Impaired β -adrenoceptor mediated venodilation in patients with diabetes mellitus

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Aims We investigated whether venoconstriction by α -adrenoceptor stimulation, and venodilation by β -adrenoceptor stimulation and nitroglycerin are altered in patients with diabetes mellitus (DM).

Methods Eight male patients with non insulin-dependent DM and eight age-matched control subjects were included. The patients had neither hypertension nor hyperlipidaemia. Noradrenaline (1 to 512 ng min⁻¹), isoprenaline (1 to 256 ng min⁻¹) and nitroglycerin (0.5 to 128 ng min⁻¹) were infused into a dorsal hand vein and its diameter was measured using a linear variable differential transformer.

Results The venoconstricting response to noradrenaline and the venodilating response to nitroglycerin in DM patients were similar to those in control subjects, while the venodilation by isoprenaline was significantly ($P < 0.05$) smaller in DM patients than in control subjects at the dose of 32 ng min⁻¹ or more [32 ng min⁻¹: 11.5% vs 29.8% (DM vs control subjects), 64 ng min⁻¹: 19.0% vs 40.1%, 128 ng min⁻¹: 25.2% vs 49.0%, 256 ng min⁻¹: 34.3% vs 56.7%].

Conclusions These data suggested that venoconstriction by α -adrenoceptor stimulation and venodilation by nitroglycerin are not altered, whereas venodilation by β -adrenoceptor stimulation might be impaired in patients with DM.

Keywords: diabetes mellitus, vein, vascular response, α - and β -adrenoceptors, nitroglycerin

Introduction

Diabetes mellitus (DM) is a prevalent disease often associated with vascular complications, such as micro-angiopathy and atherosclerosis. Although the mechanisms of vascular dysfunction in DM are poorly understood, several reports [1–5] have provided data suggesting alternations in vascular responses to vasoactive agents. Based on the findings obtained from studies of diabetic animals [1, 2] and humans [3, 4], it is suggested that endothelium-dependent vasodilation is impaired in DM. However, whether vasodilator response to nitrates and vasoconstrictor response to α -adrenoceptor agonists are altered in DM is controversial [1–5]. Furthermore, to our knowledge, it is unknown whether vasodilation induced by β -adrenoceptor stimulation is impaired in DM.

As the sympathetic nervous system plays an important role in the regulation of systemic circulation and is involved in the onset of cardiovascular events [6, 7], it is

important to investigate the functions of both α - and β -adrenoceptors in blood vessels in DM.

In the present study, we examined, using a dorsal hand vein technique [8], the vasoconstrictor response to noradrenaline, an α -adrenoceptor agonist, and vasodilator responses to isoprenaline, a β -adrenoceptor agonist, and nitroglycerin in DM patients and control subjects.

Methods

Subjects

Eight male DM patients and eight male control subjects who were not hypertensive and had normal serum cholesterol level were included in this study (Table 1). They did not take any medications except for hypoglycemic drugs for at least 7 days before the study. There were three smokers in each group, but they did not smoke for at least 24 h before the study. All the patients had non-insulin-dependent DM (NIDDM) with the history of 6 to 17 (mean 10) years, and six of them had been treated with glibenclamide (2.5–7.5 mg day⁻¹) and/or voglibose (0.4–0.6 mg day⁻¹), an α -glucosidase

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	Diabetic patients (n = 8)	Control subjects (n = 8)
Age (years)	55 (42–66)	57 (40–68)
Weight (kg)	66 (57–79)	65 (61–73)
Systolic blood pressure (mmHg)	129 (112–156)	132 (108–152)
Diastolic blood pressure (mmHg)	83 (68–88)	82 (68–90)
Fasting blood glucose (mg dl ⁻¹)	157 (122–175)*	96 (86–107)
Glycosylated hemoglobin (%)	8.3 (7.0–9.8)*	5.0 (4.7–5.5)
Total cholesterol (mm)	5.1 (4.4–5.7)	5.0 (4.3–5.7)
Creatinine (mg dl ⁻¹)	1.1 (0.8–1.3)	1.1 (0.9–1.2)

* $P < 0.01$ vs control subjects.

inhibitor. They had at least one of the following complications: retinopathy, nephropathy (less than 1.0 g of albuminuria day⁻¹) and neuropathy (with decreased nerve conduction velocity but without symptoms due to autonomic nerve disturbance). They did not have any symptoms of angina pectoris, claudication, or cerebrovascular ischaemia. This study was approved by the Ethics Committee of Jichi Medical School. All subjects gave their informed consent to participate in this study.

Dorsal hand vein technique

A linear variable differential transformer (LVDT) (Schaevitz type 025MHR, Pennsauken, N.J., U.S.A.) was used. This has a freely movable core that rests over the centre of the vein to be studied. During the study, the subject lay in supine position with one arm placed on a support sloping upward at an angle of about 30 degrees from the horizontal, inducing an emptying of the superficial hand veins. A 25-gauge needle was inserted into a dorsal hand vein and a continuous infusion of physiological saline was started. The core was placed over the centre of the vein, about 10 mm downstream from the tip of the needle. The position of the core was recorded before and after inflation of a sphygmomanometer cuff on the same arm to 45 mmHg for 1 min.

Protocol

Subjects were asked to refrain from caffeine containing and alcoholic beverages for at least 12 h before the study. The study started about 3 h after breakfast. Venoconstrictor response to increasing doses (1–512 ng min⁻¹) of noradrenaline and venodilator response to increasing doses of isoprenaline (1–256 ng min⁻¹) and nitroglycerin (0.5–128 ng min⁻¹) were obtained in this order with 5 to 8 days between studies. In the experiment measuring venoconstrictor response, 0% and 100% con-

striction were defined as the recordings with and without the cuff inflated, respectively, after 10 min infusion of saline. In the experiments measuring venodilator response, the dorsal hand vein was precontracted with concomitant noradrenaline infusion at the dose producing about 80% constriction in each subject. 100% dilation was defined as the recording with the cuff inflated before the precontraction and 0% dilation was defined as the recording of the baseline precontraction after the noradrenaline infusion for 10 min. During the drug infusion, blood pressure and pulse rate were monitored. In the experiment with isoprenaline, a higher infusion rate than 256 ng min⁻¹ was not used because of a significant increase in pulse rate. These studies were performed in a room with a controlled ambient temperature of 24–26°C.

Statistical analysis

Statistical analysis was performed using Stat View®. To compare the differences between the DM and control subjects, we analysed using Student's unpaired *t*-test, and to determine whether blood pressure or pulse rate has changed during the drug infusion in each group, we used analysis of variance (ANOVA) followed by Scheffé's multiple-range test. $P < 0.05$ was considered as significant, and the 95% confidence intervals (95% CI) for differences of the venodilations between the DM and control subjects are given.

Results

The baseline characteristics of the DM patients and control subjects are shown in Table 1. Serum glucose and glycosylated haemoglobin concentrations were higher in the DM patients, but there were no significant differences in other parameters between the two groups.

Dose-response curves for the venoconstriction by noradrenaline and for the venodilation by isoprenaline

Table 1 Characteristics of study population (mean with range).

and nitroglycerin are shown in Figure 1. At all the doses of noradrenaline infusion, there were no significant differences in the venoconstriction between the DM patients and control subjects (Figure 1a). During the noradrenaline infusion, no significant changes in blood pressure or pulse rate were observed. The dose of noradrenaline used for the precontraction was 56 (32–72) ng min^{-1} in the control subjects and 53 (32–76) ng min^{-1} in the DM patients, causing 79 (75–83)% and 81 (73–86)% constriction, respectively. There were no significant differences in these values between the two groups.

Venodilation by isoprenaline was significantly smaller

Figure 1 Dose-response curves of the venoconstricting effect of noradrenaline (a) and those of the venodilating effect of isoprenaline (b) and nitroglycerin (c) in eight DM (●) and eight control subjects (○) (mean $\pm$ s.d.). * $P < 0.05$ vs control subjects.

in the DM patients than in control subjects at the dose of 32 ng min^{-1} or more (Figure 1b). The mean and 95% CI for differences of the venodilations between the two groups were 18.2 (3.3–32.0)% at 32 ng min^{-1} , 21.1 (3.5–40.5)% at 64 ng min^{-1} , 23.7 (4.1–44.2)% at 128 ng min^{-1} and 22.5 (0.1–41.5)% at 256 ng min^{-1} . There was no significant change in blood pressure during isoprenaline infusion, however, pulse rate at 256 ng min^{-1} was significantly increased compared with that before the infusion. The increase was 6 (2–10) (mean and range) beats min^{-1} in the control subjects and 5 (2–8) beats min^{-1} in the DM patients.

At all the doses of nitroglycerin infusion, there were no significant differences in the venodilation between the two groups (Figure 1c). During the nitroglycerin infusion, no significant changes in blood pressure or pulse rate were observed.

Discussion

The new information obtained in the present study is that venodilation caused by β -adrenoceptor stimulation is impaired in patients with DM, whereas venodilation by nitroglycerin and venoconstriction by α -adrenoceptor stimulation are not altered in these patients. It is interesting that these alterations in DM patients coincide with those caused by ageing. Several reports using dorsal hand vein technique displayed that the venodilation caused by β -adrenoceptor stimulation is impaired in the elderly, while venodilation by nitroglycerin and venoconstriction by α -adrenoceptor stimulation are similar between young and elderly subjects [9–11]. Therefore, these findings might explain, in part, the observations that cardiovascular diseases occur earlier and their severity is often greater in patients with DM than in non-DM subjects [12, 13].

There is substantial evidence that endothelium-dependent vasodilation is impaired in DM [1–4]. Therefore, it is postulated that the endothelium dysfunction is relevant to the high prevalence of vascular diseases in DM patients. Besides this mechanism, impairment of β -adrenoceptor mediated vasodilation might also contribute to the pathogenesis of vascular diseases in DM patients, as the sympathetic nervous system is largely involved in the regulation of the circulatory system.

To exclude the effects of hypertension and hypercholesterolaemia on vascular reactivity, we selected patients who were not hypertensive and had normal serum cholesterol level. There were no significant differences in age, blood pressure and serum cholesterol level between the patient and control groups. However, the effects of the hypoglycaemic drugs on the results must be considered. As glibenclamide is a potent blocker of ATP-sensitive K^+ channels [14], it may influence the

vascular reactivity. *In vitro* studies using rat mesenteric arteries showed that glibenclamide did not affect the β -adrenoceptor-induced vasodilation [15], whereas the dilation of dog coronary arteries induced by isoprenaline was reported to be blunted by the simultaneous infusion of glibenclamide [16]. Further studies are needed to investigate the effect of glibenclamide on the vascular reactivity in DM patients, although, in the present study, the venodilation by isoprenaline in the DM patients receiving glibenclamide ($n=5$) was similar to that in the patients not receiving the drug ($n=3$); the mean values in the patients with vs without glibenclamide were 9.5 vs 11.9% at 32 ng min⁻¹, 17.1 vs 20.7% at 64 ng min⁻¹, 24.3 vs 25.8% at 128 ng min⁻¹, and 34.8 vs 38.0% at 256 ng min⁻¹. The effect of voglibose, an α -glucosidase inhibitor widely used for DM in Japan, on vascular reactivity is unknown but its oral bioavailability is extremely small [17], making its direct effect on the circulatory system negligible.

The patients included in this study were suffering from NIDDM with microangiopathy and/or neuropathy and had higher blood glucose level than control subjects. It is unknown whether hyperglycaemia itself, which is a major determinant of diabetic microangiopathy [18], is the cause of the findings. Further studies are needed to determine whether the findings obtained in this study are also seen in DM patients with controlled blood glucose level or in those who have no complications or a shorter history of DM.

The mechanism of the impaired β -adrenoceptor-mediated vasodilation in the DM patients is further to be determined, but *in vitro* studies have shown that β -adrenoceptor agonists may directly stimulate nitric oxide synthesis in endothelial cells [19], and the vasodilator response to isoprenaline is blunted by the inhibition of the nitric oxide synthesis in the human forearm [20]. Whether these findings are recognized in human dorsal hand veins is unknown, but it is suggested that the impaired β -adrenoceptor-mediated venodilation might be caused, at least in part, by the endothelial dysfunction, which is known to be induced by DM [1–4].

There have been conflicting data about vasoconstriction by α -adrenoceptor stimulation and vasodilation by nitrates in DM [1–5]. Our findings are consistent with the report by Eichler *et al.* [21], showing that venoconstrictor response to noradrenaline in DM patients is similar to that in control subjects, although this response is enhanced in those having symptoms due to autonomic nerve disturbance, and with the report by Huvers *et al.* [22] showing that responsiveness of veins to nitroglycerin in NIDDM is unchanged.

Systemic and hand vein responsiveness to α_1 -adrenoceptor stimulation are reported to be similar [23]. However, it is unknown whether the responsiveness of

hand veins to β -adrenoceptor stimulation and nitrates represents those of the systemic circulation.

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