Glucose-Dependent Stimulatory Effect of Glucagon-Like Peptide 1(7-36) Amide on the Electrical Activity of Pancreatic β-Cells Recorded In Vivo

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The stimulatory effect of the glucagon-like peptide (GLP)-1(7-36) amide on electrical activity in pancreatic b-cells recorded in vivo was studied. The injection of GLP-1 produces a lengthening of the active phase with respect to the silent phase, leading to a stimulation of insulin release, which produces a secondary decrease in blood glucose concentration and eventually, to the hyperpolarization of the membrane at a blood glucose level of ~5 mmol/l. The injection of GLP-1 at a glycemic level <5 mmol/l does not stimulate electrical activity. This is in contrast to the effect of tolbutamide, which stimulates electrical activity at low glucose concentrations. These results demonstrate that in vivo, the stimulatory effect of GLP-1 on insulin secretion is at least partially mediated by its effect on β-cell electrical activity. Furthermore, the glucose dependence of the effect confers to GLP-1, a security factor that supports its potential use in the treatment of type 2 diabetes. ***Diabetes*** **48:754–757, 1999**

The secretion of insulin by pancreatic β-cells is regulated by blood glucose concentration and modulated by a variety of neural and hormonal influences. Incretins are gut-derived factors that increase glucose-stimulated insulin secretion (1,2,3) of which the glucagon-like peptide (GLP)-1(7-36) amide (4,5), a factor resulting from the posttranslational processing of glucagon, has attracted much attention as a possible therapy in type 2 diabetes. This is due to the fact that the stimulatory effect of GLP-1 on insulin secretion is only exerted at stimulatory blood glucose levels (6–10). This confers on GLP-1 a security margin that sulphonylureas lack because they stimulate insulin secretion at low blood glucose concentrations. GLP-1 is secreted by L-cells in the gastrointestinal tract in response to an oral glucose load, producing a 50% increase in stimulation of insulin secretion compared with the same glucose increase achieved by intravenous infusion of the sugar (11–13). The effect of GLP-1 seems to be primarily mediated by its action on pancreatic β-cells where specific receptors have been demonstrated (14–16), although extrapancreatic effects may also play a role (17).

The stimulatory effect of GLP-1 on insulin release is the result of multiple effects on the stimulus-secretion coupling chain. The binding of GLP-1 to a G protein–coupled receptor produces an increase in cAMP levels (18) that stimulate calcium currents (19,20). This, together with the participation of calcium-induced calcium release from intracellular stores (21), brings about the well-documented increase in intracellular calcium produced by GLP treatment (19–23). Likewise, the synergistic effect of GLP-1 and glucose in inhibiting the ATP-dependent potassium channel (KATP) (24) may also contribute to increased calcium levels. In addition to the increase in calcium, increased levels of cAMP directly stimulate insulin secretion (25). Therefore, the overall stimulatory effect seems to be mediated by a synergistic calcium-cAMP interplay.

Under normal conditions, glucose elicits calcium transients in pancreatic β-cells caused by its effects on membrane potential (26). The electrical activity of pancreatic β-cells, when organized in the islets of Langerhans, consists of bursts of calcium-action potentials, the duration of the burst being in direct proportion to the blood glucose level. Such bursts are associated to intracellular calcium oscillations and produce pulsatile insulin release (27,28). Electrical recordings of pancreatic β-cells in vivo have demonstrated this characteristic burst activity, and its modulation by glucose in the 5–7 mmol/l range (29) and by sulphonylureas (30) provides a model closer to the physiological situation for checking the action of factors that affect β-cell function. Given the multiple effects of GLP-1 in pancreatic β-cells, it is important to know in vivo if GLP-1 changes electrical activity and how it is dependent on blood glucose concentration.

RESEARCH DESIGN AND METHODS

The methods used for in vivo recording have been described elsewhere (29). Albino mice (age 8–10 weeks, 25–35 g body wt) were anesthetized by intraperitoneal injection of 90 mg/kg Nembutal. The experiments were carried out according to institutional animal care guidelines. The animals were laparotomized, and the duodenal part of the pancreas dissected free from adherence. The vena cava and the abdominal aorta were cannulated caudaly for solution infusion and blood sample collection, respectively. During the experiment, the animal was laid on its back on a heated bed maintained at 37°C. For the electrical recording, the duodenal part of the pancreas was spread out on top of a platform (6 × 15 mm) covered by a 3-mm layer of Sylgard (Dow Corning, Midland, MI). The islets were impaled with high-resistance glass microelectrodes (100–150 megaohm) filled with a solution of 3 mol/l potassium citrate plus 100 mmol/l potassium chloride. The electrical activity was recorded with an

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GLP, glucagon-like peptide; KATP, ATP-dependent potassium channel.

Axoclamp-2A amplifier (Axon Instruments, Foster City, IA). Unfiltered records were acquired at a frequency of 300 Hz and stored on a microcomputer using Axotape for analysis off-line. Blood samples were collected from the aorta and analyzed for glucose concentration (Beckman glucose analyser-2). GLP-1(7-36) amide (Preproglucagon 78-107) was purchased from Sigma (St. Louis, MO) and dissolved in sterile saline solution to 6 µmol/l. A single bolus of 25 µl solution was injected, resulting in a final GLP-1 concentration of 75 nmol/l (assuming a 2-ml blood circulating volume). In control experiments, injection of the same volume of saline solution had no effect on electrical activity. Likewise, injection of the non-insulinotropic Preproglucagon 72-108 at the same concentration had no effect.

RESULTS

Figure 1 shows the effect of GLP-1 on the electrical activity of a pancreatic β-cell recorded in vivo. A continuous record of such activity, consisting of alternating depolarized and hyperpolarized phases (26,29), can be seen (Fig. 1*A*). On top of the depolarized phase, there are calcium-action potentials that are not individually resolved because of the slow time-base of the figure. The arrow indicates the time at which GLP-1 (25 µl of a 6 µmol/l solution) was injected as a single bolus through the cava vein. This produced a continuous and slow increase in the duration of the active phase with respect to the silent one. The values 7.7 and 7.4 mmol/l are the glycemia measurements made during the electrical recording. Treatment with GLP-1 produces a decrease in the glycemic level that probably reflects an increased insulin secretion associated with the increased length of the active phase. In eight experiments, the glycemic level decreased from 6.8 ± 0.4 to 6.1 ± 0.6 mmol/l (mean ± SD, $P < 0.05$) 10 min after GLP-1 injection. The time course duration of the active and silent phases is shown in Fig. 1*B*. As can be appreciated, the duration of the silent phase remains practically unchanged after the GLP-1 injection, whereas the active phase increases from a mean value of ~7 s before GLP-1 injection to ~25 s, 5 min after the injection. Figure 1*B* (bottom) shows the temporal evolution of the active versus silent phase ratio, an index of cell activity positively correlated to insulin secretion.

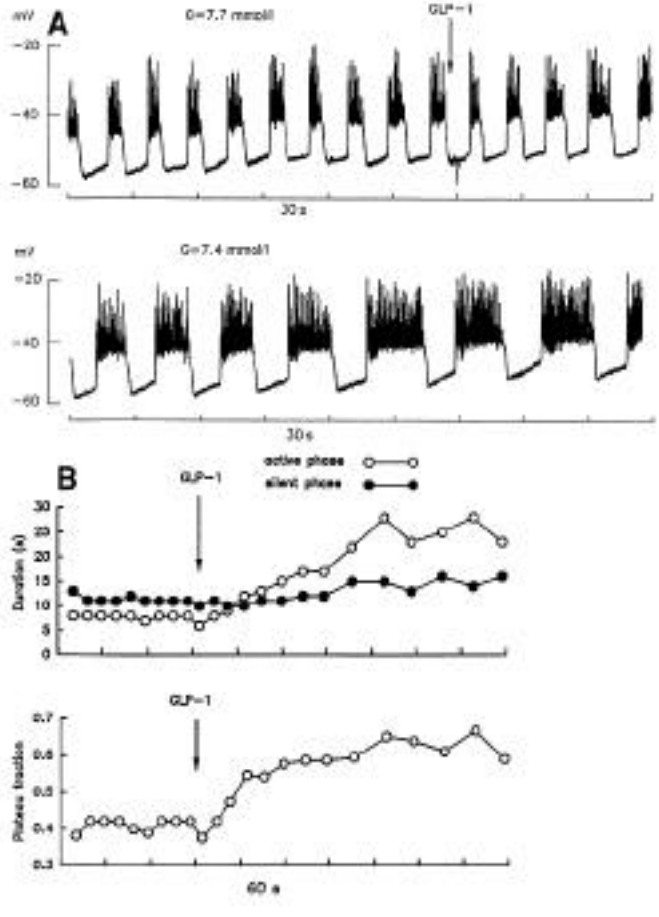

FIG. 1. Effect of GLP-1 on the electrical activity of a β-cell recorded in vivo. *A*: A continuous recording of the electrical activity is shown. The arrow indicates the time at which 25 µl of GLP-1 (6 µmol/l) was injected as a single bolus. The values 7.7 and 7.4 mmol/l are glycemic measurements made during the electrical recording. Essentially the same results were obtained in another 12 animals. *B*: The temporal evolution of the active and silent phase duration is shown. A plot of the duration of active (○) and silent (●) phases of consecutive oscillations is shown (Fig. 1*B*, top). A plot of the temporal evolution of the active/silent phase ratio is shown (Fig. 1*B*, bottom).

Figure 2 shows the effect of GLP-1 on electrical activity in an animal with an initial blood glucose concentration of 6.4 mmol/l. As in the previous experiment, GLP-1 produced an increase in electrical activity and, consequently, a gradual decrease in blood glucose concentration to a point where oscillatory electrical activity ceased and the cell became hyperpolarized. A second injection of GLP-1 at this point was ineffective and did not promote an increase in electrical activity. This glucose dependence in the action of GLP-1 is an important security factor that prevents a further decrease in blood glucose. In our experiments, GLP-1 only stimulated electrical activity at a glycemic level >5.1 ± 0.4 mmol/l ($n = 4$). This is in contrast to the action of a sulphonylurea (Fig. 3). In this experiment, the animal was treated with GLP-1, producing the effects already described, and the recording shown starts at the point when the cell became hyperpolarized. The second injection of GLP-1 did not increase electrical activity, as can be seen in the figure. However, injection of tolbutamide, despite the low blood glucose concentration, was able to depolarize the cell and to further decrease the blood glucose concentration. This experiment clearly illustrates the advantage of GLP-1 over sulphonylureas.

DISCUSSION

The experiments described here provide direct evidence of a GLP-1 stimulatory effect on the electrical activity of pancreatic β-cells in vivo. This stimulatory effect is glucose dependent, with a threshold of ~5.1 mmol/l glucose, which is consistent with the glucose-dependent insulinotropic effect reported in humans and in laboratory animals. Below this value, β-cells are hyperpolarized, and GLP-1 is not able to stimulate electrical activity. In animals treated with insulin, a transition from oscillation to continuous hyperpolarization takes place at a glycemic level of ~4.7 mmol/l (30). This value is very close to 5.1 mmol/l, indicating that GLP-1 itself does not modify the threshold for glucose-induced membrane potential oscillations. This glucose dependence is in marked contrast with the effect of tolbutamide, which is able to produce calcium-action potentials and stimulate insulin secretion at low (<4.7 mmol/l) glucose concentrations.

Previous work has shown that in isolated islets of Langerhans, GLP-1 produces a slight increase in electrical activity (20). In our case, we have shown a more pronounced stimulatory effect within the physiological range of

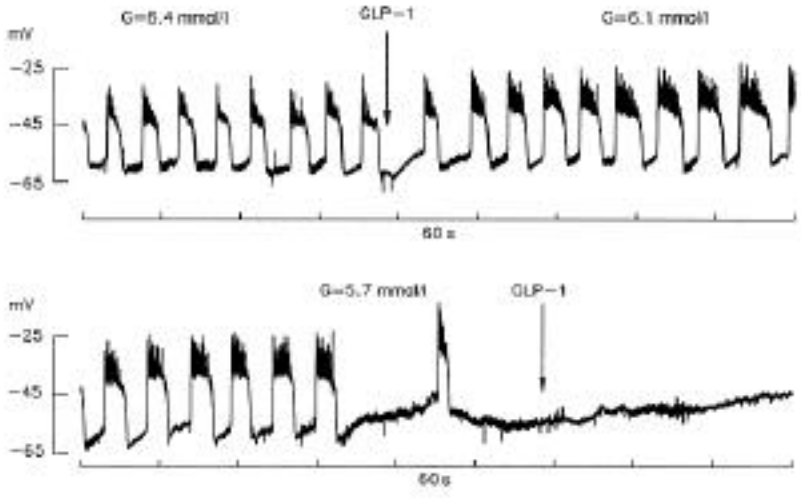

FIG. 2. Glucose dependence of the effect of GLP-1. The two panels represent a continuous record of the electrical activity. GLP-1 was injected at the times indicated by the arrows. After the second GLP-1 injection, the cell remained hyperpolarized for the rest of the recording period. Similar results were obtained in experiments in three other animals.

glycemia. The increase in electrical activity leads to a decrease in blood glucose, probably caused by an increased insulin secretion, and eventually, to cell hyperpolarization. The stimulatory effect of GLP-1 injection on electrical activity was evident after a few minutes (1–7 min) and was extended for periods of up to 25 min. The long-lasting effect of GLP-1 could only be seen in those experiments where the decrease in glycemia did not reach the threshold for membrane hyperpolarization.

Much work has been devoted to the mechanism of GLP-1 action. It is known that its receptor is coupled to a G protein, promoting an increase in cAMP levels. In pancreatic β-cells, cAMP is able to produce insulin secretion at permissive intracellular calcium concentrations. Within this framework, the increased electrical activity induced by GLP-1 may be providing the necessary intracellular calcium that permits the stimulatory effect of cAMP. Furthermore, the requirement of a certain level of intracellular calcium for the stimulatory action of cAMP ensures the glucose dependence of the GLP-1 effect. This is because the stimulatory effects on electrical activity are only apparent at glycemic levels >5.1 mmol/l. Such a stimulatory effect, reflected as an increase in the plateau fraction, may be due to a direct effect on KATP channels (24) and/or through the effect of cAMP on calcium channels (19,20).

Isolated β-cells show a marked heterogeneity in glucose-dependent insulin secretion (31,32). By contrast, studies in vivo have shown that different cells within the islet are very homogeneous (33). One important effect of GLP-1 treatment of isolated β-cells is to transfer glucose competence (24) to less-active cells. One of the possible factors that may account for the in vivo and dispersed cell differences in glucose sensitivity may be the tonic effect of GLP-1, keeping β-cells in a glucose-competent status under physiological conditions. If so, the role of GLP-1 may extend beyond its punctual insulinotropic effect.

In conclusion, our results demonstrate that GLP-1 has a direct action on the electrical activity of pancreatic β-cells in vivo that may explain the stimulatory effect on insulin secretion. Such an effect is glucose dependent, conferring to this incretin a security factor that supports its potential use in the treatment of type 2 diabetes.

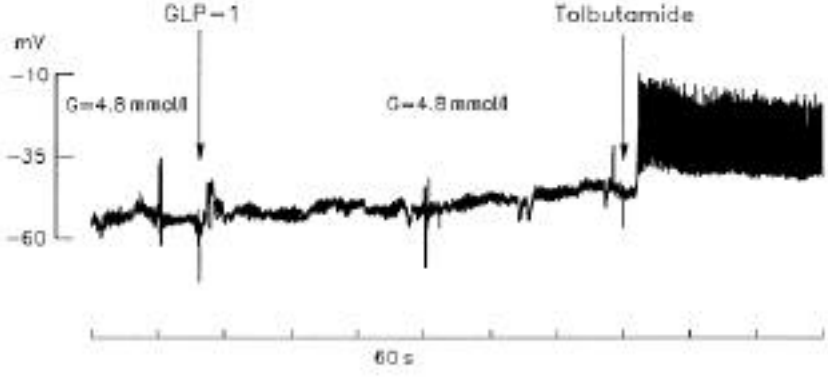

FIG. 3. Differential effects of GLP-1 and tolbutamide on electrical activity at low glucose concentrations. The beginning of the record shows a hyperpolarized membrane potential in an animal with low glucose concentration. The arrows show the effect of GLP-1 and tolbutamide injection (50 µl of a 10-mmol/l solution) on the electrical activity. Similar results were obtained in two other animals.

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