ELSEVIER

European Journal of Pharmacology 431 (2001) 119–125

ejp

www.elsevier.com/locate/ejphar

The effects of mitiglinide (KAD-1229), a new anti-diabetic drug, on ATP-sensitive K^+ channels and insulin secretion: comparison with the sulfonylureas and nateglinide

Yasuhiro Sunaga [a], Tohru Gonoi [b], Tadao Shibasaki [a], Kiyoshi Ichikawa [c], Hiroshi Kusama [c], Hideki Yano [a], Susumu Seino [a,*]

[a] *Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan*
[b] *Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University 1-8-1 Inohana, Chuo-ku, Chiba 260-8673, Japan*
[c] *Pharmacology Lab. R&D, Kissei Pharmaceutical Co. Ltd., 4365-1 Kashiwabara, Hotaka, Minamizaumi, Nagano 399-8304, Japan*

Received 11 September 2001; accepted 20 September 2001

Abstract

Mitiglinide (KAD-1229), a new anti-diabetic drug, is thought to stimulate insulin secretion by closing the ATP-sensitive K^+ (K_{ATP}) channels in pancreatic β-cells. However, its selectivity for the various K_{ATP} channels is not known. In this study, we examined the effects of mitiglinide on various cloned K_{ATP} channels (Kir6.2/SUR1, Kir6.2/SUR2A, and Kir6.2/SUR2B) reconstituted in COS-1 cells, and compared them to another meglitinide-related compound, nateglinide. Patch-clamp analysis using inside-out recording configuration showed that mitiglinide inhibits the Kir6.2/SUR1 channel currents in a dose-dependent manner (IC_{50} value, 100 nM) but does not significantly inhibit either Kir6.2/SUR2A or Kir6.2/SUR2B channel currents even at high doses (more than 10 μM). Nateglinide inhibits Kir6.2/SUR1 and Kir6.2/SUR2B channels at 100 nM, and inhibits Kir6.2/SUR2A channels at high concentrations (1 μM). Binding experiments on mitiglinide, nateglinide, and repaglinide to SUR1 expressed in COS-1 cells revealed that they inhibit the binding of [^{3}H]glibenclamide to SUR1 (IC_{50} values: mitiglinide, 280 nM; nateglinide, 8 μM; repaglinide, 1.6 μM), suggesting that they all share a glibenclamide binding site. The insulin responses to glucose, mitiglinide, tolbutamide, and glibenclamide in MIN6 cells after chronic mitiglinide, nateglinide, or repaglinide treatment were comparable to those after chronic tolbutamide and glibenclamide treatment. These results indicate that, similar to the sulfonylureas, mitiglinide is highly specific to the Kir6.2/SUR1 complex, i.e., the pancreatic β-cell K_{ATP} channel, and suggest that mitiglinide may be a clinically useful anti-diabetic drug.

Keywords: K_{ATP} channel; Sulfonylurea receptor (SUR); Mitiglinide; Nateglinide; Insulin secretion

1. Introduction

ATP-sensitive K^+ (K_{ATP}) channels are present in many tissues, including pancreatic β-cells (Cook and Hales, 1984), heart (Noma, 1983), vascular smooth muscle cells (Standen et al., 1989), and other tissues (Ashford et al., 1988; Spruce et al., 1985). K_{ATP} channels play an important role in various cellular responses such as secretion and muscle contraction by linking cell metabolism to membrane potential (Ashcroft, 1988). Classical K_{ATP} channels are now known to comprise two subunits: the Kir6.2 subunits that form the K^+-selective ion channel pore, and the SUR (SUR1 or SUR2) subunits, the receptor of sulfonylureas (Aguilar-Bryan et al., 1998; Ashcroft and Gribble, 1998; Seino, 1999). Coexpression of Kir6.2 and SUR1 in heterologous expression systems forms K_{ATP} channels with properties similar to those in native pancreatic β-cells (Inagaki et al., 1995; Sakura et al., 1995). So far, various forms of SUR2 have been identified (Chutkow et al., 1996, 1999; Inagaki et al., 1996; Isomoto et al., 1996). It is generally accepted that the Kir6.2/SUR2A channels form cardiac and probably skeletal muscle K_{ATP} channels (Aguilar-Bryan et al., 1998; Ashcroft and Gribble, 1998; Seino, 1999). Although it was proposed initially that the smooth muscle K_{ATP} channels are Kir6.2/SUR2B channels (Isomoto et al., 1996), the molecular composition of the K_{ATP} channels in native smooth muscle has not been determined. Kir6.1 and SUR2B are the constituents of the vascular smooth muscle K^+ channel (Yamada et al., 1997).

* Corresponding author. Tel.: +81-43-226-2187; fax: +81-43-221-7803.
E-mail address: seino@med.m.chiba-u.ac.jp (S. Seino).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01412-1

The K_{ATP} channels in pancreatic β-cells are critical in the regulation of both glucose-induced and sulfonylurea-induced insulin secretions (Miki et al., 1998). The binding of sulfonylureas to SUR1 probably inhibits the cooperativity of the nucleotide binding folds (NBF)-1 and -2 of SUR1 that induces the closed state of the K_{ATP} channels (Ueda et al., 1999). Recently, the non-sulfonylurea insulinotropic agent meglitinide also has been shown to inhibit the β-cell K_{ATP} channel by binding to SUR1 (Gribble et al., 1998). Mitiglinide (KAD-1229), nateglinide (A-4166), and repaglinide are new anti-diabetic drugs that are related structurally to meglitinide but are different from the sulfonylureas. Preclinical studies have shown mitiglinide to have an immediate and short-lasting effect on hypoglycemic action and to increase insulin release from both pancreatic β-cells and HIT-T15 cells (Ohnota et al., 1994, 1995). Mitiglinide has been shown to displace the bound [^{3}H]glibenclamide in the mouse insulin-secreting cell line MIN6 and to inhibit the K_{ATP} channel currents in these cells (Mogami et al., 1994), suggesting that it stimulates insulin secretion by closing the K_{ATP} channels by binding to the sulfonylurea receptors.

Determination of the tissue selectivity of anti-diabetic drugs that act through sulfonylurea receptors is critical in predicting side effects since sulfonylurea receptors are widely distributed in various tissues. The tissue specificity of some sulfonylureas has been reported (Gribble et al., 1998; Lawrence et al., 2001) but the tissue specificity of mitiglinide is not known. In the present study, we have determined selectivity of mitiglinide for various K_{ATP} channels using COS-1 cells (SV 40-transformed African green monkey kidney (AGMK) cells) coexpressing cloned Kir6.2 and SUR1, SUR2A, or SUR2B. Because it is known that chronic treatment with sulfonylureas sometimes leads to impairment of sulfonylurea-induced insulin secretion both in vivo and in vitro (Karam et al., 1986; Davalli et al., 1992; Rabuazzo et al., 1992), we also investigated the effects of chronic mitiglinide, nateglinide, or repaglinide treatment on insulin secretion and compared the results with those of the sulfonylureas, tolbutamide and glibenclamide.

2. Materials and methods

2.1. Materials

The mitiglinide, nateglinide and repaglinide were obtained from Kissei Pharmaceutical (Nagano, Japan), and dissolved in DMSO as stock solution. Glibenclamide and tolbutamide were purchased from RBI (Natick, MA, USA) and Nacalai tesque (Kyoto, Japan), respectively. [^{3}H]-labeled glibenclamide was from NEN Life Science Products (Boston, MA, USA).

2.2. Cell culture and transfection

COS-1 cells were plated at a density of 2×10^6 cells per dish (10 cm in diameter) for displacement assay of [^{3}H]glibenclamide binding and at 2×10^5 cells per dish (3.5 cm in diameter) for patch-clamp recordings. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. The full-length cDNAs encoding Kir6.2 (human or mouse) and a SUR isoform (human SUR1, hamster SUR1, rat SUR2A or rat SUR2B) were subcloned into a mammalian expression vector, pCMV6b or 6c. The Kir6.2 and SUR subunits were cotransfected with pEGFP (enhanced green fluorescent protein vector: Clontech, Palo Alto, USA), a transfec-

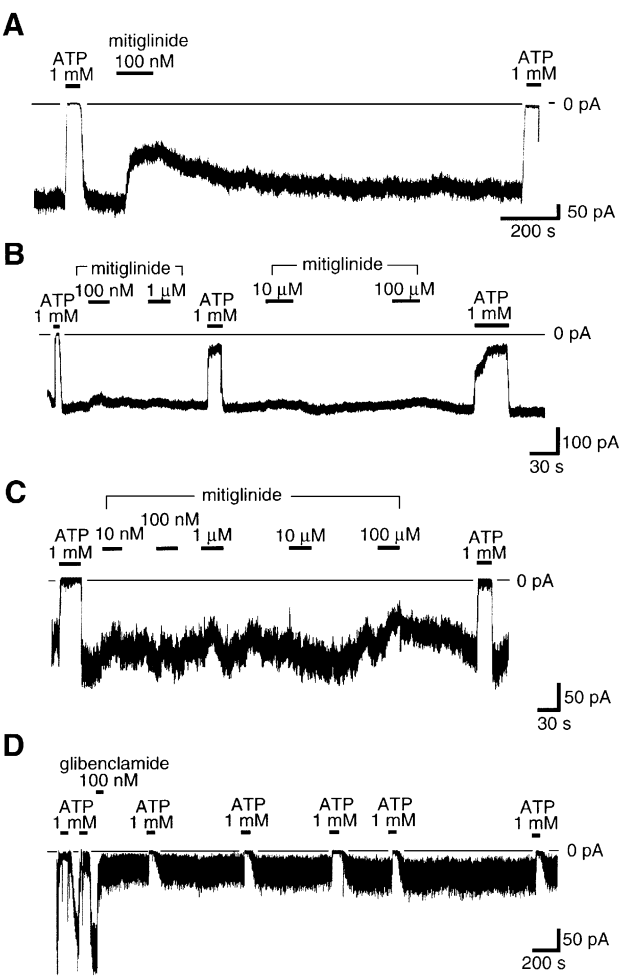

Fig. 1. Effects of mitiglinide and glibenclamide on K_{ATP} channel currents. Representative traces of channel currents recorded from COS-1 cells coexpressing mouse Kir6.2 and hamster SUR1 (A and D), mouse Kir6.2 and rat SUR2A (B), or mouse Kir6.2 and rat SUR2B (C) are shown. The effects of mitiglinide (A–C) and glibenclamide (D) on the channel currents were examined. The recordings were made using the patch-clamp technique in the excised inside-out configuration. The horizontal bars and the numbers above the currents indicate application period and concentration of test agents, respectively. Calibration bars are shown in each panel. "Zero current levels" are indicated by horizontal lines marked "0 pA".

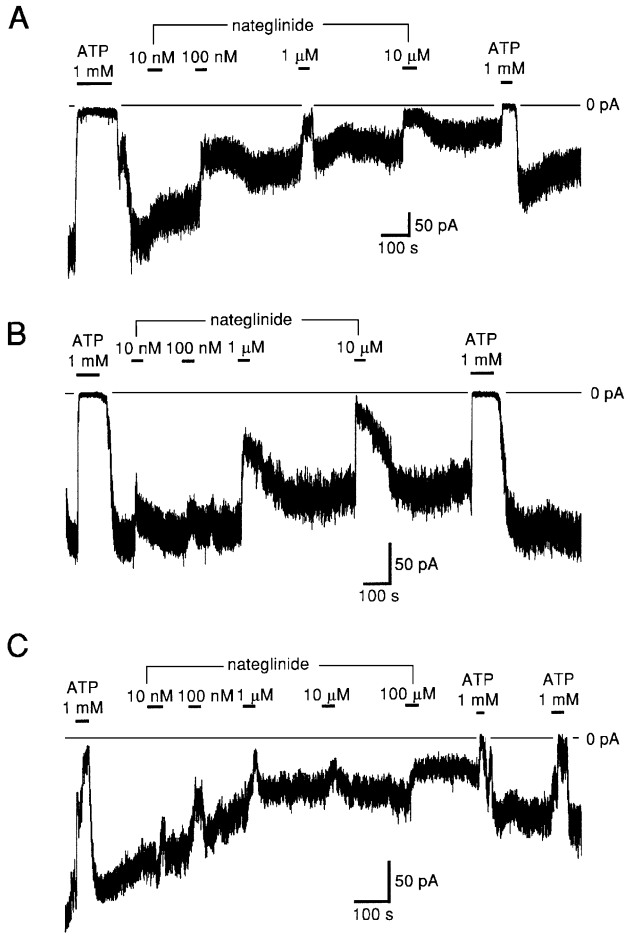

Fig. 2. Effects of nateglinide on K_{ATP} channel currents. Representative traces of channel currents recorded from COS-1 cells coexpressing human Kir6.2 and human SUR1 (A), mouse Kir6.2 and rat SUR2A (B), or mouse Kir6.2 and rat SUR2B (C) are shown. The effects of mitiglinide (A–C) on the channel currents were examined. The other details are same as those in Fig. 1.

tion marker, into COS-1 cells, using lipofectamine and Opti-MEM I reagents (In Vitrogen, Carlsbad, CA, USA).

For chronic treatment of MIN6 cells with the insulinotropic agents, the cells were plated at a density of 1×10^5 cells per dish (10 cm in diameter) and cultured in DMEM containing 25 mM glucose, 10% fetal bovine serum, and 63 μM 2-mercaptoethanol. One day after plating, mitiglinide (10 or 100 nM), nateglinide (10 μM), repaglinide (1 μM), tolbutamide (100 μM), or glibenclamide (10 or 100 nM) was added to the culture medium. The cells were cultured with the medium containing one of these drugs for 14 days, and were then plated onto a 48-well plate at a density of 1×10^5 to measure insulin secretion in response to various stimuli (Kawaki et al., 1999).

2.3. Electrophysiological analysis of K_{ATP} channels

The transfected COS-1 cells were cultured for 48–96 h before recordings and were selected by green fluorescence (Inagaki et al., 1996). Recordings were made in the excised inside-out patch-clamp mode as previously described (Inagaki et al., 1995). The bath solution contained 140 mM KCl, 2 mM EGTA, 2 mM $MgCl_2$, and 10 mM HEPES (pH = 7.3). The pipette solution contained 140 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH = 7.4). Recordings were made at 20–22 °C. K^+ conductance of K_{ATP} channels were measured as described previously (Kawaki et al., 1999).

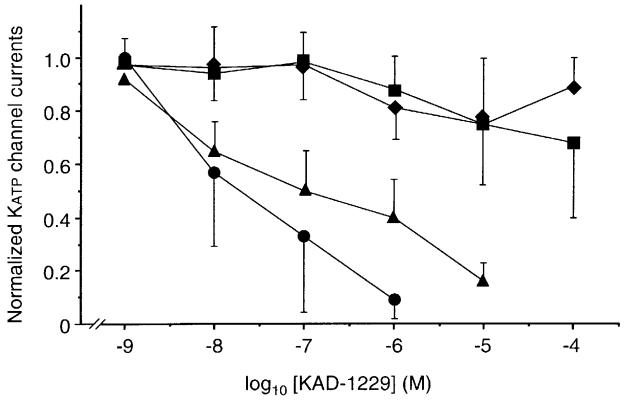

Fig. 3. Dose-dependent effects of mitiglinide on K_{ATP} channels. COS-1 cells were transfected with mouse Kir6.2 and hamster SUR1 (circle), human Kir6.2 and human SUR1 (triangle), mouse Kir6.2 and rat SUR2A (diamond), or mouse Kir6.2 and rat SUR2B (square). The K_{ATP} channel currents were normalized to the amplitude before applying mitiglinide. Data are presented as the means ± S.E. of 5–12 recording from different cells.

2.4. Inhibition of [^{3}H]glibenclamide binding to human SUR1 by various anti-diabetic drugs

Binding experiments of COS-1 cells transfected with human SUR1 were performed as described (Kawaki et al., 1999).

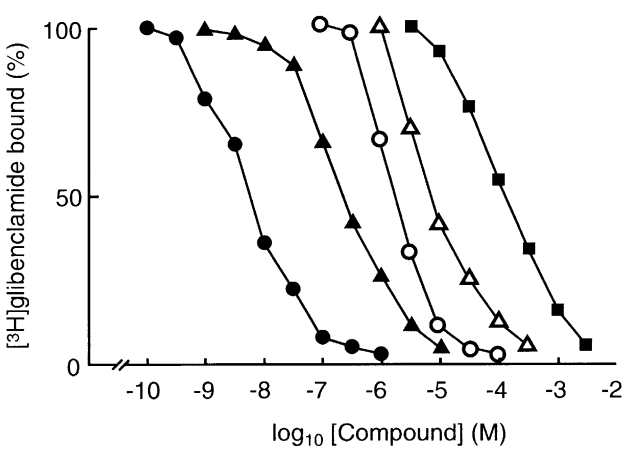

Fig. 4. Inhibition of [^{3}H]glibenclamide binding to human SUR1 by mitiglinide, glibenclamide, repaglinide, nateglinide, or tolbutamide. [^{3}H]glibenclamide binding to human SUR1 was displaced by unlabeled mitiglinide (closed triangle), glibenclamide (closed circle), repaglinide (open circle), nateglinide (open triangle), and tolbutamide (closed square). Values are the means of three independent experiments. Data are presented as the means ± S.E. of four independent experiments.

2.5. Measurements of insulin secretion and insulin content

The cells were preincubated for 30 min in KRB buffer containing 154 mM NaCl, 6.2 mM KCl, 3.3 mM $CaCl_2$, 1.5 mM KH_2PO_4, 1.6 mM $MgSO_4$, 12.4 mM $NaHCO_3$, 20 mM HEPES (pH = 7.4), and 0.2% bovine serum albumin containing 3 mM glucose. The cells were then stimulated by adding 500 μl of KRB buffer containing 25 mM glucose or 100 nM mitiglinide, 100 μM tolbutamide, or 100 nM glibenclamide in the presence of 3 mM glucose. Insulin secreted into the medium was measured by radioimmunoassay (Eiken Chemical, Tokyo, Japan). Insulin content was measured as described previously (Kawaki et al., 1999).

3. Results

Representative recordings obtained from excised inside-out patches in COS-1 cells expressing K_{ATP} channels are shown in Figs. 1 and 2. ATP inhibited almost completely the three types of K_{ATP} channel currents at a concentration of 1 mM (Figs. 1A–C and 2A–C). Mitiglinide at a concentration of 100 nM clearly inhibited the Kir6.2/SUR1 channel currents (Fig. 1A). In contrast, no significant inhibition of the Kir6.2/SUR2A channel currents with mitiglinide was observed even at the highest concentration tested (100 μM) (Fig. 1B). The Kir6.2/SUR2B channel currents also were not affected significantly by mitiglinide (Fig. 1C). The inhibitory effect of mitiglinide (100 nM) on the Kir6.2/SUR1 channel was reversible, and the current

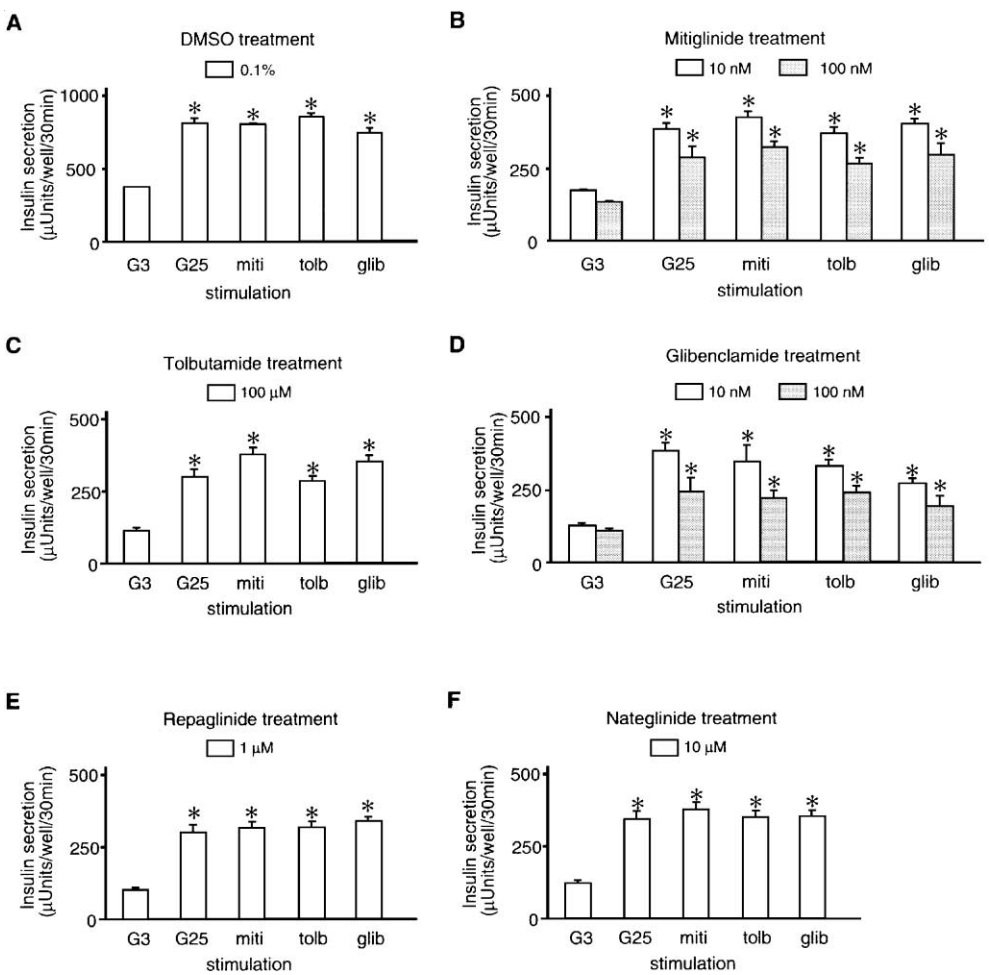

Fig. 5. Insulin secretion in MIN6 cells after chronic treatment with mitiglinide, glibenclamide, repaglinide, nateglinide, or tolbutamide. Insulin secretion in acute response to various stimuli in MIN6 cells treated with 0.1% DMSO (control) ($n = 16$) (A), with 10 or 100 nM mitiglinide ($n = 16$) (B), with 100 μM tolbutamide ($n = 12$) (C), with 10 or 100 nM glibenclamide ($n = 12$) (D), with 1 μM repaglinide ($n = 12$) (E), or with 10 μM nateglinide ($n = 12$) (F). G3, basal insulin secretion at 3 mM glucose; G25, insulin secretion stimulated by 25 mM glucose; KAD, insulin secretion stimulated by 100 nM mitiglinide in the presence 3 mM glucose; tolb, insulin secretion stimulated by 100 μM tolbutamide in the presence 3 mM glucose; glib, insulin secretion stimulated by 100 nM glibenclamide in the presence 3 mM glucose. Values are means ± S.E. $^{*}P < 0.01$ (compared with insulin secretion at 3 mM glucose).

amplitude recovered with a half recovery time of less than 2 min (115 ± 18 s) after starting washout (Fig. 1A). In contrast, the effect of glibenclamide was irreversible, and the current did not recover significantly even after continuous washing for more than 1 h ($n = 5$) (Fig. 1D). On the other hand, nateglinide inhibited both Kir6.2/SUR1 and Kir6.2/SUR2B channel currents at 100 nM (Fig. 2A), and inhibited Kir6.2/SUR2A channel currents at 1 μM (Fig. 2B,C). The dose-dependent effects of mitiglinide on K_{ATP} channel currents are shown in Fig. 3. Mitiglinide inhibited both human Kir6.2/human SUR1 K_{ATP} channel currents and mouse Kir6.2/hamster SUR1 K_{ATP} channel currents in a dose-dependent manner (IC_{50} values: 100 nM for human Kir6.2/human SUR1 channel; 22 nM for mouse Kir6.2/hamster SUR1 channel). In contrast, mitiglinide even at high concentrations (1 μM or more) showed no significant inhibitory effect on either mouse Kir6.2/rat SUR2A or mouse Kir6.2/rat SUR2B channels.

Specific binding of [^{3}H]-labeled glibenclamide to human SUR1 expressed in COS-1 cells was inhibited by unlabeled glibenclamide, mitiglinide, repaglinide, nateglinide, and tolbutamide in a concentration-dependent manner (Fig. 4). The IC_{50} values for glibenclamide, mitiglinide, repaglinide, nateglinide and tolbutamide were 7.1 and 280 nM, 1.6, 8 and 140 μM, respectively. We also found that coexpression of SUR1 with Kir6.2 lowered the IC_{50} values in all cases (data not shown).

Acute insulin secretory responses to various stimuli in MIN6 cells treated chronically with mitiglinide, repaglinide, nateglinide, tolbutamide or glibenclamide are shown in Fig. 5. As control, cells treated with vehicle (DMSO) alone were used. Basal insulin secretion in the presence of 3 mM glucose was significantly lower in MIN6 cells treated with mitiglinide, tolbutamide, glibenclamide, repaglinide, and nateglinide for 2 weeks than in control. Following chronic exposure to mitiglinide at a concentration of 10 or 100 nM, the insulin secretion in response to various stimuli was decreased compared to control. However, the insulin responses to various stimuli after chronic mitiglinide treatment were comparable to those after repaglinide, nateglinide, tolbutamide, or glibenclamide treatment. There was no significant difference in insulin content in MIN6 cells treated with each drug (Fig. 6). The K_{ATP} channel conductance, normalized by dividing by the membrane capacitance, in MIN6 cells treated with 100 nM mitiglinide was not different from that in control (mitiglinide treatment, 1.47 ± 0.44 nS/pF, $n = 10$; control, 1.01 ± 0.37 nS/pF, $n = 5$), indicating that there was no loss of K_{ATP} channels in MIN6 cells treated with mitiglinide.

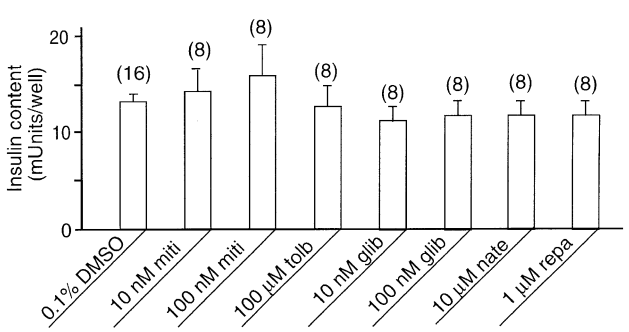

Fig. 6. Insulin content in MIN6 cells after chronic treatment with mitiglinide, glibenclamide, repaglinide, nateglinide, or tolbutamide. Insulin content of MIN6 cells after treatment with vehicle (control), 10 or 100 nM mitiglinide (miti), 1 μM repaglinide (repa), 10 μM nateglinide (nate), 100 μM tolbutamide (tolb), or 10 or 100 nM glibenclamide (glib). Numbers in parentheses indicate the number of independent experiments. Values are means ± S.E.

4. Discussion

We have shown that mitiglinide inhibits Kir6.2/SUR1 (pancreatic β-cell type) K_{ATP} channels in a dose dependent-manner, but has no significant inhibitory effect on either Kir6.2/SUR2A (cardiac and skeletal muscle type) K_{ATP} channels or Kir6.2/SUR2B K_{ATP} channels even at high concentrations (100 μM). In radioligand binding assay, mitiglinide inhibited the binding of [^{3}H]glibenclamide to SUR1 expressed in COS-1 cells. The affinity of human SUR1 for mitiglinide was about 500-fold higher than that for tolbutamide, but 40-fold lower than that for glibenclamide. The IC_{50} for the effect of mitiglinide on Kir6.2/SUR1 channel current was consistent with that found by the [^{3}H]glibenclamide displacement assay of mitiglinide to human SUR1. These results demonstrate that mitiglinide inhibits human pancreatic β-cell type K_{ATP} channels specifically by binding to SUR1.

The tissue selectivity of some anti-diabetic drugs has been demonstrated by Gribble et al. (1998): (i) tolbutamide inhibits Kir6.2/SUR1 channels but not Kir6.2/SUR2A channels; (ii) glibenclamide blocks both Kir6.2/SUR1 ($K_i = 4$ nM) and Kir6.2/SUR2B ($K_i = 27$ nM) channels; and (iii) meglitinide, a non-sulfonylurea hypoglycemic agent with a benzamide moiety, inhibits Kir6.2/SUR1 and Kir6.2/SUR2A channels at similar concentrations ($K_i =$ 0.3 and 0.5 μM, respectively). Gliclazide also has high selectivity to Kir6.2/SUR1 channels (Lawrence et al., 2001). They propose that SUR1 possesses binding sites for both drugs (a sulfonylurea binding site and a benzamide binding site), while SUR2A has only a benzamide binding site. They suggest that tolbutamide and gliclazide bind to the sulfonylurea binding site while glibenclamide binds to both sites, and that meglitinide binds only to the benzamide binding site, which implies that glibenclamide and meglitinide both bind to SUR1 and SUR2 and that tolbutamide binds only to SUR1. Recently, Reimann et al. (2001) reported that 100 nM mitiglinide blocked only Kir6.2/SUR1 channels, while 10 μM inhibited the Kir6.2/SUR1, Kir6.2/SUR2A, and Kir6.2/SUR2B channels in *Xenopus* oocytes. They suggest that SUR1 has a

high affinity site and Kir6.2 a low affinity site for mitiglinide. However, in the present study, we observed that 10 μM of mitiglinide does not inhibit either the Kir6.2/SUR2A or Kir6.2/SUR2B channels in COS-1 cells. The discrepancy between the two studies is not clear, but is probably due at least in part to the different expression systems used. On the other hand, nateglinide inhibits all the Kir6.2/SUR1, Kir6.2/SUR2A, and Kir6.2/SUR2B channels. Another meglitinide-related compound, repaglinide, already has been shown to inhibit all the Kir6.2/SUR1, Kir6.2/SUR2A, and Kir6.2/SUR2B channels (Dabrowski et al., 2001). High tissue selectivity of mitiglinide also has been found in native K_{ATP} channels (Mogami et al., 1994), so mitiglinide resembles tolbutamide in terms of tissue selectivity. In addition, mitiglinide inhibits the binding of [^{3}H]glibenclamide to SUR1. These results suggest that mitiglinide binds to the sulfonylurea binding site in SUR1 but not to the benzamide binding site in SUR2A.

The K_{ATP} channels in heart have been associated with the cardioprotective mechanism of ischemia-related preconditioning (Cole et al., 1991; Terzic et al., 1995). Furthermore, recovery of cardiac contractility after ischemia and reperfusion is significantly suppressed in the presence of glibenclamide (Shigematsu et al., 1995). It has been shown recently that although high concentrations (300 μM and 1 mM) of mitiglinide only slightly inhibit the K_{ATP} channels in cardiovascular tissues, mitiglinide at 3 μM does not inhibit the channels (Hu et al., 1999), consistent with the present study. These findings suggest that mitiglinide may not impair cardiac function after myocardial damage and that the drug may be useful clinically. Higher selectivity for the K_{ATP} channels of the pancreatic β-cells than those of cardiovascular tissues is a desirable feature for antidiabetic agents to be used in treatment of type 2 diabetes.

Sulfonylureas have been used in the treatment of type 2 diabetes mellitus for many years but several reports have shown that chronic sulfonylurea treatment produces unresponsiveness to subsequent stimulation both in vitro and in vivo (Karam et al., 1986; Davalli et al., 1992; Rabuazzo et al., 1992). In a previous study, we found that the insulin secretion in response to glibenclamide in MIN6 cells was severely impaired after chronic exposure to 10 μM glibenclamide (Kawaki et al., 1999). In the present study, we used lower concentrations of glibenclamide for chronic treatment of MIN6 cells and found that insulin secretion in acute response to mitiglinide, tolbutamide, or glibenclamide, after chronic treatment with the respective drugs was similarly retained to some extent. It has been reported that glucose-induced insulin secretion from mice islets treated with glibenclamide or tolbutamide was delayed although there was no reduction in insulin secretion (Anello et al., 1999). However, we find that glucose-induced insulin secretion is significantly suppressed in MIN6 cells treated with mitiglinide, repaglinide, nateglinide, glibenclamide or tolbutamide for 2 weeks. The discrepancy is due probably to the different experimental conditions used, including the different materials (MIN6 vs. pancreatic islets) and the different exposure periods (2 weeks vs. 18–20 h).

In conclusion, we provide direct evidence that mitiglinide binds to human SUR1 and exhibits a high degree of specificity for the pancreatic β-cell K_{ATP} channel. Because the effects of mitiglinide on the pancreatic β-cell K_{ATP} channel are reversible and the insulin secretory responses to the various stimuli after chronic exposure to the drug are similar to those of tolbutamide and glibenclamide, mitiglinide may be a clinically useful anti-diabetic drug in the treatment of type 2 diabetes.

Acknowledgements

This study was supported by Grants-in-Aid for Creative Scientific Research (10NP0201) from the Japan Science Promotion Society, and for Scientific Research from the Ministry of Education, Culture Sports, Science and Technology, and from the Ministry of Health, Labor and Welfare, Japan.

References

Aguilar-Bryan, L., Clement, J.P.T., Gonzalez, G., Kunjilwar, K., Babenko, A., Bryan, J., 1998. Toward understanding the assembly and structure of K_{ATP} channels. Physiol. Rev. 78, 227–245.

Anello, M., Gilon, P., Henquin, J.C., 1999. Alterations of insulin secretion from mouse islets treated with sulphonylureas: perturbations of Ca^{2+} regulation prevail over changes in insulin contents. Br. J. Pharmacol. 127, 1833–1891.

Ashcroft, F.M., 1988. Adenosine 5′-triphosphate-sensitive potassium channels. Annu. Rev. Neurosci. 11, 97–118.

Ashcroft, F.M., Gribble, F.M., 1998. Correlating structure and function in ATP-sensitive K^+ channels. Trends Neurosci. 21, 288–294.

Ashford, M.L., Sturgess, N.C., Trout, N.J., Gardner, N.J., Hales, C.N., 1988. Adenosine-5′-triphosphate-sensitive ion channels in neonatal rat cultured central neurones. Pfluegers Arch. 412, 297–304.

Chutkow, W.A., Simon, M.C., Le Beau, M.M., Burant, C.F., 1996. Cloning, tissue expression, and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle, and vascular K_{ATP} channels. Diabetes 45, 1439–1445.

Chutkow, W.A., Makielski, J.C., Nelson, D.J., Burant, C.F., Fan, Z., 1999. Alternative splicing of SUR2 Exon 17 regulates nucleotide sensitivity of the ATP-sensitive potassium channel. J. Biol. Chem. 274, 13656–13665.

Cole, W.C., McPherson, C.D., Sontag, D., 1991. ATP-regulated K^+ channels protect the myocardium against ischemia/reperfusion damage. Circ. Res. 69, 571–581.

Cook, D.L., Hales, C.N., 1984. Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. Nature 311, 271–273.

Dabrowski, M., Wahl, P., Holmes, W.E., Ashcroft, F.M., 2001. Effect of repaglinide on cloned beta cell, cardiac and smooth muscle types of ATP-sensitive potassium channel. Diabetologia 44, 747–756.

Davalli, A.M., Pontiroli, A.E., Socci, C., Bertuzzi, F., Fattor, B., Braghi, S., Di Carlo, V., Pozza, G., 1992. Human islets chronically exposed in vitro to different stimuli become unresponsive to the same stimuli given acutely: evidence supporting specific desensitization rather than β-cell exhaustion. J. Clin. Endocrinol. Metab. 74, 790–794.

Gribble, F.M., Tucker, S.J., Seino, S., Ashcroft, F.M., 1998. Tissue specificity of sulfonylureas: studies on cloned cardiac and β-cell K_{ATP} channels. Diabetes 47, 1412–1418.

Hu, S., Wang, S., Dunning, B.E., 1999. Tissue selectivity of antidiabetic agent nateglinide: study on cardiovascular and β-cell K_{ATP} channels. J. Pharmacol. Exp. Ther. 291, 1372–1379.

Inagaki, N., Gonoi, T., Clement, J.P.T., Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., Bryan, J., 1995. Reconstitution of I_{KATP}: an inward rectifier subunit plus the sulfonylurea receptor. Science 270, 1166–1170.

Inagaki, N., Gonoi, T., Clement, J.P.T., Wang, C.Z., Aguilar-Bryan, L., Bryan, J., Seino, S., 1996. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. Neuron 16, 1011–1017.

Isomoto, S., Kondo, C., Yamada, M., Matsumoto, S., Higashiguchi, O., Horio, Y., Matsuzawa, Y., Kurachi, Y., 1996. A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. J. Biol. Chem. 271, 24321–24324.

Karam, J.H., Sanz, N., Salamon, E., Nolte, M.S., 1986. Selective unresponsiveness of pancreatic β-cells to acute sulfonylurea stimulation during sulfonylurea therapy in NIDDM. Diabetes 35, 1314–1320.

Kawaki, J., Nagashima, K., Tanaka, J., Miki, T., Miyazaki, M., Gonoi, T., Mitsuhashi, N., Nakajima, N., Iwanaga, T., Yano, H., Seino, S., 1999. Unresponsiveness to glibenclamide during chronic treatment induced by reduction of ATP-sensitive K^+ channel activity. Diabetes 48, 2001–2006.

Lawrence, C.L., Proks, P., Rodrigo, G.C., Jones, P., Hayabuchi, Y., Standen, N.B., Ashcroft, F.M., 2001. Gliclazide produces high-affinity block of K_{ATP} channels in mouse isolated pancreatic beta cells but not rat heart or arterial smooth muscle cells. Diabetologia 44, 1019–1025.

Miki, T., Nagashima, K., Tashiro, F., Kotake, K., Yoshitomi, H., Tamamoto, A., Gonoi, T., Iwanaga, T., Miyazaki, J., Seino, S., 1998. Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. Proc. Natl. Acad. Sci. U.S.A. 95, 10402–10406.

Mogami, H., Shibata, H., Nobusawa, R., Ohnota, H., Satou, F., Miyazaki, J., Kojima, I., 1994. Inhibition of ATP-sensitive K^+ channel by a non-sulfonylurea compound KAD-1229 in a pancreatic β-cell line, MIN6 cell. Eur. J. Pharmacol. 269, 293–298.

Noma, A., 1983. ATP-regulated K^+ channels in cardiac muscle. Nature 305, 147–148.

Ohnota, H., Koizumi, T., Tsutsumi, N., Kobayashi, M., Inoue, S., Sato, F., 1994. Novel rapid- and short-acting hypoglycemic agent, a calcium(2*s*)-2-benzyl-3-(*cis*-hexahydro-2-isoindolinylcarbonyl) propionate (KAD-1229) that acts on the sulfonylurea receptor: comparison of effects between KAD-1229 and gliclazide. J. Pharmacol. Exp. Ther. 269, 489–495.

Ohnota, H., Koizumi, T., Kobayashi, M., Momose, Y., Sato, F., 1995. Normalization of impaired glucose tolerance by the short-acting hypoglycemic agent calcium (2*S*)-2-benzyl-3-(*cis*-hexahydro-2-isoindolinylcarbonyl)propionate dihydrate (KAD-1229) in non-insulin-dependent diabetes mellitus rats. Can. J. Physiol. Pharmacol. 73, 1–6.

Rabuazzo, A.M., Buscema, M., Vinci, C., Caltabiano, V., Vetri, M., Forte, F., Vigneri, R., Purrello, F., 1992. Glyburide and tolbutamide induce desensitization of insulin release in rat pancreatic islets by different mechanisms. Endocrinology 131, 1815–1820.

Reimann, F., Proks, P., Ashcroft, F.M., 2001. Effects of mitiglinide (S21403) on Kir6.2/SUR1, Kir6.2/SUR2A and Kir6.2/SUR2B types of ATP-sensitive potassium channel. Br. J. Pharmacol. 132, 1542–1548.

Sakura, H., Ammala, C., Smith, P.A., Gribble, F.M., Ashcroft, F.M., 1995. Cloning and functional expression of the cDNA encoding a novel ATP-sensitive potassium channel subunit expressed in pancreatic β-cells, brain, heart and skeletal muscle. FEBS Lett. 377, 338–344.

Seino, S., 1999. ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. Annu. Rev. Physiol. 61, 337–362.

Shigematsu, S., Sato, T., Abe, T., Saikawa, T., Sakata, T., Arita, M., 1995. Pharmacological evidence for the persistent activation of ATP-sensitive K^+ channels in early phase of reperfusion and its protective role against myocardial stunning. Circulation 92, 2266–2275.

Spruce, A.E., Standen, N.B., Stanfield, P.R., 1985. Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. Nature 316, 736–738.

Standen, N.B., Quayle, J.M., Davies, N.W., Brayden, J.E., Huang, Y., Nelson, M.T., 1989. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. Science 245, 177–180.

Terzic, A., Jahangir, A., Kurachi, Y., 1995. Cardiac ATP-sensitive K^+ channels: regulation by intracellular nucleotides and K^+ channel-opening drugs. Am. J. Physiol. 269, C525–C545.

Ueda, K., Komine, J., Matsuo, M., Seino, S., Amachi, T., 1999. Cooperative binding of ATP and MgADP in the sulfonylurea receptor is modulated by glibenclamide. Proc. Natl. Acad. Sci. U.S.A. 96, 1268–1272.

Yamada, M., Isomoto, S., Matsumoto, S., Kondo, C., Shindo, T., Horio, Y., Kurachi, Y., 1997. Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K^+ channel. J. Physiol. (London) 499, 715–720.