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Nateglinide, a non-sulfonylurea rapid insulin secretagogue, increases pancreatic islet blood flow in rats

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Abstract

We studied whether the rapid hypoglycemic action of nateglinide is associated with an increase in islet blood flow. Islet blood flow was measured using the two-colour microsphere method. Orally administered nateglinide with glucose acutely increased islet blood flow to levels greater than those after glucose alone or tolbutamide with glucose in conscious Sprague–Dawley rats (percent increase at 10 min after oral administration; nateglinide + glucose, 125 ± 25%; glucose, 33 ± 11%, $p < 0.001$; tolbutamide + glucose, 42 ± 23%, $p < 0.01$). Nateglinide administered with non-metabolisable 3-*O*-methylglucose also increased islet blood flow (61 ± 17%). The stimulated islet blood flow significantly correlated with serum insulin levels. N^G-monomethyl-L-arginine, a nitric oxide synthase inhibitor, completely inhibited the increase in islet blood flow induced by nateglinide with glucose. Intravenously administered nateglinide did not significantly affect the already increased islet blood flow in diabetic Otsuka Long–Evans Tokushima Fatty rats. Our results indicated that nateglinide acutely increased islet blood flow at least in part through a nitric oxide-dependent mechanism.

Keywords: Oral hypoglycemic agent; Tolbutamide; Nitric oxide; Microsphere; Regional blood flow; Otsuka Long–Evans Tokushima Fatty rat

1. Introduction

The pancreas has a unique vascular system separated into exocrine and endocrine parts (Bonner-Weir, 1993). Arterioles that directly enter islets of Langerhans divide into a glomerulus-like capillary network, and blood containing secreted hormones drains into venous system through neighbouring exocrine tissue, forming insuloacinar system. Islets receive abundant blood flow at the basal state, and islet vasculature regulates its amount of blood flow based on physiological and pathological conditions (Jansson, 1994). Typically, acute hyperglycemia following glucose administration increases islet blood flow (Jansson and Hellerström, 1983), whereas acute insulin-induced hypoglycemia decreases it (Iwase et al., 2001b; Carlsson et al., 2003). Animal models of type 1 or type 2 diabetes mellitus including Otsuka Long–Evans Tokushima Fatty (OLETF) rats have increased islet blood flow (Atef et al., 1994; Carlsson et al., 1996, 1998; Svensson et al., 2000; Iwase et al., 2002). The effects of anti-diabetic agents on islet blood flow were investigated in a few studies using the microsphere technique in anesthetized rats. The effects of sulfonylurea have been controversial, e.g., tolbutamide increased islet blood flow (Vetterlein et al., 1985), whereas glipizide decreased it (Jansson et al., 2003). Since these measurements were performed under induced hypoglycemic condition, it is difficult to determine whether islet blood flow was modulated by the drug per se or accompanying hypoglycemia. On the other hand, biguanide metformin increased islet blood flow without affecting blood glucose levels (Jansson, 1995). Thus, the importance of these modulations of islet blood flow by anti-diabetic agents remains unknown at present.

Nateglinide, a D-phenylalanine derivative lacking either a sulfonylurea or benzamido moiety, is a rapidly acting

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insulin secretagogue with short duration of action (Shinkai et al., 1988). Based on its pharmacological characteristic, nateglinide has been recently introduced into the treatment of postprandial hyperglycemia that strongly links to cardiovascular disease (Carroll et al., 2002). Although the distinctive early action of nateglinide is may be due to its rapid absorption from the gastrointestinal tract (Sato et al., 1991) and fast inhibition in K_{ATP} channel activity on β cells (Hu et al., 2000), the effect of nateglinide on islet blood flow has not been studied. Since islet blood hyperperfusion may lead to rapid dispersal of insulin to target tissue (Jansson, 1994), it is conceivable that an increase in islet blood flow may contribute to the rapid hypoglycemic action of nateglinide. For this purpose, we designed the present study to determine the effects of nateglinide on islet blood flow in rats during non-hypoglycemic condition.

2. Materials and methods

2.1. Effects of oral administration of nateglinide in conscious Sprague–Dawley rats

Eight-week-old male Sprague–Dawley rats of ~300 g body weight (Kyudo, Fukuoka, Japan) were used. All experiments were approved by the Animal Experimentation Ethics Committee of Kyushu University. Under ether anesthesia, a polyethylene tube (PE-50) was implanted in the ascending aorta via the right carotid artery. The femoral artery was also cannulated. Both catheters were exteriorised through the back of the neck, filled with heparinized saline, and each was plugged with a pin. Experiments were performed at least 72 h postoperatively, in an unrestrained state. The measurement of islet blood flow was performed after overnight fast as reported previously (Iwase et al., 2001b). After administration of heparin (100 IU, Shimizu Co., Shizuoka, Japan) and stabilization of arterial blood pressure as confirmed by direct recording using a pressure transducer (Nihon Koden Co., Tokyo) connected to the carotid catheter, islet blood flow was measured using 10 μm green and black microspheres (E-Z TRAC microsphere, Interactive Medical Technology, San Diego, CA). As control, a non-metabolisable glucose derivative, 3-*O*-methylglucose (Sigma Chemical Co., St. Louis, MO) or 20% glucose solution (2 g/kg) was administered by gastric gavage after the first microsphere injection. Nateglinide (50 mg/kg, Ajinomoto Co., Tokyo) or tolbutamide (250 mg/kg, Aventis Pharma Co., Tokyo) dissolved in alkalised saline was similarly administered with glucose or 3-*O*-methylglucose solution. The doses of the drugs were equipotent as determined by the previous report (Ikenoue et al., 1997). In some experiments, 25 mg/kg of N^G-monomethyl-L-arginine (L-NMMA), a nitric oxide synthase (NOS) inhibitor dissolved in saline, was administered via the carotid catheter immediately after gastric gavage of the solution. This dose of L-NMMA was reported to prevent an increase in islet blood flow induced by intravenous glucose administration (Iwase et al., 2001a). The second microsphere was injected 10 min after oral administration of the solution. The order of microsphere colour was switched every experiment. Microspheres were suspended in saline and sonicated before injection, 400,000 microspheres were injected and flushed with 350 μl saline into the ascending aorta over 25 s. Starting 10 s before the microsphere injection, the reference blood sample was withdrawn from the femoral artery catheter into a syringe at a rate of 0.5 ml/min using a constant withdrawal pump (model 120, KD Scientific Inc., Boston, MA). The number of microspheres used did not cause any adverse hemodynamic effects (Kobayashi et al., 1994; Iwase et al., 2001b). Blood was obtained for determination of blood glucose and serum insulin concentrations. Blood glucose concentration was measured by the electrode method (Glutest Ace, Kyotodaiichikagaku, Kyoto, Japan), and serum immunoreactive insulin was measured by an enzyme-linked immunosorbent assay (ELISA) commercial kit using rat insulin as a standard (Morinaga, Yokohama, Japan). The rats were then sacrificed and the whole pancreas, adrenal glands, stomach, duodenum and ileum were removed, blotted, and weighed. The whole pancreas was carefully dissected and freed of fat and lymph nodes under a stereomicroscope (Leica MZ8, Leica, Heerbrugg, Switzerland). Each pancreas was cut into small pieces and placed between object slides, then treated with the freeze-thawing technique, which allows visualization of microspheres and pancreatic islets. The percentage of islet volume was determined using the point-counting method in a blinded manner (Iwase et al., 2001b). Intersections of overlapping islets were counted at a magnification of ×400. Whole pancreas, stomach, duodenum and ileum were digested with 2 mol/l NaOH at 70 °C overnight. The microsphere contents of the organ and reference blood sample were determined by transferring parts of the samples after vigorous stirring to glass microfibre filters (GF/A, Whatman, Kent, UK) and counting the microspheres under a stereomicroscope in a blinded manner. By determining the number of microspheres present in the organ and the arterial reference sample, the blood flow values were calculated using the formula $Q_{org}=N_{org}\times 0.5/N_{ref}$, where Q_{org} is the organ blood flow (milliliter per minute), 0.5 is the withdrawal rate of the reference sample (milliliter per minute), N_{org} is the number of microspheres in the organ, and N_{ref} is the number of microspheres in the reference sample. A difference in microsphere content of <10% in the adrenal glands was considered to indicate sufficient mixing of the microspheres with the arterial bloodstream. When the islet blood flow was expressed per islet weight, islet weight was estimated by multiplying the pancreatic weight with the islet volume fraction of the whole pancreas. Fractional islet blood flow was expressed as a percentage of whole pancreatic blood flow.

2.2. Effects of intravenous administration of nateglinide in OLETF rats, a model of type 2 diabetes mellitus

Four-week-old male OLETF rats ($n=7$) and control Long–Evans Tokushima Otsuka (LETO) rats ($n=7$) were supplied by Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan) (Kawano et al., 1992). Islet blood flow was measured in 20-week-old LETO and OLETF rats under pentobarbital anesthesia (50 mg/kg, Abbott Laboratories, Chicago, IL), because OLETF rats were sensitive to various stress (K. Kawano, personal communication) and we were unable to implant chronic catheters in these rats. Body temperature was maintained at 37.5 °C using a body temperature controller (Fine Science Tools Inc., Foster City, CA). Eight minutes after the first microsphere injection, we injected 10 mg/kg nateglinide dissolved in alkalised saline (1 ml/kg) via the femoral vein catheter. The second microsphere was injected 5 min after injection of nateglinide solution. Blood flow to

Table 1
Effects of oral nateglinide administration on blood glucose, serum insulin and blood pressure in conscious Sprague–Dawley rats

	3MG	3MG+NG	Glucose	Glucose+NG	Glucose+TB
n	10	8	10	10	8
Blood glucose (mmol/l)					
Before	4.6±0.2	5.2±0.3	5.4±0.3	5.1±0.2	5.7±0.3
10 min after	5.4±0.2	4.8±0.2	10.7±0.3[a]	9.2±0.3[a,b]	9.6±0.6[a]
Serum insulin (ng/ml)					
Before	0.6±0.2	0.7±0.1	1.3±0.3	1.0±0.2	2.1±0.8
10 min after	0.8±0.2	5.6±0.6	12.5±2.3[a]	24.0±2.3[a,c]	14.2±1.4[a]
MBP (mmHg)					
Before	99±3	95±3	99±4	98±4	94±5
10 min after	97±4	100±2	98±3	98±3	97±6

Data are mean±S.E.M. [a]$p<0.001$ vs. 3MG, [b]$p<0.05$, [c]$p<0.001$ vs. glucose. 3MG, 3-*O*-methylglucose; NG, nateglinide; TB, tolbutamide; MBP, mean blood pressure.

whole pancreas, pancreatic islets, duodenum and kidney was calculated as describe above.

2.3. Statistical analysis

Values are expressed as mean±S.E.M. Student's paired *t*-test was used for comparison of data from the same animal. Student's unpaired *t*-test was used for comparison of two groups. Analysis of variance (ANOVA) and Scheffe's *F*-test as a post hoc test were used for comparison of multiple groups. Correlation coefficients were determined by univariate Spearman correlation. Differences were considered significant when the p value was less than 0.05.

3. Results

3.1. Effects of oral administration of nateglinide in conscious Sprague–Dawley rats

3-*O*-methylglucose, non-metabolisable glucose did not significantly affect serum insulin, but oral nateglinide with 3-*O*-methylglucose significantly increased serum insulin concentrations (Table 1). Blood glucose and serum insulin concentrations were significantly higher at 10 min after oral glucose load (2 g/kg body weight) compared with administration of 3-*O*-methylglucose. Nateglinide with glucose significantly suppressed the rise in blood glucose concentration, and markedly increased serum insulin concentration, which was significantly higher than administration of glucose alone. Tolbutamide with glucose did not significantly suppress the rise in blood glucose, but increased serum insulin concentrations, although the latter did not significantly differ from those noted after glucose alone. The mean blood pressure was similar between the groups.

As shown in Table 2, 3-*O*-methylglucose did not affect total pancreatic blood flow, islet blood flow or fractional islet blood flow (fraction of blood diverted through the islets). Nateglinide with 3-*O*-methylglucose did not affect total pancreatic blood flow but significantly increased islet blood flow and fractional islet blood flow. Oral glucose administration did not affect total pancreatic blood flow, but significantly increased islet blood flow and tended to increase fractional islet blood flow. Nateglinide with glucose significantly increased total pancreatic blood flow and markedly increased islet blood flow, which was significantly higher than that after the administration of glucose alone ($p<0.05$) and tolbutamide with glucose ($p<0.01$). Nateglinide with glucose significantly increased fractional islet blood flow to a level similar to that seen in nateglinide with 3-*O*-methylglucose. Tolbutamide with glucose did not alter total pancreatic blood flow, but increased islet blood flow, the degree of which was similar to that noted after administration of glucose alone. Fractional islet blood flow tended to be increased. Blood flow to the stomach, duodenum and ileum did not differ between the groups (Table 3).

As shown in Fig. 1, islet blood flow correlated significantly with serum insulin concentrations at 10 min after oral administration of 3-*O*-methylglucose, glucose, nateglinide with 3-*O*-methylglucose, nateglinide with glucose, and tolbutamide with glucose ($r=0.65$, $p<0.0001$). There were no significant correlations in the individual group. When nateglinide with 3-*O*-methylglucose was excluded from the analysis, a better correlation was observed ($r=0.79$, $p<0.0001$).

Table 2
Effects of oral nateglinide administration on pancreatic and islet blood flow in conscious Sprague–Dawley rats

	3MG	3MG+NG	Glucose	Glucose+NG	Glucose+TB
Total pancreatic blood flow (ml/min/g)					
Before	1.6±0.1	2.9±0.4	2.3±0.2	1.6±0.1	1.9±0.2
10 min after	1.2±0.1	1.9±0.4	2.1±0.2	2.3±0.2[a]	2.0±0.2
Islet blood flow (μl/min/mg)					
Before	4.7±0.3	5.9±0.7	6.0±0.5	5.4±0.3	5.4±0.6
10 min after	3.5±0.2	9.1±1.1[c]	7.7±0.7[a]	11.9±1.0[c,d]	7.2±0.8[a]
Fractional islet blood flow (% of total pancreatic blood flow)					
Before	7.9±0.7	8.5±1.0	6.5±0.4	8.4±0.5	6.7±0.8
10 min after	7.4±0.6	12.8±1.7[b]	9.1±0.7	12.7±0.9[b]	8.3±0.5

The numbers of animals are similar to those listed in Table 1. Data are mean±S.E.M. [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ vs. 3MG, [d]$p<0.05$ vs. glucose. 3MG, 3-*O*-methylglucose; NG, nateglinide; TB, tolbutamide.

Table 3
Effects of oral nateglinide administration on gastrointestinal blood flow in conscious Sprague–Dawley rats

	3MG	3MG+NG	Glucose	Glucose+NG	Glucose+TB
Stomach (ml/min/g)					
Before	1.0±0.1	0.9±0.0	1.0±0.2	1.1±0.2	1.3±0.2
10 min after	0.5±0.1	0.6±0.1	0.7±0.1	0.6±0.1	0.7±0.1
Duodenum (ml/min/g)					
Before	2.1±0.3	2.3±0.3	2.1±0.3	2.3±0.2	2.5±0.2
10 min after	2.8±0.4	3.1±0.5	3.0±0.4	2.7±0.2	2.6±0.3
Ileum (ml/min/g)					
Before	1.4±0.2	1.3±0.2	1.3±0.1	1.7±0.2	1.1±0.1
10 min after	1.3±0.2	1.4±0.3	1.5±0.3	1.6±0.2	1.1±0.2

The numbers of animals are similar to those listed in Table 1. Data are mean±S.E.M. 3MG, 3-*O*-methylglucose; NG, nateglinide; TB, tolbutamide.

3.2. Effects of pretreatment with L-NMMA, a NOS inhibitor in conscious Sprague–Dawley rats

Next, we investigated the effect of pretreatment with L-NMMA, a NOS inhibitor, on islet blood flow after oral administration of glucose or nateglinide with glucose (Table 4). Pretreatment with L-NMMA did not significantly change blood glucose or serum insulin concentrations in rats treated with glucose alone or nateglinide with glucose compared to without the pretreatment (Table 1). L-NMMA significantly increased mean blood pressure. Pretreatment with L-NMMA tended to reduce total pancreatic and islet blood flow in rats treated with glucose alone, but significantly reduced it in rats treated with nateglinide and glucose compared to without the pretreatment (Table 2). However, fractional islet blood flow did not significantly change by pretreatment with L-NMMA.

3.3. Effects of intravenous nateglinide in anesthetized OLETF rats

Lastly, we investigated the effects of intravenous nateglinide administration in obese type 2 diabetic OLETF rats (Table 5). Nateglinide did not affect blood glucose concentrations in either strain rats 5 min after intravenous injection, while it significantly increased serum IRI in both groups. Nateglinide did not affect total pancreatic blood flow. Islet blood flow and fractional islet blood flow, which were significantly higher in OLETF rats than in LETO rats as reported previously (Iwase et al., 2002), were significantly enhanced by nateglinide only in LETO rats. Duodenal and renal blood flow rates were not altered by nateglinide injection (duodenal blood flow, LETO before 1.6±0.2 ml/min/g, after 1.8±0.2 ml/min/g, OLETF before 1.7±0.1 ml/min/g, after 1.6±0.2 ml/min/g; renal blood flow, LETO before 6.0±0.7 ml/

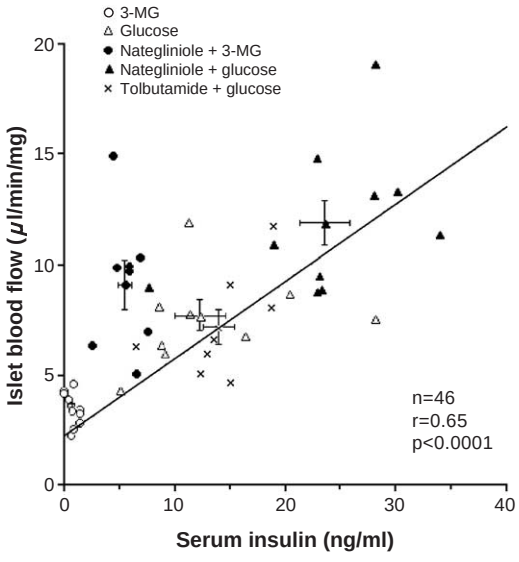

Fig. 1. Correlation between islet blood flow and serum insulin concentrations 10 min after oral administration of various compounds. Data are mean±S.E.M.

Table 4
Effect of pre-treatment with N^G-monomethyl-L-arginine (L-NMMA), a nitric oxide synthase inhibitor, on pancreatic and islet blood flow after oral administration of glucose or nateglinide with glucose in conscious Sprague–Dawley rats

	Glucose+ L-NMMA	Glucose+NG+ L-NMMA
n	6	7
Blood glucose (mmol/l)		
Before	5.5±0.6	5.1±0.3
10 min after	12.1±0.8	9.9±0.2
Serum insulin (ng/ml)		
Before	0.8±0.2	0.8±0.2
10 min after	9.8±2.6	23.7±5.5
MBP (mmHg)		
Before	100±6	104±7
10 min after	124±11[a]	123±10[b]
Total pancreatic blood flow (ml/min/g)		
Before	2.2±0.6	1.7±0.3
10 min after	1.3±0.3	1.1±0.2[c]
Islet blood flow (μl/min/mg)		
Before	5.6±0.6	5.1±0.4
10 min after	4.7±0.6	5.1±1.1[d]
Fractional islet blood flow (% of total pancreatic blood flow)		
Before	8.6±0.7	8.7±1.1
10 min after	13.4±3.7	14.1±2.8

Data are mean±S.E.M. [a]$p<0.05$ vs. glucose (Table 1), [b]$p<0.05$, [c]$p<0.01$, [d]$p<0.001$ vs. nateglinide with glucose (Tables 1 and 2). L-NMMA was administered immediately after gastric gavage of glucose alone. (glucose+L-NMMA) or glucose with nateglinide (glucose+NG+L-NMMA). L-NMMA, N^G-monomethyl-L-arginine; NG, nateglinide; MBP, mean blood pressure.

Table 5
Effects of intravenous nateglinide on pancreatic and islet blood flow in Otsuka Long–Evans Tokushima Fatty (OLETF) rats, a model of type 2 diabetes mellitus, and control Long–Evans Tokushima Otsuka (LETO) rats under anesthesia

	LETO	OLETF
n	7	7
Body weight (g)	412±12	532±9[c]
Blood glucose (mmol/l)		
Before	5.4±0.2	7.4±0.3[c]
5 min after	5.6±0.3	7.5±0.2[c]
Serum insulin (ng/ml)		
Before	3.2±0.5	13.8±3.3[b]
5 min after	12.4±3.2[d]	21.1±4.7[d]
MBP (mmHg)		
Before	114±6	93±5[a]
5 min after	120±6	104±4
Total pancreatic blood flow (ml/min/g)		
Before	1.2±0.4	1.1±0.1
5 min after	1.2±0.2	1.2±0.2
Islet blood flow (μl/min/mg)		
Before	6.4±1.2	11.6±1.3[b]
5 min after	9.0±1.6[e]	13.1±1.5
Fractional islet blood flow (% of total pancreatic blood flow)		
Before	12.8±1.4	39.5±5.3[c]
5 min after	16.1±1.3[d]	39.3±1.4[c]

Data are mean±S.E.M. [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ vs. LETO, [d]$p<0.05$, [e]$p<0.01$ vs. before. MBP, mean blood pressure.

min/g, after 5.1±0.7 ml/min/g, OLETF before 6.6±0.1 ml/min/g, after 5.7±0.6 ml/min/g).

4. Discussion

The present study demonstrated that *1)* orally administered nateglinide with glucose solution in conscious rats resulted in an acute rise in islet blood flow greater than that noted after administration of glucose alone or tolbutamide with glucose. *2)* Nateglinide with 3-*O*-methylglucose but without glucose resulted in an acute rise in islet blood flow. *3)* L-NMMA, a NOS inhibitor, prevented the increase in islet blood flow induced by nateglinide with glucose but did not affect serum insulin levels. *4)* Intravenously administered nateglinide resulted in an acute rise in islet blood flow in control LETO rats but not significantly in OLETF rats, although serum insulin increased in both strains.

The effects of insulin secretagogues on islet blood flow have been investigated using the microsphere technique in anesthetized rats treated with intravenous tolbutamide (Vetterlein et al., 1985) or glipizide (Jansson et al., 2003) in doses that induced hypoglycemia. However, conflicting results were reported, e.g., tolbutamide increased islet blood flow whereas glipizide decreased it. Since hypoglycemia per se reduced islet blood flow (Iwase et al., 2001b; Carlsson et al., 2003), it is difficult to determine whether islet blood flow was altered by the drug per se or accompanying hypoglycemia. In addition, anesthesia and concomitant catheter surgery may affect the results (Iwase et al., 2001b). In the present study, the drug was orally administered in conscious Sprague–Dawley rats and islet blood flow was measured during the early phase when hypoglycemia was avoided. However, since one colour of microspheres measures one time point only, the time course of islet blood flow is not measured. To investigate the delayed effect of tolbutamide, more time points may be required.

Abnormality of insulin secretion pattern, i.e., lack of early insulin secretion, is a common feature in patients with type 2 diabetes mellitus (Pratley and Weyer, 2001). Although the significance of impaired early insulin secretion is not fully understood, a recent study suggested that early insulin secretion is more important in modulating postprandial glycemia than previously considered (Bruttomesso et al., 1999). In this regard, nateglinide is unique in correcting the abnormal pattern of insulin secretion in some diabetic patients. In the present study, nateglinide stimulated insulin secretion more rapidly than tolbutamide with an increase of islet blood flow 10 min after oral administration. The rapidity of insulinotropic effect of nateglinide has been ascribed to rapid absorption from the gastrointestinal tract (Sato et al., 1991) and fast inhibitory action on K_{ATP} channel on β cells (Hu et al., 2000). The absorption of nateglinide was suggested to be mediated by a proton-dependent transport system, not by passive diffusion (Okamura et al., 2002). However, a recent study has demonstrated that the pharmacokinetics in gastrointestinal absorption may not fully explain the rapid onset of the hypoglycemic action of nateglinide (Okamoto et al., 2002). When similar kinetic changes in plasma drug concentration were produced in nateglinide and glibenclamide by intraportal infusion in conscious dogs, nateglinide still exerted faster insulinotropic action than glibenclamide. In vitro study using isolated perfused pancreas showed earlier insulinotropic effect of nateglinide than sulfonylurea (Hirose et al., 1995), and a patch clamp study revealed that the K_{ATP} channel blocking effect of nateglinide was also more rapid than that of sulfonylurea (Hu, 2002). To what extent an increased islet blood flow contributes to the rapid onset of insulinotropic effect of nateglinide remains unknown at present. Nateglinide rapidly enhanced insulin secretion even in L-NMMA-pre-treated rats and OLETF rats without increasing islet blood flow. It seems unlikely that the rapid insulinotropic action of nateglinide was due to an increase in islet blood flow.

K_{ATP} channels, ubiquitously present, are composed of sulfonylurea (SU) receptor and an inwardly rectifying K^+ channel (Kir6.2) (Inagaki et al., 1995; Seino, 1999). Inhibition of K_{ATP} channels in β cells induces insulin release, and stimulation of vascular K_{ATP} channels mediates vasodilatation. Although nateglinide is chemically distinct from sulfonylurea, it inhibits K_{ATP} channels of rat β cells 45 times more potently than those of smooth muscle cells from rat aorta (Hu et al., 1999). Using a mammalian cell line transfected with a Kir6.2 subunit and either of SU receptor 1 (islet type), SU receptor 2A

(cardiac type), or SU receptor 2B (vascular smooth muscle type), nateglinide specifically inhibited SU receptor 1/Kir6.2 channels through the same site on SU receptor 1, similar to tolbutamide (Chachin et al., 2003). If nateglinide had non-specific inhibitory actions on K_{ATP} channels like glibenclamide, vasoconstriction might occur (Moreau et al., 1994). Although basal islet blood flow may be naturally luxurious for insulin secretion, it is tightly regulated by nervous, hormonal, metabolic and locally produced factors independent of surrounding exocrine tissue (Jansson, 1994). In general, however, islet blood perfusion correlates with insulin secretion activity. In the present study, we demonstrated that acute stimulation of insulin release by insulin secretagogues correlated with islet blood flow (Fig. 1). It should be noted that nateglinide with 3-*O*-methylglucose showed more enhanced effect on islet blood flow relative to serum insulin levels, as indicated by the data points above the regression line in Fig. 1. Recently, Jansson et al. (2003) reported that K_{ATP} channel openers, diazoxide and more β-cell specific K_{ATP} channel openers (NNC 55-0118) increased islet blood flow while they decreased serum insulin levels. These vasodilatory effects seem more pronounced in exocrine tissue than in islets probably due to direct vascular actions. Although the significance of islet blood hyperperfusion remains to be determined, it may contribute to supply nutrients and oxygen to metabolically active endocrine cells, to prevent accumulation of metabolic waste in interstitium as well as to transport a large amount of secreted insulin to systemic circulation. Considering the importance of islet blood flow, increased islet blood flow may be advantageous for nateglinide to disperse secreted insulin into portal circulation and target tissue.

The importance of nitric oxide (NO) in the regulation of islet blood flow has been consistently reported (Svensson et al., 1994). The participation of locally produced NO in the nateglinide-induced increase in islet blood flow was investigated using a NOS inhibitor in the present study. L-NMMA completely prevented the increase in islet blood flow. Therefore, nateglinide seems to increase islet blood flow at least in part through a NO-dependent mechanism. It was reported that NOS inhibitors decreased islet blood flow and fractional islet blood flow below the basal value when glucose was injected intravenously (Svensson et al., 1994; Iwase et al., 2001a). However, when glucose was administered orally, L-NMMA did not affect fractional islet blood flow and the suppression in islet blood flow was smaller. This suggests that the regulation of islet blood flow may be different between oral and intravenous glucose administration. Previous studies indicated that the origin of the NO may be endothelial, neuronal and/or β cells (Jansson, 1994) and that NOS is constitutively expressed in cultured rat islet capillary endothelial cells (Suschek et al., 1994). Insulin has a vasodilator effect, an action mainly mediated by NO (Scherrer et al., 1994). In cultured endothelial cells, insulin-induced stimulation of protein kinase B/Akt has been proposed to phosphorylate NOS, leading to NO production (Dimmeler et al., 1999; Fulton et al., 1999). The correlation of islet blood flow and circulating serum insulin suggests that the effect of nateglinide on islet blood flow is secondary to enhanced insulin secretion. In addition, it was recently reported that NO was produced in isolated rat islet β cells in response to high glucose (15 mmol/l) using the NO-sensitive fluorescent dye (Smukler et al., 2002). Constitutive NOS (cNOS), which is activated by Ca^{2+} and calmodulin, is mainly localized in the secretory granules of β cells (Lajoix et al., 2001). Nateglinide increased immediately after an increase in intracellular Ca^{2+}, sustained its increased level (Ikenoue et al., 1997), and may activate cNOS. Since NO is highly diffusible, NO may reach vascular smooth muscle cells of afferent arterioles before entering into the islets, and may relax them by elevating cGMP levels following activation of soluble guanylate cyclase. Although small amounts of NO play a crucial role in a number of physiological functions including vascular tone, further research is required to elucidate the mechanisms of nateglinide-induced increase of islet blood flow.

Due to the sensitive characteristics of OLETF rats, islet blood flow was measured under anesthesia in response to intravenous nateglinide administration instead of oral route. This may be responsible for the lack of vasodilator effect of nateglinide. On the other hand, we previously reported that islet hyperperfusion in OLETF rats was at least in part due to a NO-dependent mechanism (Iwase et al., 2002). Therefore, it is conceivable that nateglinide failed to enhance the already increased islet blood flow in OLETF rats. Since the effects of oral hypoglycemic agents on islet blood flow have not been studied in other diabetic models to our knowledge, the mechanisms of nateglinide-stimulated insulin secretion without further increase of islet blood flow remain to be determined.

In conclusion, orally administered nateglinide with glucose solution acutely increased islet blood flow to levels greater than those after glucose alone or tolbutamide with glucose in conscious rats. Nateglinide administered with 3-*O*-methylglucose also increased islet blood flow. L-NMMA completely inhibited the increase in islet blood flow induced by nateglinide with glucose. Our results indicated that the early hypoglycemic action of nateglinide was associated with a rapid increase in islet blood flow mediated at least in part through a NO-dependent mechanism. However, it seems unlikely that this contributes to the rapid insulinotropic or hypoglycemic action of nateglinide.

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