HIV Protease Inhibitors: Suppression of Insulin Secretion by Inhibition of Voltage-Dependent K⁺ Currents and Anion Currents

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ABSTRACT

We have shown before that the human immunodeficiency virus (HIV) protease inhibitors ritonavir and nelfinavir, but not indinavir, suppress insulin secretion from mouse pancreatic B-cells via reduction of the cytosolic free calcium concentration ([Ca²⁺]ᵢ). This was not because of an effect on ATP-dependent K⁺ channels (Kₐₜₚ channels) or L-type Ca²⁺ channels. The study was intended to elucidate the mechanisms by which distinct HIV protease inhibitors decrease [Ca²⁺]ᵢ and thus evoke their adverse side effect on insulin release. Membrane potential and whole-cell currents were measured with the patch-clamp technique, and [Ca²⁺]ᵢ, was determined with a fluorescence dye. Ritonavir and nelfinavir both inhibited the same component(s) of voltage-dependent K⁺ currents with a concomitant change in action potential wave form, whereas indinavir was ineffective. Comparison with other blockers of voltage-dependent K⁺ currents revealed that suppression of distinct noninactivating current component(s) altered action potential wave form and decreased [Ca²⁺]ᵢ similar to ritonavir and nelfinavir, whereas blockade of inactivating component(s) was without effect. Complete inhibition of voltage-dependent K⁺ currents by 80 mM TEA- drastically increased [Ca²⁺]ᵢ, demonstrating that voltage-dependent K⁺ channels are not the sole target of ritonavir and nelfinavir. Accordingly, the Ca²⁺-lowering effect of ritonavir was preserved in the presence of 80 mM TEA-. This effect was mimicked by the anion channel blocker 4,4'-disothiocyanostilbene-2,2'-disulfonic acid (DIDS). Consequently, ritonavir and nelfinavir inhibited a DIDS-sensitive anion current in B-cells. We suggest that ritonavir and nelfinavir decrease insulin secretion by inhibition of voltage-dependent K⁺ channels and anion channels, which are essential to provide counterion currents for Ca²⁺ influx across the plasma membrane.

Human immunodeficiency virus (HIV) protease inhibitors are known to induce glucose intolerance or overt diabetes mellitus as a severe side effect (Behrens et al., 1999; Gatti et al., 1999; Justman et al., 2003; Woerle et al., 2003; Lien and Feinglos, 2005). Alterations of glucose metabolism and insulin signaling in HIV-infected patients are more frequent since the introduction of potent antiretroviral therapy with protease inhibitor-based regimen (Hardy et al., 2001; Vigouroux et al., 2003; Lee et al., 2005). The mechanisms of protease inhibitors that may influence glucose homeostasis are various. In adipocytes, altered adipocytokine expression, reduced insulin binding, and down-regulation of peroxisome proliferator-activated receptor gamma expression were observed (Cammalleri and Germinario 2003; Vernochet et al., 2005). Reduction of glucose transporter 4-mediated glucose uptake and promoter-activated receptor gamma expression were observed (Koster et al., 2003; Du¨fer et al., 2004). Because the adverse side effects of some of the inhibitors have been proposed to be due to inhibition of insulin secretion from pancreatic islets (Koster et al., 2003; Du¨fer et al., 2004), the present study intends to elucidate the interactions between protease inhibitors and pancreatic B-cell function.

ATP-dependent K⁺ channels (Kₐₜₚ channels) and voltage-dependent Ca²⁺ channels usually play the key role in stimulus-secretion coupling of pancreatic B-cells. ATP production by glucose metabolism inhibits the Kₐₜₚ channels, and the resulting depolarization leads to opening of Ca²⁺ channels, Ca²⁺ influx, and eventually insulin secretion (Ashcroft and Rorsman, 1989; Zhou and Misler, 1996). The Ca²⁺ action potential wave form and thus the Ca²⁺ current during an
action potential are dependent on the opening of voltage- and/or Ca\(^{2+}\)-dependent K\(^+\) channels (K\(_s\) and K\(_{ca}\) channels) (Smith et al., 1990; Göpel et al., 1999; Su et al., 2001; MacDonald and Wheeler, 2003). Additionally, volume-sensitive anion currents may be involved in the regulation of glucose-induced electrical activity of pancreatic B-cells (Kinard and Satin, 1995; Best et al., 1996; Drews et al., 1998).

We have shown recently that ritonavir and nelfinavir, but not indinavir, diminish glucose-induced insulin secretion of islets in vitro. This effect was because of a reduction in the cytosolic free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) to basal values by ritonavir and nelfinavir. This decrease in [Ca\(^{2+}\)]\(_i\) could not be counteracted by tolbutamide. Surprisingly, ritonavir had no direct effect on K\(_{ATP}\) or voltage-dependent Ca\(^{2+}\) currents (Düfer et al., 2004). Ritonavir affected the electrical activity of B-cells in a characteristic manner that was ascribed to the partial inhibition of voltage-dependent K\(^+\) currents (Düfer et al., 2004). Inhibition of a K\(^+\) current cannot, however, at first glance not explain a reduction in [Ca\(^{2+}\)]\(_i\) and insulin secretion. Therefore, we scrutinized the effects of the HIV protease inhibitors, K\(^+\) current blockers and inhibitors of the volume-sensitive anion current (VSAC) on B-cell plasma membrane potential (V\(_m\)), ion currents, and [Ca\(^{2+}\)]\(_i\). The results let us conclude that the effect of ritonavir and nelfinavir is due to the inhibition of ion currents providing counterbalancing ions for charge compensation, which is a prerequisite for Ca\(^{2+}\) influx.

Materials and Methods

Cell Preparation. Experiments were performed on islets or dispersed pancreatic B-cells from fed Naval Medical Research Institute mice, killed by cervical dislocation or CO\(_2\). The principles of laboratory animal care were followed (National Institutes of Health publication 85-23, revised 1985). Islet cells were isolated by collagenase digestion of the pancreas. Cells were dispersed in Ca\(^{2+}\)-free medium and cultured up to 4 days in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (Plant, 1988).

Solutions and Chemicals. Bath solution was composed of 140 mM NaCl, 5 mM KCl, 1.2 mM MgCl\(_2\), 2.5 mM CaCl\(_2\), and 10 mM HEPES, pH 7.4, adjusted with NaOH. Glucose was added in the concentrations indicated. For measurements of anion or voltage-dependent K\(^+\) currents, 0.1 mM tolbutamide was added to the bath solution. Whole-cell recordings of voltage-dependent K\(^+\) currents were performed with a pipette solution containing 130 mM KCl, 4 mM MgCl\(_2\), 2 mM CaCl\(_2\), 10 mM EGTA, 0.65 mM Na\(_2\)ATP, and 20 mM HEPES, pH 7.15, adjusted with KOH. The same solution was taken for measurements of anion currents, except that 2 mM Na\(_2\)ATP was used. Membrane potential recordings were registered with a pipette solution composed of 10 mM KCl, 10 mM NaCl, 70 mM K\(_2\)SO\(_4\), 4 mM MgCl\(_2\), 2 mM CaCl\(_2\), 10 mM EGTA, 20 mM HEPES, and 250 µM/ml amphotericin B, pH 7.15, adjusted with KOH.

Fura-2AM was obtained from Molecular Probes (Eugene, OR), and RPMI 1640 medium and penicillin/streptomycin were from Invitrogen (Carlsbad, CA). Ritonavir was a kind gift from Abbott (Wiesbaden, Germany). Nelfinavir and indinavir were kindly provided by Prof. Dr. S. Laufer (Tübingen, Germany). Stock solutions of these substances were prepared with dimethyl sulfoxide. All other chemicals were purchased from Sigma Chemie (Deisenhofen, Germany) or Merck (Darmstadt, Germany) in the purest form available.

Patch-Clamp Recordings. Patch pipettes were pulled from borosilicate glass capillaries (Clark Electromedical Instruments, Pangbourne, UK). They had resistances between 3 and 5 MΩ when filled with pipette solution. Membrane currents were recorded at 27°C with an EPC-9 patch-clamp amplifier and the software, Pulse (HEKA, Lambrecht/Pfalz, Germany) in the voltage-clamp mode. Membrane potential was measured in current-clamp in the perforated-patch mode. Perforation usually occurred within 10 min after seal formation (series resistance < 30 MΩ). Conventional whole-cell anion currents were measured at a holding potential of −70 mV and during 300-ms pulses from −80 to −60 mV at 15-s intervals in the presence of 100 µM tolbutamide. Currents through voltage-dependent K\(^+\) channels have been registered in the conventional whole-cell mode by 150-ms pulses from −70 to 0 mV applied every 15 s.

Analysis of Action Potentials. Full width at half-maximum amplitude of action potentials was determined in each experiment by averaging the values of the last five action potentials before changing the solution. The frequency of action potentials was calculated during the last 30 s before bath solution change.

Measurement of [Ca\(^{2+}\)]\(_i\). [Ca\(^{2+}\)]\(_i\) was measured at 37°C by the fura-2 method according to Grynkiewicz et al. (1985) using equipment and software from TILL Photonics (Gräfelfing, Germany). The cells were loaded with fura-2AM (5 µM) for 30 min. Intracellular fura-2 was excited alternately at 340 or 380 nm by means of an oscillating mirror with a diffraction grating. The excitation light was then directed through the objective (PlanNeofluar 40× objective; Carl Zeiss GmbH, Jena, Germany) by means of a glass fiber light guide and a dichroic mirror. The emitted light was filtered (LP 615 nm) and measured by a digital camera. The ratio of the emitted light intensity at 540-/380-nm excitation was used to calculate [Ca\(^{2+}\)], using an in vitro calibration with fura-2-K\(^+\) salt.

Presentation of Results. Electrophysiological experiments and [Ca\(^{2+}\)]\(_i\) are illustrated by recordings representative of the indicated number of experiments performed with different cells. At least two different cell preparations have been used for each series of experiments. If possible, the means ± S.E.M. are given in the text for the indicated number of experiments. The statistical significance of differences between means was assessed by a one-sample Student’s t test or Student’s t test for paired values when two samples were compared. Multiple comparisons were made by analysis of variance followed by Student-Newman-Keuls test. P ≤ 0.05 was considered significant.

Results

Effects of Ritonavir, Nelfinavir, and Indinavir on Electrical Activity and Voltage-Dependent K\(^+\) Channels. Comparing the effect of ritonavir, nelfinavir, and indinavir on electrical activity of pancreatic B-cells revealed that ritonavir and nelfinavir clearly changed shape and frequency of glucose-induced action potentials (Fig. 1, A and B), whereas indinavir was without effect (Fig. 1C). The addition of 10 µM ritonavir increased the average length (full width at half-maximum amplitude) of action potentials from 20 ± 3 to 1373 ± 218 ms (n = 5, P ≤ 0.005) (Fig. 1A). Concomitantly, the frequency of action potentials decreased from 91 ± 7 to 20 ± 7 AP/min (n = 5, P ≤ 0.0001). Similarly, 20 µM nelfinavir caused a broadening of glucose-induced action potentials from 34 ± 13 to 537 ± 64 ms (n = 6, P ≤ 0.001) and reduced the frequency from 134 ± 32 to 26 ± 7 AP/min (n = 6, P ≤ 0.05) (Fig. 1B). Indinavir up to 70 µM did not change shape and/or frequency of action potentials (Fig. 1C). The length of action potentials was 17 ± 3 ms before versus 17 ± 3 ms after application of indinavir (n = 4). Likewise, the frequency did not change significantly [31 ± 10 AP/min under control conditions versus 59 ± 9 AP/min with indinavir (n = 4)]. Characteristic action potentials in the presence of the respective compound are shown in the insets of Fig. 1, A to C, on an extended time scale.
The changes in electrical activity and action potential shape caused by ritonavir and nelfinavir are unlikely due to changes in $K_{ATP}$ or $Ca^{2+}$ current (Du¨fer et al., 2004) but could be associated with an alteration in voltage-dependent $K^+$ current. To evaluate this suggestion, we tested the influence of the three substances on the voltage-dependent $K^+$ current in the conventional whole-cell mode (Figs. 1, D–F, and 2A). Ritonavir and nelfinavir inhibited noninactivating component(s) of the current, while leaving inactivating component(s) (Fig. 2A; Du¨fer et al., 2004). In contrast, the voltage-dependent $K^+$ current was not influenced by indinavir (Fig. 1F). In the series with ritonavir, the peak current under control conditions in 0.5 mM glucose amounted to 500 ± 7 pA ($n$ = 7). It was reversibly reduced to 234 ± 24 pA by treatment of the cells with 10 μM ritonavir ($n$ = 7, $P$ = 0.01) (Fig. 1D). Similarly, 20 μM nelfinavir diminished the amplitude of the $K^+$ current from 441 ± 51 to 222 ± 31 pA ($n$ = 6, $P$ ≤ 0.001) (Fig. 1E). In the presence of 70 μM indinavir, the amplitude of the $K^+$ current remained almost constant with 523 ± 79 pA under control conditions versus 466 ± 87 pA with indinavir ($n$ = 7) (Fig. 1F).

Comparison of the Effects of Ritonavir, Clotrimazole, and TEA$^+$ on Voltage-Dependent K$^+$ Currents, Electrical Activity, and [$Ca^{2+}$]$_e$. To reveal whether the effects of ritonavir (and nelfinavir) can be attributed to the inhibition of distinct component(s) of the voltage-dependent $K^+$ current, we chose a low concentration of TEA$^+$ (2 mM) and the imidazole antimycotic clotrimazole. In contrast to TEA$^+$, clotrimazole also inhibits other B-cell ion currents, e.g., VSACs (Welker and Drews, 1997). Although the effects of clotrimazole on voltage-dependent $K^+$ currents, $V_m$ and [$Ca^{2+}$]$_e$, were comparable with those of ritonavir, TEA$^+$ acted differently. Figure 2 compares the effect of ritonavir with the influence of clotrimazole and 2 mM TEA$^+$. As mentioned above, ritonavir inhibited noninactivating component(s) of the voltage-dependent $K^+$ channels (Fig. 2A) and markedly changed the shape of $Ca^{2+}$ action potentials (Fig. 2D). Moreover, ritonavir caused a rapid decrease of [$Ca^{2+}$]$_e$ in the
Fig. 2. Effects of ritonavir, clotrimazole, and TEA⁺ (2 mM) on voltage-dependent K⁺ currents, glucose-induced electrical activity, and [Ca²⁺], in the presence of 15 mM glucose. Ritonavir (10 μM; A, n = 4), and 5 μM clotrimazole (B, n = 5) inhibited noninactivating component(s) of the voltage-dependent K⁺ current, whereas 2 mM TEA⁺ inhibited inactivating component(s) of the current (C, n = 7). Voltage-dependent K⁺ currents were monitored every 15 s during 50- to 150-ms voltage steps from −70 to 0 mV. Ritonavir (10 μM; D, n = 5) and 5 μM clotrimazole (E, n = 4) markedly changed the spike pattern of action potentials, which were not influenced by 2 mM TEA⁺ (F, n = 6). Ritonavir (20 μM; G, n = 8) and 5 μM clotrimazole (H, n = 9) suppressed the glucose-induced increase in [Ca²⁺], TEA⁺ (2 mM) did not affect [Ca²⁺], in the presence of 15 mM glucose (I, n = 15).

presence of 15 mM glucose, a concentration of the sugar at which [Ca²⁺]c is steadily increased or oscillations occur (Fig. 2G). The average peak value of [Ca²⁺]c amounted to 341 ± 42 nM before addition of 20 μM ritonavir and decreased to 69 ± 12 nM (n = 8, P < 0.001) in the presence of the drug. Clotrimazole also inhibits noninactivating component(s) of voltage-dependent K⁺ channels (Fig. 2B) similar to ritonavir. The amplitude of the K⁺ current was reduced from 439 ± 68 pA to 81 ± 12 pA by 5 μM clotrimazole (n = 5, P < 0.001) (Fig. 2B). Furthermore, the antymycotic drug revealed effects analogous to ritonavir on electrical activity and [Ca²⁺]c (Fig. 2, E and H). The length of action potentials was 22 ± 2 ms before addition of clotrimazole and increased to 880 ± 99 ms in the presence of 5 μM clotrimazole (Fig. 2E, n = 4, P < 0.005). Simultaneously, the frequency of action potentials decreased from 77 ± 9 to 23 ± 8 AP/min (n = 4, P < 0.05) (Fig. 2E). Figure 2H shows the effect of clotrimazole on [Ca²⁺]c. On average, the maximal peak value before addition of 5 μM clotrimazole was 334 ± 15 nM. [Ca²⁺]c irreversibly dropped to a plateau value of 199 ± 4 nM (n = 9, P < 0.0001) after addition of the antymycotic substance. TEA⁺ at a concentration of 2 mM affected inactivating component(s) of the K⁺ current (Fig. 2C). The amplitude of the current was reduced from 321 ± 55 pA under control conditions to 148 ± 28 pA in the presence of 2 mM TEA⁺ (n = 7, P < 0.001). In contrast to ritonavir and clotrimazole, TEA⁺ caused no significant changes in the spike pattern of glucose-induced action potentials (Fig. 2F). Full width at half-maximum amplitude of action potentials amounted to 23 ± 5 ms before addition of TEA⁺ versus 28 ± 7 ms in the presence of 2 mM TEA⁺ (n = 6). The frequency of action potentials was 36 ± 6 AP/min before and 51 ± 12 AP/min after addition of TEA⁺. Oscillations in [Ca²⁺]c in the presence of 15 mM glucose were not affected by application of 2 mM TEA⁺ (n = 15) (Fig. 2I).

Effect of Ritonavir on [Ca²⁺]c in the Presence of 80 mM TEA⁺. We next tested whether the effect of ritonavir on [Ca²⁺]c still occurs in the presence of 80 mM TEA⁺, a concentration at which voltage-dependent K⁺ channels are completely blocked. Surprisingly, ritonavir even decreased [Ca²⁺]c, augmented by the high concentration of TEA⁺ (Fig. 3). The addition of 80 mM TEA⁺ in the presence of 15 mM glucose caused a rapid increase in [Ca²⁺]c consisting of a peak and a sustained plateau without oscillations. Subsequent application of 20 μM ritonavir provoked a clear drop in [Ca²⁺]c (Fig. 3). In the presence of 15 mM glucose, the peak value of [Ca²⁺]c amounted to 417 ± 49 nM and increased to 668 ± 58 nM after addition of 80 mM TEA⁺. Directly after application of ritonavir, [Ca²⁺]c decreased to 90 ± 5 nM (n = 9, P < 0.001). The inset demonstrates that voltage-dependent K⁺ currents were abolished by 80 mM TEA⁺ (peak control
current was 395 ± 87 pA in this series of experiments versus -5 ± 3 pA with 80 mM TEA⁺, n = 6, P ≤ 0.001, Fig. 3, inset).

**Effect of DIDS on [Ca²⁺]c in the Presence of 80 mM TEA⁺.** So far, the results suggest that voltage-dependent K⁺ channels are not the only target of ritonavir. Another channel that is assumed to be involved in B-cell function is the volume-sensitive anion channel (Kinard and Satin, 1995; Best et al., 1996; Drews et al., 1998). Thus, we tested the effect of the anion channel blocker DIDS on [Ca²⁺]c in the presence of 80 mM TEA⁺. Figure 4 shows that we could mimic the effect of ritonavir on [Ca²⁺]c with the anion channel blocker DIDS during complete blockage of voltage-dependent K⁺ channels by 80 mM TEA⁺. The peak value of [Ca²⁺]c in the presence of 15 mM glucose was 361 ± 50 nM (data not shown) and increased after addition of 80 mM TEA⁺ to 1017 ± 169 nM (n = 8, P ≤ 0.01). The application of 100 µM DIDS caused an immediate initial drop in [Ca²⁺]c to basal values of 67 ± 9 nM (n = 8, P ≤ 0.001). In four of eight cells, this first drop was followed by a small increase or even oscillations in [Ca²⁺]c (maximal peak value of 352 ± 98 nM) similar to the action of ritonavir observed in some experiments (Düfer et al., 2004). For comparison, in each experiment with DIDS, B-cells were also treated with 20 µM ritonavir.

**Effect of Ritonavir and Nelfinavir on VSAC.** To test ritonavir and nelfinavir for an effect on VSACs, Kᵥ,ᵥ currents were blocked by 100 µM tolbutamide and DIDS-sensitive VSACs (Kinard and Satin, 1995; Drews et al., 1998) were evoked by lowering the osmolarity of the extracellular solution (Drews et al., 1998) (Fig. 5A). After decreasing the concentration of NaCl from 140 to 100 mM, the anion current developed with an average amplitude of -154 ± 20 pA. The addition of 20 µM ritonavir reversibly reduced the amplitude of the anion current to -60 ± 10 pA (n = 7, P ≤ 0.001). After wash-out of ritonavir, the current increased again to values of -145 ± 33 pA (n = 6, P ≤ 0.001). The same effect on VSACs was seen with nelfinavir (Fig. 5B). In this series of experiments, the hypoosmotic solution evoked a VSAC of -378 ± 72 pA (n = 5) and amounted to -17 ± 17 pA (n = 3, P ≤ 0.01) in the presence of 20 µM nelfinavir. A similar
Discussion

Inhibition of hormone release from B-cells may, besides effects on insulin signaling, contribute to disturbed glucose homeostasis observed in patients treated with HIV protease inhibitors (Behrens et al., 1999; Dubé, 2000; Woerle et al., 2003). We have shown before that ritonavir and nelfinavir, but not indinavir, inhibit insulin secretion of mouse pancreatic B-cells (Düfer et al., 2004). This is in agreement with observations by Schutt et al. (2004), who found insulin secretion of INS-1 tumor cells to be inhibited by ritonavir, nelfinavir, and saquinavir, but not by indinavir or amprenavir. The decrease in insulin secretion of native B-cells by ritonavir and nelfinavir is because of a decrease in [Ca\(^{2+}\)]\(_i\) (Düfer et al., 2004). However, the underlying mechanism remained obscure since neither the K\(_{\text{ATP}}\) channel nor the voltage-dependent Ca\(^{2+}\) channel were influenced by ritonavir. Moreover, intracellular stores seem not to be involved in the ritonavir-induced lowering of [Ca\(^{2+}\)]\(_i\) (Düfer et al., 2004). Measurements of the V\(_m\) revealed that ritonavir changes the wave form of action potentials and broadens the action potentials 75-fold, but they are still sensitive to the Ca\(^{2+}\) channel blocker D600. The change in action potential length was ascribed to an inhibition of distinct component(s) of voltage-dependent K\(^+\) currents by ritonavir and nelfinavir, leaving a fast inactivating component (Düfer et al., 2004). These results left us with the puzzling situation that ritonavir reduced [Ca\(^{2+}\)]\(_i\), although voltage-dependent Ca\(^{2+}\) channels appeared to be open. Additionally, at first glance, it seems not plausible that the closure of a hyperpolarizing (K\(^+\)) current should reduce Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels.

For pancreatic B-cells, the depolarization-dependent change in [Ca\(^{2+}\)]\(_i\), and not membrane depolarization, is the vital trigger signal for insulin secretion. Thus, Ca\(^{2+}\) current during Ca\(^{2+}\) action potentials must flow for a period of time sufficient to augment [Ca\(^{2+}\)]\(_i\). This is completely different from the signaling mechanism in nerve fibers where membrane depolarization, i.e., the Na\(^+\) action potential, constitutes the signal that influences cell function. During such an action potential in nerve fibers, the amount of ions crossing the membrane is too low to induce measurable changes in the intracellular ion concentration. Therefore, a change in [Ca\(^{2+}\)]\(_i\), by Ca\(^{2+}\) influx, the prerequisite for insulin secretion, requires the concomitant outflux of a cation or influx of an anion. Counterions are needed because charge separation beyond the negligible amount that charges or recharges the membrane capacitor does not occur within solutions or across membranes. Consistently, it has been shown for Ca\(^{2+}\) release in stripped skeletal muscles fibers, which also lead to a significant change in [Ca\(^{2+}\)]\(_i\), that inhibition of K\(^+\) channels in the membrane of the sarcoplasmic reticulum blocks Ca\(^{2+}\) release from these organelles (Abramcheck and Best, 1989). To understand this inhibition of a current by suppression of counterion currents, one has to consider that at any given membrane potential, the sum of all ion currents is zero (if a net current would flow, it would change V\(_m\) according to Ohm’s law). Consequently, if in a cell all ion channels except one are almost entirely inhibited, the current through the open channel must be marginal.

During glucose-induced electrical activity of mouse pancreatic B-cells, K\(_{\text{ATP}}\) channels are almost completely closed (Ashcroft and Rorsman, 1989). Apparently, only L-type Ca\(^{2+}\) channels (Plant, 1988; Schulla et al., 2003) and voltage-dependent K\(^+\) channels (Ashcroft and Rorsman, 1989; MacDonald and Wheeler, 2003) are open during the action potentials. Ritonavir inhibits the entire voltage-dependent K\(^+\) current except for a fast-inactivating component. This component is completely inactivated after 50 ms at latest and can thus only carry a marginal K\(^+\) current during the first 30 to 50 ms of the long action potentials lasting about 1 s (Fig. 1; Düfer et al., 2004). Therefore, during these ritonavir-modulated action potentials, the Ca\(^{2+}\) influx might be negligible because of the lack of the charge-compensating K\(^+\) current.

The hypothesis that the suppression of Ca\(^{2+}\) influx by ritonavir is because of counterion current inhibition is supported by other results presented here and in a previous publication (Düfer et al., 2004). Nelfinavir had very similar effects on voltage-dependent K\(^+\) currents, V\(_m\), [Ca\(^{2+}\)]\(_i\), and insulin secretion, whereas indinavir did not affect these parameters at all (Fig. 1; Düfer et al., 2004). Clotrimazole had effects analogous to ritonavir and nelfinavir on voltage-dependent K\(^+\) currents and V\(_m\) and also diminished [Ca\(^{2+}\)]\(_i\) (Fig. 2). In contrast, TEA\(^-\) at a low concentration of 2 mM also decreased the voltage-dependent K\(^+\) current to about 50% as the other substances but left a noninactivating (therefore long-lasting) component and had nearly no effect on V\(_m\) or [Ca\(^{2+}\)]\(_i\) (Fig. 2). The latter result is expected because in this case counterions for Ca\(^{2+}\) entry are still available. However, even complete suppression of voltage-dependent K\(^+\) currents does not decrease [Ca\(^{2+}\)]\(_i\). It has been demonstrated that blockage or knockout of voltage-dependent K\(^+\) channels results in an increase in insulin secretion (e.g., Henquin, 1990; Roe et al., 1996; MacDonald et al., 2001; Su et al., 2001; MacDonald et al., 2002). In addition, the augmenting effect of TEA\(^+\) on [Ca\(^{2+}\)]\(_i\), at a concentration of 80 mM, which entirely blocked voltage-dependent K\(^+\) currents (Fig. 3, inset), ruled out that K\(^+\) currents are the only currents that can provide counterions for Ca\(^{2+}\) influx in B-cells. The high concentration of TEA\(^+\) drastically increased [Ca\(^{2+}\)]\(_i\), and ritonavir was still effective (Fig. 3). Since no K\(^+\) current can compensate the charge for Ca\(^{2+}\) influx under these conditions, we investigated whether anions can act as counterions, too. This idea is supported by two observations. The unspecific anion channel blocker DIDS had the same effect as ritonavir on [Ca\(^{2+}\)]\(_i\), augmented by 80 mM TEA\(^+\) (Fig. 4). Ritonavir and nelfinavir both block VSACs (Fig. 5, A and B), which are believed to be involved in glucose-induced electrical activity (Best, 1997). This block was similarly effective as VSAC inhibition by DIDS (Fig. 5B). These results clearly demonstrate that only the combined inhibition of voltage-dependent K\(^+\) channels and VSACs, either achieved by simultaneous application of TEA\(^+\) and DIDS or by HIV protease inhibitors that suppress both currents, decreases [Ca\(^{2+}\)]\(_i\). This observation also explains the similarity in the action of ritonavir or nelfinavir and clotrimazole on [Ca\(^{2+}\)]\(_i\), because the antymycotic also concomitantly blocks voltage-dependent K\(^+\) channels (Fig. 2) and VSACs (Welker and Drews, 1997).

From the observation that HIV protease inhibitors directly affect [Ca\(^{2+}\)]\(_i\), and inhibit the effect of sulfonylureas on
[Ca^{2+}], (Düfer et al., 2004), one would suggest that insulinotropic oral antidiabetic drugs are less suitable for the treatment of HIV-infected patients than metformin and thiazolidinediones, which increase insulin sensitivity. These drugs are presently under evaluation in diabetic and glucose-intolerant HIV-infected patients treated with highly active antiretroviral therapy (Fantoni et al., 2003; Kamin et al., 2005).

In conclusion, ritonavir and nelfinavir but not indinavir directly interfere with B-cell electrical activity, [Ca^{2+}], and insulin secretion by inhibiting currents that are necessary to provide countereffects for charge compensation to allow Ca^{2+} influx during action potentials.

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References


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