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Interaction of nateglinide with K_{ATP} channel in β-cells underlies its unique insulinotropic action

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Abstract

Nateglinide is a novel insulinotropic agent for the treatment of type 2 diabetes. It is a D-phenylalanine derivative, chemically distinct from repaglinide and sulphonylureas (glyburide or glimepiride). Although each agent is known to stimulate insulin release via the signaling cascade initiated by closure of ATP-dependent K^+ (K_{ATP}) channels in pancreatic β-cells, the pharmacological effect of nateglinide is reportedly fast-acting, short-lasting, sensitive to ambient glucose and more resistant to metabolic inhibition. The aim of the present study was to elucidate the molecular mechanism(s) underlying the distinct properties of the insulinotropic action of nateglinide. By using the patch-clamp methods, we comparatively characterized the potency and kinetics of the effect of these agents on K_{ATP} channels in rat β-cells at normal vs. elevated glucose and under physiological condition vs. experimentally induced metabolic inhibition. Our results demonstrated that the mode of the action of nateglinide on K_{ATP} current was unique in (a) glucose dependency; (b) increased potency and efficacy under ATP depletion and uncoupling of mitochondrial oxidative phosphorylation than physiological condition; (c) substantially more rapid onset and offset kinetics. The data provide mechanistic rationale for the unique in vivo and ex vivo activity profile of nateglinide and may contribute to reduced hypoglycemic potential associated with excessive insulin secretion.

Keywords: Nateglinide; Pancreatic β-cell, rat; K_{ATP} channel; Glucose sensitivity; Metabolic inhibition

1. Introduction

The maintenance of homeostatic blood glucose concentration is an integrated process predominantly regulated by the antihyperglycemic hormone insulin. Glucose is taken up into the β-cell and metabolized via glycolysis, mitochondrial respiration and oxidative phosphorylation to produce ATP that, by inhibiting K_{ATP} channels, causes cell depolarization and Ca^{2+} influx through voltage-dependent Ca^{2+} channels. These events lead to an increase in intracellular Ca^{2+} and subsequent exocytosis to release insulin (Dunne and Petersen, 1991). Defects in glycolysis or mitochondrial function, which were reported to be characteristically associated with and may contribute to β-cell dysfunction in type 2 diabetes, are expected to jeopardize glucose handling and ATP synthesis resulting in a reduction of insulin release in response to physiological stimuli (Portha et al., 1988; Giroix et al., 1993; Efendic et al., 1994; Fernandez-Alvarez et al., 1994; Hughes et al., 1998). To promote insulin secretion under diseased state, agents that act to block K_{ATP} channels in pancreatic β-cells independently of glucose level can induce insulin secretion and, hence, serve as antidiabetic drugs.

Representatives of this class of antidiabetic drugs are sulfonylureas such as glyburide and glimepiride (Amaryl), the benzoic acid derivative repaglinide (NovoNorm) and the recently marketed novel D-phenylalanine derivative nateglinide (also known as A-4166). Although each agent has been shown to bind to the sulfonylurea receptor or to displace labeled sulfonylureas and, in turn, close K_{ATP} channels (Akiyoshi et al., 1995; Malaisse, 1995; Gromada et al., 1995; Fujita et al., 1996; Ikenoue et al., 1997; Fuhlendorff et al., 1998), the pharmacological action of nateglinide appeared to differ from that of other agents in several aspects: (1) a preferential first phase insulin release effect due to rapid onset (De Souza et al., 2001a,b); (2) no sustained hypoglycemia and reduced total insulin secretion due to its short duration of action (Sato et al., 1991; Kikuchi, 1996; Ikenoue et al., 1997; De Souza et al., 2001a,b; Keilson et al., 2000); (3) enhanced activity under hyperglycemic conditions due to glucose-sensitive action (Sato et al., 1991; Akiyoshi et al.,

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1995; Seto et al., 1995; Kikuchi, 1996; Ikenoue et al., 1997; Tsukuda et al., 1998); (4) resistance to metabolic suppression compared to sulfonylureas (Fujitani et al., 1997). In contrast, glyburide and repaglinide cause long-lasting hypoglycemic action under both normoglycemic and hyperglycemic conditions in animal models (Mark and Grell, 1997). Besides, sulfonylureas displayed a marked attenuation in the ability to block K_{ATP} channels in cardiac myocytes (Findlay, 1993) and rat β-cells (Mukai et al., 1998) during induced metabolic stress.

In light of our recent work showing that nateglinide acts on sulfonylurea receptor I and K_{ATP} channels in insulin-secreting cells (Hu et al., 2000), the present studies further elucidate the cellular mechanism(s) underlying the differential profile of in vivo insulinotropic actions of nateglinide and its comparators glyburide, glimepiride and repaglinide. We have comparatively characterized the effects of these agents on K_{ATP} channels in rat pancreatic β-cells with respect to the rank order of potency, the kinetics of actions, the glucose sensitivity of the actions, and the effectiveness of drugs during induced metabolic impairment. Our results have clearly distinguished nateglinide from sulfonylureas and repaglinide at the level of molecular interaction with K_{ATP} channels and, hence, for the first time, provide mechanistic explanation for the uniqueness of in vivo insulinotropic action of nateglinide.

2. Materials and methods

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

Isolation procedure was described previously (Hu et al., 2000). Briefly, islets of Langerhans were isolated from pancreas Male Sprague–Dawley rats (250–275 g) by liberase digestion (0.5 mg/ml, Boehringer Mannheim, Germany) followed by a Ficoll gradient centrifugation. The islets were then dissociated into single cells by 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 isolated β-cells were seeded in CMRL (Connaught Medical Research Laboratory) medium (Gibco, Gaithersburg, MD) supplemented with 1% fetal calf serum, 1% antibiotic–antimycotic, 10 mM glucose, and incubated at 37 °C in an atmosphere of 95% air and 5% CO_2 for 2–5 days before electrophysiological recordings. All operations were performed at room temperature (~ 22 °C) except for β-cell culture.

2.2. Electrophysiological recording of whole-cell K_{ATP} current

The K_{ATP} currents were recorded at 22 °C using the whole-cell configuration of the patch-clamp technique in the primary culture of rat pancreatic β-cells. The currents were elicited by a voltage ramp ranging from −120 to +40 mV over a 1.5-s period from a holding potential of −60 mV. At very negative voltages where the voltage-dependent K^+ channels and Ca^{2+}-activated K^+ channels were all inactivated, the remaining current was mainly composed of the voltage-independent K_{ATP} current. The inward K_{ATP} current was further magnified by using high K^+ (140 mM) symmetrical bath and pipette solutions to shift the K^+ reversal potential from the conventional −80 mV to 0 mV. Thus, the K_{ATP} currents at negative potentials could be measured in the absence of interference of any other ion current components. As the basal level of K_{ATP} currents in β-cells is usually low, diazoxide (100 μM), a known effective opener of the K_{ATP} channels in insulin-secreting cells, was applied to enhance K_{ATP} currents prior to the addition of channel blockers. The effect of drugs, each applied at five concentrations in an ascending order, under conditions mimic normoglycemia (5 mM extracellular glucose, G5) or hyperglycemia (G16) was recorded. At each concentration, approximately 20 min were allowed for a full development of inhibitory effect. The K_{ATP} currents at −90 mV was used as an index for quantitative evaluation.

In the study on the time course of drug effect on K_{ATP} currents, the ramp voltage protocol was given to the cells under investigation every 30 s repeatedly and the current amplitude was measured whenever the pulse was given until maximal drug effects were achieved.

The effectiveness of antidiabetic agents was also examined on β-cells under metabolic inhibition by extracellular treatment with dinitrophenol, an uncoupler of mitochondrial oxidative phosphorylation, and intracellular dialysis of 1 mM ATP to mimic the condition of ATP depletion. The physiological ATP concentration in β-cells is within the range of 4–6 mM (Petersen and Findlay, 1987). The bath solution throughout the study was composed of (mM) NaCl 5, KCl 140, $MgCl_2$ 2, HEPES 10, $CaCl_2$ 2, glucose 5; pH 7.4; and the pipette solution had (mM) NaCl 5, KCl, 140, $MgCl_2$ 2, HEPES 10, $CaCl_2$ 0.1, EGTA 0.6, Na_2UDP 2, K_2ATP 5 (in normal condition) or 1 (with metabolic inhibition), pH 7.4.

Experiments were performed at a 600× magnification under a Nikon inverted microscope, and cells with a diameter larger than 10 μm and well preserved granulation were used for the current recording, because β-cells were reportedly two- to threefold larger than α-cells (Pipeleers et al., 1985). The capacitance of β-cells was 12.1 ± 0.5 pF ($n = 35$). Currents recorded were amplified by a List EPC-7 amplifier (Adams and 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 analysis with software pClamp version 6.03 (Axon Instruments).

2.3. Data analysis

Drug effects were indexed with the ratio of the K_{ATP} currents (at −90 mV) posttreatment with drugs to those

pretreatment in the same β-cells. These values were used to construct concentration–response, fit with the three-parameter logistic equations, $y = a/[1+(X/X_o)^b]$. Here, y denotes the amplitude of current in the presence of drugs normalized to the control value; X_o, a and b are, respectively, IC_{50} (half-maximal blockade), maximal current amplitude and Hill coefficient. Statistical significance of the data was determined with single-tailed t-test.

3. Results

3.1. Inhibition of K_{ATP} currents in β-cells by antidiabetic agents in normal and elevated glucose

The effects of antidiabetic agents nateglinide, glyburide and repaglinide (see Fig. 1 for structure) on K_{ATP} channel activity were examined in rat pancreatic β-cells at a physiological glucose level of 5 mM as well as at an elevated glucose level of 16 mM to mimic a hyperglycemic condition. All agents caused inhibition of diazoxide (100 μM) induced K_{ATP} currents in a concentration-dependent manner at normal and elevated glucose levels. For each drug at each glucose level, five to seven experiments were pooled and averaged to form the concentration–response curves shown in Fig. 2A (5 mM glucose) and Fig. 2B (16 mM glucose). The IC_{50}s for K_{ATP}-blocking effect of these agents were obtained by least-square fitting and summarized in Table 1. Although the rank order of potency in blocking K_{ATP} channels (repaglinide > glyburide > nateglinide) remained unchanged at both glucose levels, the IC_{50}s of nateglinide decreased by threefold from 5 to 16 mM glucose ($P = 0.05$), suggesting a favorable albeit weak tendency of glucose-sensitive action. Conversely, the IC_{50}s of glyburide and repaglinide were unchanged (statistically insignificant increase) between normal and hyperglycemic condition. The results were, thus, suggestive of glucose-responsive inhibition of K_{ATP} channels by nateglinide.

3.2. Inhibition of K_{ATP} currents by antidiabetic agents in β-cells under metabolic stress

To understand how antidiabetic drugs behave under impairment of mitochondrial function and/or ATP production, we have investigated the effects of these compounds on K_{ATP} current in β-cells under ATP depletion and dinitrophenol-induced uncoupling of oxidative phosphorylation. Fig. 4A shows typical recordings of the concentration-dependent inhibition of K_{ATP} currents by nateglinide in β-cells under experimentally induced metabolic stress. Nateglinide produced a complete inhibition of K_{ATP} current at concentration of 3 μM, compared to ~ 300 μM under normal conditions (data in Fig. 4 of Hu et al., 2000). Parallel studies with glyburide, repaglinide and glimepiride indicated that all agents blocked K_{ATP} currents in a concentration-dependent manner under metabolic suppression, as can be seen in the concentration–response curves in Fig. 2C (nateglinide/glyburide/repaglinide) and Fig. 3C (nateglinide/glimepiride). For comparison, the concentration–response curve of each agent in normal β-cells is displayed in Figs. 2A and 3A, respectively. The curves for glimepiride are shown separately in Fig. 3 because they largely overlapped with the curves for glyburide and repaglinide. It was obvious from Figs. 2 and 3 that nateglinide, normally several orders less potent than repaglinide, glyburide or glimepiride, became comparably potent under metabolic inhibition. The IC_{50}s of drugs under normal and diseased conditions shown in Table 2 demonstrated quantitatively that the potency of nateglinide and glimepiride to inhibit K_{ATP} channels increased by approximately 400- and 13-

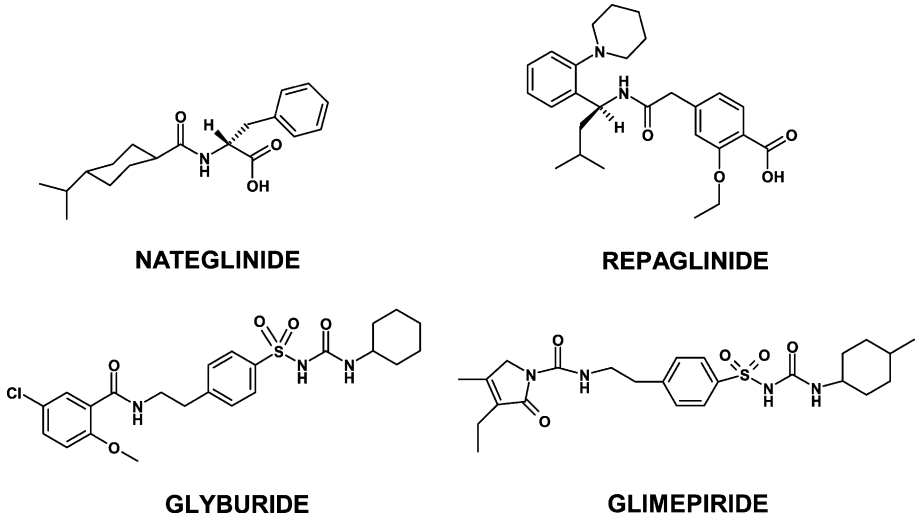

Fig. 1. Chemical structure of antidiabetic agents nateglinide, repaglinide, glyburide and glimepiride.

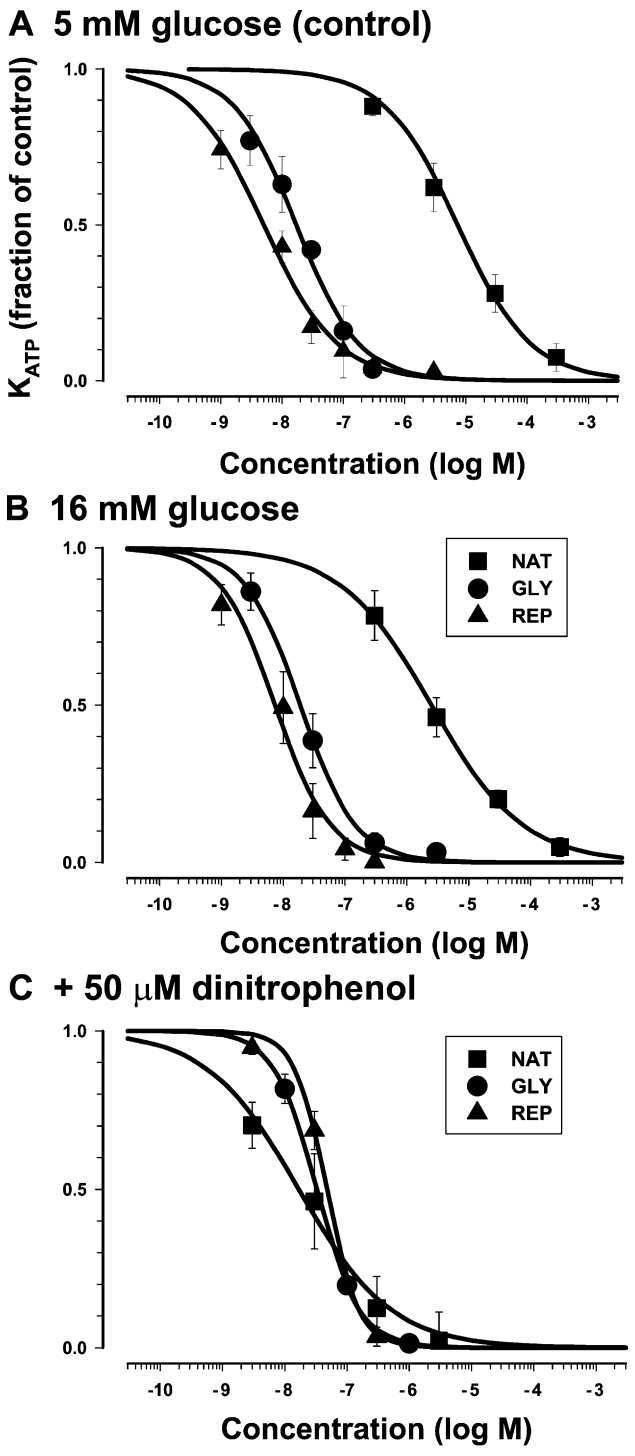

Fig. 2. Concentration–response curves for the inhibition of K_{ATP} currents by nateglinide (square), glyburide (circle) and repaglinide (triangle) in 5 mM (A, control) and 16 mM glucose (B) and in 5 mM glucose with metabolic suppression (C). Points are the amplitudes of K_{ATP} currents at −90 mV, $n=5–7$. The ordinates are K_{ATP} currents in the presence of drugs as fraction of the maximal values activated by 100 μM diazoxide in (A) and (B), or by metabolic suppression modeled with the application of 50 μM dinitrophenol plus intracellular dialysis of 1 mM ATP in (C). The abscissas indicate drug concentration (M) in a logarithmic scale. The slope coefficients for repaglinide, glyburide and nateglinide are 0.7, 0.9 and 0.7 in (A); 1.0, 1.0 and 0.6 in (B); and 1.7, 1.3 and 0.6 in (C).

Table 1
IC_{50}s of K_{ATP}-blocking effect by antidiabetic agents at 5 or 16 mM glucose

Drug	5 mM glucose (G5)	16 mM glucose (G16)	IC_{50} ratio (G5/G16)
Nateglinide	7.4 ± 0.2 μM	2.4 ± 0.2 μM	3.0 ($P=0.05$)
Glyburide	16.6 ± 0.3 nM	18.9 ± 1.6 nM	0.9 (NS)
Repaglinide	5.0 ± 1.4 nM	7.4 ± 2.5 nM	0.7 (NS)

NS, not statistically different between the data at G5 and G16.

fold, respectively, under metabolic stress, whereas the potency was unchanged with glyburide and decreased by 10-fold with repaglinide.

The profound change in the activity of antidiabetic drugs on K_{ATP} currents under metabolic inhibition was further ascertained in the following set of experiments, in which the effect on K_{ATP} currents by nateglinide and glyburide or nateglinide and repaglinide were investigated sequentially in

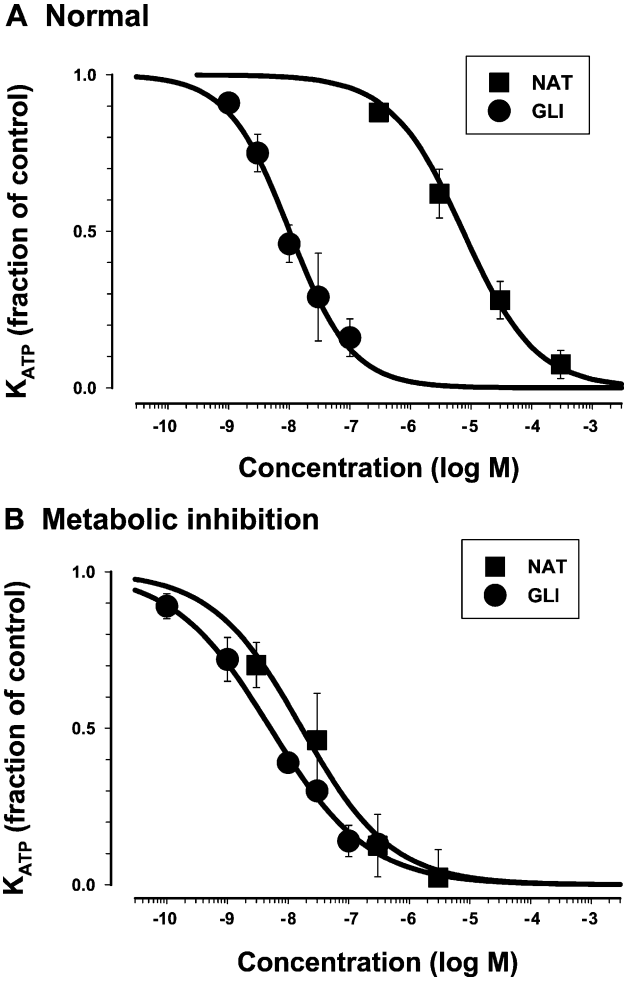

Fig. 3. Concentration–response curves of blockade of K_{ATP} current by nateglinide and glimepiride under normal condition (A) and metabolic inhibition (B). Points are mean current amplitudes in the presence of nateglinide (squares) or glimepiride (circles) as fractions of the maximal values activated by 100 μM diazoxide (A, $n=5–7$) or by 50 μM dinitrophenol in combination with intracellular ATP depletion (B, $n=4$). The slope coefficients are 0.7 and 0.9 in (A) and 0.6 and 0.7 in (B) with nateglinide and glimepiride, respectively.

Table 2
IC_{50}s of K_{ATP}-blocking effects under normal condition and metabolic inhibition

Drug	Normal (nM)	Metabolic inhibition (nM)	Ratio (Norm/MI)
Nateglinide	7400 ± 200	16.9 ± 5.2[a]	440
Glyburide	16.6 ± 0.3	32.8 ± 0.9	0.5
Glimepiride	6.3 ± 1.5	0.5 ± 0.2[b]	13
Repaglinide	5.0 ± 1.4	47.5 ± 8.4[b]	0.1

[a] $P < 0.001$ compared to the data under normal condition.
[b] $P < 0.05$ compared to the data under normal condition.

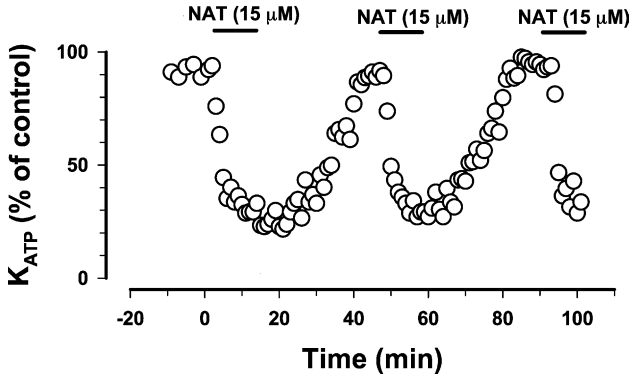

Fig. 5. Time course of the effect of repeated application of nateglinide (15 μM, ~ 2 × IC_{50} under normal condition) on K_{ATP} current. Points are the amplitudes of K_{ATP} current as percent of the maximal value activated by 100 μM diazoxide. [glucose] = 5 mM. Abscissas indicate the time (min) at which the current amplitude was measured. Duration of drug application is marked at the top. (1) Rapid onset and washout of the effect; (2) no desensitization observed.

a same cell to circumvent cell-to-cell variability. Fig. 4A shows typical recordings of dinitrophenol-induced K_{ATP} currents that were inhibited by nateglinide in a concentration-dependent manner. The efficacy and potency were substantially increased when compared with those under normal condition (see Fig. 4A in Hu et al., 2000). Fig. 4B and C show, respectively, typical recordings of K_{ATP} currents in the presence of nateglinide followed by glyburide in one cell and of nateglinide followed by repaglinide in another cell under metabolic impairment. In Fig. 4B, nateglinide at 3 μM (~ 1/2 of IC_{50} in normal condition), that would be weakly effective in blocking K_{ATP} current, became remarkably efficacious, whereas the effect of glyburide in the same cell was attenuated by metabolic inhibition such

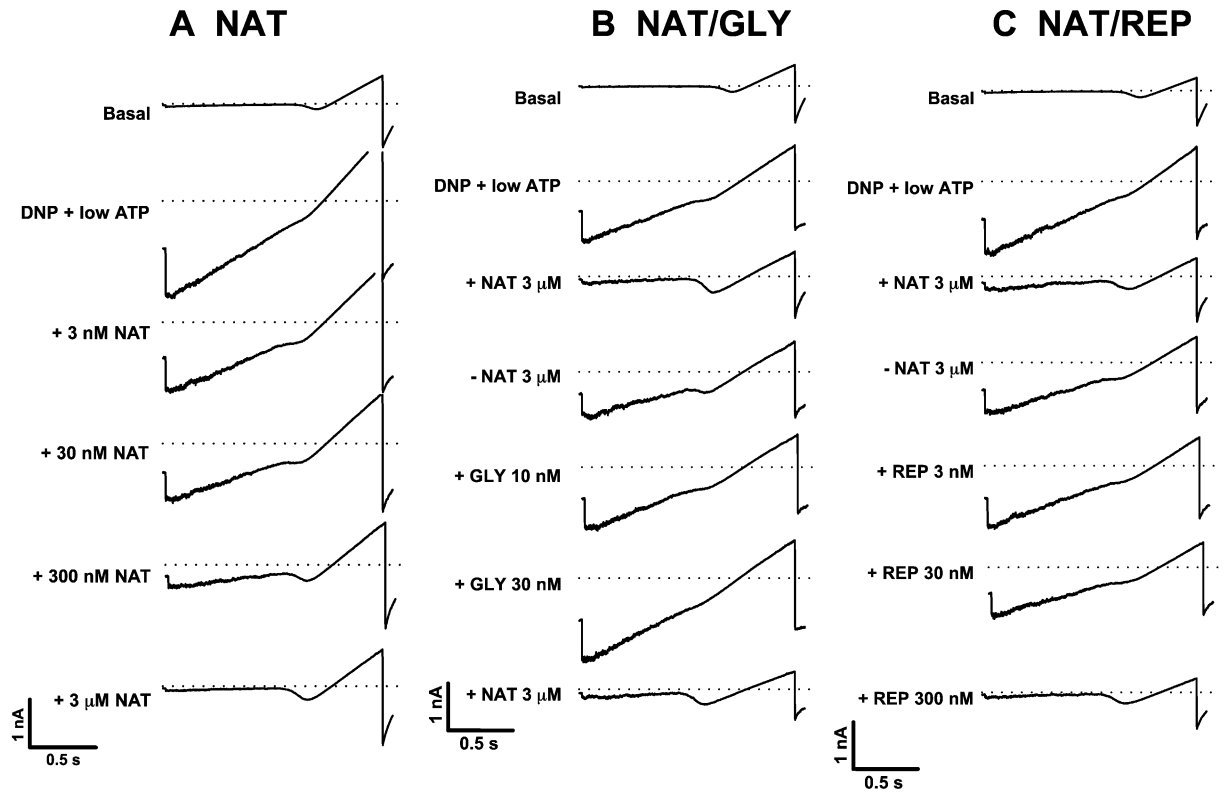

Fig. 4. (A) Concentration-dependent inhibition of metabolic stress-induced K_{ATP} currents by nateglinide. Representative recordings of K_{ATP} currents elicited by a voltage ramp (top to bottom) at basal (immediately after seal rupture), under metabolic stress induced by 1 mM intracellular ATP plus 50 μM dinitrophenol, in the presence of 3 nM, 30 nM, 300 nM and 3 μM nateglinide. (B) Glyburide failed to inhibit metabolic stress-induced K_{ATP} currents, but nateglinide was effective. Representative recordings of ramp K_{ATP} currents (top to bottom) at basal, under metabolic stress, in the presence of 3 μM nateglinide, removal of 3 μM nateglinide, in the presence of 10 and 30 nM glyburide and 3 μM nateglinide alone. (C) Repaglinide at 300 nM blocked metabolic stress-induced K_{ATP} currents to a similar extent as did 3 μM nateglinide while repaglinide was normally three orders of magnitude more potent than nateglinide. The K_{ATP} currents (top to bottom) at basal, under metabolic stress, in 3 μM nateglinide, removal of nateglinide and in 3, 30 and 300 nM repaglinide. Dotted lines indicate zero current levels. The currents at negative voltages are predominantly K_{ATP} currents. All experiments were performed in 5 mM glucose.

that it was inactive at 10 or 30 nM (~ 2 × normal IC_{50}). Subsequent application of 3 μM nateglinide almost completely blocked K_{ATP} currents. A complete blockade was not seen with glyburide until its concentration reached 300 nM (~ 20 × normal IC_{50}, data not shown). Likewise, repaglinide at 3 nM (~ 1/2 of normal IC_{50}) or 30 nM (6 × normal IC_{50}) was marginally effective in reducing K_{ATP} current (Fig. 4C). An inhibition similar to that produced by 3 μM nateglinide occurred with repaglinide at 300 nM (60 × normal IC_{50}). These experiments collectively reinforced the argument that nateglinide was remarkably resistant to metabolic inhibition.

3.3. Kinetics of K_{ATP} channel-blocking effect by antidiabetic agents

The time-course of the effect on K_{ATP} currents by 15 μM nateglinide (~ 2 × IC_{50}) is displayed in Fig. 5, in which nateglinide suppressed the current with a rapid onset and the action was readily reversed upon washout. In addition, repeated application of nateglinide did not lead to β-cell desensitization to subsequent application of the drug. In an earlier study, we have compared the time course of the K_{ATP} channel-blocking effect of nateglinide to those of glyburide and repaglinide at equipotent concentrations (Fig. 6 in Hu et al., 2000). Additional kinetic experiments of the effect

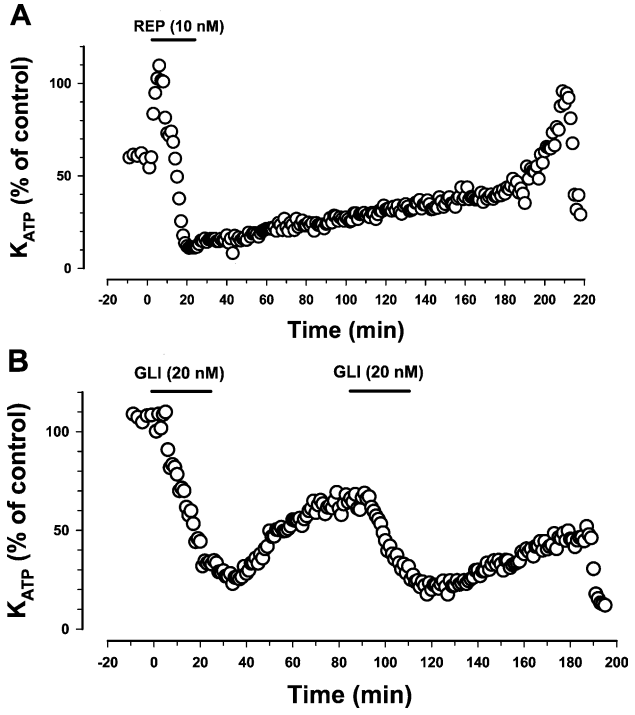

Fig. 6. Time course of the K_{ATP} channel-blocking effect by repaglinide (A) or glimepiride (B). Points denote amplitudes of K_{ATP} current as percent of the maximal values activated by 100 μM diazoxide. Abscissas indicate the time (min) at which the current amplitude was measured. Duration of drug application is marked at the top of the figures. Note the prolonged recovery of repaglinide effect (A) and the slow onset of glimepiride action (B).

Table 3
On- and off-kinetics of K_{ATP} channel blocking action

Drug	$T_{1/2}$on (min)	$T_{1/2}$off (min)
Nateglinide	4.1 ± 0.2	29.7 ± 4.2
Glyburide	4.2 ± 0.5	68.1 ± 4.0[a]
Glimepiride	22.3 ± 2.9[b]	62.2 ± 2.0[a]
Repaglinide	12.2 ± 1.8[b]	175.0 ± 1.0[b]

$T_{1/2}$on—time to a half-maximal inhibition. $T_{1/2}$off—time to a half-recovery from maximal inhibition.
[a] $P < 0.05$ compared to the data with nateglinide.
[b] $P < 0.001$ compared to the data with nateglinide.

of repaglinide (10 nM) and glimepiride (20 nM) on K_{ATP} currents were carried out in the present study. The action by repaglinide was consistently outlasted the duration of drug presence by several folds. In most cases, a complete recovery of repaglinide effect was not seen within 3 h (Fig. 6A). Fig. 6B shows the time course of the action of glimepiride on K_{ATP} currents. The effect had a slow onset and an incomplete recovery as opposed to nateglinide. A summary of the time to a half maximal inhibition ($T_{1/2}$on) and the time to a half recovery from maximal inhibition ($T_{1/2}$off) of all four drugs is given in Table 3.

4. Discussion

Nateglinide, a D-phenylalanine derivative, is a novel antidiabetic agent recently received marketing approval from over 50 countries around the world. Despite a common primary mechanism of action, nateglinide appears distinct from repaglinide, glyburide or glimepiride in preferential initial insulinotropic effect due to rapid onset; low incidence of hypoglycemia due to short duration of action; enhanced activity in hyperglycemia due to glucose-sensitive action; and resistance to metabolic inhibition (Akiyoshi et al., 1995; Seto et al., 1995; Hirose et al., 1995; Kikuchi, 1996; Ikenoue et al., 1997; De Souza et al., 2001a,b; Tsukuda et al., 1998). This study by comparatively characterizing the inhibitory effect of the antidiabetic drugs on K_{ATP} channels in rat β-cells demonstrated that nateglinide was mechanistically unique in several respects: (1) glucose sensitivity; (2) enhanced action under metabolic impairment; (3) rapid onset of action; (4) readiness of reversibility. In addition, our observation of lack of desensitization of β-cell K_{ATP} channels to repeated application of nateglinide is consistent with the results from in vitro (Lagmich et al., 1998, 1999) and in vivo (Akiyoshi et al., 1994; Fujitani et al., 1994) studies, which showed that nateglinide is superior to sulfonylurea or repaglinide in maintaining its insulinotropic efficacy following repeated treatment. Thus, the unique properties of nateglinide's insulinotropic action are attributable to basic characteristics in the interaction between nateglinide and its molecular target — K_{ATP} channels.

Our data of the effects of diabetic drugs on K_{ATP} channels and the rank order of potency under physiological condition are, in general, consistent with the reported results

on K_{ATP} channels in rat/mice β-cells or insulinoma cells (Zunkler et al., 1988; Sturgess et al., 1985; Panten et al., 1989; Gillis et al., 1989; Ashcroft and Rorsman, 1989; Schwanstecher et al., 1994; Akiyoshi et al., 1995; Gromada et al., 1995) and are also commensurate to those of in vitro stimulation of insulin release (Malaisse, 1995; Ikenoue et al., 1997). The observed glucose sensitization of nateglinide's action on K_{ATP} channels (decrease in IC_{50}) is in line with reported enhancement by nateglinide at elevated glucose of Ca^{2+} influx in rat β-cells (Fujitani and Yada, 1994), of in vitro insulin secretion from rat islets (Hu et al., 2001) and of in vivo insulin release in hamster pancreas (Seto et al., 1995). On the other hand, the glucose insensitivity of the action of glyburide or repaglinide on K_{ATP} channels is consistent with the reported in vivo results that glyburide and repaglinide cause long-lasting hypoglycemic action under both normoglycemic and hyperglycemic conditions in animal models (Mark and Grell, 1997).

Defects in glycolysis or mitochondrial function, which were reported to be characteristically associated with β-cell dysfunction in type 2 diabetes, are expected to jeopardize glucose handling and ATP synthesis resulting in a reduction of insulin release in response to physiological stimuli (Portha et al., 1988; Giroix et al., 1993; Efendic et al., 1994; Fernandez-Alvarez et al., 1994; Hughes et al., 1998). In this study, the application of dinitrophenol and dialysis of lower concentration of ATP would additively cause a decline of intracellular ATP concentration, thereby modulating the ATP-dependent cellular functions. Under these circumstances, the ability to block K_{ATP} channels by all drugs tested was altered one way or the other, suggesting the involvement of an ATP-dependent process in the activities of these drugs. Nateglinide, a relatively less potent insulinotropic agent in β-cells under physiological condition, became two orders of magnitude more potent in blocking K_{ATP} channels under metabolic stress. With an identical transformation of conditions, the potency of glyburide and repaglinide reduced by a respective 2- and 10-fold. These findings are in line with earlier results demonstrating that nateglinide acted more effectively than sulfonylureas to increase $[Ca^{2+}]_i$ in β-cells during metabolic inhibition (Fujitani et al., 1997). The enhanced efficacy of nateglinide to inhibit K_{ATP} channels in diseased state adds to the list of properties of this agent that distinguish it from other sulfonylurea receptor ligands, and may bear important clinical significance.

The cellular mechanism(s) responsible for the increase in effectiveness of nateglinide and glimepiride but not glyburide and repaglinide under metabolic stress are presently unclear, since these agents are known to bind to the same sulfonylurea receptor (Hu et al., 2000; Sunaga et al., 2001). Our observations may suggest that nateglinide binds to the sulfonylurea receptor but at a molecular site distinct from those for glyburide and repaglinide. The site for nateglinide might be functionally less susceptible to ATP depletion or, alternatively, the binding affinity for nateglinide to the sulfonylurea receptor might be enhanced at lower ATP concentration due to retarded dissociation of nateglinide, which appeared extremely rapid under normal condition (Hu et al., 2000). In this context, a common sulfonylurea receptor with distinct sites for glyburide and repaglinide have been suggested to be responsible for the common and differential insulin-stimulating processes by these two compounds (Fuhlendorff et al., 1998).

Being both sulfonylureas, glimepiride (IC_{50} of 6.3 nM) that was marginally more potent than glyburide (IC_{50} of 16.6 nM) under physiological condition, became 60 times more potent than glyburide (0.5 nM vs. 32.8 nM) under metabolic suppression. An interpretation, which may reconcile the dispute, stemmed from the converging evidence showing that glimepiride and glyburide bind, respectively, to a 65- and 140-kDa sulfonylurea binding protein, each regulating the open/closed state of a common pore-forming Kir6.2 subunit by allosteric interaction (Kramer et al., 1994, 1996). It is speculated that the binding of glimepiride to the 65-kDa subunit and of glyburide to the 140-kDa protein might have differential sensitivity to metabolic inhibition/ATP depletion at either the binding site or phosphorylation.

It seems to be contradictory that the efficacy of nateglinide to inhibit K_{ATP} channels increases at ATP depletion under metabolic stress as well as at hyperglycemia that presumably results in an increase in ATP, though the magnitude of changes in efficacy is substantially different under these two conditions. Such an inconsistency might be accounted for by several possibilities. Firstly, metabolic inhibition activates (via dephosphorylation) either an additional type of K_{ATP} channel or a part of K_{ATP} channel, which is present in a conformational state under normal conditions. These induced channels may be sulfonylurea-insensitive but nateglinide-sensitive, whereas the K_{ATP} channels regulated by glucose are present in a functional state and highly sulfonylurea-sensitive. The sulfonylurea-sensitive and -insensitive components of K_{ATP} current components under metabolic inhibition have been previously studied in more detail (Krause et al., 1995). Secondly, some unknown proteolytic substance(s) generated under metabolic inhibition may enhance the sulfonylurea receptor binding to nateglinide and/or the coupling between the sulfonylurea receptor and Kir6.2 (Mukai et al., 1998; Inagaki et al., 1995; Deutsch and Weiss, 1993). Thirdly, acute inhibition of mitochondrial function by dinitrophenol fails to deplete ATP to the extent at which K_{ATP} channels are so drastically activated. Rather, dinitrophenol directly interacts with a distinct nucleotide binding site to stimulate K_{ATP} current that is nateglinide-sensitive but glyburide-insensitive. It is conceivable to infer that in pancreatic β-cells, hyperglycemia and metabolic stress involve different action site and/or receptor/effector pathways. In fact, the amplitude of K_{ATP} current induced by metabolic inhibition is substantially greater (>twofold) than that by zero glucose (our unpublished observation) or by K_{ATP} channels openers (Krause et al., 1995). The differential behavior of nateglinide vis à vis

glyburide or repaglinide under metabolic stress and hyperglycemia may again suggest nateglinide binding to the sulfonylurea receptor at a distinct molecular site from those for glyburide and repaglinide.

The time course of in vivo hypoglycemic effect of an antidiabetic agent depends on the intrinsic characteristics of the mechanism of action as well as its pharmacokinetic profile including absorption, distribution and elimination. When administered orally prior to meal in human subjects, neither the time to reach peak plasma concentration with nateglinide (18 min) and repaglinide (34 min) nor the rate of elimination of nateglinide ($T_{1/2}$ = 39 min) and repaglinide ($T_{1/2}$ = 59 min) differed drastically from each other (Kikuchi, 1996). However, the inhibition of nateglinide on K_{ATP} channels was fast-acting with a $T_{1/2}$on of 4.1 min, in contrast to 12.2 and 22.3 min with repaglinide and glimepiride, respectively. Consistent with our findings, earlier studies showed a slow development of K_{ATP} channel blockade by second generation sulfonylureas in rat β-cells (Zunkler et al., 1988) and a kinetically slow secretory responses to glimepiride ($T_{1/2}$on ~ 60 min) in isolated pancreatic islets of mice (Schwanstecher et al., 1994). These results collectively suggest that the difference in in vivo onset of action reflected, to a greater extent, the difference in the molecular interaction between ligands and sulfonylurea/K_{ATP} channels. However, pharmacokinetic factors can not be ruled out to reconcile the discrepancy in time course of hypoglycemic action. For instance, nateglinide had a $T_{1/2}$on value of 4.1 min similar to that of glyburide (4.2 min, Table 3) but exhibited a considerably earlier insulinotropic action in vivo than glyburide (Ikenoue et al., 1997).

While the $T_{1/2}$off values of two sulfonylureas, glyburide (68.1 min) and glimepiride (62.2 min) were comparable, the values of sulfonylureas and repaglinide (175.0 min) were about two- and five-fold that of nateglinide (29.7 min), indicating that a majority of K_{ATP} channels remained at a closed state long after the sulfonylureas and repaglinide were removed. Such a mechanism-based slow recovery of the action would be a crucial element that contributes to the clinically observed long lasting hypoglycemic action of these drugs (Draeger, 1995; Rosskamp et al., 1996). In contrast, the rapid-acting and short-lasting effect of nateglinide would be beneficial in curbing the exaggerated meal-induced glucose excursion without excessive insulin release while preventing delayed hypoglycemic episodes.

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