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Effect of pioglitazone on muscle sympathetic nerve activity in type 2 diabetes mellitus with alpha-glucosidase inhibitor

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ABSTRACT

Activation of the sympathetic nervous system is augmented in patients with type 2 diabetes mellitus (DM). Pioglitazone, an anti-diabetic drug, improves insulin resistance, but its influence on sympathetic nerve activity is not clear. To identify the relationship between insulin resistance and sympathetic activity, we examined muscle sympathetic nerve activity (MSNA) in controlled type 2 DM patients with alpha-glucosidase inhibitor (GI). We measured MSNA and calculated homeostasis model assessment of insulin resistance index (HOMA-IR) in twelve DM patients treated with alpha-GI and thirteen age-matched healthy subjects. In DM patients with alpha-GI, all parameters were reexamined after three months of treatment with pioglitazone. MSNA and HOMA-IR were significantly greater in DM patients with alpha-GI compared to healthy subjects. Hemoglobin A1c did not differ in DM patients before and after pioglitazone. However, pioglitazone significantly decreased MSNA in DM patients compared with alpha-GI (21.7 ± 5.2 vs. 32.0 ± 6.8 burst/min, $p<0.01$). Furthermore, MSNA level in pioglitazone was similar to that in healthy subjects. HOMA-IR significantly decreased after pioglitazone, and a significant relationship was found between the absolute change in MSNA and HOMA-IR ($r=0.65$, $p<0.05$). These results suggest that improved insulin resistance with pioglitazone provides an additional effect on inhibition of sympathetic nerve activity.

1. Introduction

Type 2 diabetes mellitus (DM) is increasing worldwide with changes in life style and dietary habits (Pereira et al., 2005; World Health Organnization, 2007). Type 2 DM causes cardiovascular complications that are related to mortality and morbidity. Previous studies have not shown a beneficial effect of insulin and conventional sulfonylurea therapy on cardiovascular mortality and morbidity (Finfer et al., 2009; UK Prospective Diabetes Study (UKPDS), 1998). The results indicate that tight glycemic control mainly affects the cardiovascular outcome in DM patients without established macrovascular disease. Therefore, additional therapeutic strategy is warranted to prevent major cardiovascular events in type 2 DM.

Recently, augmented sympathetic nerve activity in uncomplicated DM has been identified using microneurography (Huggett et al., 2003). Underlying mechanisms of disturbed autonomic nervous system in DM are not understood clearly. The increased sympathetic outflow in DM patients has been partly assumed to result from serum insulin and glucose levels (Scherrer and Sartori, 1997; Straznicky et al., 2009; Fagius et al., 1996; Fagius, 2003). The exaggerated sympathetic outflow increases cardiovascular mortality and morbidity in humans via beta-receptor downregulation (Bohm et al., 1997), cardiac myocyte apoptosis (Communal et al., 1998), and arrhythmogenesis (Chaudhri et al., 2002). The relationship between sympathetic nerve activity and mortality in type 2 DM remains unclear, but reducing the sympathetic activation might be a potential therapeutic target in type 2 DM.

STOP-NIDDM trial showed that acarbose treatment in patients with impaired glucose tolerance was associated with a significant reduction in the risk of developing diabetes, hypertension, and cardiovascular complications (Chiasson et al., 2003). Acarbose acts as an alpha-glucosidase inhibitor (GI) and slows the digestion of starch in the small intestine without reducing insulin release (Meneilly et al., 2000) or insulin sensitivity (Matsumoto et al., 1998). On the other hand, PROactive study showed that pioglitazone, which acts on peroxisome proliferator responsive elements, reduced the composite of all-cause mortality, non-fatal myocardial infarction, and stroke in patients with type 2 diabetes at a high risk of macrovascular events (Dormandy et al., 2005). One possible explanation for the reduction in cardiovascular events is thought to be the improved serum insulin level and decreased insulin resistance. However, it is unclear whether these improvements affect sympathetic nerve activity. These observations suggest that

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pioglitazone might confer additional beneficial effect on the sympathetic nerve system in type 2 DM patients.

In this study, we hypothesized that 1) changing alpha-GI to pioglitazone would decrease sympathetic nerve activity and 2) improved insulin resistance with pioglitazone would contribute to reduction of sympathetic nerve activity, independent of lowering blood glucose. To identify the relationship between insulin resistance and sympathetic activity without blood glucose effects, we examined muscle sympathetic nerve activity (MSNA) with microneurography in controlled type 2 DM patient with alpha-GI before and after changing to pioglitazone.

2. Materials and methods

2.1. Subjects

Data were obtained from twelve controlled type 2 DM (6 men and 6 women) ranging in age from 49 to 72 years (mean 64.0 ± 6.5), who had been treated with one of alpha-GIs for more than 3 months prior to study recruitment (five with voglibose (0.9 mg/day), four with miglitol (150 mg/day), and three with acarbose (150 mg/day)). Patients with type 2 DM were diagnosed 3.8 ± 0.4 years ago and mean hemoglobin A1c (HbA1c) before medication was 7.6 ± 0.6%. The diagnosis of DM2 was confirmed as recommended by the Japan Diabetic Society (The Committee of Japan Diabetes Society for the Diagnostic Criteria of Diabetes Mellitus, 1999). Subjects had uncomplicated DM, defined as no clinical evidence of macrovascular disease and no retinopathy (normal fundoscopy), nephropathy (albumin excretion ratio <20 μg/min and normal renal function), or neuropathy (normal monofilament testing, normal nerve conductance velocity, normal vibration and reflex test, and normal Valsalva maneuver response). We excluded DM patients on combined treatment with insulin therapy and complicated with uncontrolled hypertension and/or hyperlipidemia. Of twelve patients, six were diagnosed with hypertension, and received long acting calcium channel blockade, four also received angiotensin II receptor blockade, and three were treated with statin therapy.

We recruited and screened thirteen healthy volunteers (7 men and 6 women) aged 53 to 71 years (mean 61.8 ± 4.6). None of the healthy subjects was taking medication. The screening included a physical examination, baseline electrocardiogram evaluation, echocardiography, and complete blood work examination including a 75 g oral glucose tolerance test to exclude diseases that might affect cardiovascular status, such as cardiac dysfunction, metabolic syndrome, diabetes mellitus, impaired glucose tolerance, chronic kidney disease, and hypertension.

All subjects gave written informed consent to participate in the study. The study protocol was approved by the ethics review panel of the Graduate School of Medical Science at Kanazawa University, Japan.

2.2. Measurements

Heart rate was determined from a continuous electrocardiogram. Arterial pressure was recorded continuously from the radial artery using a noninvasive tonometry monitoring system (JENTOW-7700; Nihon Colin, Komaki, Japan). The postganglionic MSNA was recorded from the left peroneal nerve, as described previously (Murai et al., 2006; Otowa et al., 2008; Murai et al., 2009). Briefly, with the subject in a comfortable supine position, the common peroneal nerve was located by palpation and stimulated electrically via the skin surface. A tungsten micro-electrode was inserted percutaneously into a motor fascicle of the peroneal nerve. The electrode was adjusted until spontaneous pulse-synchronous sympathetic burst activities could be recorded.

The electrodes were connected to a preamplifier at a gain of 1000 and to an amplifier at a gain of 70. The signal was fed through a band-pass filter (500–3000 kHz) and a resistance-capacitance integrated circuit with a time constant of 0.1 s to produce a mean voltage neurogram. During off-line analysis, multi-unit MSNA was identified based on its relationship to cardiac activity in the integrated nerve recording in a blinded fashion by an experienced investigator. Within mean voltage neurograms, the burst activity was identified as over 3 of signal to noise ratio. For each subject, multi-unit MSNA was expressed as the number per minute (burst frequency) and the number per 100 heartbeats (burst incidence).

Fasting blood glucose (FBG), immunoreactive insulin (IRI), HbA1c, brain natriuretic peptide (BNP), and lipids were measured on the same study days. The homeostasis model assessment of the insulin resistance index was calculated as HOMA-IR = fasting glucose (mg/dL) × fasting insulin (μU/mL)/405 (Bonora et al., 2000).

2.3. Study protocol

All experiments were carried out in a quiet, electrically shielded room with the subject in the supine position at the same time in the morning (9:00–12:00). After the position of tungsten electrode to obtain MSNA was determined, all subjects were rested for 15 min. The recording of MSNA, HR and continuous noninvasive BP was recorded for 15 min. Then, the patients (not healthy subjects) were changed from alpha-GI to pioglitazone (15 mg/day), which was continued for three months. After three months, all of the parameters were reexamined for the patients treated with pioglitazone. Fig. 1 shows a simultaneous recording of MSNA, arterial pressure and electrocardiogram recording in a DM patient before and after changing to pioglitazone. As the reproducibility of MSNA had been reported (Fagius and Wallin, 1993; Hoffman et al., 1998; Grassi et al., 2009), reexamination was not performed in healthy subjects group. To minimize the confounding effects of hypoglycemia on sympathetic nerve activity, anti-diabetic drugs were withheld on the morning of the study.

2.4. Statistical analysis

Values are expressed as means ± SD. Student's paired *t*-test was used for paired comparisons. Student's unpaired *t*-test was used to compare between healthy subjects and DM patients. Liner regression analysis was used to determine the relationship between the multi-unit MSNA and other variables. All calculations were performed with a personal computer using the StatView-J 5.0 statistical software package (Abacas Concepts, Berkeley, CA). *p* values below 0.05 were considered to indicate statistical significance.

3. Results

3.1. Baseline characteristics

The baseline characteristics are shown in Table 1. No significant differences between the DM patients treated with alpha-GI and the healthy subjects were observed with respect to age, height, body weight, body mass index, systolic arterial pressure, diastolic arterial pressure, and heart rate. The lipid profiles did not differ between the two groups. BNP, a marker of cardiac function, did not differ significantly in the two groups. HbA1c, FBG, and HOMA-IR were significantly higher in DM patients than in the healthy subjects, and IRI tended to increase in DM patients.

3.2. Effect of changing to pioglitazone on hemodynamics and diabetic profiles

Table 2 shows the responses of the hemodynamic and diabetic profiles to changing to pioglitazone. Changing to pioglitazone had no effect on HbA1c and FBG, which remained higher than in healthy subjects. Heart rate, systolic and diastolic arterial pressure, and BNP