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Effects of the hypoglycaemic drugs repaglinide and glibenclamide on ATP-sensitive potassium-channels and cytosolic calcium levels in β TC3 cells and rat pancreatic beta cells

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Summary The present study demonstrates the action of the hypoglycaemic drugs repaglinide and glibenclamide in cultured newborn rat islet cells and mouse βTC3 cells. In cell-attached membrane patches of newborn rat islet cells repaglinide (10 nmol/l) and glibenclamide (20 nmol/l) decrease the open probability of single ATP-sensitive K^+-channels to approximately 10 % of the activity prior to addition of the drugs in short-term experiments (< 5 min). The influence of repaglinide and glibenclamide on the ATP-sensitive K^+ current was studied using the whole-cell patch clamp configuration. A half-maximal steady-state inhibition of the ATP-sensitive K^+ currents is observed at 89 pmol/l repaglinide and at 47 pmol/l glibenclamide in whole-cell experiments of longer duration (30 min). Applying digital Ca^{2+} imaging on single βTC3 cells we found that repaglinide and glibenclamide induced a concentration-dependent increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) with a half-maximal effect at 0.5 nmol/l for both drugs in long-term experiments (30 min). The rise in $[Ca^{2+}]_i$ results from Ca^{2+} entry through voltage-dependent L-type Ca^{2+}-channels since it is inhibited by verapamil (10 μmol/l). The effect of repaglinide and glibenclamide is partly reversible (≈ 80 %). [Diabetologia (1995) 38: 1025–1032]

Key words ATP-sensitive K^+-channel, calcium, glibenclamide, non-insulin-dependent diabetes mellitus, repaglinide.

Sulphonylureas have been used in the treatment of patients with non-insulin dependent diabetes mellitus (NIDDM) for more than 30 years [1]. Sulphonylureas enhance insulin secretion from beta-cells by closure of K^+_{ATP}-channels [2, 3]. This leads to a depolarization of the cell and subsequent activation of voltage-dependent Ca^{2+}-channels. Ca^{2+} then enters the cell and initiates insulin secretion [4]. Recently, it has become evident that sulphonylureas also promote insulin secretion by an interaction with the secretory machinery [5].

Sulphonylurea treatment can result in a gradual loss of glycaemic control with time, a phenomenon called secondary failure [1, 6]. Furthermore, sulphonylurea treatment does not restore the first phase insulin secretion which is virtually absent in patients with NIDDM [7, 8]. In an attempt to circumvent these problems, and to improve blood glucose control, several new hypoglycaemic drugs have been developed. One such drug is repaglinide, a benzoic acid derivate (Fig. 1), which has been reported to enhance insulin secretion, to have a short plasma half-life and which is secreted predominantly via the bile [9]. Repaglinide has an insulinotropic effect in humans [9], although its mechanism of action in the pancreatic beta-cell remains to be established. There-

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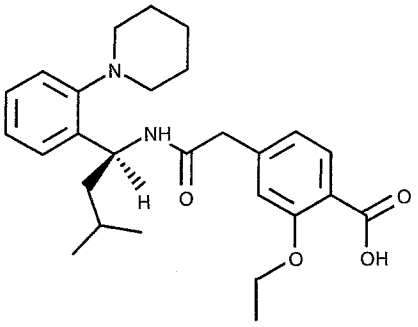
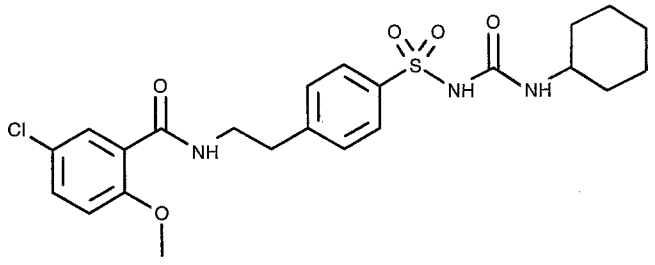
Abbreviations: K^+_{ATP}-channel, ATP-sensitive potassium channel; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; EGTA, ethylene glycol-O,O′-bis(2-aminoethyl]-N′,N,N′,N′-tetraacetic acid; IC_{50}, apparent inhibitor constant; repaglinide, ((S)-(+)-2-ethoxy-4-[2-((3-methyl-1-[2-(1-piperidinyl) phenyl]-butyl)amino)-2-oxoethyl) benzoic acid; NIDDM, non-insulin-dependent diabetes mellitus; βTC3 cells, transgenic mouse insulinoma tumour cell line.



Repaglinide



Glibenclamide

Fig. 1. Structural formulae of repaglinide and glibenclamide

fore, we investigated the effects of repaglinide on cultured newborn rat islet cells and βTC3 cells using patch-clamp techniques and digital Ca^{2+} imaging. The results were compared with the sulphonylurea glibenclamide and partly presented previously [10].

Materials and methods

Chemicals. Fura-2 acetoxymethyl ester (Fura-2/AM) and fura-2 pentapotassium salt were supplied by Molecular Probes (Eugene, Ore. USA). EGTA was obtained from Fluka (Buchs, Switzerland). Repaglinide was synthesized at Dr. Karl Thomae GmbH (Biberach an der Riss, Germany). All other chemicals were obtained from Sigma Chemical (St. Louis, Mo., USA).

Cell culture. Islet cells were isolated from newborn rats and cultured for up to 3 months, as described previously [11]. The newborn rat islet cells were used for the electrophysiological recordings whereas the βTC3 cell line, derived from a transgenic mouse insulinoma [12], was used for all Ca^{2+} measurements. The latter cell line was used because we were unable to make the newborn rat cells attach to glass coverslips, necessary for measurements of $[Ca^{2+}]_i$. The βTC3 cells were cultured at 37 °C in Dulbecco's modified Eagle medium containing 1000 mg/l D-glucose, 10 % heat-inactivated fetal calf serum, penicillin (50 U/ml), streptomycin (0.05 mg/ml), 2 mmol/l L-glutamine (Gibco, Roskilde, Denmark) and incubated in humidified 5 % CO_2 in 95 % air. Cells were passaged once each week and media were changed twice a week. All experiments were performed with cells from passage 30–45.

Measurements of electrical activity. Single-channel and whole-cell ion currents were recorded using an EPC-9 patch-clamp amplifier (Heka Elektronik, GmbH, Lambrecht, Germany), stored on video tape (JVC D860EH) via an analogue-to-digital converter (10B, Instructech Corporation, Elmont, N.Y., USA) and a low bandpass filter (500 Hz) to a chart recorder (DASH IV, Astro Med, West Warwick, R.I., USA). Pipettes were pulled from borosilicate glass (Modulohm A/S, Herlev, Denmark) on a vertical puller (BB-CH-PC Mecanex, Bromma, Switzerland), coated with Sylgard and heat polished at the tip. Pipette resistances ranged between 2 and 4 MΩ when filled with the pipette solution.

For cell-attached patch-clamp experiments the pipette solution contained (in mmol/l): 6 Na^+, 155 K^+, 2 Ca^{2+}, 1 Mg^{2+}, 137 Cl^-, 10 EGTA, 0.3 ATP, 5 HEPES, pH 7.2: the extracellular solution contained (in mmol/l): 140 Na^+, 4K^+, 2 Ca^{2+}, 1 Mg^{2+}, 150 Cl^-, 10 HEPES, pH 7.3. In cell-attached single-channel experiments the recordings were performed at the resting membrane potential of the cell and the applied voltage was 0 mV. In whole-cell experiments the same solutions were used as described above. The membrane potential was held at –70 mV and hyper- and depolarizing voltage pulses of 10 mV amplitude and 250 ms duration were applied alternately every 1.5 s [13]. At the applied membrane potentials, voltage dependent currents, i.e., the Ca^{2+} inward currents and the delayed K^+ outward current are not induced and therefore most of the current is flowing through K^+_{ATP}-channels. In addition, activation of Ca^{2+}-dependent K^+-channels was prevented by including EGTA in the pipette solution [14, 15]. Solutions were applied by gravity feed from a series of parallel pipes. Solution changes around the cell were complete within 5 s as measured by monitoring the membrane potential during a step in extracellular K^+ concentration from 4 to 45 mmol/l. The experiments were performed at room temperature.

Measurements of $[Ca^{2+}]_i$. Images of $[Ca^{2+}]_i$ in single βTC3 cells were obtained by means of a fluorescence microscope (Zeiss Axiovert 135, Oberkochen, Germany) with a Xenon lamp (75 W), a CCD camera (Dage-MTI, Michigan City, Ind., USA) with an intensifier (Genesis, Dage-MTI) and a digital image processing system (Universal imaging, West Chester, Pa., USA). The cells were loaded with 3 μmol/l fura-2/AM for 40 min at 37 °C. Before the experiment the cells were washed twice with extracellular solution (see previous section) and placed in a perfusion chamber which allows for a continuous flow of control or test solution at rates between 0.1–2 ml/min. Images were obtained by an oil-immersion UV permeable objective (Zeiss Achrostigmat 40×, 1.30 numerical aperture) and transferred to a video tape recorder (Umatic SP, VO 9600, Sony, Copenhagen, Denmark). The images were obtained by averaging eight frames using a sample rate between 0.2–0.5 Hz. The data were obtained by averaging the pixel grey values inside a 4 × 4-μm window placed in the centre of the cells.

The free intracellular Ca^{2+} concentrations were calculated from the measurements of the ratio of fluorescence intensities according to the equation [16]: $[Ca^{2+}]_i = K_d \cdot [(R\text{-}R_{min})/(R_{max}\text{-}R)] \cdot (S_{f2}/S_{b2})$. Calibrations of intracellular fura-2 signals were done by adding ionomycin to a final concentration of 1 μmol/l in order to equilibrate Ca^{2+} across the plasma membrane. The grey values obtained for R_{max} amounted in average to 241. R_{min} and the proportionality constant (S_{f2}/S_{b2}) were obtained from a high K^+-medium with fura-2 pentapotassium salt and 10 mmol/l EGTA and amounted to 27 and 9.8, respectively. A value for the apparent dissociation constant (K_d) for Ca^{2+} binding to fura-2 of 224 nmol/l was used [16]. All experiments were performed at room temperature (20–22 °C).

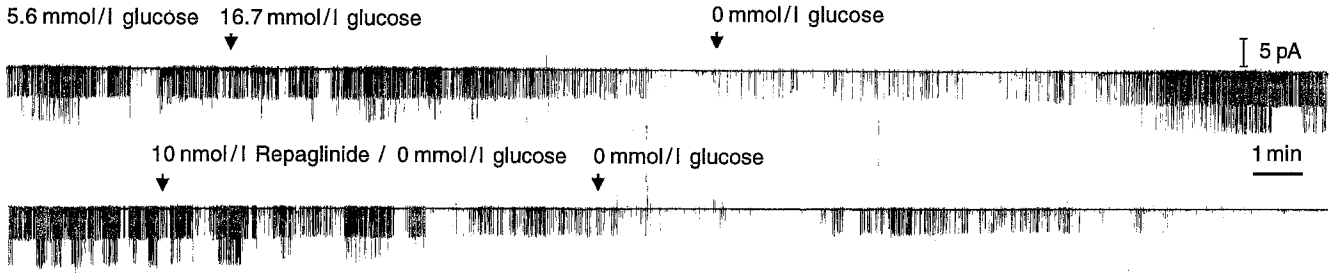


Fig. 2. Effect of glucose and repaglinide on K^+_{ATP}-channels in a cell-attached patch of cultured beta-cell from neonatal rats. Continuous tracing depicting closure of K^+_{ATP}-channels induced by 16.8 mmol/l glucose and 10 nmol/l repaglinide. The recording was performed at the resting membrane potential of the cell and the applied voltage was 0 mV with extracellular solution in the bath and intracellular solution in the pipette. The currents are displayed as downward deflections indicating that the currents are inward. This is solely due to the high K^+ concentration of the intracellular solution in the pipette and under physiological conditions the currents will be outwardly directed tending to repolarize the beta-cell. The experiment is performed at 22–24 °C

Statistical analysis

All data are expressed as means ± SEM for *n* tested cells. Curves were fitted to the data by a non-linear least-squares method using the computer program Statgraphics from STST Inc. (Rockville, Md., USA). In order to measure the inhibitory effect of repaglinide and glibenclamide on the mean current amplitude a curve was fitted to the data points by the following equation, which is a simplified version of the Hill equation assuming a cooperativity of one:

$$I/I_c = [IC_{50}]/([IC_{50}] + [S]) \quad \text{(Eq. I)}$$

where I is the change in current amplitude, I_c is the maximum change in current amplitude in the absence of drug, $[IC_{50}]$ is concentration of repaglinide or glibenclamide giving half-maximal inhibition and S is the concentration of the inhibitor. In these experiments differences in cell size were not corrected for, as we did not find any correlation between cell size and the maximum current amplitudes.

For measurements of concentration-response curves the data were fitted by means of the Michaelis-Menten equation:

$$\Delta[Ca^{2+}]_i = \Delta([Ca^{2+}]_i)max \cdot [S]/(k_s + [S]) \quad \text{(Eq. II)}$$

where $\Delta[Ca^{2+}]_i$ is the change in $[Ca^{2+}]_i$, $\Delta([Ca^{2+}]_i)_{max}$ is the maximal rise observed in $[Ca^{2+}]_i$, S is the concentration of drug and k_s is the drug concentration causing a half-maximal rise in $[Ca^{2+}]_i$.

Results

Effects of glucose and repaglinide on K^+_{ATP}-channel activity. Figure 2 shows the effect of glucose and repaglinide on individual K^+_{ATP}-channels in a cell-attached patch of membrane of cultured beta-cells from neonatal rats. From observations of channel activity over a range of voltages we found a reversal potential of –70 to –80 mV and a maximum slope conductance of approximately 70 pS in symmetrical K^+ solutions. In most experiments, glucose alone (16.7 mmol/l) totally blocked the K^+_{ATP}-channels within approx. 8 min. This effect is reversible since reducing bath glucose to 0 mmol/l results in the return of channel activity within 5–10 min. Subsequent addition of repaglinide (10 nmol/l) inhibited channel activity approximately 90 % within 1–4 min. The effect of repaglinide was not reversible within 30 min of washout. Similar results were obtained with glibenclamide, although 20 nmol/l was required in order to obtain 90 % inhibition of channel activity. The effects of repaglinide and glibenclamide are specific for K^+_{ATP}-channels, since the drugs did not affect the Ca^{2+}-sensitive K^+-channels or the smaller (≈ 15 pS) K^+-channels also present in the beta-cells (data not shown). All experiments examining the effects of repaglinide or glibenclamide were performed in a glucose-free medium in order to avoid contributions from glucose induced changes on K^+_{ATP}-channel activity and $[Ca^{2+}]_i$.

Effect of repaglinide and glibenclamide on ATP-sensitive K^+ whole-cell current. At low repaglinide and glibenclamide concentrations it becomes difficult to separate drug effect from spontaneous rundown of K^+_{ATP}-channels. Thus, in order to establish the dose-response relationship for repaglinide and glibenclamide we used a different whole-cell protocol [13]. The beta-cells were preincubated for 30 min with different concentrations of the drugs and the current was recorded at a holding potential of –70 mV applying hyper- and depolarizing pulses of 10 mV. Figure 3A illustrates the current responses in the absence of drugs and in the presence of 1 nmol/l repaglinide. After patch rupture and dialysis of the cell with a pipette solution of low ATP concentration (0.3 mmol/l) the whole-cell K^+ current gradually increased to a maximal value of approximately 500 pA in the control and approximately 50 pA in the presence of 1 nmol/l repaglinide. We found that an equilibrium state of the blocking effect of all tested drug concentrations was achieved after 30 min of incubation. Exposure of the cells to different repaglinide and glibenclamide concentrations caused a concentration-dependent decrease of the maximal current amplitude.

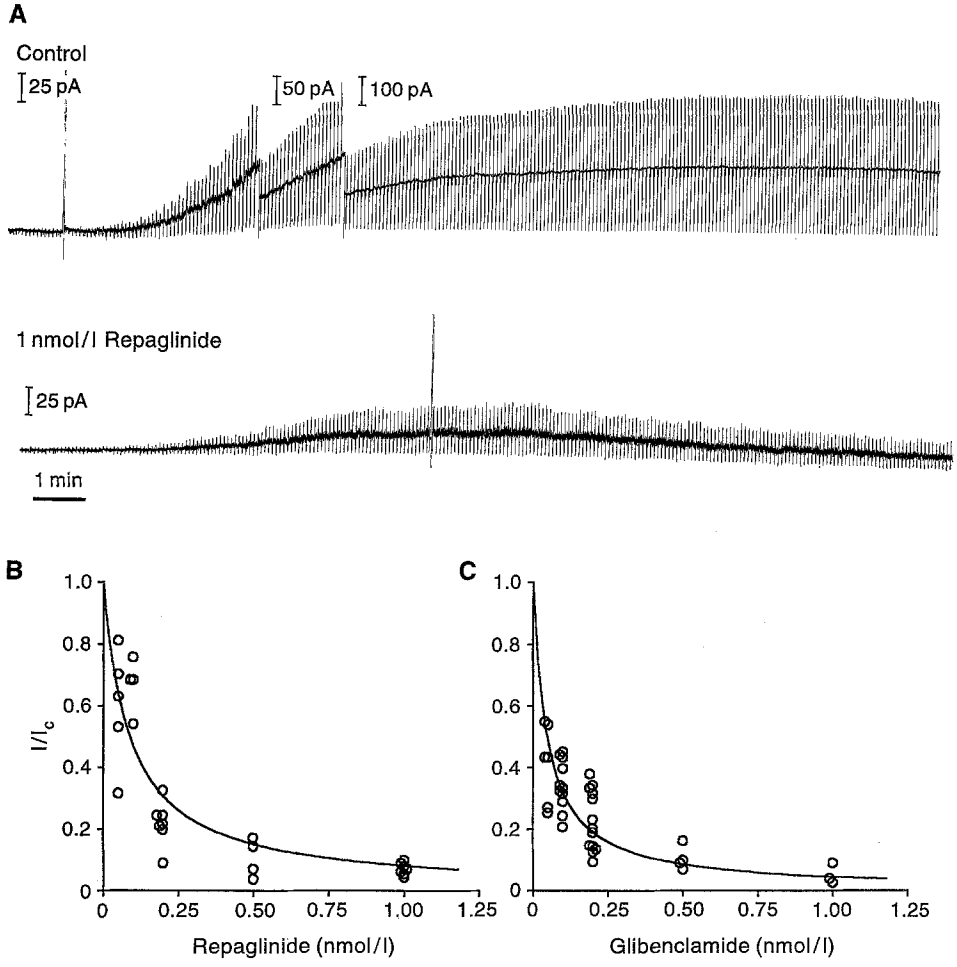


Fig. 3. **A** Effect of repaglinideon ATP-sensitive K^+ whole-cell currents. Newborn rat beta-cells were incubated for 30 min without or with repaglinide. Membrane currents were then measured with pipettes containing intracellular solution with 0.3 mmol/l ATP. Due to wash-out of ATP from the cytoplasm the current amplitude increased during the first minutes after patch rupture. Note the change in scale of the current amplitude in control. The current amplitude was determined after reaching a maximum. **B, C** Concentration dependence of inhibition of current responses for repaglinide and glibenclamide. The ordinate shows the fraction of the maximum currents in the presence and absence of inhibitor. Curves were fitted as described in Materials and methods

By fitting a curve to the data (Eq. I) an IC_{50}-value of 89 ± 13 pmol/l for repaglinide and 47 ± 4 pmol/l for glibenclamide was calculated (Fig. 3B,C).

Effects of repaglinide and glibenclamide on $[Ca^{2+}]_i$. Figure 4 shows typical time courses of the rise in $[Ca^{2+}]_i$ induced by maximally stimulating concentrations of repaglinide and glibenclamide (100 nmol/l) in βTC3 cells. The resting $[Ca^{2+}]_i$ in unstimulated βTC3 cells amounted to 189 ± 7 nmol/l ($n = 59$). The Ca^{2+} responses were sustained and 800–900 nmol/l in amplitude and had a latency of 30–60 s. In the absence of extracellular Ca^{2+} (with 100 μmol/l EGTA) addition of repaglinide did not evoke any increase in $[Ca^{2+}]_i$ but subsequent change to a Ca^{2+}-containing medium caused an immediate increase in $[Ca^{2+}]_i$ (Fig. 4C). Furthermore, the $[Ca^{2+}]_i$ response to 100 nmol/l repaglinide was completely inhibited by the addition of 10 μmol/l verapamil, a L-type Ca^{2+}-channel blocker (Fig. 4D).

By exposing βTC3 cells to different repaglinide and glibenclamide concentrations, we found that the time course of change in $[Ca^{2+}]_i$ strongly depended on the applied drug concentration. Low drug concentrations caused a very gradual increase in $[Ca^{2+}]_i$ reaching peak values after 20–30 min, whereas higher concentrations caused a faster increase in $[Ca^{2+}]_i$ (Fig. 5A, B). No such long-term change in $[Ca^{2+}]_i$ were observed in the absence of drug. Figure 5C, D shows concentration-response curves for the repaglinide- and glibenclamide-induced changes in $[Ca^{2+}]_i$ when measured at 30 min, where $[Ca^{2+}]_i$ had reached a maximum level at all tested drug concentrations. When $[Ca^{2+}]_i$ was induced to rise with repaglinide (Fig. 5C) the $\Delta([Ca^{2+}]_i)_{max}$ amounted to 731 ± 15 nmol/l with a half-maximal effective concentration of 0.53 ± 0.04 nmol/l. In the presence of glibenclamide (Fig. 5D) the $\Delta([Ca^{2+}]_i)_{max}$ amounted to 715 ± 15 nmol/l and k_s to 0.52 ± 0.04 nmol/l.

Reversibility of repaglinide and glibenclamide. We have investigated the reversibility of repaglinide and glibenclamide on the ATP-sensitive whole-cell K^+-current in newborn rat islet cells. After pretreatment with 100 nmol/l of each drug for 30 min the cells were washed with drug free medium for variable periods of time before a whole cell experiment was performed. After 250 min of washing we found approximately 80 % recovery of the maximal ATP-sensitive K^+ current in cells pretreated with 100 nmol/l repaglinide (Fig. 6A). Similar results were obtained with glibenclamide (data not shown). We have also exam-

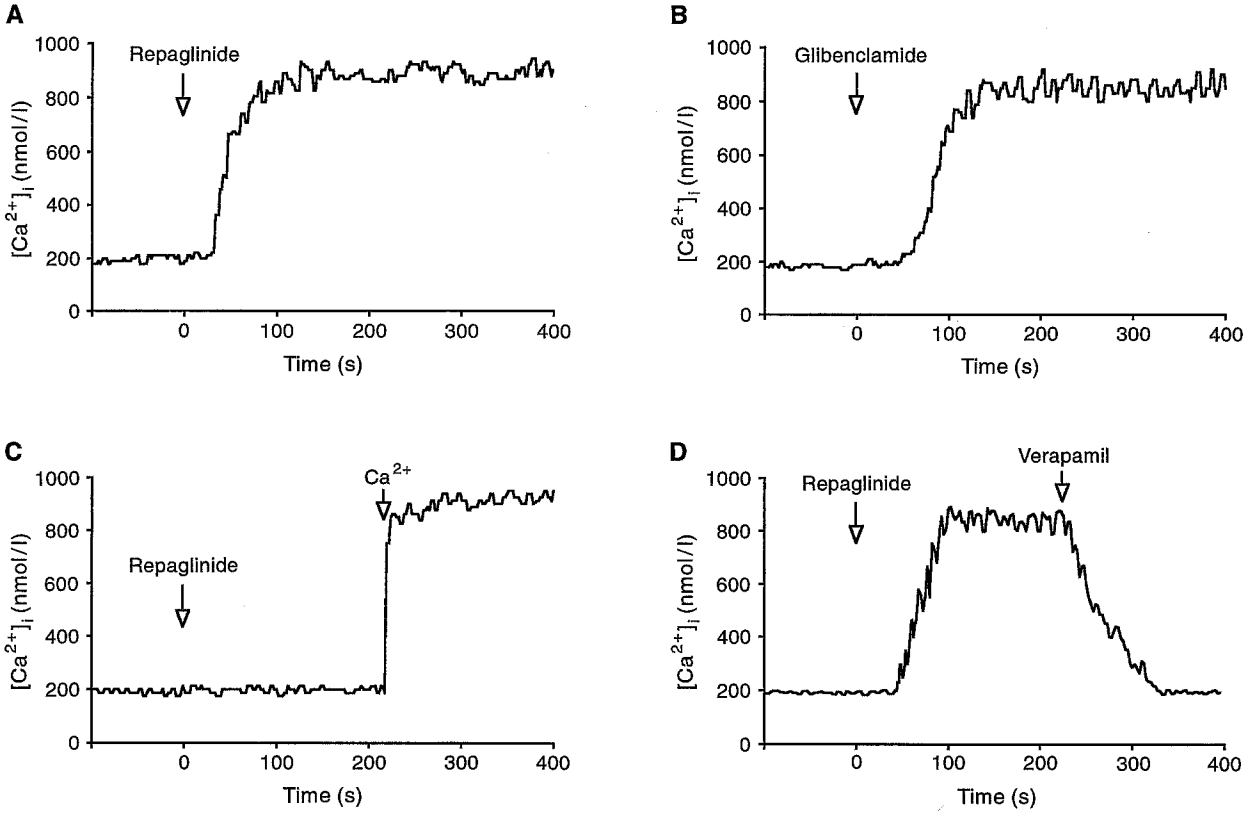


Fig. 4. A, B Time course of changes in $[Ca^{2+}]_i$ in βTC3 cells following stimulation with repaglinide or glibenclamide (100 nmol/l). **C** Inhibition of repaglinide induced changes in $[Ca^{2+}]_i$ when added to a medium without Ca^{2+} and with 100 μmol/l EGTA. Subsequent perfusion with a Ca^{2+}-containing medium induced an increase in $[Ca^{2+}]_i$. **D** Inhibition of repaglinide (100 nmol/l) evoked $[Ca^{2+}]_i$ response by 10 μmol/l verapamil when added as indicated by the arrow. The tracing is representative of 18–43 experiments

ined the reversibility of repaglinide and glibenclamide on $[Ca^{2+}]_i$ by including a washing step after the cells had reached their maximal $[Ca^{2+}]_i$ in response to drug application (100 nmol/l). In the Ca^{2+} experiments inclusion of albumin (10 mg/ml) in the washing solution enhanced the back regulation of $[Ca^{2+}]_i$. It is evident from Fig. 6B that $[Ca^{2+}]_i$ was decreased to near resting levels within 30 min of washing in a cell stimulated with repaglinide (100 nmol/l). At this time $[Ca^{2+}]_i$ was reduced to 265 ± 32 nmol/l for repaglinide and to 274 ± 44 nmol/l for glibenclamide ($n = 9$). Subsequent exposure of the same cells to repaglinide or glibenclamide (100 nmol/l) induced a new increase in $[Ca^{2+}]_i$ reaching 623 ± 37 nmol/l for repaglinide and 637 ± 46 for glibenclamide.

Discussion

The present study demonstrates that the hypoglycaemic drugs repaglinied and glibenclamide block the K^+_{ATP}-channels found in pancreatic beta-cells, thereby causing Ca^{2+} influx through voltage-dependent L-type Ca^{2+}-channels and a subsequent increase in $[Ca^{2+}]_i$. The K^+_{ATP}-channels found in the present study are similar to those previously described in beta-cells from newborn rats [17] and in other types of beta-cells [4, 18]. Repaglinide and glibenclamide caused a dose-dependent inhibition of the K^+_{ATP}-channels, which constitute the resting conductance of the beta-cell [4, 18]. Thus, 89 pmol/l repaglinide blocked the ATP-sensitive K^+-currents half-maximally whereas glibenclamide inhibited the currents half-maximally at 47 pmol/l. By comparison, Sturgess et al. [19] found that 27 nmol/l glibenclamide was needed in order to block the ATP-sensitive K^+-current in CRI-G1 insulinoma cells half-maximally. In mouse pancreatic β-cells 0.4–0.6 nmol/l glibenclamide (free concentrations) was required in order to inhibit the ATP-sensitive K^+-current half-maximally [20, 21]. However, in RIN cells 60 pmol/l glibenclamide was half-maximally effective on $^{86}Rb^+$ efflux which reflects the activity of the K^+_{ATP}-channels [22].

We have shown the presence of functional K^+_{ATP}-channels in βTC3 cells which were sensitive to inhibition by repaglinide and glibenclamide (data not shown). Thus, the observed rise in $[Ca^{2+}]_i$ in re-

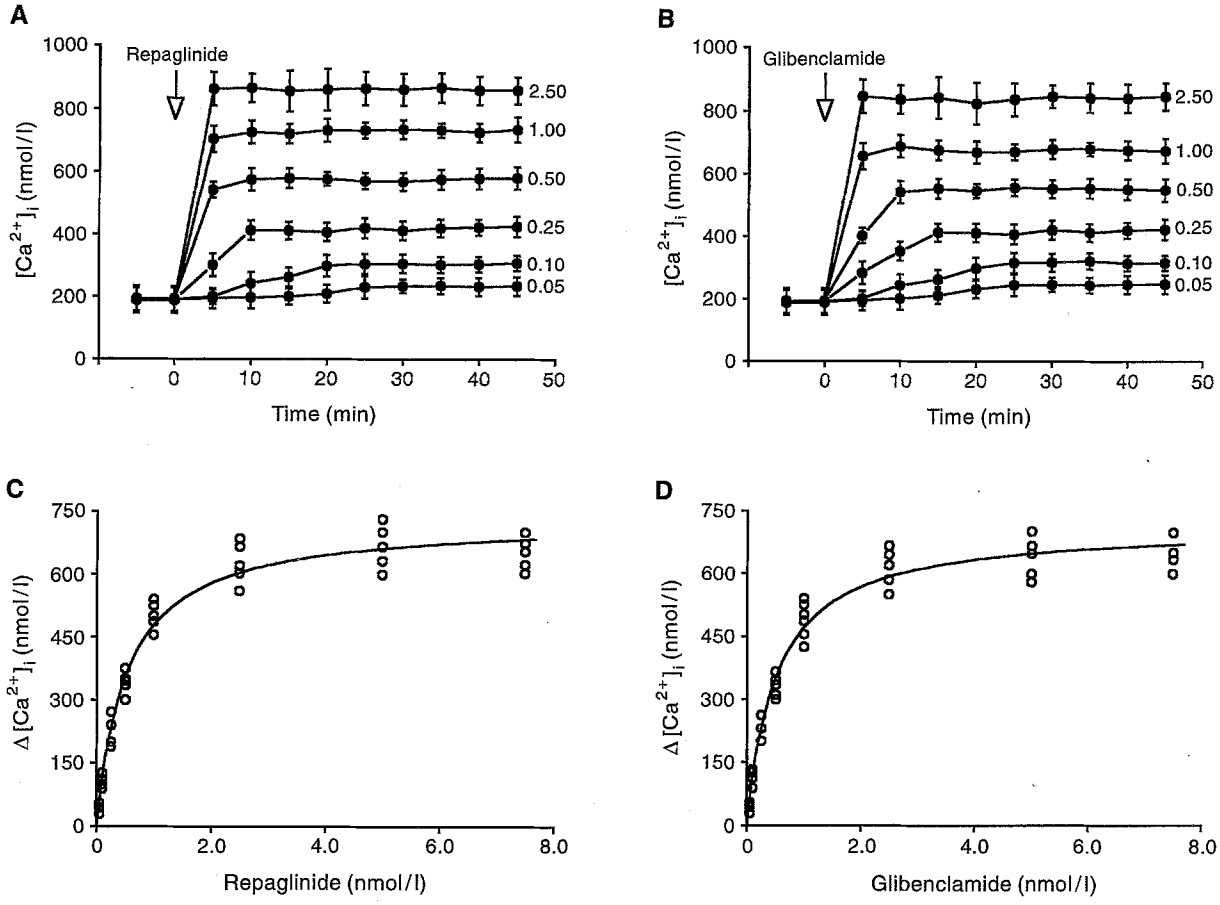


Fig. 5 (A–D). Time and concentration-dependent effects of repaglinide and glibenclamide on $[Ca^{2+}]_i$ in βTC3 cells. **A, B** Time-dependent changes in $[Ca^{2+}]_i$ after stimulation with repaglinide and glibenclamide (concentrations are shown on the right of the graphs in nanomol/l). **C, D** Concentration-response curves for the repaglinide- and glibenclamide-induced changes in $[Ca^{2+}]_i$. The ordinate shows the changes in $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$) obtained by subtracting the unstimulated $[Ca^{2+}]_i$ from that measured at min 30. Curves were fitted as described in Materials and methods

sponse to repaglinide or glibenclamide is due to inhibition of the K^+_{ATP}-channels, thereby causing membrane depolarization and Ca^{2+} influx. The extracellular source of Ca^{2+} is supported by the observation that verapamil, a L-type Ca^{2+} channel blocker, prevents the rise in $[Ca^{2+}]_i$.

Repaglinide and glibenclamide were about six and tenfold more potent in the electrophysiological experiments than in measurements of $[Ca^{2+}]_i$ ($k_s \approx 0.5$ nmol/l for both drugs). Patch-clamp experiments have revealed that complete closure of the K^+_{ATP}-channels is required for the pancreatic beta-cell to be depolarized beyond the threshold for the generation of Ca^{2+}-dependent action potentials [4]. Against this background it is clear that higher concentrations of repaglinide and glibenclamide are needed to cause a half-maximal increase in $[Ca^{2+}]_i$. However, we acknowledge that the dose-response relationships on the K^+_{ATP}-currents and $[Ca^{2+}]_i$ were determined on two different beta-cell types. Preliminary results on βTC3 cells have shown that the concentrations of repaglinide and glibenclamide causing half-maximal inhibition of the ATP-sensitive K^+ whole-cell current were approximately 100 pmol/l which is nearly identical with those obtained in the present study on newborn rat islet cells. Thus, the difference in the dose-response relationships for repaglinide and glibenclamide determined in the present study is likely to reflect true differences in potency and is probably not due to the different cell types used. Our data on the potency of repaglinide and glibenclamide to cause an increase in $[Ca^{2+}]_i$ compare favourably with the potency of glibenclamide to stimulate insulin release in mouse pancreatic islets ($EC_{50} \approx 0.5$ nmol/l) [21].

Recently, it was demonstrated that the undissociated forms of glibenclamide and related compounds are the active forms for inhibition of the ATP-sensitive K^+-currents and that the drugs probably gain access to their binding sites from the lipid phase of the beta-cell membrane [23]. In addition, an internalization of glibenclamide in rat islet cells occurs to a rather large extent where it preferentially associates with insulin secretory granules [24]. Thus, the rate of

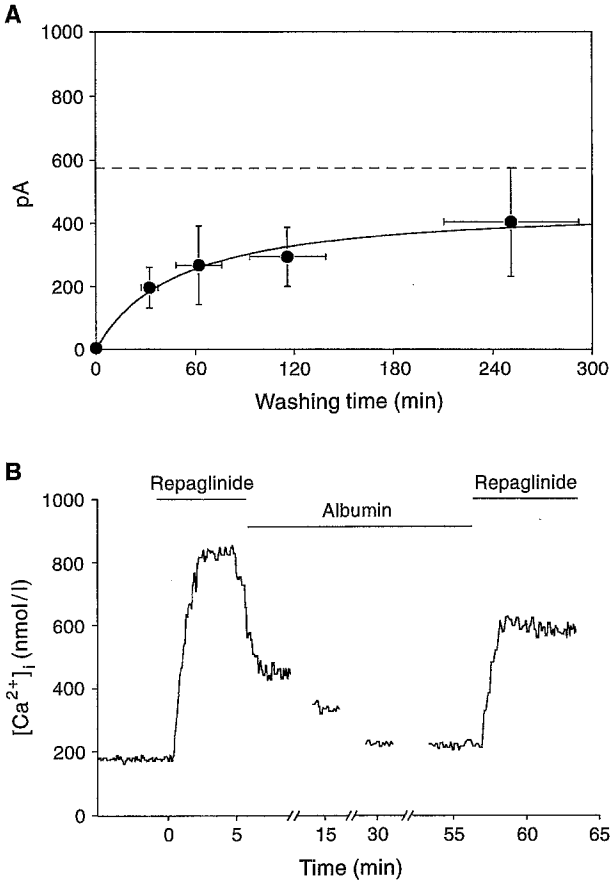


Fig. 6 (A, B). Reversibility of repaglinide on the ATP-sensitive K^+ current and $[Ca^{2+}]_i$. **A** Cells were pretreated with 100 nmol/l repaglinide for 30 min and then washed for variable periods of time in drug-free medium before a whole-cell experiment was performed. The ordinate shows the maximal whole-cell current in pA. The solid line was obtained by fitting the data points ($n = 51$) to the equation described in text. The dashed line represents the average maximal whole-cell current in control experiments (575 ± 33 pA, $n = 54$). **B** Ca^{2+} response in a βTC3 cell after exposure to a maximally stimulating concentration of repaglinide (100 nmol/l). The cell was washed with bath solution containing 10 mg/ml albumin. Subsequent addition of repaglinide (100 nmol/l) took place after 57 min. The tracing is representative of nine experiments

entry and the capacity of the cell will influence the access of the drug to its binding site. It seems likely that much larger amounts of drug accumulate in the cell than are needed to occupy all of the sulphonylurea receptors [25]. If we assume that internalization and accumulation of repaglinide also occurs, this might explain the kinetics of the onset of closure of the K^+_{ATP}-channels and increase in $[Ca^{2+}]_i$ observed in the present study. Thus, with submaximal concentrations, the time course of entry and accumulation of repaglinide and glibenclamide in the cell will influence the onset and time course of channel closure and the subsequent increase in $[Ca^{2+}]_i$. However, with maximal concentrations, repaglinide and glibenclamide will accumulate in the cell much more rapidly and occupy all binding sites, thereby inducing a more rapid rise in $[Ca^{2+}]_i$ subsequent to channel closure. The ability of repaglinide and glibenclamide to accumulate in the cell could also explain the slow reversibility on the ATP-sensitive K^+-current and $[Ca^{2+}]_i$.

In conclusion, the new non-sulphonylurea-based hypoglycaemic agent repaglinide, a benzoic acid derivate, is equipotent with glibenclamide, a second generation sulphonylurea with respect to inhibition of beta-cell ATP-sensitive K^+ currents and stimulation of an increase in $[Ca^{2+}]_i$. The close structural relationship between repaglinide and meglitinide, another hypoglycaemic drug which does not contain the sulphonylurea moiety, supports the observation first made by Zünkler et al. [20] that the the sulphonylurea group is not necessary for inhibiting K^+_{ATP}-channel activity and thus stimulating insulin secretion from the pancreatic beta-cell. It will be interesting to determine whether the non-sulphonylureas such as repaglinide and meglitinide share all properties of the sulphonylureas or if the sulphonylurea part of the molecules confers some distinct biological action.

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