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Angiotensin II, as well as 5-hydroxytriptamine, is a potent vasospasm inducer of saphenous vein graft for coronary artery bypass grafting in patients with diabetes mellitus

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ABSTRACT

Diabetes mellitus (DM) is an important risk factor for adverse outcomes of coronary artery bypass grafting. The bypass grafts harvested from patients with DM tend to go into spasm after their implantation into the coronary circulation. To clarify the contribution of 5-hydroxytriptamine (5-HT) and angiotensin II (AngII) in the bypass graft spasm, we examined the contractile reactivity to 5-HT or AngII of isolated human endothelium-denuded saphenous vein (SV) harvested from DM and non-DM patients. The 5-HT-induced constriction of the SV was significantly augmented in the DM group than in the non-DM group, which is similar to our previous report. AngII-induced constriction of the SV was also significantly augmented in the DM group than the non-DM group. Especially in the non-DM group, the AngII-induced maximal vasoconstriction was markedly lower than the 5-HT-induced one. Meanwhile, the increasing rates of AngII-induced vasoconstriction in the DM group to the non-DM group were significantly greater than those of 5-HT-induced vasoconstriction. These results indicate that 5-HT is a potent inducer of SV graft spasm in both DM and non-DM patients, while AngII is a potent inducer of SV graft spasm only in patients with DM. Furthermore, the protein level of AngII AT_1 receptor (AT_1R), but not the protein level of $5\text{-}HT_{2A}$ receptor, in the membrane fraction of the SV smooth muscle cells of DM patients was significantly increased as compared with that of the non-DM patients. These results suggest that the mechanism for hyperreactivity to AngII in the SV from DM patients is due to, at least in part, the increase in the amount of AT_1R on membrane of the SV smooth muscle cells.

1. Introduction

Coronary artery bypass grafting (CABG) remains the gold standard therapy for severe coronary artery disease involving three-vessel or left main coronary disease [1]. Especially among patients with diabetes mellitus (DM), CABG has a better prognosis than percutaneous coronary intervention [1,2]. However, DM is an established risk factor for early and late adverse outcomes after CABG [3], with the most serious associated complication being the spasm of bypass grafts after their implantation into the coronary circulation, which can lead to premature occlusion and increased perioperative morbidity [4,5]. The saphenous vein (SV) has been widely used as a conduit for CABG because of its ready availability and suppleness [6]. We previously reported that the constrictive responses induced by 5-hydroxytryptamine (5-HT) in the human endothelium-denuded SVs isolated from patients with DM were significantly augmented compared with those in veins isolated from patients without DM (non-DM) [7]. Based on these results, we hypothesized that increased reactivity to 5-HT may be responsible for vasospasm after CABG among diabetic patients.

Insulin resistance, a well-known risk factor for type 2 DM, upregulates the renin-angiotensin-aldosterone system (RAAS), which is involved in the pathogenesis of hypertension, arteriosclerosis, and ischemic heart failure [8,9]. Angiotensin II (AngII) is part of the RAAS and plays important roles in regulating the

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vascular tone and the pathogenesis of cardiovascular diseases [10]. AngII acts primarily through the angiotensin AT_1 receptor (AT_1R) to exert the most of its biological effects, including the constriction of vascular smooth muscle. AngII-induced constrictive responses of the SVs harvested from DM patients may be augmented in a similar manner to 5-HT-induced responses, because AT_1R, like the 5-HT_{2A} receptor, is a Gq-coupled receptor. However, little is known about the influence of DM on AngII-induced vasoconstriction of human SVs. Hence, we examined the effect of DM on AngII-induced vasoconstriction, in comparison to 5-HT-induced vasoconstriction, of endothelium-denuded SV grafts harvested from both DM and non-DM patients. We further determined the levels of 5-HT_{2A} receptor and AT_1R on the SV smooth muscle membrane.

2. Materials and methods

2.1. Preparation of blood vessels and contractile studies

The human SVs were obtained from patients undergoing CABG at Miyazaki Prefectural Nobeoka Hospital (Nobeoka, Japan) or surgical varicose vein treatment at Kuwabara Clinic (Miyazaki, Japan). SV samples from 15 patients with DM (DM group) and 25 patients without DM (non-DM group) were used in this study. The diabetes status of patients was accepted as diagnosed from the medical records. The hemoglobin A_{1c} (HbA_{1c}) levels of the DM group were 6.6 ± 0.1 (only 7 DM patients who were confirmed by us). We could not follow the HbA_{1c} data of the other DM patients or non-DM patients. At Miyazaki Prefectural Nobeoka Hospital, portions of each great SV graft were sectioned to the desired lengths for bypassing the occluded coronary arteries, while the remainder was used for the experiments. At Kuwabara Clinic, portions of the SV were sectioned from each patient as a surgical treatment for varix of the lower extremity, and only the non-distended areas were used for the experiments. The small SV segments were transported and their constrictive responses were measured as described previously [11,12]. In brief, isolated vessels were placed in modified Krebs buffer, which had been previously aerated with 95% O_2 and 5% CO_2, and transported promptly to our laboratory. The composition of modified Krebs buffer solution was as follows: 118.0 mM NaCl, 4.7 mM KC1, 25.0 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 1.1 mM KH_2PO_4, 2.5 mM $CaCl_2$, 0.01 mM EDTA, and 11.0 mM glucose, pH 7.4 at 37 °C. After removal of fat and connective tissue, the vessel was cut into 2-mm rings, and endothelium was removed to exclude the influences of it to contractile response. Each ring was suspended between stainless steel hooks in a 5-mL organ bath containing modified Krebs buffer maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2. One hook was connected to a force transducer (Nihon-Kohden, Japan) to record the isometric tension in a computer system (PowerLab8/30; Bio Research Center Co., Ltd., Japan). The SV rings were stretched progressively to the optimal tension (2.0 g) and allowed to equilibrate for 1 h. The buffer was changed every 30 min. After the tension of rings had completely stabilized, they were preconstricted with 60 mM KCl. Once maximum vasoconstriction was reached at a plateau, the ring was washed three times with fresh modified Krebs buffer solution. Thereafter, cumulative concentration-response curves to 5-HT (Sigma-Aldrich Co.) or AngII (Sigma-Aldrich Co.) over concentration ranges of 1 nM to 10 μM were constructed. Finally, the ring was washed three times, and the contraction in response to a second exposure to 60 mM KCl was recorded as the control contraction. The contractile reactivity of vein rings was evaluated using the percentage of the second KCl-induced vasoconstriction as 100%.

2.2. Western blot analysis of the AT_1R and 5-HT_{2A} receptor in membrane fractions

Following the contractile studies, the SV rings were flash frozen in liquid nitrogen and stored at −80 °C for Western blot analysis. Frozen SV rings were crushed with manual mill SK-200 (Tokken, inc.) and suspended in transmembrane protein extraction buffer 1 (ProteoExtract Transmembrane Protein Extraction Kit; Calbiochem-Novabiochem). Preparation for the membrane fractions and Western blot analysis was performed as described previously [11]. Equal amounts of protein (5.5 μg per lane) were separated by SDS-10% polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane Hybond-P (GE Healthcare Japan). The membrane was preincubated with 1% skim milk in Tween-Tris-buffered saline (10 mM Tris–HCl [pH 7.4], 150 mM NaCl, and 0.1% Tween-20) and reacted with rabbit anti-5-HT_{2A} receptor (1:2000, Santa Cruz Biotechnology), rabbit anti-β-actin (1:2000, Cell Signaling Technology) or mouse anti-AT_1R (1:1000, abcam) overnight at 4 °C in Immuno-enhancer Reagent A (Wako). The immunoreactive bands were reacted with horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibodies in Immuno-enhancer Reagent B (Wako), visualized using the Immuno-Star enhanced chemiluminescent detection system (Wako), and quantified using a luminoimage LAS-4000 analyzer (GE Healthcare Japan). Protein levels of 5-HT_{2A} receptor and AT_1R were normalized by the level of β-actin.

2.3. Ethics

The Ethics Committees at both Miyazaki Prefectural Nobeoka Hospital and Kyusyu University of Health and Welfare approved this study, with acceptance number 09-004 (study using human saphenous vein). All patients provided written consent to participate in the study. These experiments were conducted in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for Experiments Involving Humans.*

2.4. Statistical analysis

All the data were averaged for each patients. Thus, the numbers of subject indicated in figures are the number of patients. Statistical comparisons of mean value of 5-HT- or AngII-induced vasoconstriction were made using two-way analysis of variance (ANOVA). If significant differences in vasoconstriction between the DM and non-DM groups were found, the rank-sum test of the increasing effect of DM on the vasoconstriction induced by AngII or 5-HT was carried out using the Mann-Whitney *U* test. Statistical comparisons of 5-HT_{2A} receptor and AT_1R in the membrane fraction of the SV smooth muscle were made using Welch's *t*-test. The data are presented as mean ± standard error of the mean (SEM). Significance was assumed at $P < 0.05$. Statistical analyses were performed using SPSS 21.0 J for Windows.

3. Results

We examined the 5-HT- or AngII- induced constriction of isolated human endothelium-denuded SVs. The cumulative administration of 5-HT (1 nM–10 μM) induced the constriction of the SV in a concentration-dependent manner, however, the responses did not reach a plateau at less than 10 nM 5-HT in either the DM group ($n=9$) or the non-DM group ($n=9$) (Fig. 1A). Two-way ANOVA revealed that the 5-HT- induced vasoconstriction in the DM group was significantly greater than that in the non-DM group ($P=0.011$). The cumulative administration of AngII (1 nM–10 μM) induced the constriction of the SV in a concentration-dependent manner, and the responses reached a plateau at 300–1000 nM