Improvement of Glucose Tolerance by Nateglinide Occurs Through Enhancement of Early Phase Insulin Secretion

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Nateglinide is a new, fast-onset, short-acting hypoglycemic agent, which increases early phase insulin secretion and the total amount of insulin secreted. However, it is not clear which of these effects contribute more to the decrease in postprandial plasma glucose (PG). To further clarify the pharmacologic actions of nateglinide, we investigated the changes in PG and insulin levels during meal tolerance tests with and without nateglinide. Subjects were 10 newly diagnosed and untreated inpatients with type 2 diabetes. After diet and exercise therapy for 1 week, nateglinide at 270 mg divided 3 times a day, was started. Meal tolerance tests were performed before (baseline) and after a single nateglinide administration (day 1), after 7 days of repeated administration (day 7), and after cessation of nateglinide on day 8. Mean fasting PG was 146 ± 6 mg/dl (mean ± SEM) at baseline and 130 ± 6 mg/dL on day 7 ($P = .0004$). The 2-hour postprandial PG level was 226 ± 10 mg/dL at baseline, 145 ± 11 mg/dL on day 1 ($P = .0008$), and 190 ± 15 mg/dL on day 8 ($P = .08$, baseline; $P = .01$, day 7). The mean fasting insulin level was 5.4 ± 1.0 μU/mL at baseline and did not change significantly during the study. The 30-minute postprandial insulin level was 14.4 ± 1.9 μU/mL at baseline, 39.5 ± 4.5 μU/mL on day 1 ($P = .0004$), and 23.6 ± 3.6 μU/mL on day 8 ($P = .045$, baseline; $P = .010$, day 7). The total insulin amount, in terms of area under the curve (AUC · IRI), was 3.99 ± 0.7 × 10^3 μU/mL · min at baseline, 5.47 ± 0.8 μU/mL · min on day 1 ($P = .029$), and 6.01 ± 1.9 μU/mL · min on day 8 ($P = .047$ *v* baseline). The early phase of insulin secretion, based on the ratio of delta IRI to delta PG from fasting to 30 minutes after a meal was 0.15 ± 0.13 at baseline, 1.44 ± 0.26 on day 1 ($P = .0009$) and 0.26 ± 0.06 on day 8 ($P = .05$ *v* day 1). After cessation of nateglinide, the postprandial PG level increased immediately. Although early phase insulin secretion returned nearly to the baseline level, total insulin secretion remained at a high level. These results suggested that early phase insulin secretion contributes more than total insulin secretion to the improvement of postprandial hyperglycemia in type 2 diabetes.

THE IMPORTANCE OF postprandial glucose control is evident in the literature. Postprandial hyperglycemia has been associated with an increased risk of microvascular[1,2] and macrovascular[3,4] complications. Therefore, intervention aimed at lowering postprandial glucose levels may be important in reducing diabetic complications and mortality and may be an important focus for therapy.[5] However, conventional oral hypoglycemic agents are not without problems, because they sometimes cause episodes of hypoglycemia.

Nateglinide is a new oral hypoglycemic agent with an acyl-D-phenylalanine structure that shows a hypoglycemic effect by directly stimulating the pancreatic β cells to facilitate insulin secretion.[6-8] Because this effect appears very quickly and lasts for only a short period of time, nateglinide is considered a fast-onset, short-acting hypoglycemic agent.[9,10] Nateglinide is thought to reduce the postprandial glucose level by enhancing the early phase of insulin secretion and by increasing the total amount of insulin secreted.[10-13] However, it is not clear whether the early phase of insulin secretion or the total insulin secretion contributes more to the decrease in postprandial glucose levels. To further clarify the pharmacologic actions of nateglinide, changes in postprandial plasma glucose (PG) and serum insulin levels were measured during a meal tolerance test with and without nateglinide.

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Submitted August 24, 2000; accepted July 17, 2001.

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0026-0495/02/5101-0012$35.00/0
doi:10.1053/meta.2002.29008

SUBJECTS AND METHODS

Subjects

Ten newly diagnosed and untreated inpatients with type 2 diabetes were the subjects of this study. There were 6 women and 4 men with a mean age of 55.0 ± 4.1 years (mean ± SEM). Their body mass index was 23.9 ± 1.0, the duration of diabetes was 1.8 ± 0.5 years, fasting PG on admission was 167.0 ± 6.9 mg/dL, and hemoglobin A_{1c} (HbA_{1c}) was 8.9% ± 0.4% (Table 1). The urinary C-peptide immunoreactivity (CPR) excretion and the plasma CPR response to 1 mg of glucagon intravenous injection were measured as indices of endogenous insulin secretion. The mean urinary CPR level was 66.3 ± 6.7 μg/day, and the delta CPR value at 0 minute and 6 minutes in a glucagon injection test was 2.8 ± 0.3 ng/mL. These data suggested that the subjects had good insulin secreting ability. None of the patients had diabetic complications or liver or renal dysfunction, and there were no episodes of hypoglycemia during the study. The study was approved by the Saiseikai Kurihashi Hospital Research Committee, and all participants gave written informed consent.

Methods

Patients were under a strict medical nutrition therapy regimen of 25 to 27 kcal/kg/d and exercise for 1 week. Their fasting PG levels decreased to 146 ± 6 mg/dL. A meal tolerance test was then performed on the 7th hospital day (baseline) with an 8 to 9 kcal/kg diet, in which 60% to 65% of the total calories (%kcal) was derived from carbohydrates, 15% to 20%kcal from protein, and 20%kcal from fat. Plasma glucose and immunoreactive insulin (IRI) levels were measured at fasting and at 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 180 minutes after a meal in this test. The administration of nateglinide (Yamanouchi Pharmaceutical, Tokyo, Japan) at 270 mg divided 3 times a day was then started. Meal tolerance tests were also performed after a single administration of 90 mg of nateglinide (day 1) and after 7 days of repeated administration of nateglinide (day 7). Nateglinide was then discontinued, and a meal tolerance test was performed on day 8. The

Table 1. Clinical Characteristics on Admission

N (F/M)	10 (6/4)
Age (yr)	55.0 ± 4.1 (33-67)
Duration of diabetes (yr)	1.8 ± 0.5 (0.1-5.0)
BMI (kg/m^2)	23.9 ± 1.0 (19.3-28.1)
FPG (mg/dL)	167.0 ± 6.9 (129-202)
HbA_{1c} (%)	8.9 ± 0.4 (7.5-11.3)
GA (%)	25.2 ± 1.1 (21.4-33.4)
Delta CPR during glucagon injection test (ng/mL)	2.8 ± 0.3 (1.6-4.7)
uCPR (μg/d)	66.3 ± 6.7 (38-92)

NOTE. All data are shown as mean ± SEM (range).

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; GA, glycosylated albumin; CPR, C-peptide immunoreactivity; uCPR, 24 hour-collected urinary CPR extraction.

diets used for all meal tolerance tests were supplied by our nutrition staff and were identical in calories and composition. PG levels were measured by glucose oxidase. The levels of IRI were measured by enzyme immunoassay methods using a commercially available kit (Roche Diagnostic, Tokyo, Japan). Glycated albumin (GA) and HbA_1c were measured by high-performance liquid chromatography using GAA-2000 (Arkray, Kyoto, Japan) and HA-8150 (Arkray, Kyoto, Japan), respectively. Plasma and urinary CPR were assayed by radioimmunoassay and enzyme immunoassay, respectively.

Statistical Analysis

Statistical analysis was performed using the SAS System (SAS Institute, Cary, NC) with data generally presented as frequency or mean ± SEM. The normality of distribution of the data was evaluated by the Shapiro-Wilks test. The overall changes of PG and IRI levels were tested with repeated measureds analysis of variance. Comparison between each 2 groups was tested using multiple comparison *t* test. Two-tailed *P* values less than .05 were considered statistically significant.

RESULTS

Changes in PG Levels in the Meal Tolerance Tests

PG levels at baseline were 146 ± 6 mg/dL at fasting, 215 ± 12 mg/dL at 30 minutes after a meal, 260 ± 10 mg/dL at 1 hour, and 226 ± 10 mg/dL at 2 hours (Fig 1A). The fasting PG level on day 7 was 130 ± 6 mg/dL, which was significantly lower than baseline ($P = .01$). Postprandial PG levels were rapidly reduced after nateglinide administration on day 1 and day 7. The 2-hour postprandial PG level on day 1 was 145 ± 9 mg/dL ($P = .0008$). After cessation of nateglinide administration, the 2-hour postprandial PG level immediately increased to 190 ± 15 mg/dL ($P = .01$ *v* day 7).

The total PG, in terms of area under the curve (AUC · PG), was 39.7 ± 1.5 × 10^3 mg/dL · min at baseline, which decreased

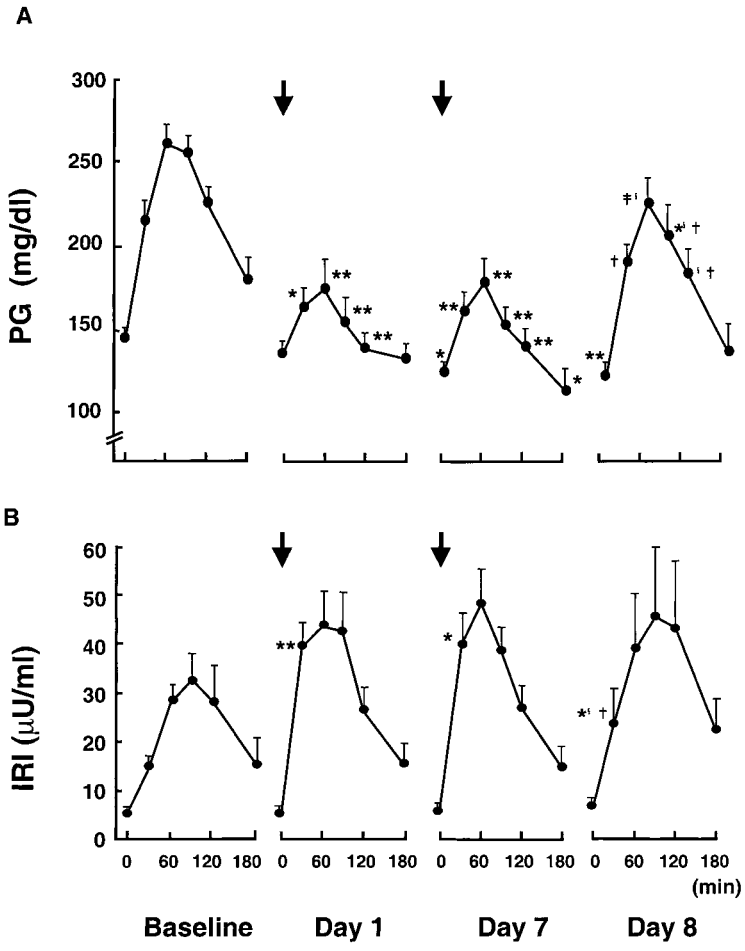

Fig 1. Profile of PG and insulin in the meal tolerance test. Meal tolerance tests were performed before (baseline) and after a single nateglinide administration (day 1), after 7 days of repeated administration (day 7), and after cessation of nateglinide on day 8. (A) PG profile during meal tolerance tests. (B) Plasma insulin profile during meal tolerance tests. Arrows show 90 mg of nateglinide administration. Data are shown as mean ± SEM. *$P < .05$; **$P < .01$ (*v* baseline); §$P < .05$ (*v* day 1); †$P < .05$; ‡$P < .01$ (*v* day 7); PG, plasma glucose; IRI, immunoreative insulin.

to 28.0 ± 1.8 × 10^3 mg/dL · min after a single administration of nateglinide (P = .0003, Fig 2A). After cessation of nateglinide, the AUC · PG increased to 34.0 ± 2.4 × 10^3 mg/dL · min (P = .03, baseline; P = .006, day 7). This AUC · PG was higher than the level on day 1 and day 7, but still lower than baseline. Glycated albumin was reduced from 25.2% ± 1.1% at baseline to 23.5% ± 1.1% on day 7 (P = .00008).

Changes in IRI Levels in the Meal Tolerance Tests

The mean fasting IRI level at baseline was 5.4 ± 1.0 μU/mL and was not significantly different on any of the days tested (Fig 1B). The 30-minute postprandial IRI level increased significantly to 39.5 ± 4.4 μU/mL after a single administration of nateglinide on day 1 compared with baseline (14.4 ± 1.9 μU/mL, P = .0004). It was 2.7 times higher after a single administration of nateglinide. The total amount of insulin secretion, in terms of area under the curve (AUC · IRI), also increased significantly to 5.47 ± 0.85 × 10^3 μU/mL · min compared with baseline (3.99 ± 0.7 × 10^3 μU/mL · min, P = .03, Fig 2B). This was an increase during nateglinide administration of 1.4 times over baseline. After discontinuing nateglinide, although the IRI at 30 minutes was markedly reduced to 23.6 ± 3.6 μU/mL (P = .010 *v* day 7), it was still higher than the baseline level (P = .045). On the other hand, AUC · IRI after nateglinide cessation was very similar to the AUC · IRI on day 7 (P = .99).

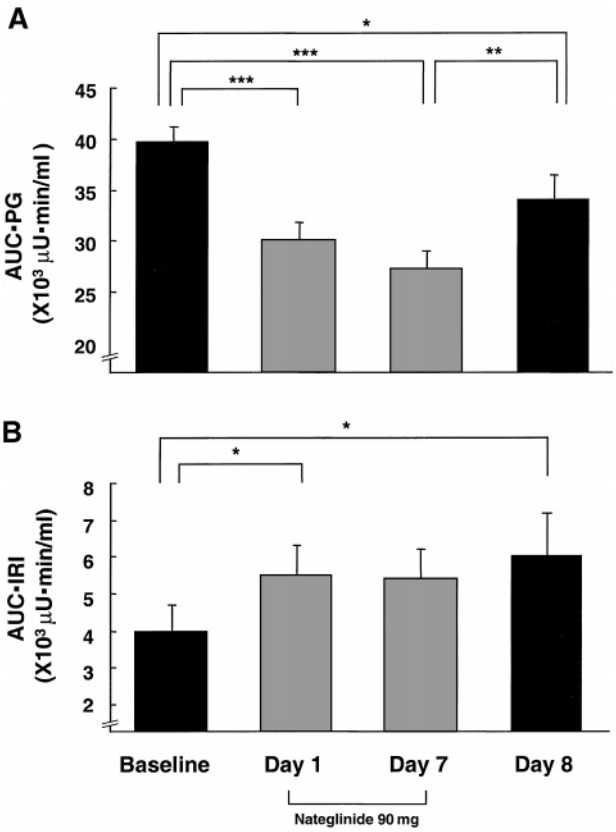

Fig 2. AUC · PG and AUC · IRI in the meal tolerance test. Meal tolerance tests were performed before (baseline) and after a single nateglinide administration (day 1), after 7 days of repeated administration (day 7), and after cessation of nateglinide on day 8. (A) Total amount of PG (AUC · PG). (B) Total amount of insulin (AUC · IRI). Data are shown as mean ± SEM. *P < .05; **P < .01; ***P < .001; PG, plasma glucose; IRI, immunoreative insulin; AUC, area under the curve.

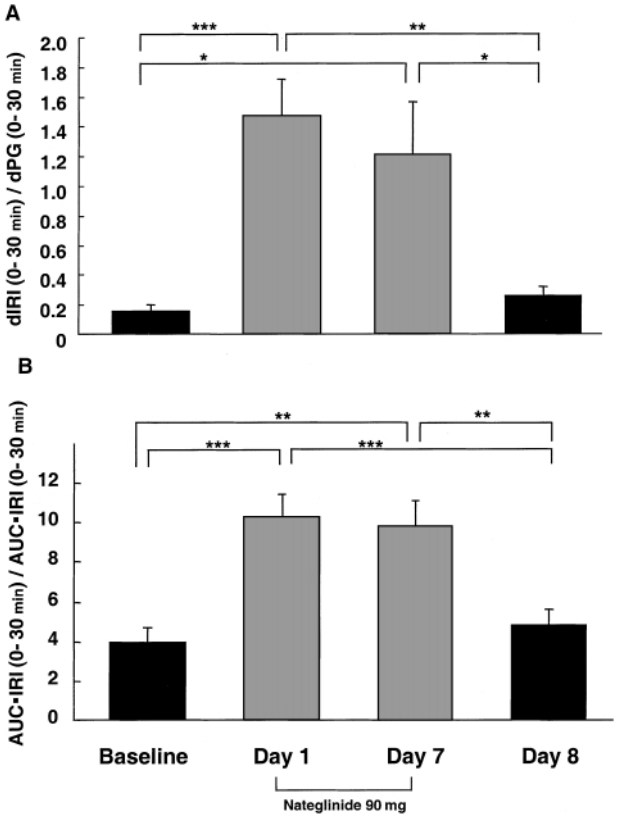

Fig 3. Comparison of early phase insulin to total insulin secretion ratio in the meal tolerance test. Meal tolerance tests were performed before (baseline) and after a single nateglinide administration (day 1), after 7 days of repeated administration (day 7), and after cessation of nateglinide on day 8. (A) The early phase of insulin secretion is based on the ratio of delta IRI to delta PG from fasting to 30 minutes after a meal. (B) The component of early phase insulin secretion to total insulin secretion was based on the ratio of AUC · IRI from fasting to 30 minutes after a meal to AUC · IRI from fasting to 180 minutes after a meal. Data are shown as mean ± SEM. *P < .05; **P < .01; ***P < .001; IRI, immunoreative insulin.

Next, we evaluated early phase insulin secreting ability (Fig 3A), based on the ratio of delta IRI to delta PG from fasting to 30 minutes after a meal.[14] This $dIRI_{30}/dPG_{30}$ ratio was 0.15 ± 0.04 at baseline, 1.44 ± 0.26 on day 1 (P = .0009), 1.16 ± 0.38 on day 7, and 0.26 ± 0.06 on day 8 (P = .29 and P = .05 *v* baseline and day 7, respectively). This index increased only during nateglinide administration.

The component of early phase insulin secretion to total insulin secretion (Fig 3B) was also investigated, which is based on the ratio of AUC · IRI from fasting to 30 minutes after a meal to AUC · IRI from fasting to 180 minutes after a meal. This ratio was 3.9% ± 0.7% at baseline, 10.2% ± 1.1% on day 1 (P = .0002), 9.7% ± 1.3% on day 7, and 4.8% ± 0.7% on day 8 (P = .99, baseline; P = .004, day 7).

DISCUSSION

The determination of PG and the insulin profile during meal tolerance tests with and without nateglinide provided 3 unique measurements for clinical appraisal of nateglinide. First, both the early phase of insulin secretion and the total amount of secreted insulin increased with nateglinide administration. Second, although a single administration of nateglinide induced immediate improvement of hyperglycemia, only a single missed dose of nateglinide also resulted in an immediate decline of its glucose-lowering ability. Third, although early phase insulin secretion was immediately reduced, total insulin secretion remained at a high level after cessation of nateglinide.

Previous studies showed that nateglinide increased early phase insulin secretion and the total amount of insulin secreted,[10-13] but did not discuss which of these effects contributed more to the lowering of postprandial glucose levels seen with nateglinide administration. The present study also shows that nateglinide increased both early phase and the total amount of insulin secretion. After cessation of nateglinide, the postprandial PG level significantly increased. On the other hand, although early phase insulin secretion returned nearly to the baseline level, AUC · IRI did not, surprisingly, decrease at all. Although the total secreted insulin level was still high, it was unable to suppress postprandial glucose sufficiently. These findings imply that once PG increases after a meal, it is difficult to suppress, even if insulin is secreted following this increase of PG. Therefore, improvement in postprandial PG seems to depend on the early secretion of insulin rather than on total insulin secretion. This is the first report, to our knowledge, demonstrating that early phase insulin secretion contributes more than total insulin secretion to the improvement of postprandial hyperglycemia by nateglinide in type 2 diabetes.

Nateglinide is a very short-acting medicine; its half-life is 1.1 to 1.3 hours, and insulin levels return to baseline values 3 to 4 hours after administration.[13] It was naturally assumed that the insulin-secreting effect and hypoglycemic effect of nateglinide would disappear overnight. However, it is interesting that insulin secretion did not return to the baseline level during the postprandial period after cessation of nateglinide, since total secreted insulin had, paradoxically, increased. This finding implies that the ability to secrete insulin may be improved by maintaining an ideal postprandial PG level for 7 days, and/or some unknown effect of nateglinide remains after its main effect dissipates. First, it is well known that although sustained hyperglycemia reduces the islets' ability to secrete insulin, this pancreatic islet desensitization is a temporary and reversible state.[15,16] Kosaka et al[17] investigated PG profiles and insulin response to a 100-g oral glucose tolerance test before and after strict glycemic control in patients with type 2 diabetes and reported an increase in insulin secretion by maintaining strict glycemic control. Therefore, β cells may be able to respond to postprandial hyperglycemia due to relief from glucose desensitization. Second, a high serum concentration of nateglinide (30 μmol/L), which can be reached with a 90-mg oral dose,[18] stimulates insulin secretion even under euglycemia.[7,19] On the other hand, a low concentration of nateglinide (0.3 μmol/L), which is reached 8 hours after administration,[18] cannot stimulate insulin secretion during euglycemia, but significantly facilitates insulin secretion at a 7.5-mmol/L or higher concentration of glucose.[20] A low concentration of nateglinide can potentiate insulin secretion induced by hyperglycemia. Therefore, there is the possibility that this very low concentration of nateglinide, which remains in the blood overnight after the last dose of nateglinide, showed a certain potency on glucose-induced insulin secretion on day 8.

Postprandial hyperglycemia has been associated with an increased risk of diabetic complications.[1-4] The Diabetes Intervention Study demonstrated that postprandial PG was an independent risk factor for mortality in patients with newly diagnosed type 2 diabetes, but fasting PG was not.[3] It has been previously reported that impaired glucose tolerance results primarily from a reduced ability to suppress hepatic glucose output secondary to abnormal pancreatic β-cell function, and late hyperinsulinemia may be the consequence of an inadequate early β-cell response rather than from insulin resistance.[21] As is often experienced clinically, postprandial hyperglycemia is characteristic of patients with an early stage of type 2 diabetes, who have the ability to maintain late phase insulin secretion, but a poor ability to secrete in the early phase.[22] Therefore, it is thought that improvement of early phase insulin secretion, in order to suppress postprandial hyperglycemia, is very important. In this study, it was demonstrated that a single dose of nateglinide significantly improves postprandial hyperglycemia. However, it was also demonstrated that this ability immediately declines after a single missed dose. This result emphasizes the importance of continuous administration.

In conclusion, the increase in early phase insulin secretion induced by nateglinide contributes more than total insulin secretion to the improvement of postprandial hyperglycemia in type 2 diabetes.

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