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Hypoglycaemic and insulinotropic effects of a novel oral antidiabetic agent, (−)-*N*-(*trans*-4-isopropylcyclohexane-carbonyl)-D-phenylalanine (A-4166)

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1 (−)-*N*-(*trans*-4-isopropylcyclohexanecarbonyl)-D-phenylalanine (A-4166), a novel oral hypoglycaemic agent is a non-sulphonylurea insulin secretagogue.

2 We investigated the insulin-releasing action and hypoglycaemic effect of A-4166 compared to sulphonylureas *in vitro* and *in vivo*.

3 A-4166 stimulated insulin secretion from rat freshly isolated pancreatic islets at concentrations from 3×10^{-6} M to 3×10^{-4} M in the presence of 2.8 mM glucose. There was no obvious difference in glucose dependency between the insulinotropic effect of A-4166 and that of glibenclamide, and no additive or synergistic effect was observed between these two drugs.

4 A-4166 displaced [^{3}H]-glibenclamide bound to intact HIT-T15 cells in a concentration-dependent manner. The K_i value was $4.34\pm0.04\times10^{-7}$ M, and the displacement potency of A-4166 was between that of glibenclamide and tolbutamide, being similar to that of gliclazide.

5 In fasted beagle dogs, A-4166 showed a dose-dependent hypoglycaemic effect after oral administration over the range 1 to 10 mg kg^{-1}. The hypoglycaemic action of A-4166 showed an earlier onset and a shorter duration than that of sulphonylureas.

6 Simultaneous measurement of plasma insulin levels revealed that the hypoglycaemic effect of A-4166 was caused by a rapid-onset and brief burst of insulin secretion.

7 The pharmacokinetic profile of A-4166 was consistent with the changes of the blood glucose and plasma insulin levels.

8 Although the *in vitro* insulin-releasing effect of A-4166 was similar to that of sulphonylureas, its hypoglycaemic effect was more rapid and shorter-lasting, associated with rapid absorption and clearance. Thus, A-4166 may be useful in suppressing postprandial hyperglycaemia in patients with non-insulin-dependent diabetes mellitus.

Keywords: A-4166; sulphonylurea; insulin secretion; islets of Langerhans

Introduction

Impaired insulin secretion from pancreatic β-cells is one of the major abnormalities in patients with non-insulin-dependent diabetes mellitus (NIDDM). This particularly involves postprandial insulin secretion (Polonsky *et al*., 1988; Porte, 1991). To compensate for defective insulin release, sulphonylureas have been widely used for more than 40 years in the treatment of NIDDM. These drugs exert an antidiabetic action primarily by direct stimulation of insulin release from pancreatic β-cells, followed by a reduction of the blood glucose level. However, sulphonylureas do not appear to be able to ameliorate impairment of the first phase of glucose-stimulated insulin secretion (Shapiro *et al*., 1989; Groop *et al*., 1991). Furthermore, there are several disadvantages to sulphonylurea therapy: first, some patients suffer from excessive hypoglycaemia (Asplund *et al*., 1983; Ferner & Neil, 1988; Seltzer, 1989) because of the long duration of action of these drugs. Second, failure to respond to these agents after chronic therapy, which is known as secondary failure, is also a common problem in the management of NIDDM patients (Groop *et al*., 1989; 1986).

(−)-*N*-(*trans*-4-isopropylcyclohexanecarbonyl)-D-phenylalanine (A-4166, Figure 1), a D-form derivative of phenylalanine, is a novel oral hypoglycaemic agent (Shinkai *et al*., 1988; 1989). Recent *in vitro* studies have revealed that A-4166 promotes insulin secretion from the rat isolated perfused pancreas in a concentration-dependent manner (Fujitani *et al*., 1996). It has also been demonstrated that A-4166 acts on pancreatic β-cells directly and specifically blocks ATP-sensitive K^+ channels (Akiyoshi *et al*., 1995), and that it increases cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) by stimulating extracellular Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels in single β-cells (Fujitani & Yada, 1994). These observations have suggested that blockade of ATP-sensitive K^+ channels in the plasma membrane of β-cells is a key step in the stimulation of insulin secretion by A-4166, as well as by sulphonylureas (Henquin, 1990; Panten *et al*., 1996). On the other hand, it has not been determined whether there is any precise difference in the insulin-releasing effect of A-4166 and sulphonylureas, and little is known about the binding of this agent to pancreatic β-cells. In addition, there is not enough information about the characteristics of its hypoglycaemic effect.

The present study demonstrated that A-4166 exhibits an insulin-releasing effect via the sulphonylurea receptor, which showed no apparent difference from that of sulphonylureas. However, the *in vivo* hypoglycaemic effect of A-4166 was different from that of sulphonylureas, with an early and short-term decrease of blood glucose due to its rapid absorption and clearance. These results suggest that A-4166 could be beneficial in clinical use to prevent postprandial hyperglycaemia without causing prolonged hypoglycaemia in NIDDM patients.

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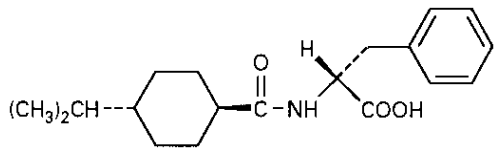

Figure 1 Structure of A-4166.

Methods

Isolation of islets

Male Wistar rats (weighing 360–420 g) were allowed free access to a normal laboratory diet until the experiment. The animals were anaesthetized with pentobarbitone sodium (50 mg kg^{-1}) and the pancreas was distended with collagenase solution via the bile duct. Then the pancreas was removed from each rat and digested at 37°C for 15 min. Islets were manually separated from the remaining exocrine tissue with a siliconized Pasteur pipette under a stereomicroscope and were immediately used for the following experiments. The isolation medium was Krebs-Ringer bicarbonate solution containing (in mM): NaCl 129, KCl 4.7, $NaHCO_3$ 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 2.8 and HEPES 10 (pH adjusted to 7.4 with NaOH).

Insulin secretion studies

Batches of five size-matched islets were preincubated at 37°C for 60 min in 0.5 ml of Dulbecco's modified Eagle's medium (DMEM) supplemented with 2.8 mM glucose, 20 mM HEPES, and 0.1% bovine serum albumin. The islets were then incubated at 37°C for 30 min in the same medium with test materials, after which aliquots of medium were stored at −20°C until assayed for insulin. Insulin concentrations were determined by the double-antibody procedure using a radioimmunoassay kit (Amersham, Buckinghamshire, U.K.).

Hypoglycaemic agents were dissolved in dimethyl sulphoxide and the same final concentration of the solvent was added to the control medium. We verified that dimethyl sulphoxide had no influence on islet secretory function at this concentration (data not shown).

Measurement of $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was measured in single islet β-cells according to a previously described method (Fujitani & Yadra, 1994) with some modifications. In brief, isolated islets were dispersed into single cells in Ca^{2+} depleted Krebs-Ringer bicarbonate solution with 1 mM EGTA, after which the cells were plated on coverslips and cultured 1–4 days in Eagle's minimum essential medium containing 5.6 mM glucose.

Then the cells on coverslips were loaded with 1 μM fura-2/acetoxymethylester (Grynkiewicz *et al.*, 1985) for 30 min at 37°C in DMEM containing 2.8 mM glucose. Subsequently, the cells were mounted in a superfusion chamber, placed on the stage of an inverted TMD microscope (Nikon, Tokyo, Japan), and superfused at 1 ml min^{-1} at 37°C with DMEM containing 0.1% BSA and test compounds. Following excitation at 340 and 380 nm every 3 s, fura-2 fluorescence at 510 nm was detected by an intensified charge-coupled device camera, and the ratio was obtained by use of an Argus-50 sytem (Hamamatsu Photonics, Hamamatsu, Japan) (Yada *et al.*, 1994). The ratios were converted to $[Ca^{2+}]_i$ values by using a calibration curve. We used the data from cells which showed a response to glucose that was typical of β-cells.

Cell culture

HIT-T15 cells were cultured at 37°C in RPMI 1640 tissue-culture medium containing 100 units ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 0.25 μg ml^{-1} amphotericin B, 10% heat-inactivated foetal calf serum in an atmosphere of 5% CO_2 and 95% air. Cells were passaged once each week and harvested with trypsin/EDTA. All experiments were performed with cells from passages 81–86.

Binding experiments

HIT-T15 cells grown in culture flasks were scraped and washed twice with phosphate-buffered saline (PBS, pH 7.4). For the equilibrium binding assay, the cells were resuspended at a density of 1.5×10^7 ml^{-1} in PBS and then incubated for 2 h at room temperature with 0.1–20 nM [^{3}H]-glibenclamide at a density of 1.5×10^6 per 0.4 ml assay volume. Incubation was terminated by rapid filtration under vacuum with a cell harvester (FilterMate, Packard Instrument) and Whatman GF/C glass fibre filters, and the cells were washed four times with PBS. Then the ^{3}H radioactivity of the filters was counted in a liquid scintillation counter (TopCount, Packard) after the addition of 20 μl of scintillation fluid. Non-specific binding was determined as residual binding in the presence of 1 μM unlabelled glibenclamide.

Competitive inhibition assays were performed with 1 nM [^{3}H]-glibenclamide and various concentrations of the other drugs. Buffer concentrations and incubation conditions were the same as those for the direct binding assays. Binding inhibition was expressed as a percentage of the specific binding of [^{3}H]-glibenclamide.

In vivo *experiments in fasting normal dogs*

Normal male beagles weighing 8.8 to 12.4 kg were fasted for 16 h before the experiments. Drugs suspended in 0.5% methylcellulose were administered orally via a stomach tube in a volume of 1 ml kg^{-1} body weight. Blood samples were taken from a foreleg vein before and at 15, 30, 45, 60, 90, 120, 180, 240, and 360 min after the administration of drugs or vehicle for determination of the blood glucose and plasma insulin levels as well as the drug concentrations. These experiments were performed at Ina Research Inc. (Nagano, Japan).

Whole blood glucose was measured by the glucose oxidase method with a Dri-Chem 2000 glucose autoanalyser (Fuji Medical System, Tokyo, Japan). Plasma insulin levels were measured by the RIA mentioned above. The plasma levels of A-4166 and sulphonylureas were determined by high-performance liquid chromatography. The peak concentration (C_{max}) was defined as the highest value recorded and the time to reach the peak concentration (t_{max}) was obtained in the same way. The elimination half-life ($t_{1/2}$) was estimated by regression analysis using the mean values after t_{max}.

Data analysis

In the radioligand binding studies, the equilibrium dissociation constant (K_d) and the maximal binding capacity (B_{max}) were calculated from Scatchard plots. The concentration of unlabelled ligand causing 50% of displacement of specific binding (IC_{50}) was determined by log-probit linear regression analysis. This was then converted to an inhibitory constant (K_i) by use of the Cheng-Prusoff equation: $K_i = IC_{50}(1 + L/K_d)$ (Cheng & Prusoff, 1973) where L is the ligand concentration and K_d is the apparent dissociation constant.

Materials

A-4166 and nitrendipine were synthesized at the Central Research Laboratories of Ajinomoto, Inc. (Kanagawa, Japan). Gliclazide was purchased from Dainippon Pharmaceutical Co. (Osaka, Japan) or Farmaceutica Milanese S.P.A. (Milano, Italy). We obtained glibenclamide, tolbutamide, collagenase (Type V), and diazoxide from Sigma (St. Louis, MO, U.S.A.), bovine serum albumin (fraction V) from Boehringer (Mannheim,

Germany), foetal bovine serum from GIBCO (Uxbridge, Middlesex, U.K.), fura-2-free acid and fura-2/acetoxymethylester from Dojin Chemicals (Kumamoto, Japan), and [^{3}H]-glibenclamide (1739.0 GBq mmol^{-1}) from Daiichi Kagakuyakuhin Co. (Tokyo, Japan). The HIT-T15 insulinoma cell line was purchased from Dainippon Pharmaceutical Co.

Statistical analysis

Results are presented as the mean ± s.e. mean. Statistical analysis of differences between mean values was undertaken by Dunnett's multiple comparison test or Student's *t* test. Differences were accepted as significant at $P<0.05$.

Results

Effect of A-4166 and sulphonylureas on insulin secretion from rat isolated pancreatic islets

In Figure 2, the relationship between the insulin response to A-4166 and the drug concentration is compared with those for glibenclamide, gliclazide, and tolbutamide in the presence of 2.8 mM glucose. A significant insulinotropic effect of A-4166 was observed at a concentration of 3×10^{-6} M. Glibenclamide, gliclazide, and tolbutamide significantly induced insulin release at concentrations of 3×10^{-8} M, 1×10^{-6} M, and 3×10^{-5} M, respectively. The maximal insulinotropic effect displayed by each agent was of the same order of magnitude.

From the data illustrated in Figure 2, it was calculated that A-4166 was about 100 times less potent than glibenclamide on a molar basis. In the following *in vitro* studies comparing these two compounds, we used supramaximal concentrations of A-4166 and glibenclamide (30 μM and 0.3 μM, respectively).

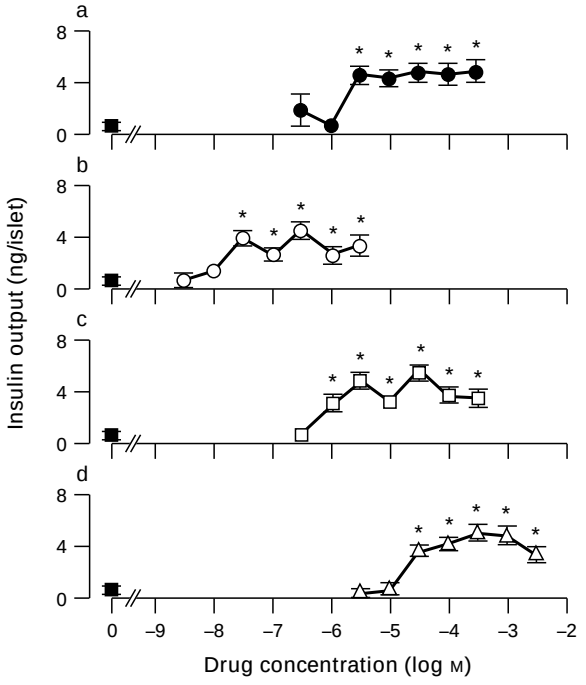

Figure 2 Dose-response relationship for the insulinotropic effect of (a) A-4166, (b) glibenclamide, (c) gliclazide and (d) tolbutamide on rat isolated pancreatic islets. Islets were isolated from male Wistar rats by collagenase digestion. Batches of five islets were incubated for 30 min at 37°C in medium containing various concentrations of drugs. The control (2.8 mM glucose only) is denoted by (■). Results are expressed as the mean and vertical lines show s.e.mean ($n=4-9$). *$P<0.05$ vs. control by Dunnett's multiple comparison test.

Inhibitory effect of diazoxide and nitrendipine on A-4166-induced insulin release and the $[Ca^{2+}]_i$ response

Blockade of ATP-sensitive K^+ channels and subsequent opening of voltage-dependent L-type Ca^{2+} channels in the plasma membrane of β-cells are key steps in the stimulation of insulin secretion by sulphonylureas (Henquin, 1990). To examine whether these ion channels were related to the effect of A-4166, the influence of diazoxide, an opener of the ATP-sensitive K^+ channel (Trube *et al*., 1986), or 1 μM nitrendipine, a blocker of voltage-dependent L-type Ca^{2+} channels, was examined in isolated islets.

A-4166-induced insulin secretion was completely inhibited by 400 μM diazoxide or 1 μM nitrendipine (Figure 3a). In the same medium, these inhibitory effects were also observed with respect to the A-4166-induced $[Ca^{2+}]_i$ response of single rat pancreatic β-cells (Figure 3b and c). The same results were also obtained with respect to the insulinotropic effect of glibenclamide (data not shown).

Insulinotropic effect of A-4166 and glibenclamide at various glucose concentrations

Figure 4 illustrates the effects of A-4166 and glibenclamide on insulin secretion at various glucose concentrations. The rate of insulin secretion in the presence of glucose alone progressively increased as the glucose concentration was raised from 0 to 300 mg dl^{-1}. A-4166 and glibenclamide exerted a similar insulinotropic action in the absence or presence of various concentrations of glucose. Both compounds exerted a significant stimulant effect over the range of 0 to 150 mg dl^{-1} glucose, but no significant effect on insulin secretion could be detected at higher glucose levels (200–300 mg dl^{-1}). The failure of these drugs to exhibit a marked effect at high glucose levels was not due to saturation of the secretory process because the addition of forskolin (10 μM), an adenylate cyclase activator (Malaisse *et al*., 1984), induced further augmentation of insulin release under these conditions (data not shown).

Combined secretory effect of A-4166 and glibenclamide

To investigate whether there was an additive or synergistic effect of A-4166 and gibenclamide, isolated islets were incubated in medium containing both these agents. Simultaneous exposure to A-4166 and glibenclamide did not cause a further increase of insulin release in comparison with that produced by either drug alone (Figure 5). On the other hand, addition of forskolin (10 μM) as a positive control caused a large increase in the release of insulin compared to the response observed with each of these drugs alone.

Displacement of [^{3}H]-glibenclamide from intact HIT-T15 cells by A-4166

Figure 6a shows equilibrium binding of [^{3}H]-glibenclamide to intact HIT-T15 cells at room temperature. Non-specific binding was determined in the presence of 1 μM unlabelled glibenclamide. The Scatchard plot of specific binding indicated one saturable binding site with a dissociation constant (K_d) of 0.29 nM and a maximal binding capacity (B_{max}) of about 8100 sites/cell (Figure 6a, inset).

Increasing concentrations of A-4166, gliclazide, tolbutamide, and unlabelled glibenclamide inhibited the specific binding of [^{3}H]-glibenclamide to intact HIT cells (Figure 6b). The K_i values were calculated from the half-maximum inhibitory concentration (IC_{50}), the K_d value of [^{3}H]-glibenclamide (0.29 nM), and its free concentration, to be $4.34\pm0.04\times10^{-7}$M, $6.55\pm0.80\times10^{-7}$M, $1.50\pm0.13\times10^{-5}$M, and $8.63\pm0.27\times10^{-10}$, respectively.

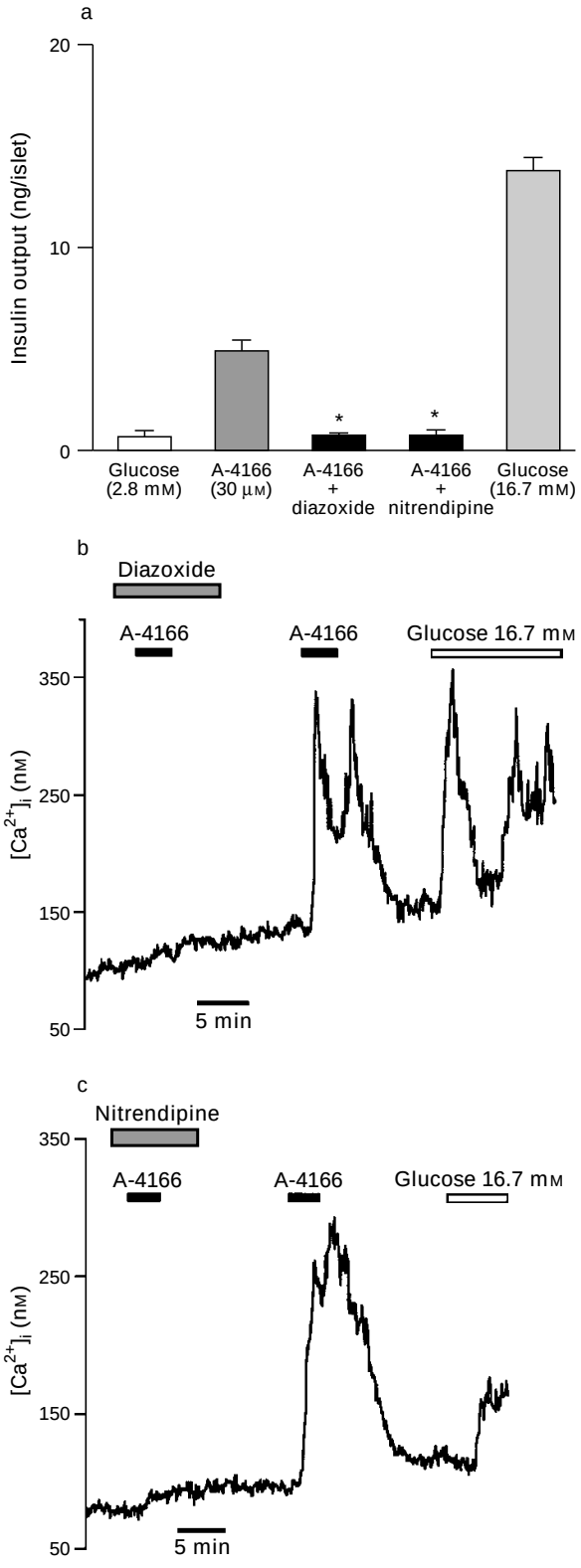

Figure 3 Inhibitory effect of diazoxide and nitrendipine on A-4166-induced insulin release from isolated islets and A-4166-induced $[Ca^{2+}]_i$ increase in single β-cells. (a) Groups of five islets were incubated for 30 min at 37°C in medium containing A-4166 (30 μM) alone or with diazoxide (400 μM) or nitrendipine (1 μM). Data are expressed as the mean ± s.e. mean ($n = 6-12$). $^*P < 0.05$ vs. 30 μM A-4166 by Student's t test. (b, c) Single rat pancreatic β-cells were superfused with DMEM containing 2.8 mM glucose and 0.1% BSA at 37°C. The horizontal bars above the tracings indicate the duration of exposure to A-4166, diazoxide or nitrendipine. The result is representative of 6 and 7 similar experiments, respectively.

Hypoglycaemic effect of A-4166 and sulphonylureas in fasting normal dogs

Figure 7 shows a comparison of A-4166, glibenclamide, gliclazide, and tolbutamide with regard to their relative hypoglycaemic potency in fasted beagle dogs. The hypoglycaemic potency was estimated from the maximum blood glucose reduction after oral administration of one dose. A-4166, as well as sulphonylureas, reduced the blood glucose levels in a dose-dependent manner. All compounds exhibited a high correlation between the maximum blood glucose decrease and the dose administered. The hypoglycaemic potency of A-4166 was about three times higher than that of tolbutamide, about three times less than that of gliclazide, and about 50 times less than that of glibenclamide.

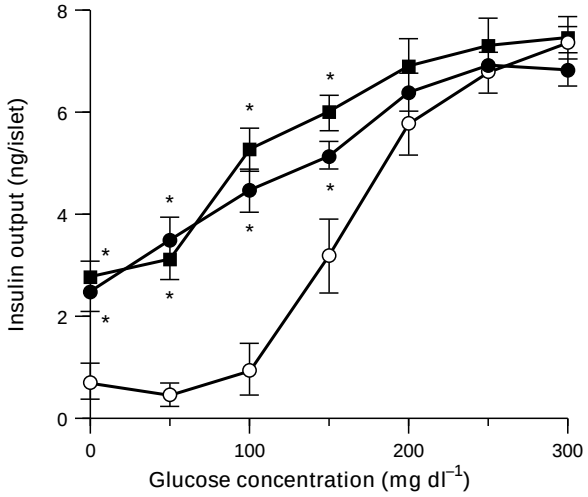

Figure 4 Effect of glucose concentration on insulin release from rat isolated islets by A-4166 and glibenclamide. Batches of five islets were incubated for 30 min at 37°C in medium containing glucose at the given concentrations. Insulin secretion was induced by 30 μM A-4166 (●), 0.3 μM glibenclamide (■) and glucose alone (○). Results are expressed as the mean and vertical lines show s.e.mean ($n = 5-7$). $^*P < 0.05$ vs. control by Dunnett's multiple comparison test.

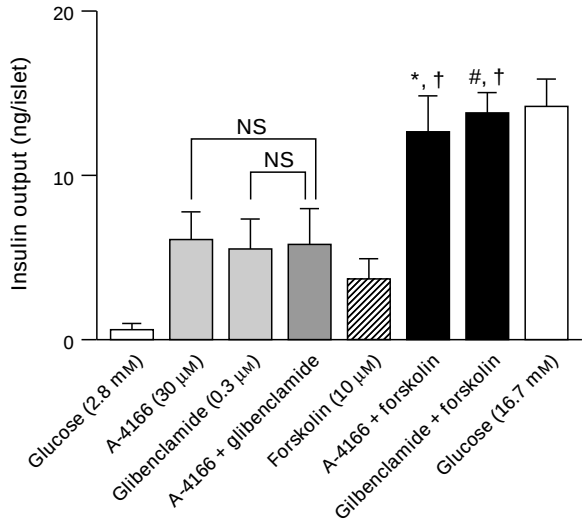

Figure 5 Insulin release elicited by the combination of A-4166 and glibenclamide. Batches of five islets were incubated with various agents for 30 min at 37°C. Data are expressed as the mean and vertical lines show s.e.mean ($n = 6-7$). Statistical analysis was performed by Student's t test. $^*P < 0.05$ vs. 30 μM A-4166. $^{\#}P < 0.05$ vs. 0.3 μM glibenclamide $^{\dagger}P < 0.05$ vs. 10 μM forskolin.

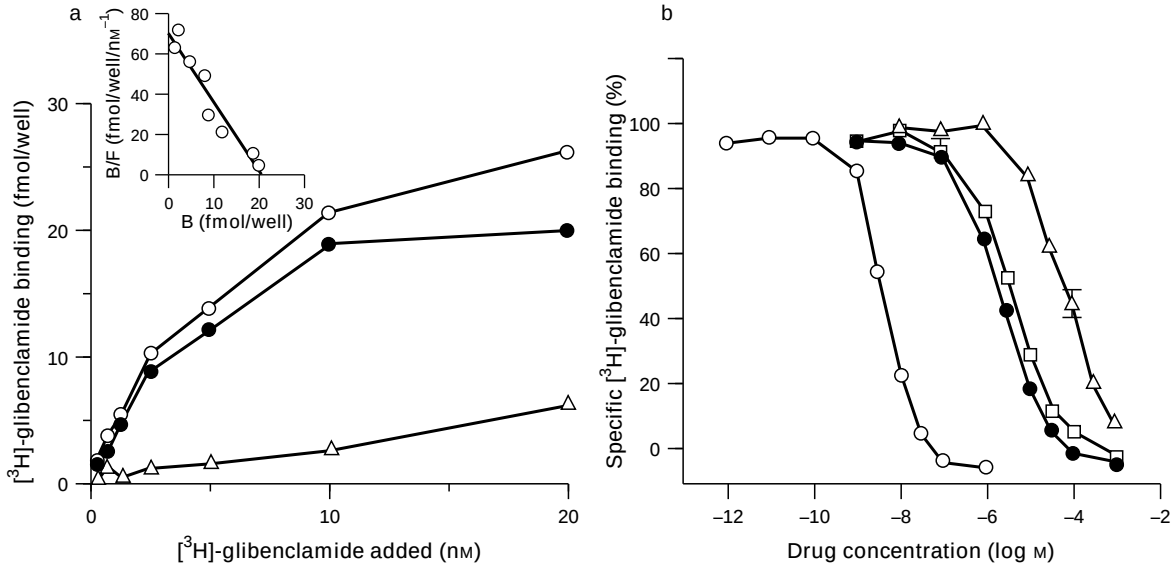

Figure 6 Equilibrium binding of [^{3}[H]-glibenclamide to intact HIT cells and inhibition of [^{3}H]-glibenclamide binding by A-4166 and sulphonylurea compounds. (a) Aliquots of whole HIT cells (1.5×10^7 cells ml^{-1}) were incubated with [^{3}H]-glibenclamide at concentrations from 0.3 to 20 nM for 2 h at room temperature. Bound and free radioligands were separated by filtration through Whatman GF/C filters. Specific binding (●) was determined by subtracting the non-specific binding (△) (counts remaining in the presence of 1 μM unlabelled glibenclamide) from the total binding (○). Each data point is the mean of duplicate determinations. Inset, Scatchard analysis of specific [^{3}H]-glibenclamide binding to intact HIT cells. The regression line was calculated by linear regression analysis. (b) Inhibition of [^{3}H]-glibenclamide binding by A-4166 (●), gliclazide (□), tolbutamide (△), and glibenclamide (○) was measured at room temperature in 0.2 ml of PBS containing [^{3}H]-glibenclamide (1 nM), whole HIT cells (1.5×10^7 cells ml^{-1}) and the indicated concentrations of displacing drugs. Results are presented as a percentage of the specific binding of [^{3}H]-glibenclamide in the absence of other drugs. Data are expressed as the mean and vertical lines show s.e.mean ($n = 3$).

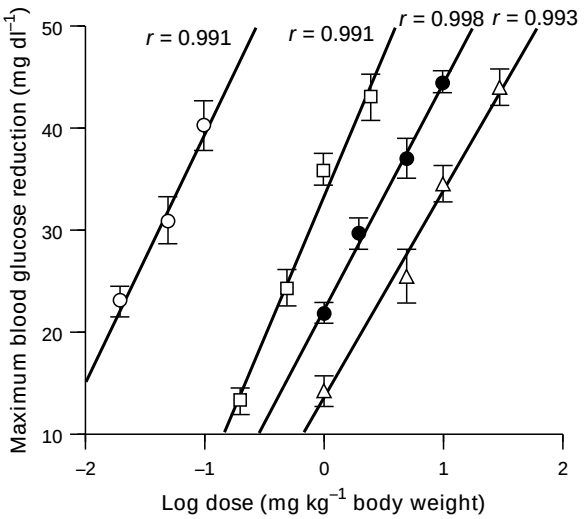

Figure 7 Comparison of A-4166 (●), glibenclamide (○), gliclazide (□), and tolbutamide (△) with regard to their relative hypoglycaemic potency in fasted beagle dogs after oral administration. The abscissa scale represents the logarithm of the dose (mg kg^{-1}) and the ordinate scale represents the decrease of blood glucose. Each point represents the mean maximal blood glucose reduction obtained in each animal after administration of one dose.

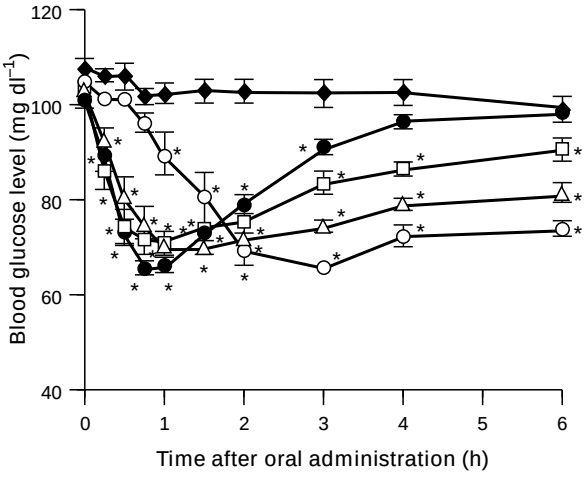

Figure 8 Blood glucose profiles after oral administration of A-4166 (●), glibenclamide (○), gliclazide (□),tolbutamide (△), and vehicle (◆) in fasted beagle dogs. Blood samples were taken before and at 15, 30, 45, 60, 90, 120, 180, 240 and 360 min after oral administration of the drugs or vehicle. Values represent the mean and vertical lines show s.e. mean of six dogs. $^*P < 0.05$ vs. vehicle treatment by Dunnett's multiple comparison test.

Blood glucose, plasma drug, and plasma insulin levels after oral administration of A-4166 to fasting normal dogs

The blood glucose profiles obtained after administration of A-4166 (5 mg kg^{-1}), glibenclamide (0.1 mg kg^{-1}), gliclazide (1 mg kg^{-1}), and tolbutamide (10 mg kg^{-1}), at approximately equipotent dosages, are shown in Figure 8. The plasma drug concentrations and plasma insulin levels measured simultaneously are shown in Figure 9 and Figure 10, respectively.

A-4166 exhibited a rapid and short-term hypoglycaemic effect (Figure 8). The peak effect was observed at about 1 h after oral administration, and the blood glucose level was almost normalized within 6 h. Gliclazide and tolbutamide also induced a rapid decrease of blood glucose. In contrast, glibenclamide showed a delayed onset of its hypoglycaemic action. All three sulphonylureas had a sustained hypoglycaemic effect for 6 h.

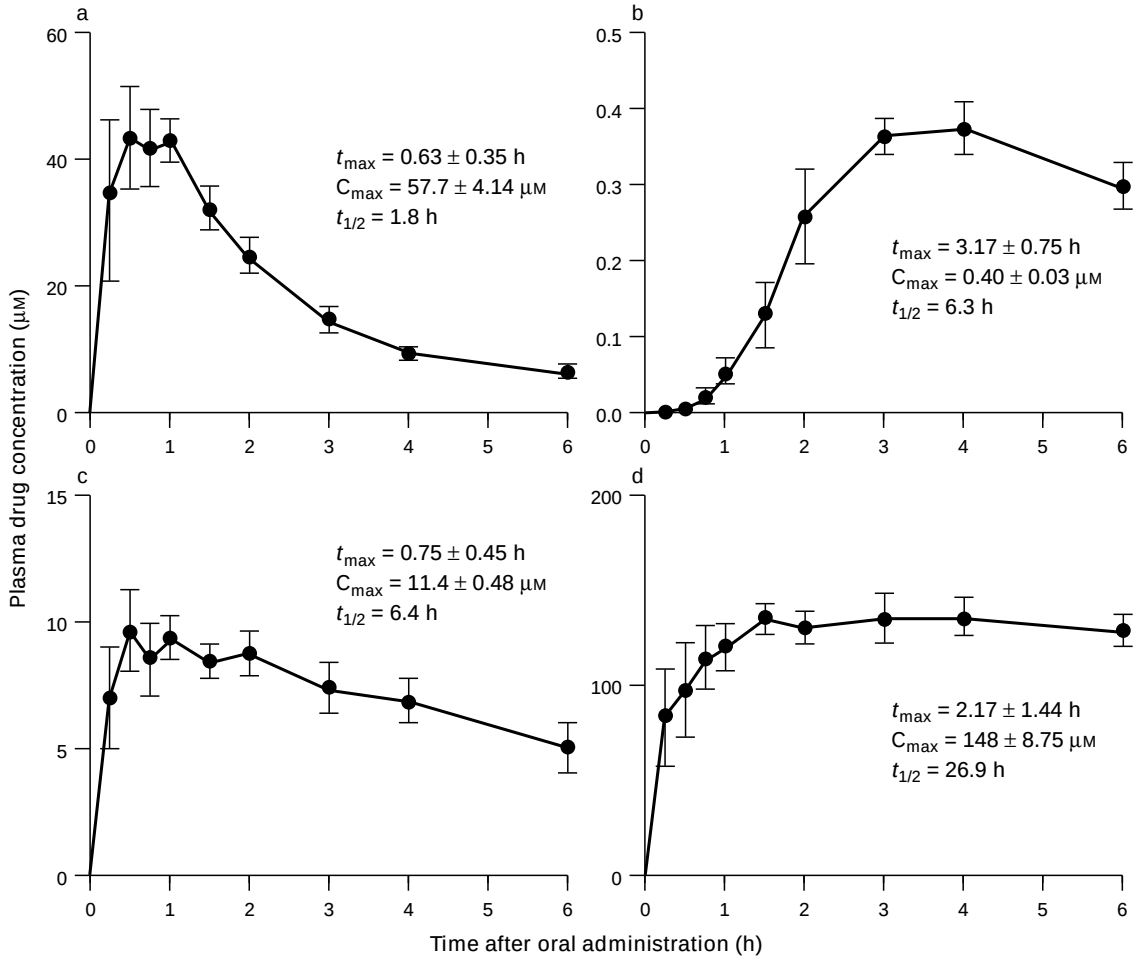

Figure 9 Plasma drug concentration profiles after oral administration of (a) A-4166, (b) glibenclamide, (c) gliclazide and (d) tolbutamide in fasted beagle dogs. Plasma drug levels were determined by high performance liquid chromatography. All data are expressed as the mean and vertical lines show s.e. mean for six dogs.

The hypoglycaemic effects of these four agents corresponded well with their pharmacokinetic profiles. After administration, A-4166 was detected in the plasma immediately, reached a maximum level within 1 h, and then progressively decreased (Figure 9a). On the other hand, the plasma concentration of glibenclamide increased steadily and reached a peak at around 3 h after administration (Figure 9b). Gliclazide and tolbutamide exhibited slowly decreasing and sustained plasma concentrations, respectively (Figure 9c and d).

Shortly after administration, A-4166 stimulated insulin release and this effect diminished within 2 h (Figure 10a). Both gliclazide and tolbutamide also produced a rapid onset of insulin release (Figure 10c and d), although it was not statistically significant in the case of tolbutamide. In contrast, glibenclamide induced endogenous insulin secretions in a delayed and sustained manner (Figure 10b).

Discussion

The present study showed that A-4166 has an earlier onset and shorter lasting hypoglycaemic action than sulphonylureas *in vivo*, despite having an *in vitro* insulinotropic mechanism which was indistinguishable from sulphonylureas.

Simultaneous measurement of blood glucose and plasma drug concentrations in fasted beagle dogs clearly demonstrated that A-4166 exhibited a markedly different pharmacokinetic profile from the sulphonylureas, which coincided well with its hyopglycaemic effect. Thus, the *in vivo* pharmacodynamic profile of A-4166, an early and short-term action, is likely to result from its rapid absorption and clearance rather than from an intrinsic difference of the insulin releasing mechanism.

The effect of A-4166 on insulin release was also consistent with its hypoglycaemic action, so the A-4166-induced blood glucose-lowering effect seems to be due entirely to an insulin-releasing effect. Conversely, the hypoglycaemic effects of sulphonylureas do not appear to be merely related to their insulin-releasing action, because the reduction of glucose levels did not directly correspond to their *in vivo* insulinotropic effects. This was especially true for gliclazide and tolbutamide, which caused a sustained reduction of blood glucose levels without sustained secretion of insulin. Several studies have demonstrated that sulphonylureas exert an antidiabetic effect not only through stimulating insulin release but also by increasing the effect of insulin (Lebovitz, 1984, Beck-Nielsen *et al*., 1988). Our present observations may also reflect the involvement of extrapancreatic actions in the blood glucose lowering effect. However, it is not unanimously accepted that the extrapancreatic effects significantly contribute to the hypoglycaemic action in the therapy of diabetic patients (Joost, 1985). It has been demonstrated in some studies that sulphonylurea compounds suppress glucagon secretion from pancreatic α-cells (Sako *et al*., 1986, Leclercq-Meyer *et al*., 1991). Therefore, the possibility that suppression of glucagon secretion may contribute to the prolonged hypoglycaemic effect of sulphonylureas cannot be excluded.

An early defect in NIDDM is the delay of acute insulin release after food intake, which causes prolonged elevation of postprandial glucose levels. The loss of this first phase

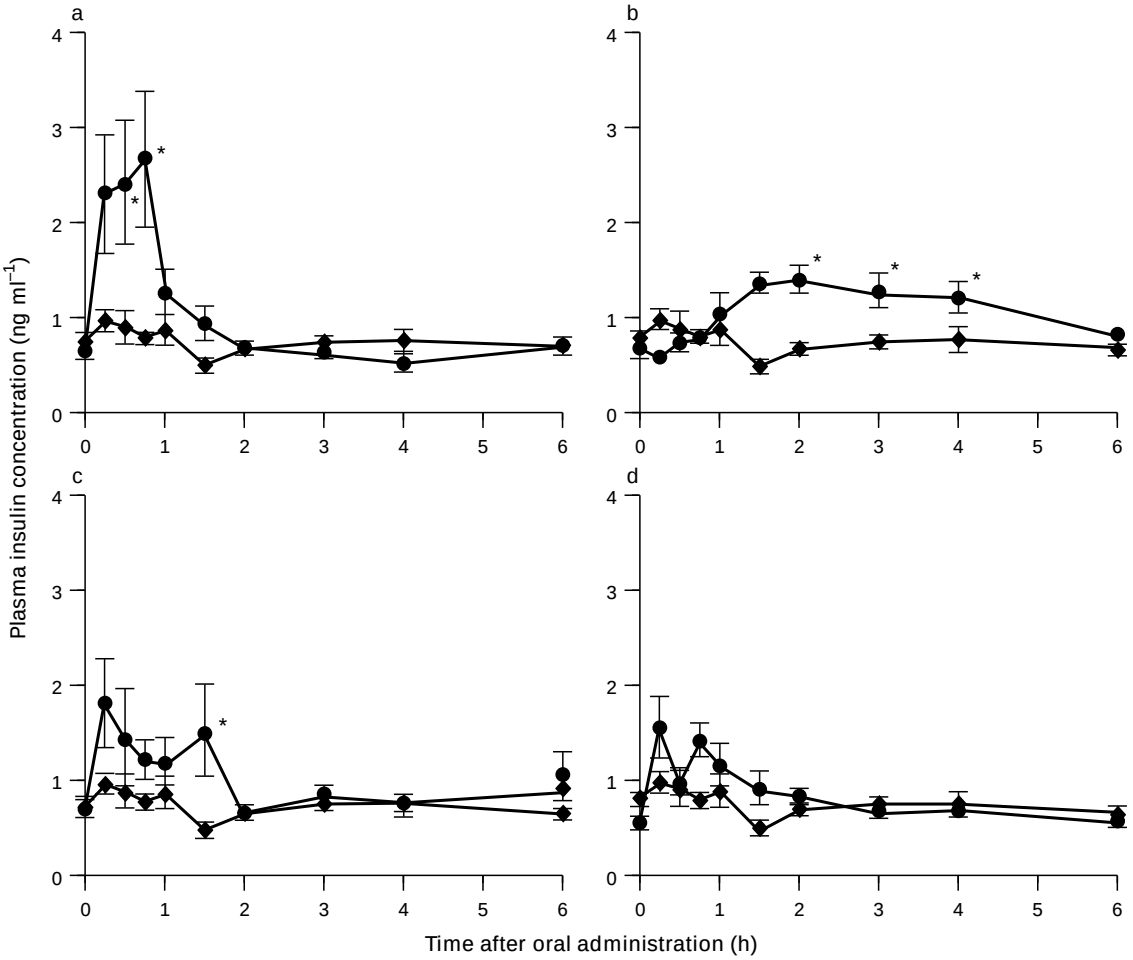

Figure 10 Plasma insulin profiles after oral administration of (a) A-4166, (b) glibenclamide, (c) gliclazide and (d) tolbutamide (●) or vehicle (◆) in fasted beagle dogs. All data are expressed as the mean and vertical lines show s.e.mean for six dogs. $^{*}P<0.05$ vs. vehicle treatment by Dunnett's multiple comparison test.

of insulin secretion causes a marked delay in the inhibition of hepatic glucose production and leads to impaired glucose tolerance (Luzi & DeFronzo, 1989), so this deficiency is regarded as being related to the pathogenesis of NIDDM (Kosaka *et al*., 1994). In addition, suppressing postprandial hyperglycaemia is considered to be very important to prevent diabetic complications. Intensive treatment using insulin, with the aim of maintaining blood glucose concentrations close to the normal range, effectively delays the onset and slows the progression of diabetic complications in patients with diabetes mellitus (The DCCT Research Group, 1993; Ohkubo *et al*., 1995). The importance of the prevention of postprandial hyperglycaemia is also emphasized by newly developed α-glucosidase inhibitors, which delay carbohydrate digestion and absorption and reduce the postprandial glycaemic rises (Lebovitz, 1992). Data from recent study indicate that long-term suppression of postprandial hyperglycaemia with acarbose, an α-glucosidase inhibitor, retarded the development of diabetic polyneuropathy in the BB/W-rat (Sima & Chakrabarti, 1992).

Considering our present findings that A-4166 exerts a rapid hypoglycaemic effect because of the prompt enhancement of insulin secretion, this novel agent should be able to compensate for the impaired first-phase response and thus suppress postprandial hyperglycaemia. Indeed, A-4166 has been shown to suppress hyperglycaemia after glucose loading in animals with or without diabetes mellitus (unpublished observations). In addition, the efficacy of A-4166 on the suppression of postprandial hyperglycaemia has also been demonstrated in NIDDM patients (Kikuchi *et al*., 1994). Therefore, this drug will be able to minimize the fluctuations of blood glucose and thus prevent diabetic complications.

In addition, A-4166 induced a hypoglycaemic effect of shorter duration than that produced by any of the sulphonylureas examined. The major disadvantage of sulphonylurea therapy is the occurrence of hypoglycaemic episodes (Asplund, *et al*., 1983; Ferner & Neil, 1988; Seltzer, 1989). The incidence of hypoglycaemia among patients treated with chlorpropamide or glibenclamide is higher than that in patients on tolbutamide, i.e., the incidence is a function of the biological half-life of these agents (Seltzer, 1989). In contrast, A-4166 could be expected to exert an antidiabetic effect without inducing hypoglycaemic episodes because of its short duration of action.

The occurrence of secondary failure is also an important problem in the management of NIDDM (Groop *et al*., 1986; 1989). The causes of the failure of sulphonylurea therapy are still unknown, but one hypothesis is that exhaustion of β-cells leads to unresponsiveness to these drugs (Greco *et al*., 1992). Chronic exposure to sulphonylureas is known to have a suppressive effect on β-cell function *in vitro* and *in vivo* (Filipponi *et al*., 1983; Davalli *et al*., 1992). The long-term *in vitro* effect of A-4166 on islet cells has not been examined. However, we speculate that A-4166 causes less damage to β-cells and has less incidence of β-cell exhaustion *in vivo* than sulphonylureas, since its plasma half-life was much shorter than that of sulphonylureas. Indeed, we previously found that gliclazide could neither enhance insulin secretion nor reduce blood glucose levels when administration was repeated six hours after the first

dosage, while A-4166 exhibited a hypoglycaemic effect on both occasions in neonatal streptozotocin-induced NIDDM model rats (Akiyoshi *et al*., 1994).

In spite of its different *in vivo* hypoglycaemic effect to that of sulphonylureas, A-4166 exhibited a similar *in vitro* insulinotropic action. A-4166-induced insulin secretion was inhibited by opening of ATP-sensitive K^+ channels or by blockade of L-type Ca^{2+} channels, and there was no additive effect between supramaximal concentrations of A-4166 and glibenclamide. Moreover, A-4166 displaced the binding of tritiated glibenclamide to intact HIT-T15 cells in a dose-dependent manner as did several sulphonylureas, and the rank order of displacement potency was largely consistent with that of insulinotropic activity. These observations indicate that A-4166 binds to the sulphonylurea receptor on the surface of pancreatic β-cells and stimulates insulin secretion through the same mechanism as sulphonylureas. On the other hand, it has also been shown that A-4166 is more effective than tolbutamide in increasing $[Ca^{2+}]_i$ in β-cells under metabolic suppression (Yada & Fujitani, 1994). Thus, the details of the differences between A-4166 and sulphonylureas still remain to be investigated.

In conclusion, our findings suggest that A-4166 exhibits an earlier and shorter-lasting hypoglycaemic effect than sulphonylureas, although it stimulates insulin secretion through the same binding site. Despite a similar *in vitro* insulinotropic effect to sulphonylureas, A-4166 seems to be a promising antidiabetic agent which may be able to suppress the postprandial increase of blood glucose without causing hypoglcaemic episodes or secondary failure in NIDDM patients owing to its rapid absorption and clearance.

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