Comparison of Insulinotrophic Actions of Nateglinide With Glibenclamide Dissociated From Absorption in Conscious Dogs

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Nateglinide is more rapidly absorbed than glibenclamide. Therefore, the different absorption kinetics of both drugs were eliminated by intraportal administration in conscious fasted dogs. The plasma insulin profiles were compared under similar kinetic changes in plasma drug concentrations. After a priming dose of nateglinide (1 mg/kg · 5 min) or glibenclamide (40 μg/kg · 5 min), plasma drug concentrations reached a peak at 4 minutes (nateglinide, 80 ± 5 μmol/L, n = 6 and glibenclamide, 263 ± 60 nmol/L, n = 6) followed by a sustained level at approximately 30% of the peak concentration at 30 minutes. Nateglinide led to a rapid and constant reduction in arterial glucose of approximately 30% basal, while glibenclamide promoted a gradual decrease to approximately 50% basal at 120 minutes. An increase in plasma insulin level by nateglinide of 4 times basal (218 ± 58 pmol/L *v* 47 ± 3 pmol/L, *P* < .05, n = 6) occurred at 6 to 10 minutes followed by sustained release of 1.4 times basal (67 ± 15 pmol/L, n = 6). The insulin surge was more than doubled (484 ± 209 pmol/L, n = 6) under a euglycemic clamp. Insulin release by glibenclamide increased gradually reaching 10-fold basal (449 ± 166 pmol/L, n = 6) at 60 minutes. This was not enhanced during a euglycemic clamp. Lowering the primed doses of nateglinide resulted in a diminished peak plasma insulin concentration. In contrast, glibenclamide caused only a slower increase, but eventually reaching a similar peak. By increasing the continuous infusion of nateglinide, the sustained insulin release was not altered. Glibenclamide, but not nateglinide, evoked prompt and sustained insulin release in the continuing presence of the other. These results are consistent with the concept that nateglinide produces a quick, but very short-lived, interaction with sulfonylurea (SU)-receptors on plasma membrane by free access of the drug from the cell exterior. In contrast, glibenclamide promotes a slow and longer interaction with the receptor by distribution of the drug into the cell inferior. We conclude, therefore, that not only the different kinetics of gastrointestinal (GI) absorption, but also the inherent difference in the interaction with β cells is attributed to the different insulin release characteristics between nateglinide and glibenclamide in vivo.

A NOVEL ORAL hypoglycemic agent, nateglinide, is an acyl-derivative of D-phenylalanine, N-[trans-4-isopropyl-cyclohexy-carbonyl]-D-phenylalanine, structurally distinct of sulfonylureas.[1,2] Nateglinide binds to the glibenclamide-binding sites on plasma membranes of HIT-15, RIN-m5F, and HEK-293 cells[3-6] and rapidly blocks potassium adenosine triphosphate (K_{ATP})-channel activity like glibenclamide,[7] which results in the opening of voltage-dependent Ca^{++} channels,[8,9] eventually leading to insulin release in rat pancreatic β cells[4,9] or HIT-15 cells.[5,10] Nevertheless, there is considerable dissimilarity to keep the pharmacokinetics and mode of action in parallel between the drugs. Nateglinide is more rapidly absorbed into and quickly excreted from blood than glibenclamide.[11,12] Nateglinide is restricted to the outer surface of the plasma membrane,[13] whereas glibenclamide is internalized by the pancreatic β cells.[14-17] Furthermore, nateglinide immediately dissociates from sulfonylurea (SU)-receptors, whereas glibenclamide slowly releases the binding.[6] Nateglinide evokes a more rapid and shorter-lasting insulin release than glibenclamide after oral administration in rats, dogs, and humans.[5,11,12] It appears that there are approximate symmetrical relationships with either drug between plasma glucose and either plasma drug or insulin profile.[5] However, it remains unclear whether the different insulin release profile is due primarily to the different absorption kinetics. Therefore, in this study, we eliminated the different gastrointestinal (GI) kinetics of absorption by using a primed continuous administration of each drug into the portal vein in fasted conscious dogs. The insulin release profiles for the 2 compounds were compared with or without euglycemic clamp to address whether the interaction of the drugs with β cells is the same in vivo under similar kinetic changes in the plasma drug level.

MATERIALS AND METHODS

Animals

Experiments were performed in conscious beagles (7 to 12 kg) of either sex normally fed daily a commercial pellet of meat and chow (Labo D standard meat; Nosan Co, Yokohama, Japan, 24% protein, 66.5% carbohydrate, 8.4% fat, and 1.1% fiber based on dry weight) once daily. The dogs were fasted for 18 hours. The protocols were approved by the Institute Animal Care Committee.

Surgical Procedures

Two weeks before each experiment, a laparotomy was performed in beagles under general anesthesia (35 mg/kg pentobarbital sodium), and polyethylen catheters (Nipro Co, Osaka, Japan) were inserted into a mesenteric vein, the portal vein, and a femoral artery. The mesenteric catheter used for blood sampling was inserted via the superior mesenteric vein, and the tip was placed at the point at which the vessel enters the liver. The tip of the other mesenteric catheter, which was used for glucose infusion into the portal vein, was placed approximately 4 cm downstream from the root of the superior mesenteric vein. The femoral

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catheter for blood sampling was inserted into the left femoral artery through an incision made in the left inguinal region. After those processes had been performed, they were filled with saline containing heparin (50 U/mL, Novo-Nordisk Pharma, Copenhagen, Denmark). The free end of the catheters was exteriorized 5 to 10 cm below the base of the skull. Two weeks after surgery, blood was drawn to determine leukocyte count, hematocrit, and liver enzyme activities. Only dogs with a good appetite and a normal blood count and liver enzymes were used in the study. On the day of the experiment, contents of each catheter were aspirated, and the catheters were flushed with saline. Each dog rested quietly in a Pavlov harness for the duration of the study.

Experimental Design

Study 1: Primed administration of nateglinide or glibenclamide in the fasting state. Animals were randomly assigned to the following 4 groups. In group 1, 1 mg/kg of nateglinide was infused (at time 0) into the portal vein over 5 minutes during continuous fast. In group 2, after nateglinide was infused as in group 1, plasma glucose levels were maintained at basal levels by infusing glucose via a cephalic vein using an artificial pancreas (Model STG-22, Nikkiso Co, Tokyo, Japan). In group 3, 40 μg/kg of glibenclamide was administered and otherwise was the same as group 1. In group 4, glibenclamide was infused as in group 3 under euglycemic conditions. Blood samples were drawn simultaneously from a femoral artery and the portal vein every 10 minutes during the basal period, every 2 minutes during the first 10 minutes, every 10 minutes during the 10- to 30-minute period, and finally every 15 minutes during the remainder of the experimental period. The total volume of blood drawn did not exceed 15% of the total blood volume for any of the animals, because 2 vol of saline was replaced for each volume of blood drawn.

Study 2: Primed-continuous administration of either nateglinide or glibenclamide. Animals were randomly assigned to 1 of 2 groups. In group 1, a primed (350 μg/kg · 5 min) continuous (2 μg/kg · min or 10 μg/kg · min) infusion of nateglinide was performed over 180 minutes under euglycemic conditions using an artificial pancreas, and glibenclamide (40 μg/kg · 5 min) was added at 90 minutes. In group 2, during a primed (15 μg/kg · 5 min) continuous (85 ng/kg · min) infusion of glibenclamide over 180 minutes, nateglinide (1 mg/kg · 5 min) was coadministered at 90 minutes. Blood samples were drawn between 0 and 90 minutes, as well as between 90 and 180 minutes at the same intervals of study 1.

Analytical Procedure

Plasma glucose concentration was determined by the glucose oxidase method using a Beckman glucose analyzer (Fullerton, CA). Immunoreactive insulin was measured by the Phadeseph Insulin Test (Pharmacia Diagnostics, Uppsala, Sweden). Serum concentration of nateglinide or glibenclamide was measured by high-performance liquid chromatography (HPLC).[18,19] The areas under the curves (AUCs) of plasma concentrations of glucose, insulin, and nateglinide were determined using the trapezoidal method.

Chemicals

Nateglinide was obtained from Ajinomoto Co, Ltd (Tokyo, Japan). Glibenclamide was obtained from Aventis Pharma Co, Ltd. (Tokyo, Japan).

Statistical Analysis

Data are reported as the mean ± SE. Statistical analyses were performed using Student's *t* test for normally distributed data and the Mann-Whitney 2-sample rank test for non-normally distributed data. A value of $P < .05$ was considered to be statistically significant.

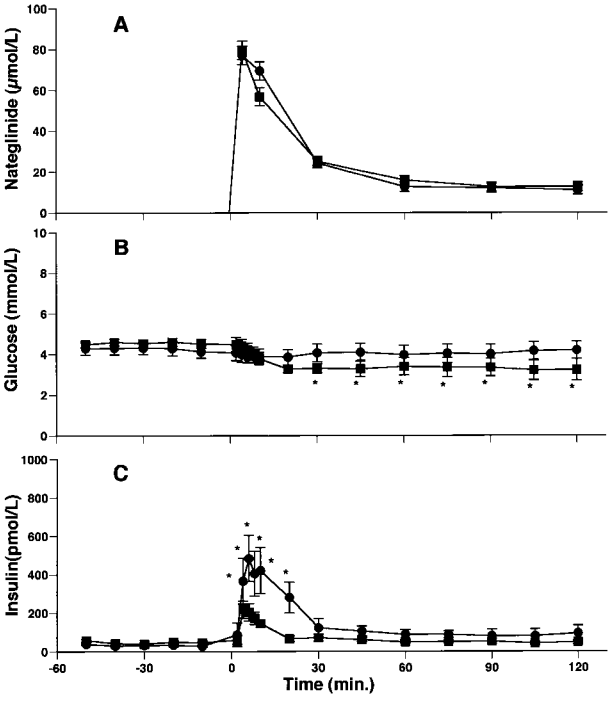

Fig 1. Changes in plasma nateglinide (A), glucose (B), and insulin (C) concentrations in a femoral artery after primed administration of nateglinide (1 mg/kg · 5 min) into the portal vein at time 0 with (●) and without (■) euglycemic clamp. Results are expressed as means ± SE for 6 dogs. *$P < .05$; significant difference with and without clamp.

RESULTS

Study 1

Primed administration of nateglinide or glibenclamide. Figure 1 shows changes in plasma nateglinide, glucose, and insulin concentrations in a femoral artery before and during the 120-minute period after intraportal infusion of 1 mg/kg of nateglinide over 5 minutes with and without euglycemic clamp. In fasted dogs, the plasma nateglinide concentration reached the maximum plasma concentration of 80 ± 5 μmol/L (n = 6) at 4 minutes followed by a rapid decline to 31% the maximal values (24 ± 2 μmol/L) at 30 minutes. Thereafter, it decreased gradually to the lowest level of 15% of the peak concentration at 120 minutes (Fig 1A). The plasma glucose concentration began to decrease at 4 minutes from the baseline of 4.5 ± 0.1 mmol/L and reached the nadir of 3.3 ± 0.2 mmol/L (71% basal) by 20 minutes and maintained this level during the remainder of the experiment (Fig 1B). The mean $AUC_{(0\text{-}120\ minutes)}$ of plasma glucose concentration decreased by 25% (67 ± 12 mmol/L · h). The plasma insulin concentration increased from the baseline to the peak value at 4 minutes of approximately 5 times basal (218 ± 58 pmol/L, $P < .05$ *v* 47 ± 3 pmol/L) and rapidly declined at 20 minutes to the constant level of 1.4 times basal (67 ± 15 pmol/L) (Fig 1C). The mean early plasma insulin $AUC_{(0\text{-}30\ minutes)}$ was approximately 6-fold higher than for the 30- to 120-minute period (3,695 ± 806 *v* 632 ± 288 pmol/L · h, $P < .05$).

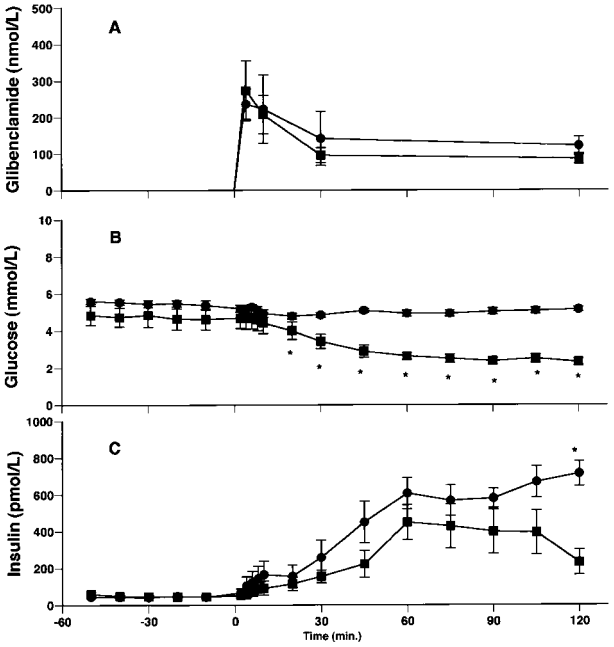

Fig 2. Changes in plasma glibenclamide (A), glucose (B), and insulin (C) concentrations in a femoral artery after primed administration of glibenclamide (40 μg/kg · 5 min) into the portal vein at time with (●) and without (■) euglycemic clamp. Results are expressed as means ± SE for 6 dogs. *$P < .05$; significant difference with and without clamp.

When the decrease in plasma glucose levels after the priming of nateglinide was interrupted by the infusion of glucose, the plasma glucose levels did not alter from the baseline over 120 minutes (Fig 1B). The mean value of plasma glucose over 120 minutes was 4.0 ± 0.1 mmol/L (n = 6) with a coefficient of variation (CV) of 4.9%. The plasma nateglinide profile was identical to that in fasting (Fig 1A). The plasma insulin level increased immediately to a peak at 6 minutes of approximately 15 times basal (484 ± 209 pmol/L *v* 32 ± 2p mol/L, $P < .05$) and decreased to sustained levels of 2.6 times basal at 30 minutes (Fig 1C). The peak and sustained levels of plasma insulin were 2.2 times and 1.2 times higher in the euglycemic state, respectively. The early plasma insulin $AUC_{(0\text{-}30\ minutes)}$ increased approximately 10 times compared with the 30- to 120-minute period (13,912 ± 6,517 *v* 1,348 ± 729 pmol/L · h, $P < .05$). Only the mean $AUC_{(0\text{-}30\ minutes)}$ was approximately 4-fold higher in the euglycemic clamp period ($P < .05$).

Figure 2 shows the plasma glibenclamide, glucose, and insulin profiles before and after primed administration of 40 μg/kg of glibenclamide over 5 minutes with and without euglycemic clamp. As shown in Fig 2A, the plasma glibenclamide concentration began to increase immediately and reached the maximum concentration at 4 minutes of 263 ± 60 nmol/L (n = 6). It declined to 78% of maximum (208 ± 53 nmol/L) at 10 minutes and 37% of maximum (96 ± 20 nmol/L) at 30 minutes without further changes. The plasma glucose concentration continued to decrease gradually from the baseline of 4.7 ± 0.2 mmol/L to a nadir of 2.3 ± 0.2 mmol/L (49% basal) at the end of the experiment (Fig 2B). The mean $AUC_{(0\text{-}120\ minutes)}$ of plasma glucose concentration decreased by 36% (103 ± 22 mmol/L · h). The decrease was 35% greater than that seen with nateglinide ($P < .05$). The plasma insulin concentration increased gradually to a peak of 10 times basal (449 ± 166 pmol/L *v* 49 ± 4 pmol/L, $P < .05$) at 60 minutes and decreased slightly thereafter (Fig 2C). The $AUC_{(0\text{-}30\ minutes)}$ of early plasma insulin response to glibenclamide was approximately 16% of the following $AUC_{(30\text{-}120\ minutes)}$ (3,170 ± 1,547 *v* 17,921 ± 6,766 pmol/L · h, $P < .05$).

During the euglycemic clamp study, the plasma glucose level did not change from the baseline after glibenclamide administration (CV, 4.9%) (Fig 2B). The plasma glibenclamide level was similar to that without the clamp (maximum, 236 ± 41 nmol/L; 30 minutes, 141 ± 74.4 nmol/L, n = 6) (Fig 2A). The plasma insulin level increased gradually to nearly saturating levels at 60 minutes of 14 times basal (606 ± 127 *v* 44 ± 42 pmol/L, $P < .05$) (Fig 3C). There was no significant difference with or without the euglycemic clamp in plasma insulin levels at all time points except at 120 minutes ($P < .05$). The mean $AUC_{(0\text{-}120\ minutes)}$ of the plasma insulin level was not different compared either with or without the euglycemic clamp (14,234 ± 5,053 *v* 24,640 ± 3,271 pmol/L · h, *P* = not significant [NS]).

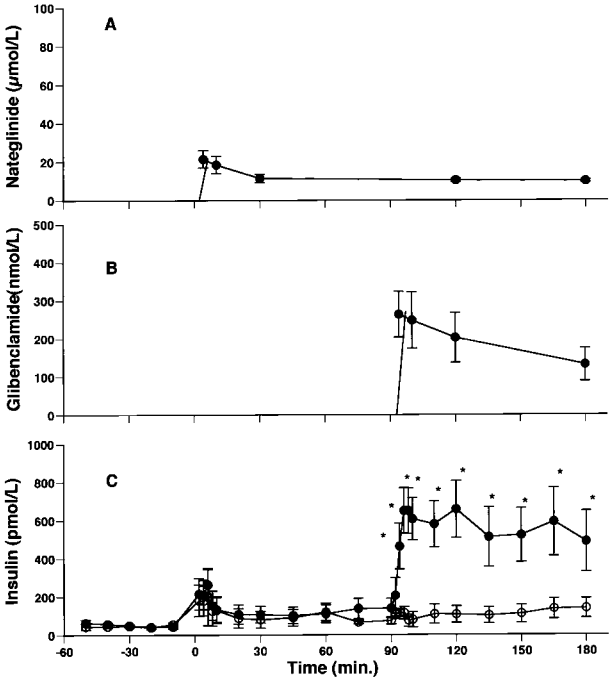

Fig 3. Changes in plasma nateglinide (A), glibenclamide (B), and insulin (C) concentrations during primed (350 μg/kg · 5 min) continuous (2 μg/kg · min) infusion of nateglinide over 90 minutes from time 0 followed by combined primed administration of glibenclamide (40 μg/kg · 5 min) with nateglinide from 90 to 180 minutes. Combined primed administration of glibenclamide (●), control (primed continuous administration of nateglinide alone) (○). Results are expressed as means ± SE for 6 dogs. Significant difference *v* control values; *$P < .05$.

Study 2

Primed continuous administration of nateglinide or glibenclamide. Figure 3 shows plasma nateglinide, glibenclamide, and insulin profiles during primed (350 μg/kg · 5 min) continuous (2 μg/kg · min) infusion of nateglinide over 180 minutes with and without the addition of glibenclamide (40 μg/kg · 5 min) at 90 minutes under euglycemic clamp conditions. The basal plasma glucose level did not change significantly (CV, 4.9%). The plasma nateglinide concentration reached a maximum of 22 ± 5 μmol/L (n = 6) at 4 minutes followed by a decrease to a plateau of 12 ± 2 μmol/L at 30 minutes (Fig 3A). The peak and sustained levels of the drug were lowered by approximately 70% and 20% compared with the primed administration of 1 mg/kg under the euglycemic state (peak value, 77 ± 2 pmol/L,mean values$_{30\text{-}120\ minutes}$; 15 ± 1 pmol/L). The plasma insulin level increased to a peak of approximately 5 times basal (263 ± 150 *v* 50 ± 4 pmol/L) at 6 minutes, returning rapidly to the sustained level of 2.2 times basal (108 ± 50 pmol/L) at 20 minutes (Fig 3C). The peak concentration was about half compared with 1 mg/kg of nateglinide administration during the euglycemic state (484 ± 209 pmol/L, $P < .05$), but the following sustained concentration was comparable (mean$_{30\text{-}120\ minutes}$: 119 ± 18 *v* 97 ± 12 pmol/L, P = NS).

At 94 minutes, 4 minutes after the addition of glibenclamide, the plasma drug level reached a peak of 294 ± 93 nmol/L with a very slow decrease to 45% of the peak value (132 ± 43 nmol/L) at 180 minutes (Fig 3B). The plasma insulin level started to increase further at 94 minutes and attained at 96 minutes the maximum of 4.7 times the predose value at 90 minutes (651 ± 223 pmol/L *v* 90 minutes; 139 ± 50 pmol/L, $P < .05$) and leveled off thereafter (Fig 3C).

In a separate experiment, the continuous administration of nateglinide was increased 5 times (10 μg/kg · min) without change of the primed dose (350 μg/kg · 5 min) to keep the plasma concentration of nateglinide high enough. The insulin release augmented no more than that seen with control (nateglinide: 2 μg/kg · min) (maximum, 263 ± 150 *v* 200 ± 150 pmol/L, P = NS; mean value$_{30\text{-}90\ minutes}$, 132 ± 18 *v* 119 ± 18 pmol/L, P = NS ,n = 5). Upon addition of glibenclamide, the plasma insulin level started to increase further at 94 minutes and almost reached the peak at 96 minutes of about half of the control values (nateglinide, 10 μg/kg · min: 348 ± 72 *v* 2 μg/kg · min: 651 ± 223 pmol/L, $P < .05$).

Figure 4 shows plasma glibenclamide, nateglinide, and insulin profiles during primed (15 μg/kg · 5 min) and continuous (85 ng/kg · min) administration of glibenclamide over 180 minutes with or without the addition of nateglinide (1 mg/kg · 5 min) at 90 minutes under euglycemic clamp conditions. The plasma glucose level did not alter significantly from the baseline of 5.6 ± 0.3 mmol/L throughout the experimental period (CV, 4.9%). The plasma glibenclamide level was maximal at 4 minutes of 47 ± 12 nmol/L (n = 6) without alteration thereafter (Fig 4A), which corresponded to approximately 20% of the peak value and 31% of the sustained level achieved by 40 μg/kg of glibenclamide. Without nateglinide, the plasma insulin level increased to 224 ± 40 pmol/L at 60 minutes and reached a saturating level of 548 ± 75 pmol/L at 120 minutes

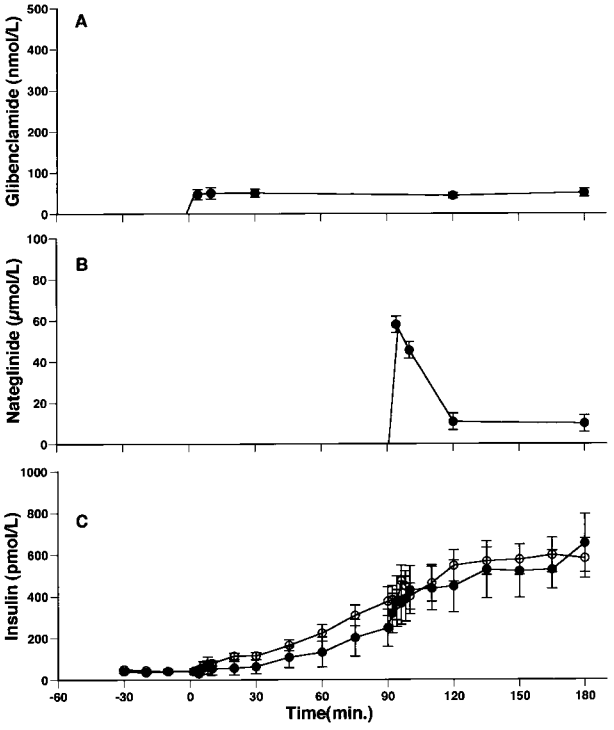

Fig 4. Changes in plasma glibenclamide (A), nateglinide (B), and insulin (C) concentrations during primed (15 μg/kg · 5 min) continuous (85 ng/kg · min) infusion of glibenclamide over 90 minutes from time 0 followed by combined primed administration of nateglinide (1 mg/kg · 5 min) with nateglinide from 90 to 180 minutes. Combined primed administration of nateglinide (●), control (primed continuous administration of glibenclamide alone) (○). Results are expressed as means ± SE for 6 dogs. Significant difference *v* control values.

(Fig 4C). The 60-minute value corresponded to about one third of the peak level, and the 120-minute value corresponded to the peak level achieved at 60 minutes by the primed administration of 40 μg/kg glibenclamide (607 ± 85 pmol/L). After the addition of nateglinide (at 90 minutes), the plasma nateglinide concentration attained a peak level at 94 minutes of 58 ± 2 μmol/L and linearly decreased to the equilibrating levels of 10 ± 1 μmol/L by 120 minutes (Fig 4B). However, there was no difference in the plasma insulin level with or without the addition of nateglinide.

DISCUSSION

The primed dose of nateglinide and glibenclamide in study 1 was determined by the dose-response studies after oral administration of nateglinide and miscellaneous sulphonylureas in fasted dogs.[11,12] There was a linear relationship between maximal decreases in fasting plasma glucose level (%) and doses of respective drugs, wherein 1 mg/kg of nateglinide or 40 μg/kg of glibenclamide evoked 15% decreases in the basal glucose level. In study 1, the primed intravenous administration of the same doses of nateglinide and glibenclamide over 5 minutes evoked approximately 30% and 50% reduction in basal plasma levels, and approximately 25% and 35% decreases of basal AUCs of plasma glucose levels, respectively. Therefore, these

doses appeared to be nearly equipotent for hypoglycemic action under our experimental conditions. The plasma drug concentrations peaked at 4 minutes and declined to 30% to 37% of the peak at 30 minutes (Figs 1A and 2A). The peak levels were about 2.5 times of the maximally effective levels of both drugs for insulinotrophic action (nateglinide, 30 to 35 μmol/L; glibenclamide, ~100 nmol/L).[12,20,21] These levels are in the range of pharmaceutical use.[20,21] In study 2, the primed and continuous doses of glibenclamide were identical to those in the studies of Groop et al[21,22] in humans, and the doses of nateglinide were in accord with the corresponding dose for glibenclamide. As a result, plasma nateglinide and glibenclamide levels reached the maximum at 4 minutes of 28% and 18% of the peak in study 1 and were followed by sustained levels of 15% and 18% of the peak at 30 minutes, respectively (Figs 1A, 2A, 3A, and 4A). It seems that their pharmacokinetics are largely similar.

Nateglinide and glibenclamide provoked the maximal insulin responses of comparable levels under the euglycemic clamp in study 1. However, nateglinide produced an acute increase in the plasma insulin level, whereas glibenclamide resulted in a gradually increasing release even under the similar changes in plasma drug concentrations. The mean early plasma insulin $AUC_{(0\text{-}30\ minutes)}$ was approximately 6 times of the following $AUC_{(30\text{-}120\ minutes)}$ after nateglinide administration without clamp, but the ratio was reversed after the administration of glibenclamide. These results indicate that the difference in plasma insulin profile is unlikely to be only a consequence of the pharmacokinetic differences in GI absorption. The time course of the K_{ATP}-channel blocking effect of both drugs has been demonstrated being similar ($t_{1/2}$; 4.1 minutes *v* 4.2 minutes) in rat β cells.[6] As the arterial insulin concentration changed similarly with the portal vein concentration (data not shown), the dynamic changes in plasma insulin is thought to reflect the effect of the drugs on insulin secretion from pancreatic β cells. Together, the considerable difference in the insulin release profiles between the 2 drugs strongly argues that glibenclamide plays a relatively minor role in the interaction with SU-receptors/K_{ATP}-channel on the plasma membrane.

The dissimilarity is particularly predominant in the defect of the acute insulin release by glibenclamide in vivo (Figs 2 and 4). However, when β cells were exposed to the media supplemented with glibenclamide at 1 to 10 μmol/L in the presence of 0.2% to 0.5% albumin in vitro, biphasic insulin release was observed.[17,23,24] It is known that free concentrations of glibenclamide, which is effective on single β cells and islets, is also in the range of the therapeutic plasma concentration of the free drug.[23] Therefore, a plausible explanation of this heterogeneity is that the plasma free concentration of glibenclamide is very low in vivo. The in vitro binding ratio of nateglinide to serum or plasma protein has been reported to be similar to that of glibenclamide[12] or tolbutamide,[25] a rapid insulin secretagogue like nateglinide.[4,23] However, protein binding of tolbutamide, but not glibenclamide, is highly dependent on the total drug concentration in the range of therapeutic doses.[23,26] Tolbutamide and nateglinide, but not glibenclamide, are anionic species, which get more free at higher pH.[26] Binding of glibenclamide, but not tolbutamide, increases markedly with decreasing temperature.[26] These results suggest that considerable amounts of nateglinide are free in blood in vivo, whereas most glibenclamide in the extracellular space binds to protein and does not interact directly with SU-receptors on plasma membrane.

With lowering of the primed doses, nateglinide decreased the peak of the plasma insulin level (Figs 1 and 3), whereas glibenclamide slowed down the increase, but eventually raised it to the similar saturating level at 120 minutes (Figs 2 and 4). This result is consistent with the study using a perifusion of mouse islets with the decreasing plasma glibenclamide level.[23] Nateglinide is known to restrict the outer surface of the β-cell plasma membrane,[13] whereas glibenclamide penetrates the cell inferior.[15] The diffusion of the drug from intracellular stores into plasma membrane is presumed to be slow.[6,23] These findings lead to the concept that nateglinide is freely accessible to the SU-receptors on the plasma membrane, whereas glibenclamide mostly binds to the intracellular membrane compartments, which would retard the increase of the plasma membrane content of the drug.[15,23] Indeed, rat or mouse islets have been reported to accumulate progressively intracellular glibenclamide in a curvilinear fashion.[14,15] This will lead to the progressive suppression of the K_{ATP}-channel activity or the direct promotion of exocytosis of insulin containing granules.[16,27-31]

The clearance of the drug from blood appears different between nateglinide and glibenclamide. The plasma nateglinide level was lowered to approximately 15% of the peak concentration, while the glibenclamide level remained at approximately 40% of the peak at 120 minutes after the primed administration (Figs 1A and 2A). The $t_{1/2}$ of nateglinide and glibenclamide was found to be 1.8 and 6.3 hours in fasting dogs, respectively.[5] These findings raise the possibility that the action of glibenclamide is more sustained than nateglinide by lesser excretion of the drug. However, our results do not support that view. The nateglinide-induced insulin release profile was not different when the continuous infusion rate was increased 5 times. The plasma concentration of glibenclamide reflects neither plasma-free drug nor intracellular concentration. Sustained blockage of the K_{ATP} channel has been reported to induce the reinforced inactivation of voltage-gated L-type Ca^{2+} channel activity.[32,33]

Nateglinide, but not glibenclamide, potentiated the insulin release under the euglycemic clamp conditions compared with that without the clamp (Figs 1 and 2): the peak and the mean plasma insulin levels increased 2 times and 4 times, respectively. This result suggests that nateglinide, but not glibenclamide, may increase glucose sensitivity of β cells around the basal glucose level. Indeed, it has been reported that nateglinide shifted a half-maximally stimulating plasma glucose level (ED_{50}) for insulin release from 7.2 mmol/L to 3.8 mmol/L in vitro,[34] whereas glibenclamide increased insulin responsiveness in the range of plasma glucose from 5 to 10 mmol/L with comparable ED_{50} (10 *v* 8 mmol/L).[24] The concentration-response curve of glucose for the K_{ATP}-channel current in β cells showed that their amplitude was highly reduced with increasing concentrations of glucose up to 5 mmol/L.[6] Together, rapid binding to SU-receptors of nateglinide and increases in ATP/adenine diphosphate (ADP) provided by basal glucose metab-

olism may evoke the synergistic closure of K_{ATP}-channel activity.

The transformation from slow to rapid insulin release by glibenclamide in the presence of nateglinide (Figs 3C and 4C) trivially contradicts the competitive binding of the 2 drugs to the same receptor sites on the plasma membrane.[3,5,6] However, this discrepancy may be attributed to the different dissociation of these drugs from binding receptors. Nateglinide, but not glibenclamide, dissociated very rapidly from the receptors ($t_{1/2}$: ~1 second *v* 2.9 minutes).[6] The effect of nateglinide on the K_{ATP}-channel was rapidly and completely reversed after the drug was removed, whereas the effect of glibenclamide was longer lasting.[6] Therefore, glibenclamide will take the place of nateglinide on receptors immediately, because the power of displacement of glibenclamide for binding receptors is 380 to 450 times stronger than nateglinide.[3,6] As a result, further closure of K_{ATP}-channel and/or synergistic stimulation of direct exocytosis by both drugs via some unknown pathways including cyclic adenosine monophosphate (cAMP)[35] or protein kinase C (PKC) activation will accelerate insulin release.[28,29,36] It is also worth noting that the plasma glibenclamide level reached a similar peak rapidly, but decreased very slowly compared with that seen with glibenclamide alone (Figs 2A and 3B). The peak level of plasma insulin was also sustained in contrast to the gradual increase by glibenclamide alone (Figs 2C and 3C). Presumably, the continuous presence of nateglinide interrupts glibenclamide with penetrating plasma membrane and facilitates the interaction with the SU-receptors on the plasma membrane, leading to the rapid onset of insulin release. However, insulin release by the addition of glibenclamide was halved when the continuous infusion rate of nateglinide was increased 5 times. Therefore, the synergistic effect may depend upon the optimal plasma concentration of nateglinide. In contrast, the blockade by glibenclamide of insulin response to added nateglinide (Fig 4C) can be explained by the strong occupation for the receptors on the plasma membrane and the slow reversibility of K_{ATP}-channel activity by glibenclamide.[37]

In summary, the current study indicates that there are several differences in the kinetics of insulin release between nateglinide and glibenclamide even under similar kinetic changes in plasma drug levels of the nearly equipotent doses. (1) Nateglinide evoked a rapid and short-lasting insulin release, whereas glibenclamide showed progressively increasing insulin release; (2) with lowering of the primed dose, nateglinide reduced the peak of the insulin level, whereas glibenclamide only slowed down the increase; (3) the sustained plasma level of nateglinide appeared not to affect sustained insulin release; (4) nateglinide, but not glibenclamide, enhanced insulin release under a euglycemic clamp compared with no clamp; and finally (5) glibenclamide, but not nateglinide, induced an immediate and sustained insulin release in the continuous presence of the other. These kinetic changes in insulin release by nateglinide in vivo is consistent with the concept that nateglinide induces rapid and short interaction with SU-receptors/K_{ATP}-channels on the plasma membrane by the free access from the extracellular space. The remaining difference in the insulin release pattern between the 2 drugs under similar pharmacokinetics suggests the different interaction with SU-receptors/K_{ATP}-channels of glibenclamide distributed into intracellular membrane. We conclude that the kinetic difference in insulin release between nateglinide and glibenclamide is not only the consequence of the different GI absorption, but also the inherent difference in the interaction of the drugs with pancreatic β cells.

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