taken as the first 10-min period and the second phase as the second 10-min period of high glucose stimulation. Data are for growth-arrested (C) and nonarrested (D) cells and are the mean ± SEM of four determinations for all sets of experiments. Differences are not significant ($P > 0.05$) for nonarrested cells and are significant ($P < 0.01$) for growth-arrested cells.

munoassayable GLP-1 could be detected in proliferating or growth-arrested cells. Also, the maximal inhibitory capacity of exendin-(9–39) on basal cAMP appears greater, although the difference is not statistically significant, in growth-arrested compared with proliferating cells (49% *vs.* 36% of cAMP decrease for growth-arrested and proliferating βTC-Tet cells, respectively) when preproglucagon mRNA is no longer detectable.

Previous reports evaluating the antagonistic activity of exendin-(9–39) did not observe this inhibitory effect on basal intracellular cAMP levels. This was tested with the insulinoma lines RINm5F (21) and HIT (26) or with fibroblasts stably transfected with the cloned human GLP-1 receptor (22). The present results may be due to species differences, as βTC-Tet cells originate from mice, or possibly because βTC-Tet cells have a much more differentiated phenotype than the other β-cell lines studied. This is in particular demonstrated by their remarkably preserved glucose dose-dependent insulin secretory activity (13) (our unpublished observations).

Importantly, the inhibitory effect of exendin-(9–39) on cAMP production was correlated with an approximately 40% reduction in insulin secretory activity when perifusion experiments were performed in the presence of the peptide.

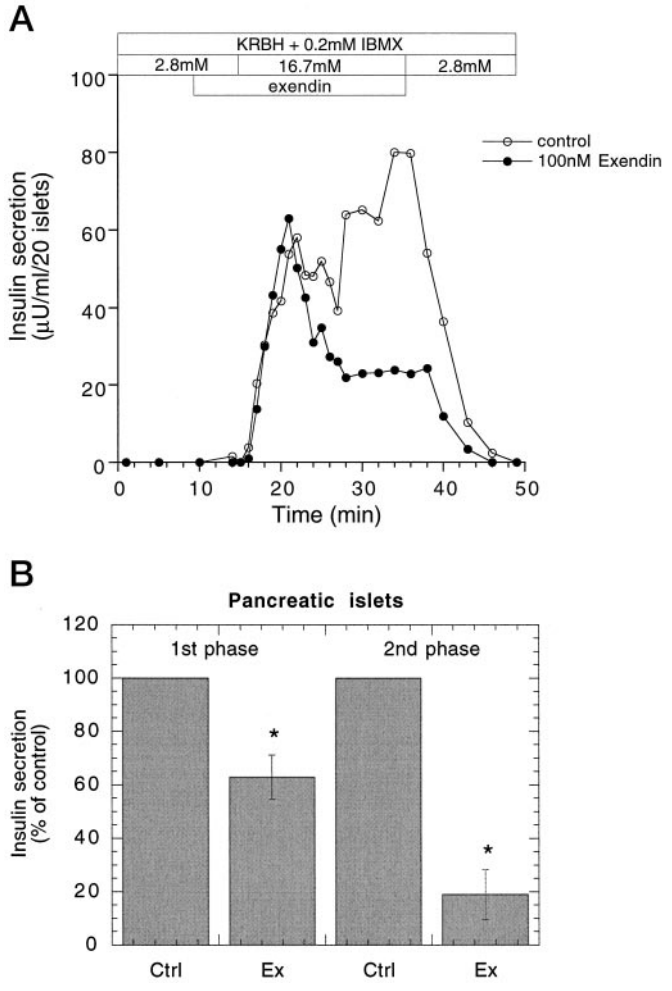

FIG. 6. Exendin-(9–39) inhibits glucose-induced insulin secretion in isolated mouse pancreatic islets. A, Insulin secretory activity of mouse pancreatic islets was measured in perifusion experiments in the presence or absence of exendin-(9–39) as described for βTC-Tet cells. The same inhibition of glucose-induced insulin secretion was observed. B, Quantitation of the area under the curve (AUC) of glucose-stimulated insulin secretion in the presence and absence of exendin-(9–39). Data are presented for first and second phase secretions. The first phase is taken as the first 10-min period and the second phase as the second 10-min period of high glucose stimulation. Data are the mean ± SEM of four determinations. Differences are significant ($P < 0.01$).

A similar inhibitory effect was also observed when perifusions were carried out with isolated mouse pancreatic islets. In both conditions the first phase of secretion appeared unchanged, and only the second phase was reduced. This was observed when the peptide was introduced at the same time as well as several minutes before switching to the high glucose solution. However, when the βTC-Tet cells were not in the growth-arrested state, this inhibitory effect on insulin secretion was not observed, although the effect on basal cAMP levels were present. Why a similar decrease in cAMP levels observed in both arrested and proliferating cells is not reflected by a similar change in secretory activity is not known. One can speculate that the stimulatory effect of cAMP, mediated by activation of protein kinase A, depends on the phosphorylation of multiple protein targets participating in the control of insulin granule exocytosis. In growth-arrested cells, which are more differentiated than proliferating cells, a protein may be expressed that exerts a negative control on exocytosis but whose effect can be relieved by phosphorylation. A decrease in the phosphorylation of this protein due to a decrease in basal cAMP may have a dominant inhibitory effect on the secretory activity of the cells. Although the exact mechanism is still hypothetical at this stage, proliferating and growth-arrested βTC-Tet cells provide a unique system to study this regulatory event.

Our present results showing an important effect of exendin-(9–39) on reducing the basal intracellular level of cAMP indicate that this peptide is an inverse agonist of the GLP-1 receptor. Consequently, this implies that the mouse receptor exists in β-cells in a constitutively activated state. Such a basal level of activity of G protein-coupled receptors has been previously reported for the β_2-adrenergic receptor (27, 28) or the δ-opioid receptor (29). This was demonstrated by the capability of specific ligands, referred to as inverse agonists (30), to decrease the basal level of cAMP production or the guanosine triphosphatase activity of G proteins linked to the receptors. The *in vivo* role of such a basal constitutive activity of the β_2-adrenergic receptor or other receptors is not clearly established. Overexpression of the β_2-adrenergic receptor in cardiomyocytes, however, led to increased basal cAMP and increased atrial contractility and ventricular function even in conditions in which the receptor was apparently not stimulated by endogenous ligands (31). This suggested that the constitutive activity of this receptor, at least when present at higher than normal levels, could modify the function of an organ. Our study is the first indication of a constitutive activity of the GLP-1 receptor in β-cells. Furthermore, this constitutive activity appears important in the glucose-stimulated insulin secretory response and, therefore, in maintaining the glucose competence of the β-cells.

These data may help understand previously published data that demonstrated that in mice with a homozygous null mutation of the GLP-1 receptor, fasting blood glucose levels were surprisingly elevated (14). The same study also reported that after an ip glucose tolerance test, the homozygous mutant mice displayed an abnormal glycemic response. This was unexpected because the ip injection of glucose should not activate the secretion of GLP-1 in either control or knockout mice and therefore should lead to the same glucose tolerance curves. As the glycemic levels were higher in GLP-1 receptor$^{-/-}$ mice, this suggests that the absence of a constitutively activated receptor leads to a reduced basal intracellular cAMP level and therefore a decreased glucose competence of the β-cells. Our conclusions on the constitutive activity of the unliganded receptor could also explain in part observations reported by D'Alessio *et al.* (25). These researchers showed that the fasting blood glucose levels of baboons could be increased by exendin-(9–39), but not by anti-GLP-1 antibodies. In fasting, the circulating GLP-1 levels are already very low, and further immunoneutralization of the peptide has no effect on basal glycemia, whereas exendin-(9–39), acting directly at the receptor level, promoted this increase in glycemia. These effects, however, were accompanied only by a small, nonsignificant decrease in plasma insulin and a significant increase in glucagon levels.

Whether the GLP-1 receptor is constitutively active in

β-cells from other animal species and in humans needs to be directly tested. Our data nevertheless suggest an important role for the GLP-1 receptor not only in the postprandial stimulation of glucose-induced insulin secretion when plasma levels of both glucose and this hormone are elevated due to nutrient ingestion but also in the glucose competence of β-cells when the level of extracellular glucose rises in the absence of added gluco-incretin hormones.

References

1. **Creutzfeld W, Ebert W, Nauck M, Stöckmann F** 1983 Disturbances of the entero-insular axis. Scand J Gastroenterol [Suppl] 83:111–119
2. **Göke R, Fehmann H-C, Göke B** 1991 Glucagon-like peptide-1 (7–36) is a new incretin/enterogastrone candidate. Eur J Clin Invest 21:135–144
3. **Göke R, Conlon JM** 1988 Receptors for glucagon-like peptide-1(7–36)amide on rat insulinoma-derived cells. J Endocrinol 116:357–362
4. **Thorens B** 1992 Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide I. Proc Natl Acad Sci USA 89:8641–8645
5. **Usdin TB, Mezey E, Button DC, Brownstein MJ, Bonner TI** 1993 Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. Endocrinology 133:2861–2870
6. **Thorens B** 1994 The gluco-incretin hormone glucagon-like peptide-1 and its β cell receptor. In: Draznin B, LeRoith D (eds) Molecular Biology of Diabetes, part I. Humana Press, Totowa, pp 357–379
7. **Thorens B, C. Widmann** 1996 Structure and function of the glucagon-like peptide-1 receptor. In: Lefèbvre PJ (ed) Glucagon III. Springer, Berlin, pp 255–273
8. **Ämmälä C, Ashcroft FM, Rorsman P** 1993 Calcium-independent potentiation of insulin release by cyclic AMP in single β cells. Nature 363:356–358
9. **Schuit FC, Pipeleers DG** 1985 Regulation of 3′,5′-monophosphate levels in the pancreatic B cell. Endocrinology 117:834–840
10. **Gremlich S, Porret A, Hani E-H, Cherif D, Vionnet N, Froguel P, Thorens B** 1995 Cloning, functional expression and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. Diabetes 44:1202–1208
11. **Moens K, Heimberg H, Flamez D, Huypens P, Ling Z, Pipeleers D, Gremlich S, Thorens B, Schuit F** 1996 Expression and functional activity of glucagon, glucagon-like peptide-1, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. Diabetes 45:257–261
12. **Abrahamsen N, Nishimura E** 1995 Regulation of glucagon and glucagon-like peptide-1 receptor messenger ribonucleic acid expression in cultured rat pancreatic islets by glucose, cyclic adenosine 3′,5′-monophosphate, and glucocorticoids. Endocrinology 136:1572–1578
13. **Efrat S, Fusco-DeMane D, Lemberg H, Al Emran O, Wang X** 1995 Conditional transformation of a pancreatic β-cell line derived from transgenic mice expressing a tetracycline-regulated oncogene. Proc Natl Acad Sci USA 92:3576–3580
14. **Scrocchi LA, Brown TJ, MacLusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ** 1996 Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide-1 receptor gene. Nat Med 2:1254–1258
15. **Efrat S, Linde S, Kofod H, Spector D, Delannoy M, Grant S, Hanahan D, Baekkeskov S** 1988 Beta-cell lines derived from tansgenic mice expressing a hybrid insulin gene-oncogene. Proc Natl Acad Sci USA 85:9037–9041
16. **Widmann C, Bürki E, Dolci W, Thorens B** 1994 Signal transduction by the cloned glucagon-like peptide-1 receptor. Comparison with signalling by the endogenous receptors of β cell lines. Mol Pharmacol 45:1029–1035
17. **Bozou JC, Amar S, Vincent JP, Kitabgi P** 1986 Neurotensin-mediated inhibition of cyclic AMP formation in neuroblastoma N1E115 cells: involvement of the inhibitory GTP-binding component of adenylate cyclase. Mol Pharmacol 29:489–496
18. **Chomczynski P, Sacchi N** 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159
19. **Heinrich G, Gros P, Lund PK, Bentley RC, Habener JF** 1984 Pre-proglucagon messenger ribonucleic acid: nucleotide and encoded amino acid sequences of the rat pancreatic complementary deoxyribonucleic acid. Endocrinology 115:2176–2181
20. **Eng J, Kleinman WA, Singh L, Singh G, Raufman J-P** 1992 Isolation and characterization of exendin-4, an exendin-3 analogue, from heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. J Biol Chem 267:7402–7405
21. **Creutzfeld W, Nauck M** 1992 Gut hormones and diabetes mellitus. Diabetes Metab Rev 8:149–177
22. **Thorens B, Porret A, Bühler L, Deng S-P, Morel P, Widmann C** 1993 Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9–39) an antagonist of the receptor. Diabetes 42:1678–1682
23. **Kolligs F, Fehmann H-C, Göke R, Göke B** 1995 Reduction of the incretin effect in rats by the glucagon-like peptide 1 receptor antagonist exendin (9–39) amide. Diabetes 44:16–19
24. **Wang Z, Wang RM, Owji AA, Smith DM, Ghatei MA, Bloom SR** 1995 Glucagon-like peptide-1 is a physiological incretin in rat. J Clin Invest 95:417–421
25. **D'Alessio DA, Vogel R, Prigeon R, Laschansky E, Koerker E, Eng J, Ensinck JW** 1996 Elimination of the action of glucagon-like peptide 1 causes an impairment of glucose tolerance after nutrient ingestion by healthy baboons. J Clin Invest 97:133–138
26. **Marie J-C, Boissard C, Skoglund G, Rosselin G, Breant B** 1996 Glucagon acts through its own receptor in the presence of functional glucagon-like peptide-1 receptors on hamster insulinoma. Endocrinology 137:4108–4114
27. **Samama P, Pei G, Costa T, Cotecchia S, Lefkowitz RJ** 1994 Negative antagonists promote an inactive conformation of the β2-adrenergic receptor. Mol Pharmacol 45:390–394
28. **Chidiac P, Hebert TE, Valiquette M, Dennis M, Bouvier M** 1994 Inverse agonist activity of the β-adrenergic receptor. Mol Pharmacol 45:490–499
29. **Costa T, Herz A** 1989 Antagonists with negative intrinsic activity at the δ opioid receptors coupled to GTP-binding proteins. Proc Natl Acad Sci USA 86:7321–7325
30. **Milligan G, Bond RA, Lee M** 1995 Inverse agonism: pharmacological curiosity or potential therapeutic strategy? Trends Pharmacol Sci 16:10–13
31. **Milano CA, Allen LF, Rockman HA, Dolber PC, McMinn TR, Chien KR, Johnson TD, Bond RA, Lefkowitz RJ** 1995 Enhanced myocardial function in transgenic mice overexpressing the β2-adrenergic receptor. Science 264: 582–586