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Effect of insulinotropic agent nateglinide on Kv and Ca^{2+} channels in pancreatic β-cell

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Abstract

Novel insulinotropic agent nateglinide stimulates insulin via binding to sulfonylurea receptor and closing the ATP-dependent K^+ (K_{ATP}) channels in pancreatic β-cells, leading to an increase in $[Ca^{2+}]_i$ for exocytosis. The voltage-dependent Ca^{2+} channel and the delayed rectifier K^+ (Kv) channels are also present in β-cells and their activities determine the configuration of action potential and hence contribute to the regulation of $[Ca^{2+}]_i$ and insulin secretion. This study, by using the patch-clamp method in whole cell configuration, comparatively characterized the direct effects of sulfonylurea receptor ligands including nateglinide, glyburide, and repaglinide on Kv and Ca^{2+} channels. Each agent inhibited Kv currents in a concentration-dependent manner with effective concentration range two to three orders higher than that for blocking K_{ATP} channels. A marginal stimulation of Ca^{2+} current was observed with all drugs, while repaglinide at concentration greater than 300 nM inhibited Ca^{2+} current. The direct effects of these antidiabetic agents on Kv and Ca^{2+} channels may act concertedly with their primary action on K_{ATP} channels in regulating $[Ca^{2+}]_i$ and the stimulus–secretion coupling.

Keywords: Nateglinide; Insulinotropic agent; Pancreatic β-cell

1. Introduction

Regulation of insulin secretion involves the coordinated control of ion channels in the β-cell membrane. The initial response of β-cells to glucose or sulfonylurea receptor ligands is closure of the ATP-sensitive K^+ (K_{ATP}) channel. This leads to membrane depolarization followed by the activation of Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Dean and Matthews, 1970). The resultant increase in cytoplasmic Ca^{2+} activity is the key element that directly triggers exocytosis and hence insulin secretion (Prentki and Wollheim, 1984; Rorsman et al., 1984). The membrane potential is restored by the activity of voltage-dependent K^+ (Kv) channels, whose time course of activation and inactivation serves to entrain the frequency and modulate the duration of action potential of β-cells and thereby regulating insulin release (Boyd, 1988; Satin et al., 1989; Bokvist et al., 1990; Smith et al., 1990). Given that the interplay of voltage-gated inward Ca^{2+} and outward Kv channels determines the configuration of action potential (Henquin and Meissner, 1984; Rorsman and Trube, 1986), modification of the activity of these ion channels would ultimately contribute to determine the pattern of electrical spikes that is presumably associated to oscillatory nature of insulin response. In essence, Kv channel is reportedly implicated in the regulation of glucose responsiveness (Philipson, 1999; Philipson et al., 1994).

Nateglinide (also known as A-4166) is a recently approved nonsulfonylurea insulinotropic agent for the treatment of type II diabetes. Analogous to the known antidiabetic agents, glyburide or repaglinide, nateglinide is capable of inducing insulin secretion by binding to sulfonylurea receptor and blocking K_{ATP} channels in pancreatic β-cells (Sturgess et al., 1985; Gromada et al., 1995; Akiyoshi et al., 1995; Hu et al., 1999, 2000). The structures of these agents are shown in Fig. 1. By closing K_{ATP} channels leading to membrane depolarization, these agents could secondarily/indirectly affect voltage-dependent Ca^{2+} and Kv channels. It is, however, unclear whether these agents also act on Ca^{2+} and Kv channels directly and independently of their action on K_{ATP} channels, since stimulation of Ca^{2+} channel and/or inhibition of Kv channel in β-cells could all contribute to the insulinotropic efficacy of these agents (Satin and Smolen, 1994; Philipson, 1999). To this end, we have extended our earlier investigation of nateglinide and its comparators glyburide

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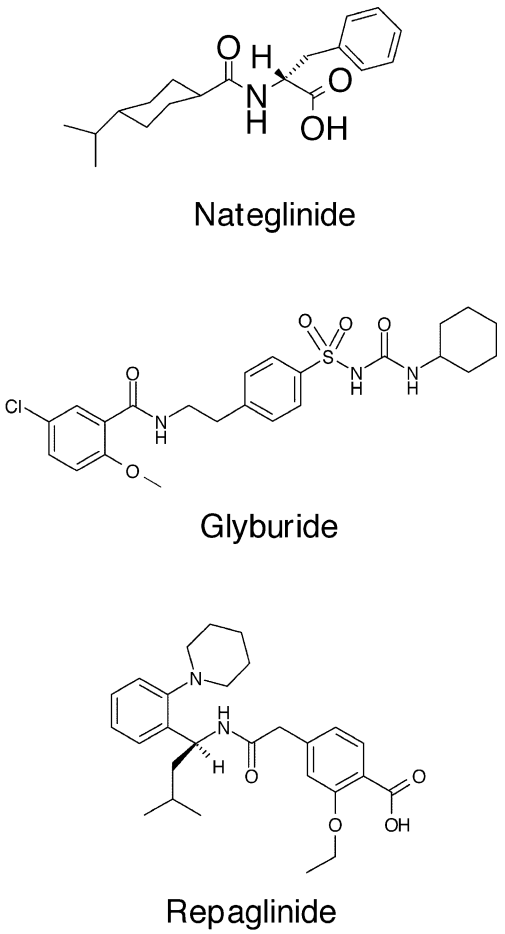

Fig. 1. Chemical structure of antidiabetic agents nateglinide, glyburide, and repaglinide.

and repaglinide on K_{ATP} channels to further elucidation of direct effects of these drugs on Kv and Ca^{2+} in intact pancreatic β-cells using patch-clamp method in whole-cell configuration. Each current component to be evaluated was physiologically and/or pharmacologically isolated from other ionic currents in β-cells to allow the observation of independent effect of the drugs. The results indicated that all these drugs had minor but direct effect on Kv and Ca^{2+} channels in addition to their action on K_{ATP} channels. The responses of multiple ion channels to the antidiabetic drugs may act in concert to contribute to the regulation of insulin secretion.

2. Materials and methods

2.1. Isolation of pancreatic islets and preparation of β-cells

Male Sprague–Dawley rats weighing 250–275 g were anesthetized with Na pentobarbital i.p. at 120 mg/kg. Islets of Langerhans were isolated from pancreas by liberase digestion (0.5 mg/ml, Boehringer Mannheim, Germany) followed by a Ficoll gradient centrifugation as a modification of the method of Lacy and Kostianovsky (1967). The islets were then dissociated into single cells with the treatment of protease (0.5 mg/ml, type IX, Sigma, St. Louis, MO). The buffer used in the entire isolation procedure consisted of (mM): NaCl 5, KCl 140, $MgCl_2$ 2, HEPES 10, $CaCl_2$ 2, glucose 5 (pH 7.4). The individual cells were seeded in CMRL (Cornaught Medical Research Laboratories) medium (Gibco, Gaithersburg, MD) supplemented with 1% fetal calf serum, 1% antibiotic–antimycotic, and 10 mM glucose, and incubated at 37 °C in an atmosphere of 95% air and 5% CO_2 for 2–5 days before the electrophysiological recording. All operations were performed at room temperature (~22 °C) except for β-cell culture.

2.2. Electrophysiological recording of voltage-dependent Ca^{2+} and Kv currents

The voltage-dependent Ca^{2+} and Kv currents were recorded at 22 °C using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981) in the primary culture of rat pancreatic β-cells. All experiments were performed under a normoglycemic glucose level of 5 mM.

The voltage-gated L-type Ca^{2+} currents were generated by depolarizing voltage steps ranging from −40 to +50 mV with an increment of 10 mV from a holding potential of −60 mV. The duration of pulses was 70 ms. Several measures were taken to eliminate the contamination of other types of ion channels. The activity of K_{ATP} channels was suppressed by dialyzing β-cells with 5 mM ATP in pipette solution. The activity of Ca^{2+}-activated K^+ channels and Kv channels was removed by replacing K^+ in bath and pipette solutions with Cs^+ supplemented with K^+ channel blockers like 4-aminopyridine (4-AP) and tetraethylammonium. Under these circumstances, the currents recorded are virtually composed of the activity of Ca^{2+} channels only.

The Kv currents were elicited either by voltage steps or ramps. The step depolarization pulses were incremented by 10 mV between −80 and +40 mV from a holding potential of −60 mV with a duration of 400 ms. The ramp voltage protocol also ranged between −80 and +40 mV over a period of 1400 ms from a holding potential of −60 mV. The ramp voltage was so chosen that it correlated with the voltage step protocol and the corresponding ramp currents would thus depict an instantaneous current–voltage relationship for the step Kv currents. To remove the contamination of Ca^{2+}-activated K^+ channels, the bath solution contained nominally zero Ca^{2+}. Besides, β-cells were dialyzed with a pipette solution containing 5 mM ATP to maximally block the K_{ATP} currents. The amplitude of ramp Kv current at +20 mV that is significantly below the activation threshold for the Ca^{2+}-activated K^+ channels was used as an index for quantitative evaluation of Kv

Table 1
Bath and pipette solutions for recording the voltage-gated Ca^{2+} and Kv currents

	Ca^{2+}		Kv	
	Bath (mM)	Pipette (mM)	Bath (mM)	Pipette (mM)
NaCl	120	–	140	5
KCl	–	–	5	140
CsCl	5	140	–	–
$MgCl_2$	1	2	1	2
HEPES	10	10	10	10
$CaCl_2$	3	0.1	–	0.1
EGTA	–	0.6	–	0.6
K_2ATP	–	5	–	5
Na_2UDP	–	2	–	2
TEACl	10	–	–	–
4-AP	5	–	–	–
Glucose	5	–	5	–
pH	7.4		7.4	

current. The compositions of the solutions for recording each current component are shown in Table 1.

Corning 35-mm culture dishes, in which β-cells were grown, were fitted with Sylgard O-ring to serve as recording chambers. Cells were perfused continuously with appropriate control or testing bath solutions at a constant rate of ~ 1.5 ml/min. The volume of the chambers were maintained at ~ 0.3 ml. Experiments were performed at $\times 600$ magnification under a Nikon inverted microscope. As β-cells were reported to be two- to threefold larger than α-cells (Pipeleers et al., 1985), only cells with a diameter larger than 10 μm and well-preserved granulation were used for the current recording. The capacitance of β-cells was 12.1 ± 0.5 pF ($n = 35$). Patch-clamp electrodes were pulled from Kimax-51 capillary tubes. The resistance of electrodes after fire polishing was between 3 and 4 MΩ. All currents recorded were amplified by a List EPC-7 amplifier (Adams & List Assoc., Darmstadt, Germany), digitized at 4 kHz with a TL-1-125 DMA interface (Axon Instruments, Foster City, CA) and stored on a Compaq Microcomputer for later analysis with software pClamp version 6.03 (Axon Instruments). The junction potential between the electrodes and the bath solution was compensated by the DC offset on the amplifier.

2.3. Data analysis

The amplitude of Kv currents at +20 mV of either ramp or step protocol was measured as index for quantitative evaluation. The magnitude of drug effect was determined by comparing the currents posttreatment to pretreatment in the same β-cell. The remaining currents after blockade by drugs at various concentrations were calculated as fractions of control to construct concentration–response curves. The data were fit to logistic equation $y = 1/1 + (X/\mathrm{Xo})^a$, in which "Xo" and "$a$" were, respectively, IC_{50} (concentration for a half-maximal blockade) and Hill coefficient. Statistical significance of the data was determined with *t*-test (single tailed).

3. Results

3.1. Concentration-dependent inhibition of Kv currents by nateglinide, glyburide, and repaglinide

In our earlier studies (Hu et al., 1999, 2000), the effects of antidiabetic agents nateglinide (0.3–300 μM), glyburide

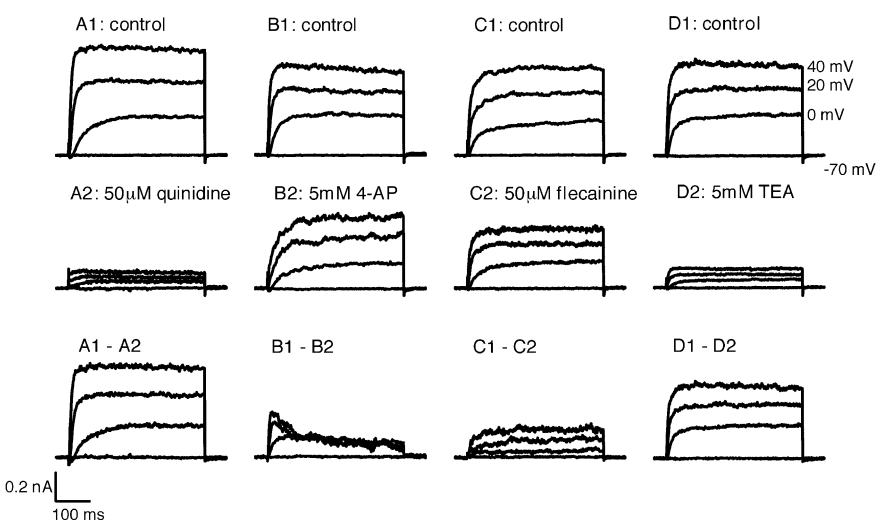

Fig. 2. Typical records showing the blockade of Kv currents by channel blockers, quinidine (column A), 4-AP (column B), flecainine (column C), and tetraethylammonium (TEA) (column D). Four current traces in each panel were elicited, respectively, by depolarizing pulses to −70, 0, +20, and +40 mV (as marked in D1). Recordings in panels A1, B1, C1, and D1 are Kv currents in control, and those in A2, B2, C2, and D2 are the currents in the presence of 50 μM quinidine, 5 mM 4-AP, 50 μM flecainine, and 5 mM TEA, respectively. The current components susceptible to blockers are derived by digital subtraction of currents in the presence from those in the absence of drugs, and are shown in the bottom row. Thus, A1–A2, B1–B2, C1–C2, and D1–D2 demonstrate, respectively, the quinidine-, 4-AP-, flecainine- and TEA-sensitive Kv components.

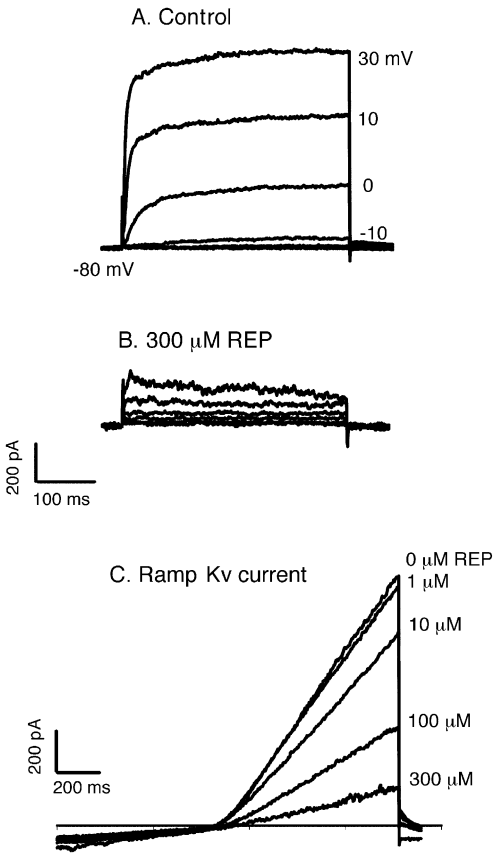

Fig. 3. Typical records demonstrating that Kv currents in rat pancreatic β-cells were blocked by repaglinide. A and B show, respectively, the Kv current traces elicited by depolarizing steps to −10, 0, +10, and +30 mV from a holding potential of −60 mV in control (A) and in the presence of 300 μM repaglinide (REP, B). C shows Kv currents elicited by a voltage ramp (from −80 to +40 mV from a holding potential of −60 mV) in control and in the presence of 1, 10, 100, and 300 μM REP, as indicated in the figure. Straight line indicates the zero current level. The amplitude of currents at +20 mV was measured as an index to performed quantitative analysis. All experiments were performed in 5 mM glucose.

(3–300 nM), and repaglinide (1–300 nM) on K_{ATP} currents were examined in rat pancreatic β-cells at a physiological glucose level of 5 mM. The results showed that all agents inhibited K_{ATP} currents in a concentration-dependent manner with a respective IC_{50} of 7.4 ± 0.2 μM, 16.6 ± 0.3 nM, and 5.0 ± 1.4 nM for nateglinide, glyburide, and repaglinide, indicating a rank order of potency as repaglinide > glyburide > nateglinide.

When the activities of the K_{ATP} channels and the Ca^{2+}-activated K^+ channels were physiologically blocked as described in Section 2, the remaining outward currents in pancreatic β-cells were predominantly composed of current through the delayed rectifier Kv channels. The notion was evidenced by the observations that: (1) the recorded outward Kv currents were little affected by nifedipine-induced inhibition of Ca^{2+} entry or omission of extracellular Ca^{2+} (results not shown); and (2) the currents were sensitive to several typical Kv channel blockers like quinidine, 4-AP, flecainine, and tetraethylammonium (Fig. 2), but were unresponsive to charybdotoxin and margatoxin up to 500 nM or apamin up to 10 μM (data not shown). These data indicate that under the experimental condition, the current activated between −80 and +40 mV with an activation threshold around −20 mV was Kv current by nature.

To study the effects of antidiabetic agents on Kv currents, the ramp voltage protocol was used (also ranging between −80 and +40 mV from a holding potential of −60 mV to correlate to the step voltage protocol), which measured the instantaneous current–voltage relationship of the Kv current. Nateglinide was found to inhibit Kv current in a concentration-dependent fashion though with a threshold concentration around 100 μM, considerably high than that for closing K_{ATP} channel (about 1 μM, Hu et al., 2000). It was not possible to test nateglinide at concentrations higher than 1 mM due to poor solubility. Parallel studies with glyburide and repaglinide showed that both agents produced suppression of Kv currents in a manner similar to that of nateglinide. Fig. 3 shows typical records

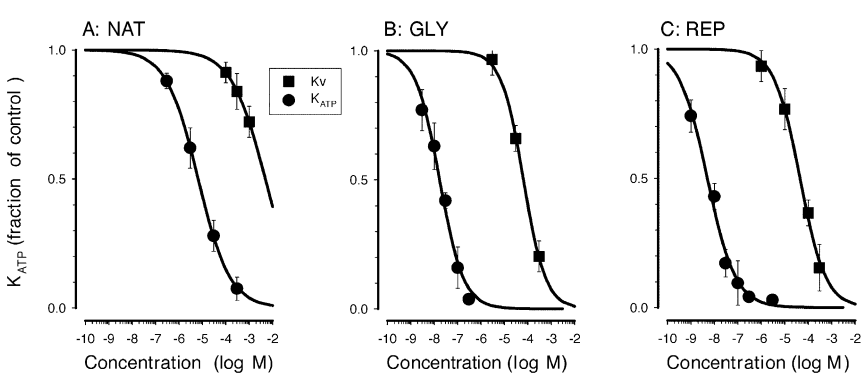

Fig. 4. Paired concentration–response curves for the inhibition of K_{ATP} and Kv currents by nateglinide (NAT, A), glyburide (GLY, B), and repaglinide (REP, C). Data of K_{ATP} currents derived from our earlier study (Hu et al., 2000) and Kv currents are represented, respectively, by circles and squares. Points are the mean of five to seven experiments. The ordinates are the remaining K_{ATP} or Kv currents in the presence of drugs as fraction of the control; and the abscissas indicate the concentration of drugs (M) in a logarithmic scale. The slope coefficients for K_{ATP} and Kv were, respectively, 0.7 and 0.6 for nateglinide (A), 0.9 and 0.9 for glyburide (B), and 0.7 and 0.8 for repaglinide (C).

Table 2
IC_{50}s of inhibition of K_{ATP} and Kv currents (μM)

Compound	K_{ATP}	Kv	IC_{50} ratio (Kv/K_{ATP})
Nateglinide	7.4 ± 0.2	4800 ± 300[a]	~650
Glyburide	0.0166 ± 0.0003	64.9 ± 6.5 μM[a]	~4000
Repaglinide	0.005 ± 0.001	44.7 ± 4.8 μM[a]	~9000

[a] $p < 0.005$, significantly different from the IC_{50} values with K_{ATP} channels.

of repaglinide-induced inhibition of Kv currents. The inhibition of Kv currents was demonstrated in response to both step (Fig. 3A and B) and ramp voltage pulses (Fig. 3C), and was obviously concentration dependent. The concentration–response curves for blocking Kv currents by test hypoglycemic agents were formed and shown in pair with the corresponding concentration–response curves for K_{ATP} currents in Fig. 4 (for data with K_{ATP} currents, see Fig. 5 of Hu et al., 2000). The IC_{50}s of nateglinide, glyburide, and repaglinide to block Kv currents were, respectively, 4.8 ± 0.3 mM, 64.9 ± 6.5 μM, and 44.7 ± 4.8 μM. For better comparison, the IC_{50}s of all three test agents on K_{ATP} and Kv currents are summarized in Table 2, in which a separation of the potencies (two to three orders in magnitude) between the effect on K_{ATP} and Kv currents is demonstrated.

3.2. Stimulation of voltage-dependent Ca^{2+} currents by nateglinide, glyburide, and repaglinide

When the membrane potential was held at −60 mV, depolarizing voltage steps elicited an inward Ca^{2+} current, which showed a fast activation followed by a very slow inactivation (top row of Fig. 5A–C). The maximal value of the Ca^{2+} current–voltage relationship was reduced by about 75% in the presence of 10 μM nifedipine (data not shown), a pharmacological characteristic typical of the voltage-dependent L-type Ca^{2+} channels. Nateglinide, glyburide, and repaglinide were all found to marginally stimulate Ca^{2+} currents by increasing its amplitude without altering current kinetics. Having a rapid onset, the effect was obviously direct and not a consequence of blockage of K_{ATP} channels by these agents, especially when K_{ATP} channels were largely blocked by intracellular dialysis of 5 mM ATP under our experimental conditions. The records in Fig. 5A and B show that the magnitude of activation by nateglinide at 3 μM (~0.5× of its IC_{50} for blockade of K_{ATP}) was comparable to that by glyburide at 100 nM (~6× of its IC_{50} for blockade of K_{ATP}). Repaglinide was extremely potent in activating Ca^{2+} current with a threshold concentration below 300 pM that was less than 1/10 of its IC_{50} for blockade of K_{ATP} channel (5 nM). The enhancement of Ca^{2+} current was also visible in the current–voltage relationship shown in the bottom row of Fig. 5. A surprising observation was that repaglinide at concentration equal or higher than 300 nM (60× of its IC_{50} for blockade of K_{ATP} channels) almost completely blocked Ca^{2+} current (data not shown). Thus, repaglinide produced a concentration-related dual action on Ca^{2+} current in rat β-cells. The inhibitory effect might, to some extent, compromise the ability of repaglinide to increase $[Ca^{2+}]_i$ via closure of K_{ATP} channel. Such a depression of Ca^{2+} current has not been observed with nateglinide and glyburide, though the augmentation of Ca^{2+} current by these two agents tended to saturate at concentration 50×–100× of their respective IC_{50}s for blockade of K_{ATP} channels.

4. Discussion

The insulin-secreting β-cells of the pancreatic islets of Langerhans are electrically excitable and changes in the

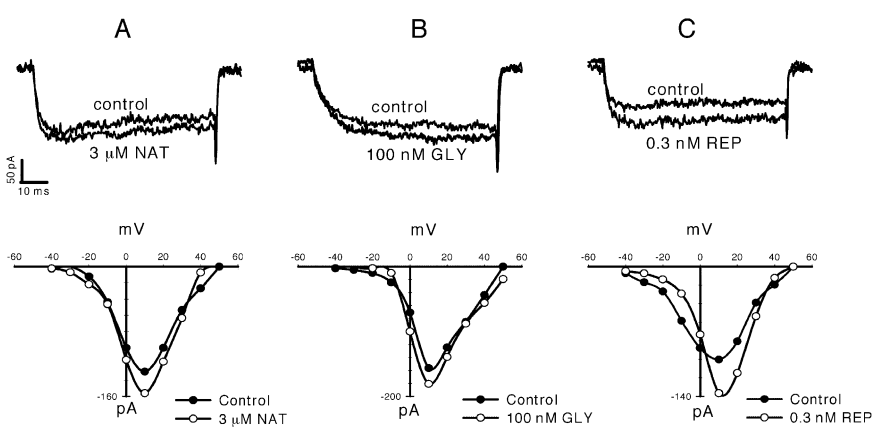

Fig. 5. Stimulation of voltage-dependent Ca^{2+} current by nateglinide (column A), glyburide (column B), and repaglinide (column C). In all columns, the upper panels show the original records of Ca^{2+} currents at +10 mV in control and in the presence of 3 μM nateglinide (A), 100 nM glyburide (B), and 0.3 nM repaglinide (C); whereas the lower panels show the respective current–voltage (*I–V*) relationship in the absence and presence of drugs. In all *I–V* curves, the ordinates give the amplitude of peak Ca^{2+} currents (pA) and the abscissas denote the membrane potentials at which the currents were measured.

membrane potential play an important role in coupling the metabolism of glucose to the discharge of the insulin-containing granule. The regulation of insulin secretion from pancreatic β-cells is a highly integrated process involving several plasma membrane ion channels such as K_{ATP} channels and voltage-gated Kv and Ca^{2+} channels. K_{ATP} channel, being functional at rest, is responsible for setting resting membrane potential, and its role in the initiation of insulin secretion has been convincingly established (Ashcroft, 1988; Dunne and Petersen, 1991). Conversely, the Ca^{2+} and Kv channels are not functional in β-cells at rest, but are activated only when the cell membrane is depolarized. Once being functional, these channels act in concert to shape the electrical spikes/Ca^{2+} action potential, which are associated in some way with the pulsatile insulin secretion (Henquin and Meissner, 1984; Dunne and Petersen, 1991; Dunne et al., 1994).

This study has profiled, for the first time, the independent actions of novel antidiabetic agent nateglinide and two comparators glyburide and repaglinide on voltage-gated Kv and Ca^{2+} channels, though all agents have been known to act primarily on K_{ATP} channels. In the study, the currents composed of each channel type were physiologically and/or pharmacologically isolated from other ionic currents to allow the observation of direct effect of test drugs. The results demonstrated that these drugs were capable of directly affecting not only K_{ATP} channels (Hu et al., 1999, 2000), but also voltage-gated Ca^{2+} and Kv channels.

The importance of voltage-dependent Ca^{2+} channel in the process of insulin secretion in β-cells has been well documented (Ashcroft and Rorsman, 1989; Misler et al., 1992). The activation of the channel is usually triggered by the closure of K_{ATP} channels and subsequent membrane depolarization. It is mainly responsible for the generation and maintenance of slow-wave electrical activity that is closely associated to pulsatile secretion (Dunne et al., 1994). Direct stimulation of the Ca^{2+} channel by its agonist BAY K 8644 has been reported to markedly increase insulin secretory response to glucose in the isolated perfused pancreas (Iwashima et al., 1993). In this study, the observed weak stimulatory effect on Ca^{2+} channels by nateglinide, glyburide, and repaglinide might, to some extent, contribute to the Ca^{2+}-induced exocytosis because (1) a marginal activation of Ca^{2+} influx through Ca^{2+} channels could significantly increase $[Ca^{2+}]_i$ due to large Ca^{2+} gradient across cell membrane (extracellular 10^{-3} M vs. intracellular 10^{-8}–10^{-7} M); and (2) the effect occurred in a concentration range comparable to those for blocking K_{ATP} channels. Our results contradicted to those reported by Fuhlendorff et al. (1998) who found that repaglinide did not change Ca^{2+} current in voltage-clamped β-cells. Moreover, repaglinide at 300 nM (only 60 × of its IC_{50} for K_{ATP} blockade) or higher was found to drastically block Ca^{2+} current, an effect that might counteract its ability to increase $[Ca^{2+}]_i$ via closure of K_{ATP} channels and thereby compromising the insulinotropic action at higher concentration of the drug. Although the mechanism for such a dual effect of repaglinide on Ca^{2+} channels remains unclear at present, it is not uncommon that a Ca^{2+} channel modulator exerts complex influence on Ca^{2+} current. Bay K 8644, for example, was also found to exert a dual effect on Ca^{2+} influx (Barger, 1999).

The physiological role of the Kv channel in pancreatic β-cells is to restore cell membrane to a hyperpolarized state after a Ca^{2+} action potential. It thus serves as a determinant of the frequency and duration of Ca^{2+}-dependent action potential in β-cells (Satin et al., 1989; Bokvist et al., 1990). Enhanced level of Kv channel activity has been reported to impair insulin secretion though excessively shortening of action potential duration (Philipson et al., 1994). The opposite was also true, as evidenced by the observation that blockade of Kv channel by tetraethylammonium induced a drastic increase in synchronous oscillations of $[Ca^{2+}]_i$ and membrane potential in insulinoma cells, resulting in a pronounced potentiation of insulin secretion (Roe et al., 1996). Although nateglinide, glyburide, and repaglinide were all found in the present study to be able to inhibit Kv channels in a concentration-dependent manner, this effect is unlikely to contribute significantly to the therapeutic action of these drugs due to the irrelevance of the effective concentrations (two to three orders higher in magnitude compared to those in blocking K_{ATP} channels). In line with our data, glyburide has been reported to inhibit 4-AP-sensitive delayed rectifier in human cardiac myocytes (Schaffer et al., 1999). The data of IC_{50} ratio (Kv/K_{ATP}) shown in Table 2 suggest that the apparent specificity for ion channel type differs among antidiabetic agents tested. Repaglinide appears highly selective for the K_{ATP} channels, while nateglinide in the therapeutic concentration range might act on K_{ATP} and, to a lesser extent, Kv channels. This additional mechanism of action of nateglinide may be linked to its glucose-dependent insulinotropic action observed in our in vitro studies (Hu et al., in press), in which we showed that stimulation of insulin release by nateglinide but not glyburide or repaglinide was enhanced at higher glucose. It is possible that the greater membrane depolarization induced by elevated glucose would promote the activation of Kv channels, whose blockade by nateglinide could be supplement to the blockade of K_{ATP} channels leading to enhanced insulinotropic action.

The finding of this study revisited the notion that specific Kv channel blockade could be a potential approach to promote insulin secretion. Information relevant to this notion has been accumulated from a number of earlier works: (1) the expression of Kv1- and Kv2-related transcripts has been identified in rodent pancreatic β-cells (Betsholtz et al., 1990; Roe et al., 1996; Kalman et al., 1998), and the heteromultimer composed of these subunits may constitute the native Kv channels; (2) Kv channel (but not large-conductance Ca^{2+}-activated K^+ channel) is the

only type of K^+ channel observed to be associated with spike action potential (Smith et al., 1990), though a slow and apamin/charybdotoxin-insensitive Ca^{2+}-activated K^+ channel has recently been shown to contribute to rhythmic firing of action potential (Gopel et al., 1999); (3) Kv1.5 overexpression in transgenic islets and β-cells was reported to be associated with impaired glucose responsiveness (Philipson et al., 1994); and (4) the dose–response relationship of insulin secretion stimulated by TEA and 4-AP (Kv blockers) correlates directly with the block of Kv currents (Roe et al., 1996). In addition, blockade of Kv channels is less likely to cause basal hypoglycemia, since Kv channel is not operational at rest.

In conclusion, the antidiabetic agents nateglinide, glyburide, and repaglinide all exert direct effects on three prominent ion channels, K_{ATP}, Kv, and Ca^{2+} channels, in adult rat β-cells. Although the effective concentration range or the magnitude of action precluded the latter two types of ion channels from being the primary targets for the antidiabetic action of these agents, the effects of these drugs on Kv and Ca^{2+} channels may contribute, under certain conditions, to the regulation of Ca^{2+}-dependent exocytosis.

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