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GLUCAGON-LIKE PEPTIDE-1 MODULATES Ca^{2+} CURRENT BUT NOT K^+_{ATP} CURRENT IN INTACT MOUSE PANCREATIC B-CELLS

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Summary. The influence of GLP-1 on electrical activity and ion currents of mouse pancreatic B-cells was studied with intracellular microelectrodes and the whole-cell configuration of the patch-clamp technique. In the presence of 15 mmol/l glucose 5, 50 and 100 nmol/l GLP-1 slightly increased electrical activity. This effect may be caused by the slowing of Ca^{2+} channel inactivation observed with GLP-1. Thus, changes in Ca^{2+} channel kinetics are suggested to contribute to the insulinotropic action of the hormone. The most prominent effect of GLP-1 on the membrane potential was the conversion of irregular electrical activity into regular oscillations of the membrane potential. At the threshold concentration for insulin secretion (7 mmol/l glucose) GLP-1 did not alter the membrane potential. Accordingly, in patch-clamp experiments GLP-1 had no effect on the whole-cell K^+_{ATP} current.

The intestinal hormone glucagon-like-peptide-1 (GLP-1) is one of the most efficient secondary stimuli of insulin secretion [1]. GLP-1 may act on glucose homeostasis by several mechanisms. It stimulates insulin secretion in a glucose-dependent manner [2, 3]. Furthermore, GLP-1 enhances insulin biosynthesis and proinsulin gene expression [4], inhibits glucagon release [3] and probably partly mimics peripheral actions of insulin [5-7]. Islets cells express GLP-1 receptors which bind the hormone with high specificity [8]. GLP-1 is considered to be a promising agent in the treatment of non-insulin-dependent diabetes mellitus [9-11]. Therefore, knowledge about its action on electrical activity, which normally couples changes in glucose metabolism to insulin secretion [12], is of great interest.

We report in this study that in intact B-cells GLP-1 increases electrical activity by slowing Ca^{2+} channel inactivation. This phenomenon which contributes to the insulinotropic action of GLP-1 can be attributed to a rise in cAMP concentration [13-16]. Recently, it has been reported that GLP-1 acts synergisticly with glucose on metabolically controlled K^+_{ATP} channels [17]. Our data do not support this concept.

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MATERIALS AND METHODS

The experiments were performed on islets of fed female NMRI mice (25-30 g), killed by cervical dislocation. For the membrane potential measurements a piece of pancreas was fixed in a perifusion chamber and islets were microdissected by hand. The potential difference across the cell membrane was determined using high resistance microelectrodes [18]. The B-cells were identified by the characteristic oscillations of cell membrane potential which they display in the presence of 15 mmol/l glucose. The extracellular fluid was composed of (in mmol/l) 120 NaCl, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 24 $NaHCO_3$, gassed with 95% O_2 and 5% CO_2 to maintain a pH of 7.4 at 37^0 C. Patch-clamp experiments were performed with islets isolated by collagenase digestion of the pancreas. Islet cells were dispersed in Ca^{2+}-free medium and cultured up to 2 days. K^+_{ATP} current whole-cell recordings were performed with nystatin in the pipette solution (150-250 µmol/l). The pipette solution also contained (in mmol/l): 10 KCl, 10 NaCl, 70 K_2SO_4, 4 $MgCl_2$, 2$CaCl_2$, 10 EGTA, 20 HEPES, pH 7.15 adjusted with KOH. Bath solution was composed of (in mmol/l): 140 NaCl, 5.6 KCl 1.2 $MgCl_2$, 2.6 $CaCl_2$, 0.5 glucose, 10 HEPES, pH 7.4 adjusted with NaOH. In experiments where the initial K^+_{ATP} current amplitude was too small to observe a possible effect of GLP-1 a low concentration of diazoxide (20 µmol/l) was added. Currents measured at a holding potential of -70 mV and during 100-300 ms pulses to -80 mV and -60 mV at 15 s intervals were almost entirely K^+_{ATP}-currents, which were blockable by tolbutamide (100 µmol/l) [19]. Ca^{2+} channel currents were measured using a bath solution containing (in mmol/l): 115 NaCl, 20 TEACl, 10 $CaCl_2$, 1.2 $MgCl_2$, 0.1 tolbutamide, 15 glucose, 10 HEPES, pH 7.4 adjusted with NaOH. The pipette solution was (in mmol/l): 70 Cs_2SO_4, 10 NaCl, 10 KCl, 7 $MgCl_2$, 10 HEPES, pH 7.4 with NaOH. The nystatin concentration in the pipette was between 200 and 250 µmol/l. Currents were elicited every 15 s by 50 ms or 300 ms voltage steps from the holding potential of -70 mV to 0 mV. Patch-clamp experiments were carried out at 25^0 C. Measurements were started when the perforation of the patch resulted in a series resistance less than 30 MΩ.
GLP-1 (7-37) and GLP-1 (7-36)amide was obtained from Peninsula, Heidelberg, FRG. Statistical evidence has been evaluated by Student's t-test. Statistical significance was accepted for $P \leq 0.05$.
In several experimental series we tested GLP-1(7-36)amide and GLP-1(7-37). Since we did not observe any difference in the effectiveness, the experiments were pooled. This is in agreement with a report on healthy humans showing that the effects of both peptides are indistinguishable [20].

RESULTS AND DISCUSSION

The membrane potential was recorded from individual B-cells in intact islets. When islets were perifused with a medium containing 15 mmol/l glucose, B-cells exhibited typical repetitive oscillations of the membrane potential (Fig. 1, a). Addition of GLP-1 led to a slight increase in electrical activity. On average, the fraction of plateau phase (percentage of time with spike activity) increased from 37 ± 1 % to 46 ± 3 %, 49 ± 3 % and 56 ± 4 % with 5, 50 and 100 nmol/l GLP-1, respectively (n=4) (Fig. 1, b). Such a small increase in electrical activity does not point to a marked alteration in the activity of K^+_{ATP} channels. To further elucidate this point we tested the influence of GLP-1 in the presence of 7 mmol/l glucose. In mouse B-cells this is the threshold concentration for the induction of slow waves and insulin release [21]. Under this condition around 25 % of cells exhibit slow waves [21], most ion channels are closed and membrane resistance is high [22]. The additional closure of a very small number of K^+_{ATP} channels should then lead to membrane depolarization and induction of electrical activity. In the four cells tested the membrane potential hyperpolarized in the presence of 7 mmol/l glucose to -59 ± 1 mV and remained stable. No

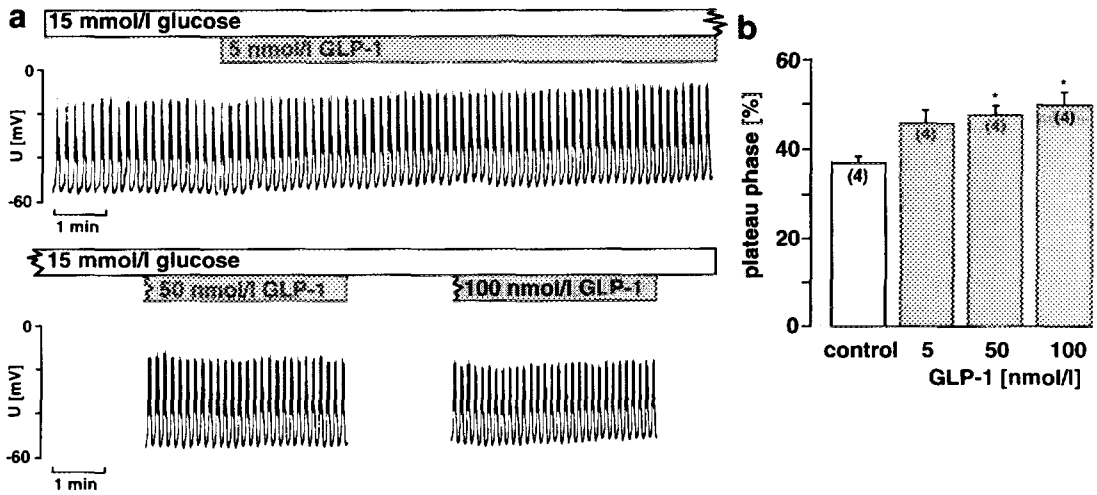

Fig. 1. Effect of different concentrations of GLP-1 on the membrane potential of mouse pancreatic B-cells in the presence of 15 mmol/l glucose. *a*: Representative recording showing the effect of accumulative addition of 5, 50 and 100 nmol/l GLP-1 on the membrane potential. Each GLP-1 concentration was added for 10 min. The lower panel shows the last four min during the addition of 50 nmol/l and 100 nmol/l GLP-1, respectively. *b*: Summary of the effect of the three different concentrations of GLP-1 on the percentage of plateau phase. *Values are significantly different from control values for (n)= number of experiments.

change (-60 ± 2 mV) was observed after addition of 50 nmol/l GLP-1 (3 experiments) or 100 nmol/l GLP-1 (1 experiment) (Fig. 2, a). We also tested GLP-1 on whole-cell K^+_{ATP} currents measured with single collagenase digested cells in the perforated patch configuration (Fig. 2, b). On average, the current elicited by a 10 mV depolarizing pulse from the holding potential of -70 mV was 30.0 ± 9.2 pA before and 30.4 ± 9.0 pA after addition of 50 nmol/l GLP-1 (n=6). Recently, it has been reported that GLP-1 acts synergisticly with glucose to close K^+_{ATP} channels [17] which is in contrast to our observations. However, in this study cells have been selected which have lost, at least in part, their responsiveness to glucose for an unknown reason. Thus, these observations do not elucidate the action of GLP-1 in intact B-cells and it remains obscure whether they reflect conditions comparable to a diabetic state.

It is known that GLP-1 enhances the concentration of cAMP in islet cells [13, 14]. Likewise, it has been shown previously that agents which increase the intracellular cAMP concentration enhance electrical activity in B-cells [15, 23, 24]. We assume that the observed slight increase in electrical activity could be attributed to the increase in cAMP concentration. Accordingly, the most striking effect of GLP-1 on electrical activity has been observed when the initial activity was irregular as it occurs in certain cells. Fig. 3 shows two out of five experiments illustrating that addition of GLP-1 converts irregular into regular oscillations of the membrane potential. Again this is consistent with the observation that after increasing the cAMP concentration by addition of forskolin irregular electrical activity became very regular [25]. However, as for GLP-1 it is unlikely that the effect of cAMP on electrical activity is due to a modulation of K^+_{ATP} channel activity. For rat insulinoma

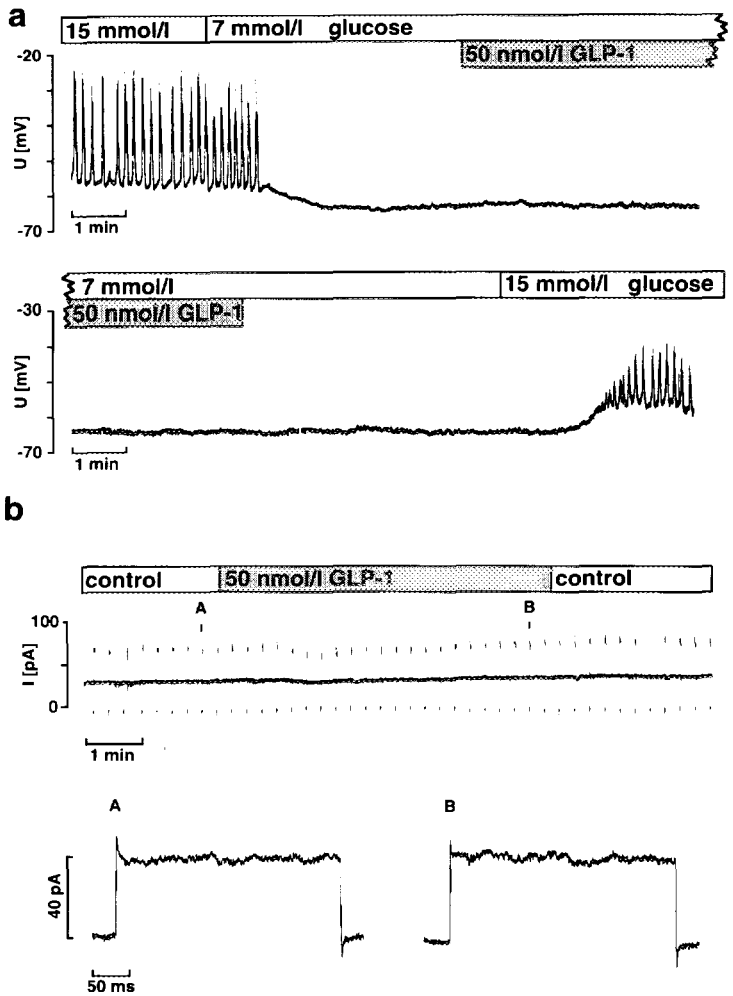

Fig. 2. *a*: Membrane potential recorded from pancreatic B-cells in the presence of 15 and 7 mmol/l glucose alone and after subsequent addition of GLP-1. The lower panel is the direct continuation of the upper panel. This record is representative of results obtained in four different experiments. *b*: Whole-cell K^+_{ATP} current recorded from pancreatic B-cells with the perforated patch technique in the absence and presence of 50 nmol/l GLP-1. The current is shown at the holding potential of -70 mV (solid line) and during 300 ms steps to -80 and -60 mV (lower dashed trace and upper dashed trace,respectively). The lower panel shows the outward current at A and B with an extended time scale. The record is representative of 6 similar experiments.

(RINm5F) cells it has been ruled out that cAMP or the protein kinase A alters the activity of K^+_{ATP} channels [26].

Electrical activity can be altered also by a change in the activity or kinetic of the voltage-dependent Ca^{2+} current. Moreover, several reports on islet cells or B-cell derived tumour cells suggest an action of GLP-1 on Ca^{2+} channels because $[Ca^{2+}]_i$ rises in response to GLP-1 and this increase is inhibited by L-type Ca^{2+} channel blockers [27-29]. Our results demonstrate that GLP-1 slowed the inactivation of the whole-cell Ca^{2+} current (Fig. 4). On average, the integrated Ca^{2+} current [$\int I_{Ca} dt$] elicited by a 300 ms pulse increased from 14.4

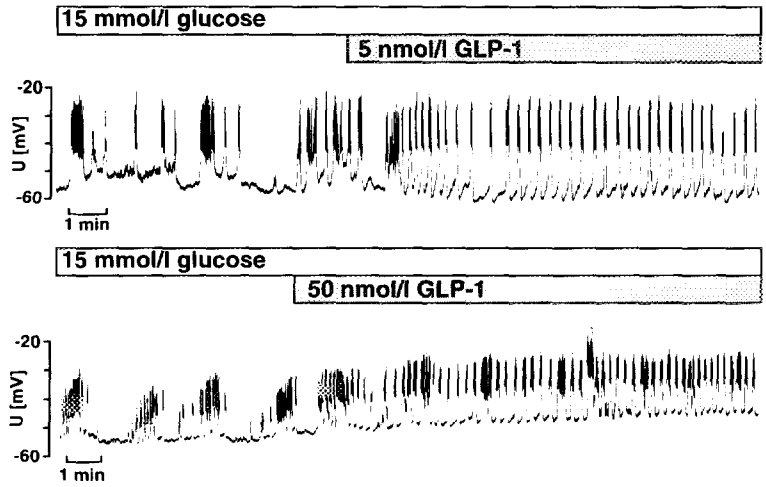

Fig. 3. Effect of GLP-1 on the membrane potential in cells spontaneously exhibiting irregular electrical activity in the presence of 15 mmol/l glucose. The figure shows two examples of cells with irregular activity and the effect of GLP-1 on the pattern of electrical activity. Similar results have been observed in five experiments.

± 2.6 pC to 17.7 ± 3.0 pC after addition of 50 nmol/l GLP-1 (n=6, P<0.05). The peak current remained unchanged (-136 ± 19 pA under control conditions and -133 ± 21 pA after addition of 50 nmol/l GLP-1). An increase of the integrated Ca^{2+} current after addition of 8-Br-cAMP or forskolin to single B-cells has been previously reported [16]. This effect was reversed by the protein kinase A inhibitor Rp-cAMPS. Thus, it seems reasonable to

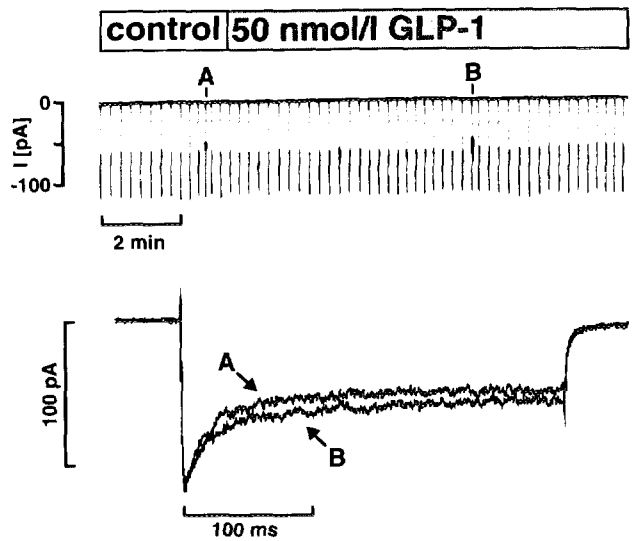

Fig. 4. Effect of 50 nmol/l GLP-1 on Ca^{2+} currents through voltage-dependent Ca^{2+} channels. Currents were measured with the perforated patch technique. Currents were elicited every 15 s by 50 ms voltage steps from the holding potential of -70 mV to 0 mV. In A and B the pulse length was 300 ms. The lower panel shows the current at A and B with an extended time scale. One representative recording out of six with similar results.

conclude that the effect of GLP-1 on Ca^{2+} current inactivation is attributable to the well-established increase in the cAMP concentration [13, 14]. This conclusion is also consistent with the observation that cAMP increases $[Ca^{2+}]_i$ by promoting Ca^{2+} influx in a pancreatic tumour B-cell line [30, 31]. The main effect of cAMP on insulin secretion, however, is its influence on a late step in stimulus-secretion coupling. It has been shown that its interaction with the secretory machinery accounts for around 80 % of the effect on secretion [16].
Our results demonstrate that GLP-1 slightly increases electrical activity and slows Ca^{2+} channel inactivation in pancreatic B-cells, but does not affect the K^+_{ATP} current. It is assumed that the effects of GLP-1 are mediated by cAMP and that they contribute to the insulinotropic effect of GLP-1 which is, however, predominantly due to the action of cAMP on a late step in stimulus-secretion coupling in B-cells.

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