Adenosine Triphosphate–Sensitive Potassium Channels Are Involved in Insulin-Mediated Glucose Transport in Humans

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We investigated the influence of treatment with nicorandil, a K-channel opener currently used for angina, on glucose homeostasis in patients with non–insulin-dependent diabetes mellitus (NIDDM) and coronary artery disease (CAD). Adenosine triphosphate (ATP)-sensitive K (K-ATP) channels are present in various tissues, including pancreatic B cells and skeletal muscle, and are the putative targets of this agent. Nine NIDDM patients with CAD and five healthy subjects participated in the study. Fasting plasma levels (mean ± SEM) of glucose (144 ± 11 to 180 ± 22 mg/dL, $P < .05$) and insulin (5.8 ± 1.6 to 7.0 ± 1.8 μU/mL, $P < .05$) and hemoglobin A_{1c} (7.54 ± 0.47 to 8.11 ± 0.55%, $P < .01$) increased significantly in nine NIDDM patients after treatment with nicorandil at a dose of 5 mg three times daily for 2 to 8 months. Glucose tolerance as examined by an identical meal test deteriorated ($P < .001$), but the insulin response did not change significantly. A washout of nicorandil for 1 to 4 months restored glucose tolerance almost to pretreatment levels in four patients. A 5- to 7-day trial of nicorandil (5 mg three times daily) in five healthy subjects resulted in a marginal to twofold increase in fasting plasma insulin, reflecting the progression of insulin resistance. In addition, three healthy subjects showed a substantial reduction in the glucose infusion rate (GIR) required in the euglycemic-hyperinsulinemic clamp study. Since the therapeutic dose of nicorandil did not affect pancreatic B-cell function but caused insulin resistance in both healthy and NIDDM subjects, we conclude that K-ATP channels play a regulatory role in insulin-mediated glucose transport in humans.

CORONARY ARTERY DISEASE (CAD) is common among patients with non–insulin-dependent diabetes mellitus (NIDDM), although it is not known whether long-term good glycemic control affects CAD in these patients. Recently, the relationship between treatment with hypoglycemic sulfonylureas (SUs) and an increased risk of CAD in NIDDM patients has attracted renewed interest due to emerging evidence regarding SU receptors (SURs) and adenosine triphosphate (ATP)-sensitive potassium (K-ATP) channels in cardiovascular tissues.[1,2]

K-ATP channels are now known to be complexes composed of at least two subunits, a weak inward rectifier channel (Kir6.2) and a SUR.[3-5] In the proposed model, the Kir6.2 protein confers the properties of conductance and ion selectivity, whereas the SUR serves as the receptor for ATP and pharmacological modulators of channel function such as glibenclamide and the so-called K-channel openers.

On the other hand, there are several lines of evidence that SUs have insulin-like actions on glucose metabolism via SURs expressed in extrapancreatic tissues.[6-8] Based on these findings, it is speculated that K-ATP channels play a role not only in insulin secretion but also in glucose uptake by peripheral tissues.

Nicorandil (*N*-[2-hydroxyethyl]nicotinamide nitrate [ester]), a K-channel opener, is now widely used for the treatment of angina in Japan and Europe. However, in our experience, the use of this agent in NIDDM patients with CAD causes a deterioration of glycemic control. To gain insight into the mechanism of its effect, we examined the effects of nicorandil treatment on glycemia, glucose tolerance, and insulin secretion in NIDDM patients with CAD and healthy subjects. Here, we report that K-ATP channels play a physiological role in insulin-mediated glucose transport in humans.

SUBJECTS AND METHODS

Subjects

Nine NIDDM patients with angina pectoris or previous myocardial infarction and five healthy subjects participated in the study. The study purpose was explained to all subjects, and their consent was obtained before participation. The clinical features of these subjects are presented in Table 1.

Studies in NIDDM Patients

After an overnight fast, all nine patients ingested 300 mL of a semiliquid mixed meal (OkunosA; Okuno, Tokyo, Japan) equivalent to 300 kcal, which included 43.5 g carbohydrate, 15.3 g protein, and 8.4 g fat. This meal test was conducted before and after 2 to 8 months of treatment with nicorandil (5 mg orally three times per day). Four of nine patients underwent an additional third meal test 1 to 4 months after discontinuation of nicorandil.

Studies in Healthy Subjects

Three separate studies were performed in healthy subjects. First, the fasting plasma concentration of insulin, C-peptide, glucose, and free fatty acids (FFAs) was examined over 5 consecutive days of nicorandil administration (5 mg three times per day) following a 3-day baseline determination in two subjects. In addition, the plasma cortisol level was measured in pooled samples taken before and after treatment with nicorandil. Second, to determine the effects on glucose tolerance, a pair of meal tests were used in another three subjects before and after receiving nicorandil (5 mg three times per day) for 7 days. Third, the effects of a 7-day trial of nicorandil (5 mg three times per day) on whole-body insulin sensitivity were assessed using the euglycemic-hyperinsulinemic clamp method in these three subjects. Details of the clamp method have been described previously.[9] With our method, the mean difference in the glucose infusion rate (GIR) determined twice each for 13 subjects with varying degrees of glucose tolerance was 20.8% (range, 0.8 to 41.3%). None of the patients were taking any medications known to interfere with glucose tolerance, including

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Table 1. Clinical Features of the Subjects

Feature	Mean ± SEM
NIDDM patients with CAD	
No. (male/female)	9 (7/2)
Age (yr)	63.2 ± 3.4
BMI (kg/m²)	23.5 ± 0.9
Duration of NIDDM (yr)	13.4 ± 2.8
Treatment (diet:SU:insulin)	4:5:0
Healthy subjects	
No. (M/F)	5 (4/1)
Age (yr)	41.0 ± 2.8
BMI (kg/m²)	23.6 ± 0.8

Abbreviation: BMI, body mass index.

thiazide diuretics or steroid hormones. All medications were continued unchanged throughout the study.

Plasma glucose and FFA concentrations were determined enzymatically. Plasma insulin, C-peptide, and cortisol levels were measured using commercial radioimmunoassay kits.

Statistical analysis was performed using two-way ANOVA with repeated measures, the Mann-Whitney test, and the paired t test as appropriate. All data are expressed as the mean ± SEM.

RESULTS

During 2 to 8 months of treatment with nicorandil, the mean body weight of nine patients with NIDDM did not change (62.9 ± 2.6 *v* 63.1 ± 2.6 kg, nonsignificant [NS]), whereas the mean glycated hemoglobin (HbA_{1c}) increased from 7.54 ± 0.47 to 8.11 ± 0.55% ($P < .01$; Fig 1). The mean fasting plasma glucose increased from 144 ± 11 to 180 ± 22 mg/dL ($P < .05$) after nicorandil treatment (Fig 2A). The plasma glucose excursion in response to ingestion of an identical test meal was also higher after nicorandil treatment ($P < .001$). Figure 2B shows that the mean fasting plasma insulin was higher after treatment with nicorandil (5.8 ± 1.6 *v* 7.0 ± 1.8 μU/mL, $P < .05$), and the insulin response was apparently higher after treatment, but this effect was not statistically significant. The ratio of the increase in insulin over glucose at 30 minutes (0.17 ± 0.05 *v* 0.14 ± 0.03) and the sum of insulin levels (58.7 ± 10.5 *v* 63.6 ± 9.1 μU/mL) during the meal test were unchanged after nicorandil treatment. Following a nicorandil washout period for 1 to 4 months, the plasma glucose response to the test meal decreased almost to the pretreatment level ($P < .001$ among the three groups; Fig 3). The plasma insulin response and sum of insulin concentrations tended to decrease, although there were no significant differences between any of the groups. On the other hand, two healthy subjects showed minimal statistically insignificant increases in fasting plasma insulin and C-peptide over the 5-day nicorandil trial compared with the baseline levels (Table 2). Plasma cortisol levels did not differ after nicorandil treatment. In another three healthy subjects, there were no changes in plasma glucose or insulin responses to the meal after a 7-day nicorandil trial, but again, fasting plasma insulin was increased compared with the baseline levels (Table 3).

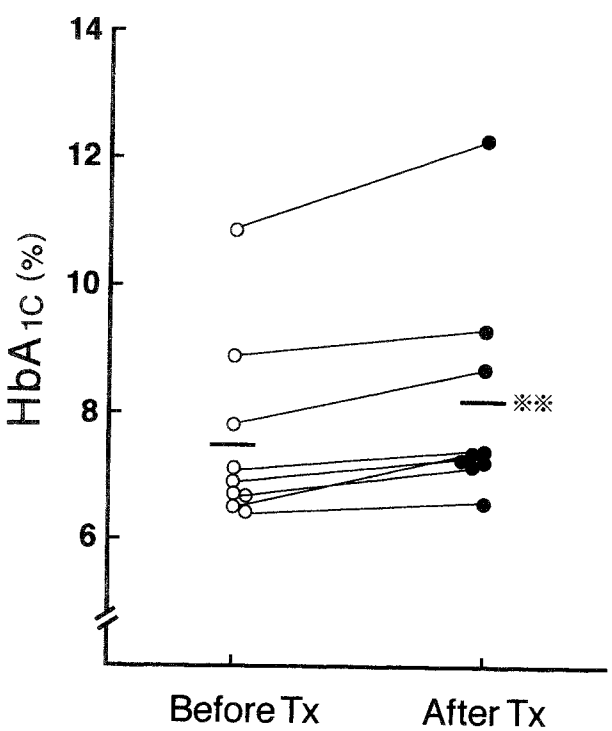

Fig 1. Changes in HbA_{1c} levels before and after nicorandil treatment (Tx) for 2-8 months in 9 NIDDM patients with CAD. **$P < .01$ (paired t test).

The euglycemic-hyperinsulinemic clamp study in the three healthy subjects showed a substantial decrease in the GIR in each case (6.9 to 4.0, 4.9 to 2.8, and 5.2 to 3.8 mg/kg/min) after administration of nicorandil for 7 days (Fig 4).

DISCUSSION

K-ATP channels have been identified in a variety of cells, where they appear to couple the metabolic state to the membrane excitability.[10-12] These K-ATP channels are thought to be the targets of so-called K-channel openers, which modulate ATP sensitivity of the channels.[13]

Nicorandil, one of the K-ATP channel openers currently used for the treatment of angina, has been reported to be highly specific for coronary artery smooth muscle cells. In previous in vitro studies,[14,15] a slight inhibition of insulin release was found only at a high concentration of nicorandil, and thus is unlikely to occur in vivo. Indeed, the present study demonstrates that nicorandil treatment did not decrease, but instead increased, fasting plasma insulin in both NIDDM patients and healthy controls. These observations confirm that the therapeutic dose of nicorandil does not interfere with pancreatic B-cell function. Nevertheless, glucose metabolism worsened after commencement of nicorandil treatment, as manifested by an elevated HbA_{1c} level and reduced glucose tolerance during the meal test. In addition, healthy control subjects showed an increase in fasting plasma insulin during a short-term nicorandil trial. Since the fasting insulin level is of clinical significance as an indicator of insulin resistance, these findings strongly suggest that nicorandil treatment can cause insulin resistance of peripheral tissue in both NIDDM patients and healthy subjects. This is supported by the results of the glucose clamp study.

Pulido et al[8] recently reported that in perfused rat hindquarter muscle preparations, gliclazide, a second-generation SU, produced dose-dependent stimulation of glucose uptake in the absence of insulin, and its effect was totally reversed by diazoxide, a potent K-ATP channel opener. Similar effects of

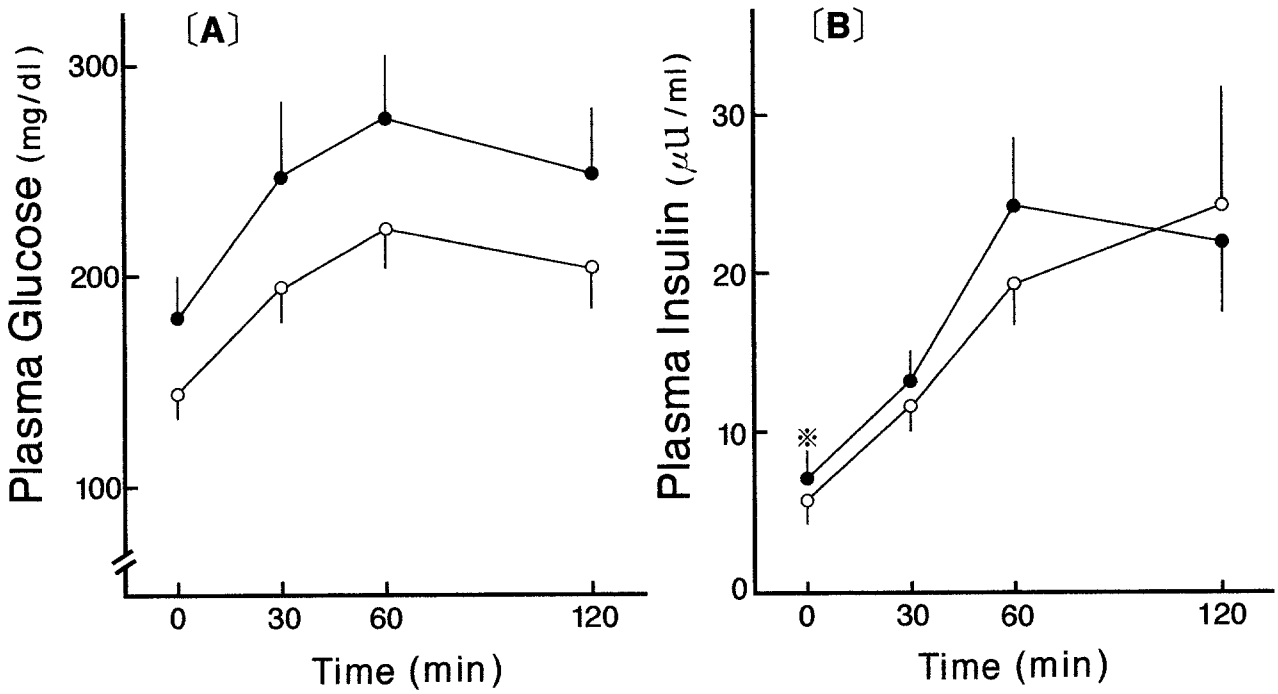

Fig 2. Changes in the plasma concentration of glucose (A) and insulin (B) during the meal test before and after nicorandil treatment for 2-8 months in 9 NIDDM patients with CAD. (○) Before treatment; (●) after treatment. The difference in glucose tolerance was significant ($P < .001$, 2-way ANOVA), but the insulin response was NS. *$P < .05$ (paired t test).

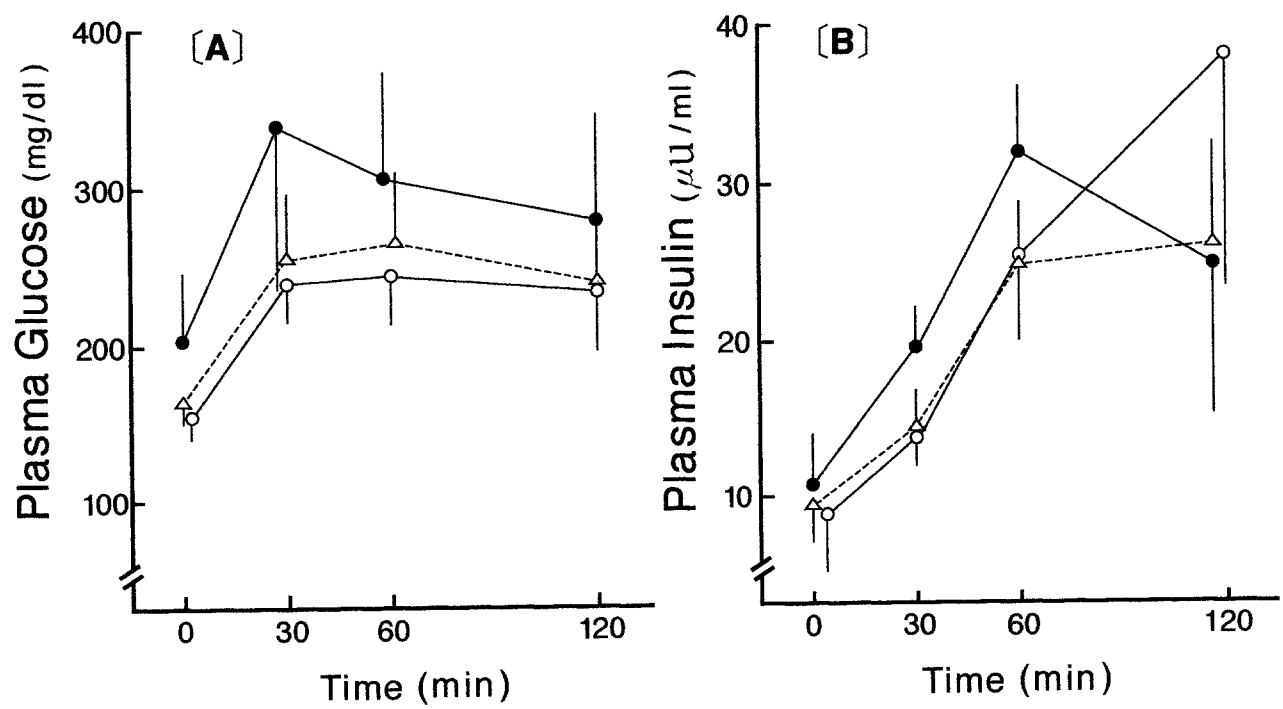

Fig 3. Changes in the plasma concentration of glucose (A) and insulin (B) during the meal test on 3 occasions, ie, before (○), during (●), and 1-4 months after discontinuation (△) of nicorandil treatment, in 4 NIDDM patients with CAD. There was a significant difference in glucose tolerance among the 3 groups ($P < .001$, 2-way ANOVA), but not in the insulin response.

Table 2. Fasting Plasma Glucose, Insulin, C-Peptide, FFA, and Cortisol Before and During 5-Day Administration of Nicorandil (5 mg three times per day) in Two Healthy Subjects

	Study Day							
	Before Nicorandil			During Nicorandil				
Parameter	−3	−2	−1	1	2	3	4	5
Case 1								
Glucose (mg/dL)	86	89	90	94	72	90	86	89
IRI (μU/mL)	5	5	7	8	7	6	8	7
CPR (ng/mL)	1.5	1.4	2.0	2.3	2.3	1.5	2.0	2.2
FFA (mmol/L)	0.24	0.29	0.17	0.58	0.33	0.32	0.22	0.26
Cortisol (μg/dL)		16.1				14.9		
Case 2								
Glucose (mg/dL)	74	78	76	78	73	74	80	82
IRI (μU/mL)	3	3	4	5	4	4	4	6
CPR (ng/mL)	1.1	0.9	1.1	1.3	1.3	1.1	1.2	1.3
FFA (mmol/L)	0.19	0.26	0.21	0.16	0.16	0.22	0.27	0.20
Cortisol (μg/dL)		17.5				18.3		

NOTE. Plasma cortisol was determined in the pooled sample. Values were not significantly different before and during nicorandil administration (Mann-Whitney test).

Abbreviations: IRI, immunoreactive insulin; CPR, C-peptide immunoreactivity.

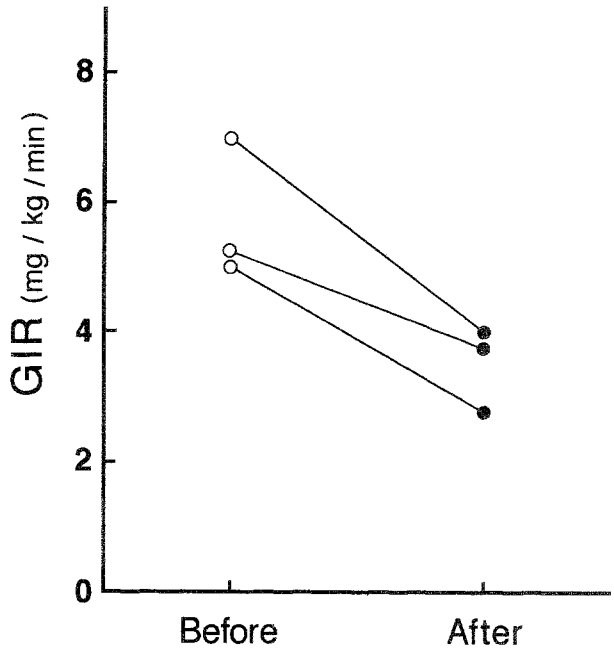

Fig 4. Changes in the GIR for the euglycemic-hyperinsulinemic clamp before and after nicorandil administration for 7 days in 3 healthy control subjects.

other SU agents were reported in L6 skeletal muscle cells[7] and insulin-resistant rat adipocytes.[6] Previous in vitro studies with SUs yielded two patterns of response: insulin-independent stimulation of glucose uptake[16,17] and potentiation of insulin-stimulated glucose uptake with no effect on the basal rate.[18,19] These results were dependent on the tissue type and the culture model system used, as well as the specific drug used. The results of the present study indicate that nicorandil treatment exerts an inhibitory effect on insulin-stimulated glucose disposal, probably via activation of K-ATP channels in insulin-responsive tissues (mainly skeletal muscle). However, the present results do not agree completely with those reported by Pulido et al,[8] who showed that the stimulatory effect of insulin on muscle glucose uptake was not affected by the presence of diazoxide. Such discrepancies may be due to differences in sensitivity to different K-channel openers (diazoxide *v* nicorandil), as well as differences in the species examined (rat *v* human). Furthermore, in the present study, treatment with nicorandil generated comparable effects on glycemia and glucose tolerance in both SU-treated and nontreated NIDDM patients (data not shown), suggesting that nicorandil binding sites are distinct from those of SUs.

Table 3. Plasma Glucose and Insulin During the Meal Test Before and After Administration of Nicorandil (5 mg three times per day) for 7 Days in Three Healthy Subjects

	Time (min)							
	Glucose (mg/dL)				Insulin (μU/mL)			
Subject No.	0	30	60	120	0	30	60	120
3								
Before	97	125	140	87	6.4	30.6	63.9	22.3
After	82	106	82	70	10.8	41.4	49.8	16.6
4								
Before	87	117	87	70	5.0	29.4	36.3	11.1
After	88	121	76	94	9.9	34.8	23.1	17.2
5								
Before	99	125	103	93	15.1	43.8	44.9	21.2
After	93	124	88	89	18.0	55.7	46.3	16.9

It is not yet clear whether K-ATP channels play a role in insulin-mediated glucose metabolism in peripheral tissues. In this regard, it is interesting that midaglizole, an α_2-adrenergic blocker, was shown to promote not only insulin secretion but also glucose disposal during a hyperinsulinemic-euglycemic clamp in NIDDM patients.[20] These effects of midaglizole were recently attributed to the inhibition of K-ATP channels by the imidazoline moiety of this compound instead of the antagonism of α_2-adrenoceptors.[21] In addition, novel antidiabetic agents with insulin-sensitizing properties such as troglitazone[22] and englitazone[23] have been shown to inhibit K-ATP channel activity in an insulin-secreting cell line. Thus, it is possible that the K-ATP channel-activating agent nicorandil could have the opposite effect on insulin-mediated peripheral glucose uptake.

It seems relevant that in skeletal muscle, an increase in the cytosolic Ca^{2+} concentration in the absence or presence of depolarization results in enhanced permeability of the cell membrane to glucose.[24] Recently, hyperglycemia per se has been shown to facilitate glucose transport via an insulin-independent and Ca^{2+}-dependent mechanism.[25] Similarly, the muscle contraction-induced increase in glucose transport is known to be associated with the release of Ca^{2+} from the sarcoplasmic reticulum.[26] Pinacidil, another potassium channel opener, has been suggested to inhibit a step involved in Ca^{2+} release from or refilling of the sarcoplasmic reticulum.[27] Thus, nicorandil likely has effects similar to pinacidil. In this context, insulin-stimulated GLUT4 translocation to the plasma membrane is thought to be a molecular process similar to the

exocytosis of insulin secretory granules in pancreatic B cells, both of which are dependent on a rapid increase in the intracellular Ca^{2+} concentration. Nitric oxide (NO) may account, in part, for the effect of nicorandil, because this agent contains a terminal nitrate that can generate NO.[28] However, contradictory results have been reported regarding the effects of NO on glucose transport.[29,30] Further studies are needed to elucidate the mechanism responsible for the present clinical observations.

In conclusion, using nicorandil as a therapeutic tool, the present results indicate that K-ATP channels play a regulatory role in insulin-mediated glucose transport in humans.

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