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Nateglinide, a D-phenylalanine derivative lacking either a sulfonylurea or benzamido moiety, specifically inhibits pancreatic β-cell-type K_{ATP} channels.

Motohiko Chachin, Mitsuhiko Yamada Akikazu Fujita, Tetsuro Matsuoka, Kenji Matsushita and Yoshihisa Kurachi

Department of Pharmacology II, Faculty of Medicine & Graduate School of Medicine, Osaka University (M.C, M.Y., T.M., K.M., Y.K.): and Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Science, Osaka Prefecture University (A.F.);

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a) Nateglinide selectively inhibits pancreatic K_{ATP} channel

b) Dr. Yoshihisa Kurachi, Department of Pharmacology II, Faculty of Medicine & Graduate School of Medicine, Osaka University 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan.

Tel . : 81-6-879-3510 ; Fax : 81-6-879-3519 ; E-mail: ykurachi@pharma2.med.osaka-u.ac.jp

c)

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K_{ATP}, ATP-sensitive K^+; SUR, sulfonylurea receptor; Kir, inwardly rectifying K^+ channel ; NBD, nucleotide binding domain; TMs, transmembrane segment; HEK, human embryonic kidney;

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ABSTRACT

A novel antidiabetic agent, nateglinide, is a D-phenylalanine derivative lacking either a sulfonylurea or benzamido moiety. We examined with the patch-clamp method the effect of nateglinide on recombinant ATP-sensitive K^+ (K_{ATP}) channels expressed in HEK293T cells transfected with a Kir6.2 subunit and either of a sulfonylurea receptor (SUR) 1, SUR2A and SUR2B. In inside-out patches, nateglinide reversibly inhibited the spontaneous openings of all three types of SUR/Kir6.2 channels. Nateglinide inhibited SUR1/Kir6.2 channels with high and low affinities (K_i = 75 nmol/L and 114 μmol/L) but SUR2A/Kir6.2 and SUR2B/Kir6.2 channels only with low-affinity (K_i = 105 and 111 μmol/L, respectively). Nateglinide inhibited the K_{ATP} current mediated by Kir6.2 lacking C-terminal 26 amino acids only with the low affinity (K_i = 290 μmol/L) in the absence of SUR. Replacement of serine at position 1237 of SUR1 to tyrosine (SUR1(S1237Y)) specifically abolished the high-affinity inhibition of SUR1/Kir6.2 channels by nateglinide. MgADP or MgUDP (100 μmol/L) augmented the inhibitory effect of nateglinide on SUR1/Kir6.2 but not SUR1(S1237Y)/Kir6.2 or SUR2A/Kir6.2 channels. This augmenting effect of MgADP was also observed with the SUR1/Kir6.2(K185Q) channel which was not inhibited by MgADP, but not with the SUR1(K1384A) /Kir6.2 channel which was not activated by MgADP. These results indicate that therapeutic concentrations of nateglinide (~10 μmol/L) may selectively inhibit pancreatic type SUR1/Kir6.2 channels through SUR1 especially when the channel is activated by intracellular MgADP, even though the agent does not contain either a sulfonylurea or benzamido moiety.

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ATP-sensitive K^+ (K_{ATP}) channels are inhibited by intracellular ATP and activated by ADP and thus provide a link between the cellular metabolic state and excitability (Ashcroft, 1988; Terzic et al., 1995). These channels are associated with such cellular functions as insulin secretion, cardiac preconditioning, vasodilatation and neuroprotection (Ashcroft, 1988; Terzic et al., 1995; Nichols et al., 1996; Quayle et al., 1997; Yamada et al., 2001; Miki et al., 2002). K_{ATP} channels are composed of an ATP-binding cassette protein, sulfonylurea receptor (SUR) and an inwardly rectifying K^+ channel (Kir) subunit, Kir6.0 (Aguilar-Bryan et al., 1995; Inagaki et al., 1995, 1996 and 1997; Sakura et al., 1995; Clement et al., 1997; Shyng and Nichols, 1997). Detailed functional analyses of K_{ATP} channels composed of Kir6.0 and either of three types of SUR (SUR1, SUR2A, and SUR2B) indicates that SUR1, SUR2A, and SUR2B represent pancreatic, cardiac and vascular smooth muscle types of SUR, respectively (Aguilar-Bryan et al., 1995; Inagaki et al., 1995, 1996; Isomoto et al., 1996; Yamada et al., 1997). SUR1/Kir6.2, SUR2A/Kir6.2, and SUR2B/Kir6.2 channels exhibit the same single-channel conductance but different responses to K^+ channel openers and MgADP because SUR regulates the channel's gating in response to these substances (Inagaki et al., 1995, 1996; Isomoto et al., 1996; Matsuoka et al., 2000).

SUR types also determine the response of K_{ATP} channels to sulfonylurea and benzamido derivatives (Gribble et al., 1998b). Sulfonylurea derivatives tolbutamide and gliclazide provoke

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selective high-affinity inhibition of SUR1/Kir6.2 channels (Gribble et al., 1998b; Gribble and Ashcroft, 1999). However, glibenclamide containing both sulfonylurea and benzamido moieties, and meglitinide possessing a benzamido but not a sulfonylurea moiety, provoke high-affinity inhibition of all SUR1/Kir6.2, SUR2A/Kir6.2 and SUR2B/Kir6.2 channels (Gribble et al., 1998b; Ashfield et al., 1999). Therefore, some of the compounds used as hypoglycemic agents may cause an adverse cardiovascular effect by cross-reacting with cardiovascular K_{ATP} channels (Bernauer, 1997; Cleveland et al., 1997; UK Prospective Diabetes Study Group, 1998).

The phenylalanine derivative, nateglinide (N-[(trans-4-isopropylcyclohexyl)-carbonyl]-D-phenylalanine, A-4166), is a novel oral hypoglycemic agent. Although nateglinide lacks either a sulfonylurea or a benzamido moiety (Akiyoshi et al., 1995; Ikenoue et al., 1997; Gribble et al., 2001), it stimulates insulin secretion by inhibiting β-cell K_{ATP} channels. However, its effects on distinct types of K_{ATP} channels have not been examined in details. In this study, we compared the effects of nateglinide on SUR1/Kir6.2, SUR2A/Kir6.2 and SUR2B/Kir6.2 channels with the patch-clamp method. We show that nateglinide provokes selective, reversible, high-affinity inhibition of the SUR1/Kir6.2 channel through the same site on SUR1 as tolbutamide. Intracellular MgADP apparently enhances the inhibitory effect of nateglinide because nateglinide suppresses the stimulatory but not inhibitory effect of the nucleotide on the channel. This is the first report to show that a compound lacking a sulfonylurea moiety exhibits the same mode of action as sulfonylurea derivatives. A part of this

study has been published in an abstract form (Chachin et al., 2002).

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Materials and Methods

Molecular Biology

The cDNAs of mouse Kir6.2 and different SURs were used (Isomoto et al., 1996; Matsuoka et al., 2000). The coding region of each cDNA was individually subcloned into expression vector pcDNA3 (Invitrogen, San Diego, CA). SUR1 whose serine at position 1237 was substituted with tyrosine (SUR1 (S1237Y)), SUR1 whose lysine at position 1384 was substituted with alanine (SUR1(K1384A)), and Kir6.2 whose lysine at position 185 was substituted with glutamine (Kir6.2(K185Q)) were constructed using the GeneEditor™ *in vitro* site-directed mutagenesis system (Promega Corp., Madison, WI). Kir6.2 whose C-terminal 26 amino acids were truncated (Kir6.2ΔC26) was made by introducing a stop codon at the appropriate position by site-directed mutagenesis (Tucker et al., 1997). The nucleotide sequence of all mutated SUR1, Kir6.2 and Kir6.2ΔC26 genes were confirmed by DNA sequencing.

Functional Coexpression of SURs and Kir6.2 cDNAs

Using LipofectAMINE (Life Technologies, Gaitheresburg, MD, USA), human embryonic kidney (HEK) 293T cells were transfected simultaneously with the plasmid containing Kir6.2, a plasmid containing either SUR1, SUR2A or SUR2B, and pCA-GFP (S65A) bearing a gene for green fluorescence protein (GFP). The plasmid containing Kir6.2ΔC26 was transfected into HEK293T

cells alone with pCA-GFP (S65A). The cells expressing GFP were identified by fluorescence microscopy and used for electrophysiology.

Electrophysiology

The currents through K_{ATP} channels expressed in the transfected HEK293T cells were recorded with patch-clamp techniques using an Axopatch 200A amplifier (Axon instruments Inc., Foster City, CA). Patch pipettes were fabricated from borosilicate capillaries, and their tips were coated with Sylgard™ (Dow Corning, Midland, MI) and heat-polished. Pipettes were filled with solution containing 145 mmol/L KCl, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$ and 5 mmol/L HEPES-KOH (pH = 7.4). The bath was perfused with modified Tyrode solution containing 136.5 mmol/L NaCl, 5.4 mmol/L KCl, 1.8 mmol/L $CaCl_2$, 0.53 mmol/L $MgCl_2$, 5.5 mmol/L glucose and 5.5 mmol/L HEPES-NaOH (pH = 7.4). After formation of the cell-attached configuration, the bath was perfused with 'internal' solution containing 145 mmol/L KCl, 5 mmol/L EGTA, 2 mmol/L $MgCl_2$ and 5 mmol/L HEPES-KOH (pH = 7.3). The concentration of free Mg^{2+} in this solution was 1.4 mmol/L. After patch excision, the internal side of the patch membrane was perfused with the internal solution containing nucleotides and/or drugs. When nucleotides were added to the internal solution, the free Mg^{2+} concentration was adjusted to 1.4 mmol/L by supplementing $MgCl_2$. K_{ATP} channel currents in inside-out patch membranes were recorded at –60 mV at room temperature (. 25•C). The data were recorded on videocassette tapes through a PCM converter (VR-10B,

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Instrutech Co., Long Island, NY). For analysis, the stored data were reproduced through the same PCM converter, low pass-filtered at 1 kHz (-3 dB) with an 8-pore Bassel filter (Frequency Devices, Haverhill, MA), digitized at 5 kHz with an AD converter (ITC16, Instrutech Co., Long Island, NY) and analyzed on a personal computer (Power Macintosh G3, Apple, Cupertino, CA) using commercially available software (Patch Analyst Pro., MT Corporation, Hyogo, Japan).

Data analysis

The channel activity was estimated by measuring a mean current amplitude or NP_o (N is the number of functional channels, and P_o is the open probability of each channel). The channel activity in the presence of nateglinide was expressed as a fraction of the channel activity recorded in the absence of the drug (relative channel activity). Nateglinide concentration–response curves were fit with the following equations according to Gribble et al. (1998b).

$$\text{Relative Channel Activity} = x*y \qquad (1)$$

x is a term describing the high-affinity site, and y is that describing the low-affinity site.

$$x = L + \frac{(1-L)}{1 + ([\text{Nateglinide}]/K_{i1})^{h1}} \qquad (2)$$

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$$y = \frac{1}{1 + ([\text{Nateglinide}]/K_{i2})^{h2}} \qquad (3)$$

where [Nateglinide] is the nateglinide concentration in mol/L; K_{i1} and K_{i2} are the nateglinide concentrations at which the magnitude of inhibition is half the maximum at the high- and low-affinity sites, respectively; h_1 and h_2 are the Hill coefficients (slope factors) for the high- and low-affinity sites, respectively; and L is the fraction remaining when the high-affinity inhibitory sites are maximally occupied. When only a single inhibitory site is present, the equation reduces to the following:

Relative Channel Activity = y (4)

All statistical values are indicated as mean ± s.e.m. The statistical difference was evaluated by Student's t test. Statistical probability of $P < 0.05$ was taken as a significant difference.

Drugs

A 100 mmol/l stock solution of nateglinide was prepared in dimethyl sulfoxide. Drugs were diluted to the desired concentrations in the internal solution. The final concentration of dimethyl sulfoxide was • 0.3%, at which the vehicle by itself did not affect the K_{ATP} channel currents

measured in the inside-out patch membranes (n = 5). Nateglinide was a kind gift from Ajinomoto Co. Ltd. (Tokyo, Japan). ATP and ADP were purchased from Sigma Chemical Co. (St. Louis, MO). UDP was obtained from Boehringer Mannheim (Mannheim, Germany). Other chemicals and materials were purchased from commercial sources.

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Results

Comparison of Effects of Nateglinide on SUR1/Kir6.2, SUR2A/Kir6.2 and SUR2B/Kir6.2 Channel Currents.

In order to determine the tissue selectivity of nateglinide, we first compared the effects of nateglinide on SUR1/Kir6.2, SUR2A/Kir6.2 and SUR2B/Kir6.2 channels expressed in HEK293T cells. These constructs represent respectively the β-cell, cardiac and smooth muscle types of K_{ATP} channel. Figures 1A-C (left column) show the effects of nateglinide on spontaneous openings of each type of K_{ATP} channel in inside-out patch membranes. The application of 0.1 μmol/L nateglinide to the intracellular side of patch membranes reversibly inhibited SUR1/Kir6.2 channel currents by 35.3 ± 3.3% (n = 8). However, nateglinide inhibited SUR2A/Kir6.2 (n = 5) and SUR2B/Kir6.2 channel currents (n = 4) in a concentration-dependent manner only at concentrations higher than 10 μmol/L. The relationship between the nateglinide concentration and the normalized amplitude of SUR1/Kir6.2 channel currents was best fit with the two-site model (Eqs. (1) to (3) in Materials and Methods) with K_{i1} of 75 nmol/L and K_{i2} of 114 μmol/L (Fig. 1A (right column)). By contrast, the drug inhibited SUR2A/Kir6.2 and SUR2B/Kir6.2 channel currents only with low affinity with K_{i2} of 105 μmol/L and 111 μmol/L, respectively (Eqs. (3) and (4) in Materials and Methods) (Figs. 1B-C (right column)). Thus, nateglinide inhibited the pancreatic type of K_{ATP} channel (SUR1/Kir6.2) with high affinity, and it caused low-affinity inhibition of all three types of K_{ATP} channel.

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Inhibitory Effect of Nateglinide on Kir6.2ΔC26 and SUR1(S1237Y)/Kir6.2 Channels.

Sulfonylurea and benzamido derivatives are thought to cause low-affinity inhibition of K_{ATP} channels through their action upon the Kir6.2 subunit and not through the SUR (Gribble et al., 1998b; Gribble and Ashcroft, 1999; Reimann et al., 2001). Figure 2A shows the effect of nateglinide on channel currents mediated by a Kir6.2 subunit whose C-terminal 26 amino acids had been removed (Kir6.2ΔC26) and which forms active K_{ATP} channels in the absence of SUR (Tucker et al, 1997). Nateglinide inhibited Kir6.2ΔC26 channel currents which were recorded in the absence of a SUR only at high concentrations (> 10 μmol/L). The complete concentration-response relationship for this reaction could not be obtained because > 300 μmol/L of the drug could not be dissolved in water. The fitting of Eq. (4) to the available data suggested a K_{i2} of 290 μmol/L. This value was of the same order as those obtained for the low-affinity inhibition of SUR1/Kir6.2, SUR2A/Kir6.2 and SUR2B/Kir6.2 channels by nateglinide, suggesting that the low-affinity site resides on the Kir6.2 subunit.

These results suggest that SUR1 mediated the high-affinity inhibition of K_{ATP} channels by nateglinide. It is known that substitution of serine at position 1237 in the cytoplasmic linker between transmembrane segments 15 and 16 of SUR1 with the corresponding amino acid tyrosine from SUR2A (SUR1(S1237Y)) abolishes the high-affinity inhibition of SUR1/Kir6.2 channels by tolbutamide (Ashfield et al., 1999). Thus, we examined the effect of this mutation on the action of

nateglinide (Fig. 2B). Nateglinide inhibited SUR1(S1237Y)/Kir6.2 channel currents only with low-affinity (K_{i2} = 72 μmol/L), indicating that the same amino acid residue in SUR1 mediates the high-affinity inhibition of SUR1/Kir6.2 channels by nateglinide and tolbutamide.

Effect of Intracellular MgADP on Nateglinide-induced Inhibition of K_{ATP} Channels.

It is known that intracellular MgADP enhances inhibition of SUR1/Kir6.2 channel currents by tolbutamide (Zünkler et al., 1988; Schwanstecher et al., 1992; Gribble et al., 1997a and 1998b). Because the same amino acid residue in SUR1 mediates the high-affinity inhibition of SUR1/Kir6.2 channels by nateglinide and tolbutamide, we examined whether the inhibitory effect of nateglinide was also augmented by MgADP (Fig. 3A). MgADP (100 μmol/L) increased opening of SUR1/Kir6.2 channels in the absence of nateglinide but decreased them in the presence of 10 μmol/L nateglinide (Fig. 3A). Whereas 10 μmol/l nateglinide inhibited SUR1/Kir6.2 channel currents by 53.9±4.0% in the absence of MgADP, in its presence the drug inhibited the currents by 86.1+0.9% (Fig. 3D) ($p = 0.0014$). Thus nateglinide also appeared to inhibit the channel currents more strongly in the presence of MgADP than in its absence.

To examine whether the potentiation by MgADP of the effect of nateglinide upon SUR1/Kir6.2 was related to the high-affinity site for the drug, we repeated the experiment using SUR1(S1237Y)/Kir6.2 channels (Fig. 3B). In this case, MgADP (100 μmol/L) increased the channel current both in the absence and presence of nateglinide (100 μmol/L). Nateglinide (10

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μmol/L) inhibited the channel current by 21.3±5.3% and 20.5±7.6% in the absence and presence of 100 μmol/L MgADP, respectively (Fig. 3D) ($p = 0.94$). Therefore, the potentiating effect of MgADP required the interaction of nateglinide with the serine at position 1237 of SUR1 and not with the Kir6.2 subunit. Consistent with this notion, nateglinide (100 μmol/L) did not inhibit SUR2A/Kir6.2 channel currents even in the presence of MgADP (100 μmol/L) (Fig. 3C), and MgADP did not potentiate the inhibitory effect of nateglinide (10 and 100 μmol/L) on SUR2A/Kir6.2 channels (Fig. 3D) ($p = 0.79$ and 0.26, respectively).

Nateglinide Abolishes the Stimulatory Effect and does not Affect the Inhibitory Effect of MgADP on SUR1/Kir6.2 Channels.

MgADP has both stimulatory and inhibitory effects on SUR1/Kir6.2 channels (Gribble et al., 1997b). The former effect is mediated by SUR1 whereas the latter is mediated by Kir6.2 (Gribble et al., 1997b). Figure 3A indicates that the stimulatory effect of the nucleotide dominated the inhibitory effect in the absence of nateglinide but *vice versa* in the presence of the drug. We therefore examined the effect of nateglinide on the separate stimulatory and inhibitory effects of the nucleotide.

First, we examined the effects of MgADP and nateglinide on K_{ATP} channels composed of SUR1 and a Kir6.2 whose lysine at position 185 was substituted to glutamine (Kir6.2(K185Q)). This mutation reduces the sensitivity of Kir6.2ΔC26 channels to MgATP by ~40 times (Tucker et al.,

1998), and MgATP (1 mmol/L) did not inhibit but stimulated SUR1/Kir6.2(K185Q) channel currents (Fig. 4A). This is probably because attenuation of the inhibitory effect of MgATP on the Kir subunit revealed the stimulating effect of the nucleotide acting through SUR1 (Gribble et al., 1998a; Bienengraeber et al., 2000). MgADP (100 μmol/L) strongly increased SUR1/Kir6.2(K185Q) channel currents in the absence of nateglinide but had little effect in the presence of 10 μmol/l of the drug. This indicated that nateglinide had abolished the stimulatory effect of MgADP. Nateglinide inhibited SUR1/Kir6.2(K185Q) channel currents by 59.6±4.0% and 83.8+3.8% in the absence and presence of MgADP, respectively (Fig. 4D). This difference was statistically significant ($p = 0.008$).

Next, we examined whether MgUDP also potentiated the effect of nateglinide. This nucleotide in particularly interesting because MgUDP stimulates but does not inhibit SUR1/Kir6.2 channels (Fig. 4B). MgUDP (100 μmol/L) strongly increased the channel current in the absence of nateglinide but not in the presence of 10 μmol/l of the agent. Nateglinide inhibited SUR1/Kir6.2 channel currents by 56.6±4.3% and 81.1±4.7% in the absence and presence of MgUDP, respectively (Fig. 4D) ($p = 0.018$).

Taken together these results indicate that nateglinide abolished the stimulatory effects of the nucleotides on K_{ATP} channel currents. This effect may then be sufficient to account for the apparent potentiating effect of MgADP on the inhibition of SUR1/Kir6.2 channels by nateglinide.

To test this hypothesis, we used SUR1 where lysine 1384 in the Walker A motif in NBD2 was substituted with alanine (SUR1(K1384A)) (Fig. 4C). This mutation extinguishes

MgADP-induced activation of SUR1/Kir6.2 channels (Gribble et al., 1997b). MgADP (100 µmol/L) inhibited SUR1(K1384A)/Kir6.2 channels by 58.2±8.6% and by 69.7±5.2% in the absence and presence of nateglinide (10 µmol/L), respectively (n = 4). Nateglinide inhibited SUR1(K1384A)/Kir6.2 channel currents by 60.5±6.3% and 71.2±3.8% in the absence and presence of MgADP, respectively (Fig. 4D). This difference was not statistically significant (p = 0.22). Thus, nateglinide did not affect the inhibitory effect of MgADP, and MgADP did not affect the inhibitory effect of nateglinide.

In conclusion, these results show that inhibition of SUR1/Kir6.2 channels was enhanced by the addition of MgADP to nateglinide because the drug abolished the stimulatory effect of the nucleotide. The thus isolated inhibitory effect of the nucleotide was then added to that of the drug.

Discussion

Nateglinide is a novel type of oral hypoglycemic agent that does not contain either a sulfonylurea or benzamido moiety (Akiyoshi et al., 1995; Ikenoue et al., 1997; Gribble et al., 2001). It was recently licensed and launched for therapy of patients with type II diabetes. We examined the effect of nateglinide on SUR1/Kir6.2, SUR2A/Kir6.2 and SUR2B/Kir6.2 channels expressed in a mammalian cell line. We found that nateglinide specifically inhibited SUR1/Kir6.2 channels with high affinity. On the other hand, Sunaga et al. (2001) reported that less than 1 μmol/L nateglinide partially inhibited all SUR1/Kir6.2, SUR2A/Kir6.2 and SUR2B/Kir6.2 channels. Although the reason for this discrepancy is not clear, Hu et al. (1999) reported that nateglinide inhibited native K_{ATP} channels in rat pancreatic β-cells ~100 times more potently than those in the smooth muscle cells, consistent with our observation. Oral hypoglycemic agents cross-reacting with extrapancreatic K_{ATP} channels potentially cause undesirable side effects. Indeed, it is still under dispute whether or not sulfonylurea and benzamido derivatives increase the risk of cardiovascular mortality (Bernauer, 1997; Cleveland et al., 1997; UK Prospective Diabetes Study Group, 1998). Taking the therapeutic plasma concentration of nateglinide (~10 μmol/L) into account, we conclude that nateglinide would not cause detrimental cardiovascular side effects (Hu et al., 1999; Gribble et al., 2001).

It is reported that physiological concentrations of MgADP enhance the inhibitory effect of sulfonylurea derivatives on native β-cell K_{ATP} channels and reconstituted SUR1/Kir6.2 channels but

not SUR2A/Kir6.2 channels (Zünkler et al., 1988; Schwanstecher et al., 1992; Gribble et al., 1997a and1998b). This was also the case for nateglinide (Figs. 3A and C). This characteristic would further enhance the selectivity of nateglinide for pancreatic K_{ATP} channels. The cumulative effects of nateglinide and MgADP upon the channels can be explained as follows. MgADP has dualistic effects upon K_{ATP} channels, evoking both stimulation and/or inhibition (Gribble et al., 1997b). Nateglinide inhibits SUR1/Kir6.2 channels and also suppresses the stimulatory effect of MgADP (Fig. 4A). The inhibitory effect of MgADP is not affected by nateglinide (Fig. 4C). Both compounds then have inhibitory effects and as a result, the channel activity becomes even smaller in the presence of nateglinide and MgADP than in the presence of nateglinide alone (Fig. 4C).

The high-affinity inhibition of the channels by nateglinide was eliminated by the S1237Y mutation of SUR1 (Fig. 2A). This mutation also abolishes the high-affinity inhibition of SUR1/Kir6.2 channels by the sulfonylurea derivative tolbutamide, but not inhibition evoked by the benzamido derivative meglitinide (Ashfield et al., 1999). This mutation also abolishes the binding of [^{3}H]glibenclamide to membranes expressing SUR1 (Ashfield et al., 1999). Because glibenclamide bears both sulfonylurea and benzamido moieties, it is likely that S1237 forms an essential part of the binding site for sulfonylurea derivatives. It would therefore seem that nateglinide inhibits SUR1/Kir6.2 channels through the same site as sulfonylurea derivatives even though it lacks a sulfonylurea moiety. Interestingly, nateglinide was a much more potent inhibitor of SUR1/Kir6.2 channels than tolbutamide and as potent as another sulfonylurea derivative, gliclazide (Gribble et al.,

1998b; Gribble and Ashcroft, 1999). Mitiglinide is another antidiabetic agent which lacks either a sulfonylurea or benzamido moiety but causes high-affinity inhibition of SUR1/Kir6.2 channels which is abolished by the S1237Y mutation (Reimann et al., 2001). Mitiglinide therefore resembles nateglinide in this regard. But mitiglinide also inhibits SUR2A/Kir6.2 and SUR2B/Kir6.2 channels with high affinity which must be mediated by a site on SUR2 which is different from the "sulfonylurea site" (Reimann et al., 2001).

It is not clear at present how nateglinide interacts with the sulfonylurea site on SUR1. Conformation analysis revealed that hypoglycemic agents such as nateglinide, glibenclamide, glimepiride, meglitinide, mitiglinide, repaglinide, and S3075 with diverse molecular structures nevertheless display a comparable U shape with hydrophobic cycles placed at the extremity of each hand and a peptidic bond placed at the bottom of U (Lins et al., 1995). Thus, molecular modeling of the agents is unable to define the particular molecular structure of the interface between nateglinide and SUR1. The molecular structure of SUR1 needs to be identified to further understand the molecular mechanism underlying the interaction between nateglinide and SUR1.

In conclusion, we have shown that nateglinide specifically inhibits SUR1/Kir6.2 channels with high affinity. Although nateglinide lacks a sulfonylurea moiety, its effect on SUR1/Kir6.2 channels resembles that of tolbutamide with respect to the S1237Y mutation and the interaction with intracellular MgADP. Nateglinide was, however, much more potent than tolbutamide. Because nateglinide has a clinically useful pharmacokinetic profile to compensate for the impaired first phase

insulin response and thus suppress postprandial hyperglycemia, this compound seems to be an effective antidiabetic agent with less undesirable extrapancreatic side effects.

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Footnotes

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In preparation of this manuscript, Hansen AM et al. reported that nateglinide inhibits SUR1/Kir6.2 channels with the half-maximum inhibitory concentration of 800 nmol/L and that this inhibition is abolished by the S1237Y mutation of SUR1, consistent with our present observations.

Figure legend

Fig. 1. Inhibitory effects of nateglinide on SUR1/Kir6.2, SUR2A/Kir6.2 and SUR2B/Kir6.2 channel currents.

Inhibitory effects of nateglinide on SUR1/Kir6.2 (A), SUR2A/Kir6.2 (B) and SUR2B/Kir6.2 (C) channel currents were measured at –60 mV in inside-out patches. The pipette contained 145 mmol/L K^+. Left column, inhibitory effects of nateglinide on spontaneously opening SUR1/Kir6.2, SUR2A/Kir6.2 and SUR2B/Kir6.2 channels. MgATP and nateglinide were added to the bath solution as indicated by bars. Right column, concentration-response relationships for inhibition of each type of K_{ATP} current by nateglinide. The relative channel activity represents the channel activity normalized to that seen in the absence of the drug. The symbols and bars represent the mean and s.e.m., respectively. The number of observations for each point was 8, 5 and 4 in A, and B and C, respectively. (A) The concentration-response curve was fit with the two-site model (Eqs. (1)-(3) in the Text) with K_{i1} = 75 nmol/L, h_1 = 0.8, K_{i2} = 114 μmol/L, h_2 = 1.3 and L = 0.37. (B) The concentration-response curve was fit with the single-site model (Eqs. (3) and (4)) with K_{i2} = 105 μmol/L and h_2 = 1.2. (C) The concentration-response curve was fit with the single-site model with K_{i2} = 111 μmol/L and h_2 = 1.1.

Fig. 2. Inhibitory effect of nateglinide on Kir6.2ΔC26 and SUR1(S1237Y)/Kir6.2 channel currents.

Left column, inhibitory effects of nateglinide on spontaneously opening Kir6.2ΔC26 (A) and

SUR1(S1237Y)/Kir6.2 (B) channels were measured at –60 mV in inside-out patches. ATP and nateglinide were added to the bath solution as indicated by bars. Right column, concentration-response relationships for inhibition of Kir6.2ΔC26 (A) and SUR1(S1237Y)/Kir6.2 (B) channel currents by nateglinide. Symbols and bars indicate the mean and S. E. The number of observation at each point was 5 in each of the graphs. The lines were fit with the single-site model with K_{i2} = 290 μmol/L and h_2 = 0.9 for Kir6.2ΔC26 channels, and K_{i2} = 72 μmol/L and h_2 = 0.7 for SUR1(S1237Y)/Kir6.2 channels. The dashed line in the graph in B depicts the concentration-response curve for the inhibition of SUR1/Kir6.2 channel currents by nateglinide shown in Fig 1 A.

Fig. 3. Effect of nateglinide on SUR1/Kir6.2, SUR1(S1237Y)/Kir6.2 and SUR2A/Kir6.2 channels in the presence and absence of 100 μmol/L MgADP.

The inhibitory effects of nateglinide on SUR1/Kir6.2 (A), SUR1(S1237Y)/Kir6.2 (B) and SUR2A/Kir6.2 (C) channels were measured in inside-out patches. ATP, ADP, and nateglinide were added to the bath solution as indicated by bars. D. The relative channel activity in the presence of 10 or 100 μmol/L nateglinide without (open bars) and with (closed bars) 100 μmol/L MgADP. The channel activity in the presence of nateglinide is expressed as a fraction of that observed in the absence of the drug. The columns and bars indicate the mean and s.e.m, respectively. **$p < 0.01$; and NS, not significantly different .

Fig. 4. Effect of nateglinide on SUR1/Kir6.2(K185Q), SUR1/Kir6.2 and SUR1(K1384A)/Kir6.2 channels in the presence and absence of nucleotides.

Effects of nateglinide on SUR1/Kir6.2(K185Q) (A), SUR1/Kir6.2 (B), and SUR1(K1384A)/Kir6.2 (C) channel currents were measured in inside-out patches. D. The relative channel activity in the presence of 10 μmol/L nateglinide without nucleotides (open columns), with MgADP (closed columns) and with MgUDP (hatched column). The channel activity in the presence of nateglinide is expressed as a fraction of that observed in the absence of the drug. The columns and bars indicate the mean and s.e.m, respectively. *$p < 0.05$; **$p < 0.01$; and NS, not significantly different .

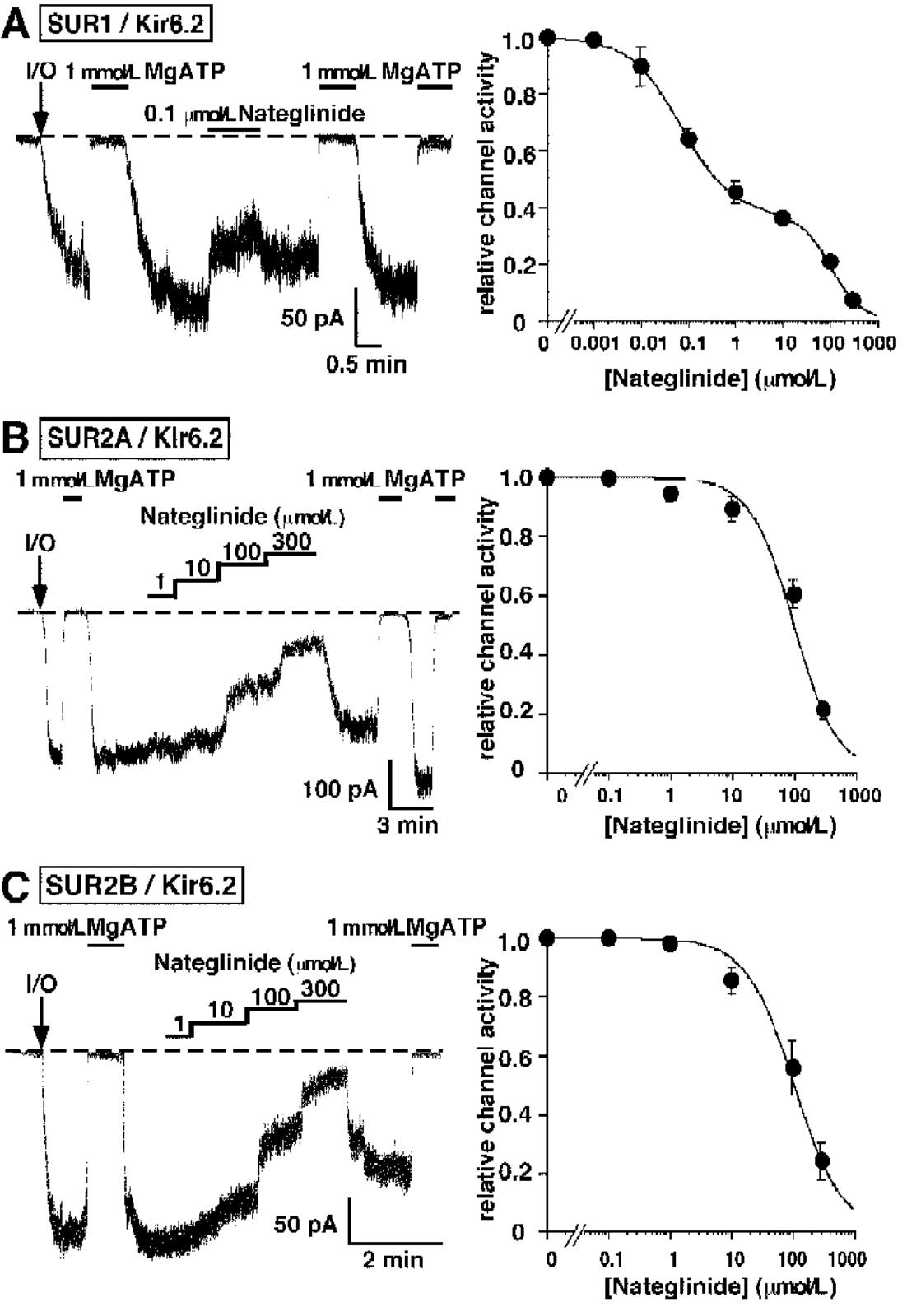
A
SUR1 / Kir6.2
I/O 1 mmol/L MgATP
1 mmol/L MgATP
0.1 μmol/L Nateglinide
50 pA
0.5 min
relative channel activity
1.0
0.8
0.6
0.4
0.2
0
0 0.001 0.01 0.1 1 10 100 1000
[Nateglinide] (μmol/L)
B
SUR2A / Kir6.2
1 mmol/L MgATP
1 mmol/L MgATP
Nateglinide (μmol/L)
I/O
1
10
100
300
100 pA
3 min
relative channel activity
1.0
0.8
0.6
0.4
0.2
0
0 0.1 1 10 100 1000
[Nateglinide] (μmol/L)
C
SUR2B / Kir6.2
1 mmol/L MgATP
1 mmol/L MgATP
Nateglinide (μmol/L)
I/O
1
10
100
300
50 pA
2 min
relative channel activity
1.0
0.8
0.6
0.4
0.2
0
0 0.1 1 10 100 1000
[Nateglinide] (μmol/L)

Fig. 1

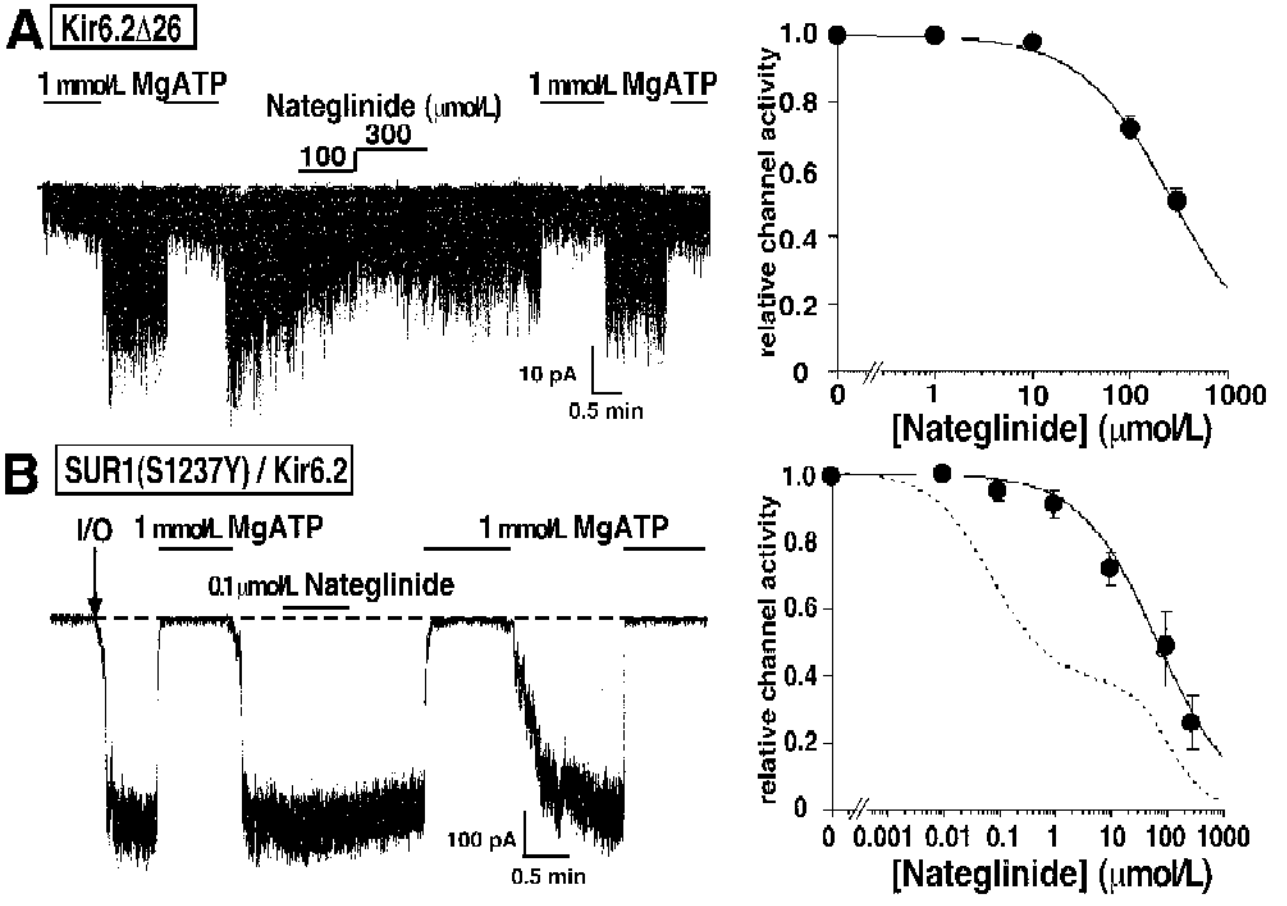
A
Kir6.2Δ26
1 mmol/L MgATP
Nateglinide (μmol/L)
100
300
1 mmol/L MgATP
10 pA
0.5 min
relative channel activity
1.0
0.8
0.6
0.4
0.2
0
0
1
10
100
1000
[Nateglinide] (μmol/L)
B
SUR1(S1237Y) / Kir6.2
I/O
1 mmol/L MgATP
1 mmol/L MgATP
0.1 μmol/L Nateglinide
100 pA
0.5 min
relative channel activity
1.0
0.8
0.6
0.4
0.2
0
0
0.001
0.01
0.1
1
10
100
1000
[Nateglinide] (μmol/L)

Fig. 2

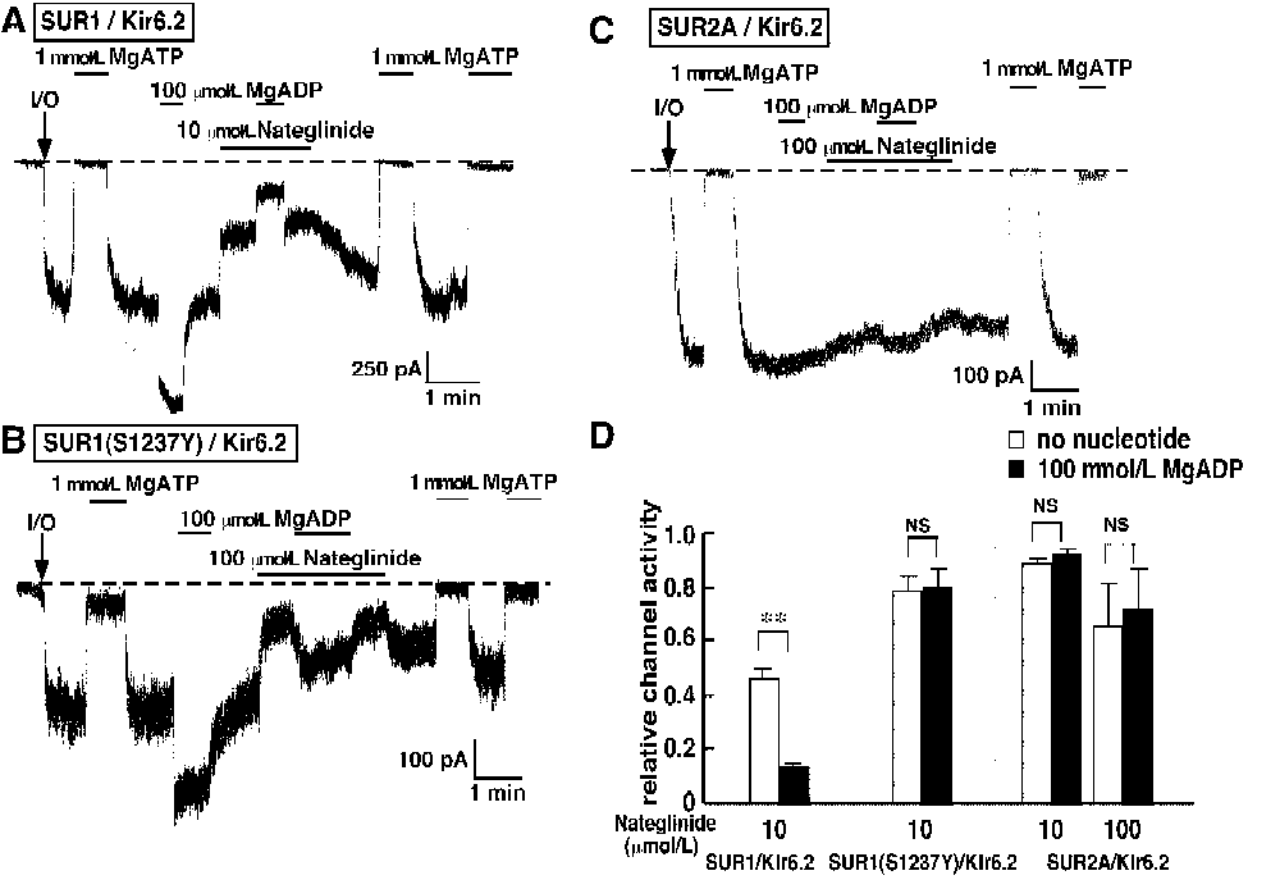
A
SUR1 / Kir6.2
1 mmol/L MgATP
1 mmol/L MgATP
100 μmol/L MgADP
I/O
10 μmol/L Nateglinide
250 pA
1 min
B
SUR1(S1237Y) / Kir6.2
1 mmol/L MgATP
1 mmol/L MgATP
I/O
100 μmol/L MgADP
100 μmol/L Nateglinide
100 pA
1 min
C
SUR2A / Kir6.2
1 mmol/L MgATP
1 mmol/L MgATP
I/O
100 μmol/L MgADP
100 μmol/L Nateglinide
100 pA
1 min
D
no nucleotide
100 mmol/L MgADP
relative channel activity
1.0
0.8
0.6
0.4
0.2
0
**
NS
NS
NS
Nateglinide (μmol/L)
10
10
10
100
SUR1/Kir6.2
SUR1(S1237Y)/Kir6.2
SUR2A/Kir6.2

Fig. 3

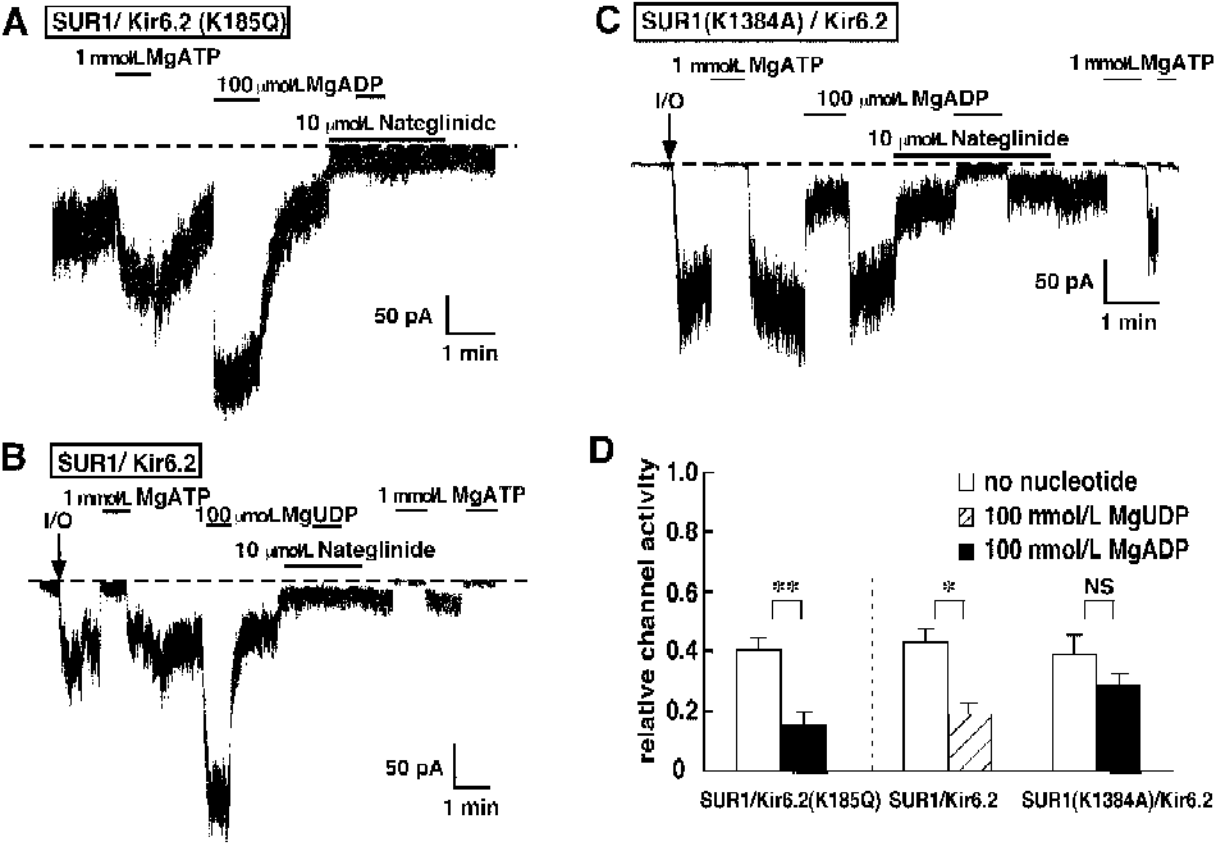
A
SUR1/ Kir6.2 (K185Q)
1 mmol/L MgATP
100 μmol/L MgADP
10 μmol/L Nateglinide
50 pA
1 min
B
SUR1/ Kir6.2
1 mmol/L MgATP
I/O
100 μmol/L MgUDP
1 mmol/L MgATP
10 μmol/L Nateglinide
50 pA
1 min
C
SUR1(K1384A) / Kir6.2
1 mmol/L MgATP
1 mmol/L MgATP
I/O
100 μmol/L MgADP
10 μmol/L Nateglinide
50 pA
1 min
D
relative channel activity
1.0
0.8
0.6
0.4
0.2
0
no nucleotide
100 mmol/L MgUDP
100 mmol/L MgADP
**
*
NS
SUR1/Kir6.2(K185Q)
SUR1/Kir6.2
SUR1(K1384A)/Kir6.2

Fig. 1