Biochemical Pharmacology, Vol. 52, pp. 407–411, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
PPI S0006-2952(96)00242-0

Studies on the *N*-[(*trans*-4-Isopropylcyclohexyl)-carbonyl]-D-phenylalanine (A-4166) Receptor in HIT T-15 Cells

DISPLACEMENT OF [^{3}H]GLIBENCLAMIDE

Tomonobu Fujita,† Yoshiko Seto,* Nobuo Kondo‡ and Ryuichi Kato*§*

*Pharmaceutical Institute and §Department of Pharmacology, Keio University School of Medicine, Tokyo 160; and ‡Central Research Laboratories, Ajinomoto Co., Inc., Life Science Laboratories, Yokohama 244, Japan

ABSTRACT. A-4166 is a new type of oral hypoglycemic agent that does not contain a sulfonylurea moiety. To clarify the mechanism of insulin secretion by A-4166, a specific receptor for A-4166 was investigated in a hamster pancreatic β cell line (HIT T-15), using [^{3}H]A-4166 or [^{3}H]glibenclamide as a ligand. The saturation binding of [^{3}H]A-4166 to HIT cell membranes was not observed up to 10 μM. In the displacement study, unlabeled A-4166 inhibited [^{3}H]A-4166 binding to HIT cell membranes, but glibenclamide did not. On the other hand, A-4166 inhibited [^{3}H]glibenclamide binding to the sulfonylurea receptor (K_i = 248 nM). A-4166 inhibited ^{86}Rb efflux from HIT cells (IC_{50} = 350 nM). The EC_{50} for insulin secretion by A-4166 was 20 μM in HIT cells when they were incubated for 30 min in Krebs–Ringer bicarbonate buffer containing 16 mM HEPES supplemented with 5 mg/mL BSA in the absence of glucose. These data demonstrate the possibility of the presence of two kinds of binding sites for A-4166: one of them is the sulfonylurea receptor, and the other might be a binding site specific for A-4166. BIOCHEM PHARMACOL 52;3:407–411, 1996.

KEY WORDS. A-4166; receptor; insulin secretion; sulfonylurea; HIT

A new oral hypoglycemic agent, A-4166[‖], is a D-phenylalanine derivative that does not contain a sulfonylurea moiety [1–3]. The hypoglycemic action of A-4166 appears rapidly and does not last as long as that of sulfonylureas. In perfusion studies of the isolated rat pancreas, A-4166 stimulated insulin secretion and had little effect on glucagon secretion in the presence of 8 and 11 mM glucose [4, 5]. The major mechanism of the hypoglycemic action by A-4166 is due to the rapid onset of insulin release [6]. In rat pancreatic β cells, A-4166 increased $[Ca^{2+}]_i$ by stimulating Ca^{2+} influx through the L-type Ca^{2+} channel in a concentration-dependent manner (3–30 μM), but the effect disappeared in the presence of diazoxide [7]. These observations suggested that inhibition of K_{ATP} channels was involved in the increase of $[Ca^{2+}]_i$ in β cells by A-4166. From these findings, it was proposed that the mechanism of insulin secretion by A-4166 is similar to that by sulfonylureas. The mechanism of insulin secretion by sulfonylureas is as follows: sulfonylureas bind to the receptor on pancreatic β cells, inhibit ATP-sensitive potassium (K_{ATP}) channels for decreasing K^+ efflux, lead to membrane depolarization, open a voltage-dependent calcium channel, and increase $[Ca^{2+}]_i$. The rise in $[Ca^{2+}]_i$ induces the exocytosis of insulin [8, 9].

On the other hand, the insulin release by A-4166 was faster than that by glibenclamide in an *in situ* perfusion system using the Syrian hamster pancreas. The stimulating action on insulin release by A-4166 was also different from that by glibenclamide in response to the inhibitory effect of diazoxide on insulin release [10]. Therefore, we have studied whether A-4166 would bind to a receptor specific for itself or to a sulfonylurea receptor. In the present paper, we describe the binding of A-4166 to a specific site and to the sulfonylurea receptor in membranes of a hamster pancreatic β cell line (HIT T-15).

MATERIALS AND METHODS

Chemicals

A-4166 was synthesized in the Central Research Laboratories of the Ajinomoto Co., Ltd., Kawasaki, Japan, and [^{3}H]A-4166 (19.4 Ci/mmol) was specially synthesized at Amersham (Amersham, Buckinghamshire, U.K.). ^{86}RbCl was purchased from Amersham. [^{3}H]Glibenclamide (50.9 Ci/mmol) was purchased from NEN Research Products (Boston, MA, U.S.A.). Glibenclamide, tolbutamide, and

† Corresponding author: Dr. Tomonobu Fujita, Division of Chemotherapy, Pharmaceutical Institute, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan. Tel. 81-3-3353-1211, Ext. 2736; FAX 81-3-3225-1479.

‖*Abbreviations:* A-4166, *N*-[(*trans*-4-isopropylcyclohexyl)-carbonyl]-D-phenylalanine; MOPS, 3-(*N*-morpholino)propanesulfonic acid; and KRB–HEPES buffer, Krebs–Ringer bicarbonate buffer containing 16 mM HEPES.

Received 14 August 1995; accepted 20 February 1996.

bovine serum albumin were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other reagent grade chemicals were also purchased from the Iwai Kagakuyakuhin Co., Ltd. (Tokyo, Japan).

Preparation of HIT Cell Membranes

HIT cells (passages 80–84) were cultured in 100-mm dishes using RPMI 1640 medium containing glucose (2 g/L), kanamycin (60 mg/L) and 10% fetal bovine serum at 37° in an atmosphere of humidified air and CO_2 (95:5). The cells were harvested and stored at −80° until preparation of crude membrane fractions. The membrane fractions (900–100,000 *g*) were prepared from HIT cells as described by Gaines *et al.* [11]. A 1 mM concentration of phenylmethylsulfonyl fluoride was used instead of 1 mM diisopropyl fluorophosphate. The membranes were resuspended in a buffer (20 mM MOPS, 1 mM phenylmethylsulfonyl fluoride, pH 7.4) and then were frozen immediately in liquid nitrogen until binding studies. Proteins were determined by a dye-binding assay (Bio-Rad protein assay, Bio-Rad, Richmond, CA, U.S.A.) with BSA as a standard.

Binding Assay for [^{3}H]A-4166

HIT cell membranes (82 μg of protein) were incubated with various concentrations of [^{3}H]A-4166 in 0.5 mL/tube of the binding buffer (50 mM MOPS, 0.1 mM $CaCl_2$, pH 7.4) for 2 hr at room temperature. Nonspecific binding was determined in the presence of 1 mM unlabeled A-4166. Bound and free [^{3}H]A-4166 were separated by rapid filtration through Whatman GF/B filters. The filters were washed once with 5 mL of ice-cold 50 mM Tris–HCl buffer, pH 7.4. Radioactivity was determined by liquid scintillation spectrometry. In the displacement study, the membranes were incubated with 0.1 μM [^{3}H]A-4166 and various concentrations of test substance for 2 hr at room temperature. The separation was performed as described above.

Binding Assay for [^{3}H]Glibenclamide

To determine total binding, HIT cell membranes (82 μg of protein) were incubated for 2 hr at room temperature with various concentrations of [^{3}H]glibenclamide in 1 mL of the binding buffer. Nonspecific binding was determined in the presence of 1 μM unlabeled glibenclamide. Binding was terminated by rapid filtration through Whatman GF/B filters followed by five washes with 5 mL of ice-cold water. K_D and B_{max} values were estimated by Scatchard analysis. Displacement study was performed using 1.25 nM [^{3}H]glibenclamide and various concentrations of the test substance. The incubation and separation were done as described above.

^{86}Rb Efflux Measurement

^{86}Rb efflux experiments were performed as described by Schmid–Antomarchi *et al.* [12] with a minor modification. HIT cells (5×10^5 cells) were added into 24-well plates and cultured for 2–4 days. On the day before an experiment, 0.1 μCi/well of ^{86}RbCl was loaded into cells overnight. Then the cultured medium was changed to an efflux buffer (124 mM NaCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 10 mM KCl, and 20 mM HEPES, pH 7.4) supplemented with ^{86}RbCl in the presence of 2-deoxyglucose (1 mM) and oligomycin (1.25 μg/mL), and the cells were incubated for 20 min at 37°. The incubated solution was removed and replaced with the efflux buffer containing a test substance. After a 5-min incubation, the solution was removed, and the intracellular ^{86}Rb contents were measured.

Measurement of Insulin Release

HIT cells (passages 75–80) were added in 24-well plates at a density of 2.5×10^5 cells/well and cultured for 3 days. Twenty-four hours before an experiment, the cultured medium was changed to a fresh RPMI 1640 medium containing 1 g/L of glucose. In the experiment to determine insulin release, cells were washed twice with KRB–HEPES buffer containing 5 mg/mL BSA in the absence of glucose. After preincubation in the same buffer for 60 min at 37°, the buffer was removed. Buffer (0.5 mL) containing an indicated concentration of test substances was added to the wells, and the cells were incubated for 30 min at 37°. The supernatants were collected, briefly centrifuged to remove cell debris, and stored at −80°. Immunoreactive insulin was measured by radioimmunoassay, using an Eiken kit (Eiken, Tokyo, Japan).

RESULTS

Binding Assay

A specific receptor for A-4166 was investigated in HIT cell membranes using [^{3}H]A-4166 as a ligand. Binding of [^{3}H]A-4166 in the membranes increased in a concentration-dependent manner, but saturable binding was not observed up to 10 μM (data not shown). The results from a displacement study using 0.1 μM [^{3}H]A-4166 are shown in Fig. 1. The binding of [^{3}H]A-4166 to the membranes was inhibited concentration dependently by unlabeled A-4166 at a maximal concentration of 1 mM, which was limited by the solubility of A-4166. Glibenclamide and tolbutamide did not inhibit the binding of [^{3}H]A-4166. The total binding of [^{3}H]A-4166 to the membranes was about 0.6% of the total count. The amount of nonspecific binding was about two-thirds of the total binding. An attempt to reduce the nonspecific binding did not succeed.

The binding of A-4166 to a sulfonylurea receptor in HIT cell membranes was then studied using [^{3}H]glibenclamide. To confirm the presence of high-affinity sulfonylurea receptors in the HIT cell membranes, a value for the dissociation constant (K_D) of glibenclamide was determined. Total and nonspecific bindings were examined in the presence and absence of 1 μM glibenclamide. The K_D for glib-

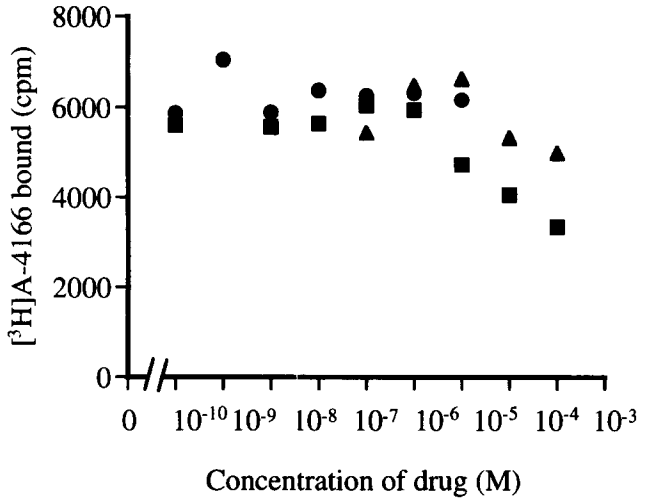

FIG. 1. Displacement of [³H]A-4166 in HIT cell membranes by A-4166 and sulfonylureas. [³H]A-4166 (0.1 μM) was added to 82 μg of HIT cell membranes in the presence of various concentrations of glibenclamide (●), unlabeled A-4166 (■), and tolbutamide (▲) and was incubated for 2 hr at room temperature. The figure shows a typical pattern in three displacement studies.

enclamide was 0.29 nM, and the maximum number of binding sites was 560 fmol/mg protein as determined by Scatchard analysis (data not shown).

[³H]Glibenclamide was used at a concentration of 1.25 nM for displacement analysis of A-4166. A-4166 concentration dependently inhibited [³H]glibenclamide binding to HIT cell membranes (Fig. 2). The IC_{50} for A-4166 was 1.5 μM, and the inhibition constant (K_i) was calculated to be 248 nM. The IC_{50} and K_i values for tolbutamide were 100 and 16.5 μM, respectively. The affinity of A-4166 for the sulfonylurea receptor was approximately 67 times as high as that of tolbutamide and about 380 times as low as that of glibenclamide in the present binding assay.

The number of sulfonylurea receptors in the HIT cell membranes did not change throughout these studies. The insulin responses to A-4166 and glibenclamide in HIT cells were not altered by the increasing passages from 75 to 84.

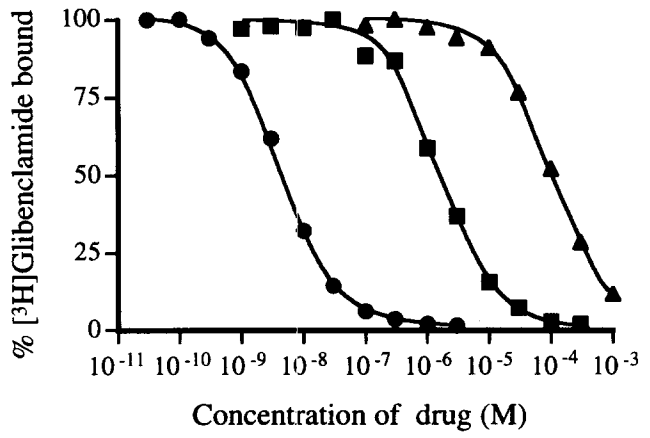

FIG. 2. Displacement of [³H]glibenclamide in HIT cell membranes by A-4166 and sulfonylureas. [³H]Glibenclamide (1.25 nM) was added to 82 μg of HIT cell membranes in the presence of various concentrations of unlabeled glibenclamide (●), A-4166 (■), and tolbutamide (▲) and incubated for 2 hr at room temperature. Results represent the means of three separate experiments.

Effect of A-4166 on ^{86}Rb Efflux

The effect of A-4166 on K_{ATP} channels was examined in HIT cells prelabeled with ^{86}Rb. After intracellular ATP concentrations were depleted by 2-deoxyglucose and oligomycin, the closing activity of K_{ATP} channels by A-4166 was estimated after a 5-min incubation. As shown in Fig. 3, A-4166, glibenclamide, and tolbutamide concentration dependently inhibited ^{86}Rb efflux. The IC_{50} values for ^{86}Rb efflux of glibenclamide, A-4166, and tolbutamide were 8 nM, 350 nM, and 25 μM, respectively.

Effect of A-4166 on Insulin Secretion in HIT Cells

The effect on insulin secretion of A-4166 was examined in HIT cells that were incubated with various concentrations of A-4166 in a buffer containing 0.5 mg/mL BSA. Figure 4 shows the concentration-dependent curves of insulin secretion in HIT cells. A-4166 stimulated insulin secretion at

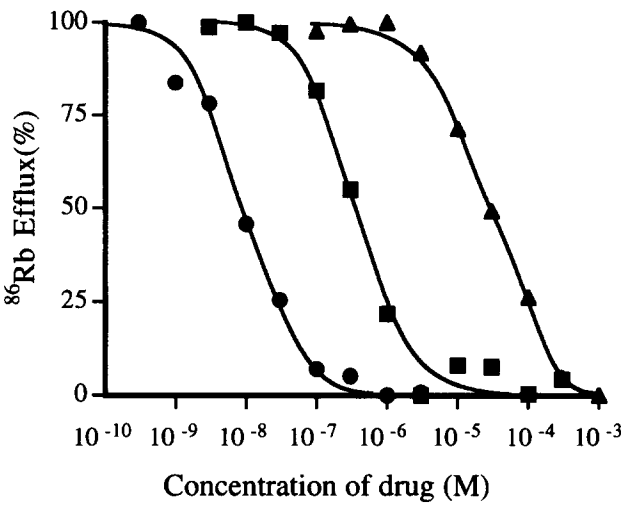

FIG. 3. Inhibition of ^{86}Rb efflux from HIT cells by A-4166 and sulfonylureas. ^{86}Rb (0.1 μCi/well) was loaded onto cells as described in Materials and Methods. To deplete the intracellular ATP, cells were preincubated with oligomycin (1.25 μg/mL) and 2-deoxy-D-glucose. After the preincubation, medium was replaced with various concentrations of glibenclamide (●), A-4166 (■), and tolbutamide (▲). The ^{86}Rb remaining in the cells was measured by Cerenkov counting after a 5-min incubation. The full range efflux was determined with and without 3 μM glibenclamide. Results represent the means of six separate experiments.

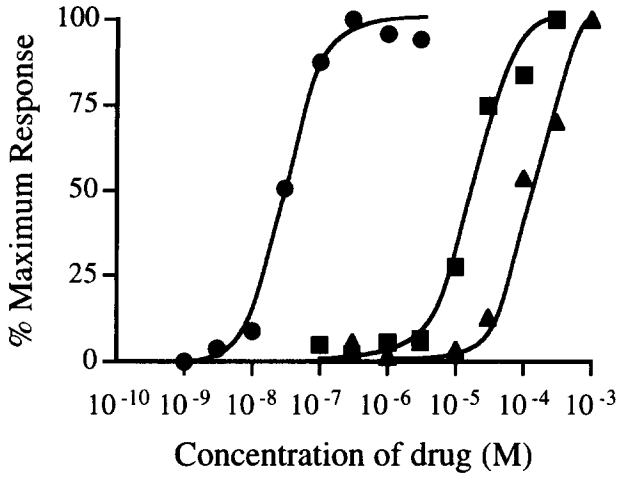

FIG. 4. Effect of A-4166 on insulin release in HIT cells. After preincubation with glucose-free KRB–HEPES buffer containing 5 mg/mL BSA for 60 min, HIT cells were incubated with various concentrations of glibenclamide (●), A-4166 (■), and tolbutamide (▲) in buffer for 30 min. Values are the means of two separate experiments.

concentrations between 3 and 300 μM, and the EC_{50} was 20 μM. The EC_{50} values of glibenclamide and tolbutamide were 30 nM and 100 μM, respectively, under the same conditions.

DISCUSSION

A-4166 is a new type of oral hypoglycemic agent that does not contain a sulfonylurea moiety, and whose hypoglycemic action very quickly occurs and disappears in experimental animals and humans [6, *]. It is thought that this characteristic hypoglycemic action of A-4166 is due to the direct and quick stimulation of insulin secretion and its rapid termination. To clarify the mechanism of insulin secretion by A-4166, we examined a specific receptor for A-4166 in HIT cells.

The binding of [^{3}H]A-4166 to HIT cell membranes was observed to be concentration dependent, but the binding was not saturated by 10 μM A-4166. In a displacement study using 0.1 μM [^{3}H]A-4166, the binding of [^{3}H]A-4166 was inhibited by unlabeled A-4166, but not by glibenclamide and tolbutamide. This finding suggests the presence of a low-affinity binding site for A-4166. However, it was hard to determine the values of K_D and B_{max} for the binding site because of poor reproducibility owing to its low affinity and high incidence of nonspecific binding. On the other hand, insulin secretion and increase in $[Ca^{2+}]_i$ by A-4166 were detectable even at a 3 μM concentration in the presence of BSA. As A-4166 will bind to serum albumin, free drug may be decreased to under 10% of the concentration used (unpublished observation). The maximal concentration (10 μM) of A-4166 employed in the binding assay was free drug. Therefore, this concentration was sufficient to stimulate the insulin secretion by A-4166 in the presence of BSA. However, it will be difficult to relate the low-affinity binding site with the stimulating action of A-4166 on insulin secretion directly.

A-4166 also inhibited the binding of [^{3}H]glibenclamide. Glibenclamide may inhibit the binding of [^{3}H]A-4166, although such inhibition was not observed. However, the number of sulfonylurea receptors was only a small percentage of the binding sites for A-4166. The inhibition of the binding of [^{3}H]A-4166 by glibenclamide was hidden by error-ranges of [^{3}H]A-4166 binding. For the same reason, we might not be able to detect a small amount on a high-affinity binding site.

Evidence from $[Ca^{2+}]_i$ and patch-clamp studies has suggested that the mode of action of A-4166 on insulin secretion is related to the K_{ATP} channels [7, 13]. Investigators have demonstrated that sulfonylurea binds to a high-affinity sulfonylurea receptor that is closely related to the K_{ATP} channel in pancreatic β cells [11, 12, 14, 15]. In our displacement study using [^{3}H]glibenclamide, A-4166 bound to the sulfonylurea receptor in HIT cells. A-4166 inhibited ^{86}Rb efflux from HIT cells. The inhibition of [^{3}H]glibenclamide binding to membranes and of ^{86}Rb efflux by A-4166 was shown at a similar range of concentrations. The IC_{50} of ^{86}Rb efflux by A-4166 agreed with that of K_{ATP} channel currents in whole cell patch [13]. The relation between the IC_{50} of ^{86}Rb efflux and the K_i of the sulfonylurea receptor by A-4166 showed good correlation with sulfonylureas [12]. The EC_{50} of insulin release by A-4166 was about 80 times higher than that of binding to the sulfonylurea receptor. The inconsistency in the IC_{50} and EC_{50} values of A-4166 may be explained by the same reason that, in glibenclamide, the concentration of free drug was decreased by binding to albumin, which was added to prevent insulin absorption during experiments [11, 15].

A-4166 is a non-sulfonylurea hypoglycemic agent. It is reported that several hypoglycemic compounds, which do not possess a sulfonylurea structure, inhibit K_{ATP} channels and stimulate insulin secretion [16–22] and that some of them inhibit the K_{ATP} channel by acting on a sulfonylurea receptor. From these results, it is demonstrated that A-4166 also acts on sulfonylurea receptors and stimulates insulin secretion in the same manner as sulfonylureas.

The characteristics of A-4166 in the *in vivo* experiments, i.e. the rapid onset and short duration of hypoglycemic action, can not be explained simply by these observations in pancreatic β cells. Its pharmacokinetic profile may also be related to the rapid absorption and metabolism of A-4166. We previously reported that the insulin release by A-4166 was faster than that by glibenclamide in an *in situ* perfusion system using the Syrian hamster pancreas. The stimulating action on insulin release by A-4166 was also different from that by glibenclamide in response to the

* Kikuchi M, Kuzuya T, Akanuma Y and Kosaka K, Early phase II study of a novel oral hypoglycemic agent, AY4166, in Japan. *15th International Diabetes Federation Congress, Kobe, Japan, 6–11 Nov. 1994*, 418, 1994.

inhibitory effect of diazoxide on insulin release [10]. Therefore, the specific binding site with low affinity for A-4166 may play a role in that function.

We thank Dr. T. Nakaki, Keio University, for helpful advice and encouragement.

References

1. Seto Y, Dan K and Fukuma Y, New hypoglycemic agents, *N*-(*p*-alkylbenzoyl)-D-phenylalanines. In: *Best Approach to the Ideal Therapy of Diabetic Mellitus* (Eds. Y. Shigeta, H. Lebovitz, J. Gerich and W. Malaisse), pp. 371–374. Elsevier Science Publishers BV, Amsterdam, 1987.
2. Shinkai H, Toi K, Kumashiro I, Seto Y, Fukuma M, Dan K and Toyoshima S, *N*-Acylphenylalanines and related compounds. A new class of oral hypoglycemic agents. *J Med Chem* **31:** 2092–2097, 1988.
3. Shinkai H, Nishikawa M, Sato Y, Toi K, Kumashiro I, Seto Y, Fukuma M, Dan K and Toyoshima S, *N*-(Cyclohexylcarbonyl)-D-phenylalanines and related compounds. A new class of oral hypoglycemic agents. 2. *J Med Chem* **32:** 1436–1441, 1989.
4. Hirose H, Maruyama H, Ito K, Seto Y, Kido K, Koyama K, Dan K, Saruta T and Kato R, Effects of *N*-[*trans*-4-isopropylcyclohexyl)-carbonyl]-D-phenylalanine (A-4166) on insulin and glucagon secretion in isolated perfused rat pancreas. *Pharmacology* **48:** 205–210, 1994.
5. Hirose H, Maruyama H, Seto Y, Ito K, Fujita T, Dan K, Kanda N, Saruta T and Kato R, Effects of D-phenylalanine-derivative hypoglycemic agent A-4166 on pancreatic α- and β-cells: Comparative study with glibenclamide. *Pharmacology* **50:** 175–181, 1995.
6. Sato Y, Nishikawa M, Shinkai H and Sukegawa E, Possibility of ideal blood glucose control by a new oral hypoglycemic agent, *N*-[(*trans*-4-isopropylcyclohexyl)-carbonyl]-D-phenylalanine (A-4166), and its stimulatory effect on insulin secretion in animals. *Diabetes Res Clin Pract* **12:** 53–59, 1991.
7. Fujitani S and Yada T, A novel D-phenylalanine-derivative hypoglycemic agent A-4166 increases cytosolic free Ca^{2+} in rat pancreatic β-cells by stimulating Ca^{2+} influx. *Endocrinology* **134:** 1395–1400, 1994.
8. Ashcroft SJH and Ashcroft FM, The sulfonylurea receptor. *Biochim Biophys Acta* **1175:** 45–59, 1992.
9. Boyd AE III, Aguilar-Bryan L, Bryan J, Kunze DL, Moss L, Nelson DA, Rajan AS, Raef H, Xiang HD and Yaney GC, Sulfonylurea signal transduction. *Recent Prog Horm Res* **47:** 299–316, 1991.
10. Seto Y, Fujita H, Dan K, Fujita T and Kato R, Stimulating activity of A-4166 on insulin release in *in situ* hamster pancreatic perfusion. *Pharmacology* **51:** 245–253, 1995.
11. Gaines KL, Hamilton S and Boyd AE III, Characterization of the sulfonylurea receptor on beta cell membranes. *J Biol Chem* **263:** 2589–2592, 1988.
12. Schmid-Antomarchi H, De-Weille J, Fosset M and Lazdunski M, The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated K^+ channel in insulin-secreting cells. *J Biol Chem* **262:** 15840–15844, 1987.
13. Akiyoshi M, Kakei M, Nakazaki M and Tanaka H, A new hypoglycemic agent, A-4166, inhibits ATP-sensitive potassium channels in rat pancreatic β-cells. *Am J Physiol* **268:** E185–E193, 1995.
14. Niki I, Kelly RP, Ashcroft SJH and Ashcroft FM, ATP-sensitive K-channels in HIT T15 β-cells studied by patch-clamp methods, 86Rb efflux and glibenclamide binding. *Pflügers Arch* **415:** 47–55, 1989.
15. Panten U, Burgfeld J, Goerke F, Rennicke M, Schwanstecher M, Wallasch A, Zünkler BJ and Lenzen S, Control of insulin secretion by sulfonylureas, meglitinide and diazoxide in relation to their binding to the sulfonylurea receptor in pancreatic islets. *Biochem Pharmacol* **38:** 1217–1229, 1989.
16. Ashcroft FM, Kerr AJ, Gibson JS and Williams BA, Amantadine and sparteine inhibit ATP-regulated K-currents in the insulin-secreting beta-cell line, HIT-T15. *Br J Pharmacol* **104:** 579–584, 1991.
17. Garrino MG, Schmeer W, Nenquin M, Meissner HP and Henquin JC, Mechanism of the stimulation of insulin release *in vitro* by HB 699, a benzoic acid derivative similar to the non-sulphonylurea moiety of glibenclamide. *Diabetologia* **28:** 697–703, 1985.
18. Garrino MG and Henquin JC, Highly potent and stereoselective effects of the benzoic acid derivative AZ-DF 265 on pancreatic β-cells. *Br J Pharmacol* **93:** 61–68, 1988.
19. Ronner P, Higgins TJ and Kimmich GA, Inhibition of ATP-sensitive K^+ channels in pancreatic β-cells by nonsulfonylurea drug linogliride. *Diabetes* **40:** 885–892, 1991.
20. Ronner P, Hang TL, Kraebber MJ and Higgins TJ, Effect of the hypoglycaemic drug (−)-AZ-DF-265 on ATP-sensitive potassium channels in rat pancreatic β-cells. *Br J Pharmacol* **106:** 250–255, 1992.
21. Muller M, De-Weille JR and Lazdunski M, Chlorpromazine and related phenothiazines inhibit the ATP-sensitive K^+ channel. *Eur J Pharmacol* **198:** 101–104, 1991.
22. Sturgess NC, Kozlowski RZ, Carrington CA, Hales CN and Ashford MLJ, Effects of sulphonylureas and diazoxide on insulin secretion and nucleotide-sensitive channels in an insulin-secreting cell line. *Br J Pharmacol* **95:** 83–94, 1988.