BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2012.01867.x
www.brjpharmacol.org

RESEARCH PAPER

Characteristics of the actions by which 5-hydroxytryptamine affects electrical and mechanical activities in rabbit jugular vein graft

Takashi Maekawa[1], Kimihiro Komori[1], Junko Kajikuri[2] and Takeo Itoh[2]

[1]*Division of Vascular Surgery, Department of Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan, and* [2]*Department of Pharmacology, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan*

Correspondence
Takeo Itoh, Department of Pharmacology, Graduate School of Medical Sciences, Nagoya City University, Nagoya 467-8601, Japan. E-mail: titoh@med.nagoya-cu.ac.jp

Keywords
5-hydroxytryptamine; 5-HT_{2A} subtype receptor; 5-$HT_{1B/1D}$ subtype receptor; 5-HT_7 subtype receptor; autologous vein graft; endothelium; jugular vein

Received
4 June 2011
Revised
19 December 2011
Accepted
10 January 2012

BACKGROUND AND PURPOSE
The vasomodulating actions of 5-HT in vein grafts, and the underlying mechanisms, remain to be fully clarified. Here, we characterized the actions by which 5-HT affects electrical and mechanical activities in rabbit autologous jugular vein grafts.

EXPERIMENTAL APPROACH
Smooth muscle cell (SMC) membrane potential and isometric tension were measured in vein grafts 4 weeks after implantation into carotid arteries. Changes in the expression of 5-HT receptor subtypes and in myosin heavy chain isoforms (SM1, SM2 and SMemb) were examined by immunohistochemistry and Western blot analysis.

KEY RESULTS
The walls of grafted veins displayed massive increases in the number of SM1- and SM2-positive SMCs. 5-HT induced a large depolarization and contraction that were each reduced by both 5-HT_{2A}- and 5-$HT_{1B/1D}$-receptor antagonists. The 5-HT-induced contraction was not modified by a 5-HT_7-receptor antagonist. The 5-HT_7-receptor-selective agonist AS 19 did not induce relaxation during the contraction to prostaglandin $F_{2\alpha}$. Immunohistochemical and Western blot analyses revealed that immunoreactive responses against 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors were increased in the vein graft.

CONCLUSIONS AND IMPLICATIONS
5-HT is able to induce a large contraction in rabbit autologous jugular vein grafts through (i) an increased number of differentiated contractile SMCs; (ii) an increased number of SMCs expressing contractile 5-HT_{2A}- and 5-$HT_{1B/1D}$ receptors; and (iii) a down-regulation of the function of the relaxant SMC 5-HT_7 receptors. These changes in the vein graft may help it to resist the higher pressure present on the arterial side of the circulation.

Abbreviations
AS 19, (2S)-(+)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin; GR55562, 3-[3-(dimethylamino)propyl]-4-hydroxy-N-[4-(4-pyridinyl)phenyl]benzamide dihydrobromide; hydroxyfasudil, 1-(1-hydroxy-5-isoquinolinesulfonyl) homopiperazine; L-NNA, N^{ω}-nitro-L-arginine; MHC, myosin heavy chain; SB200646, N-(1-methyl-1H-indol-5-yl)-N′-3-pyridinylurea; SB269970, (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine; SMC, smooth muscle cell; TCB-2, (4-bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine hydrobromide; Y-26763, (−)-(3S,4R)-4- (N-acetyl-N-hydroxyamino)-6-cyano-3, 4-dihydro-2, 2-dimethyl-2H-1-benzopyran-3-ol; Y-27632, (*R*)-(+)-*trans*-4-(1-aminoethyl)-N-(4-pyridyl)cyclohexanecarboxamide dihydrochloride monohydrate

Introduction

5-HT induces a variety of electrical and mechanical responses in blood vessels. For example, 5-HT induces a contraction with a membrane depolarization in smooth muscle cells (SMCs) in some arteries (Harder and Waters, 1983; Garland, 1985; 1987), suggesting that membrane depolarization plays a role in 5-HT-induced contraction in those arteries. In contrast, 5-HT induces an endothelium-dependent NO-mediated relaxation without a change in the SMC membrane potential in the porcine coronary artery (Frieden and Bény, 1995), indicating that 5-HT acts on the endothelium to induce vascular relaxation in that artery. We recently found in the rabbit normal jugular vein that 5-HT induces SMC hyperpolarization and relaxation, with each being independent of the presence of endothelium (Itoh and Kajikuri, 2011). These findings suggest that 5-HT can induce contraction/relaxation through membrane-potential-dependent or -independent mechanisms, depending on the type of blood vessel.

The receptor subtypes mediating the effects of 5-HT on mechanical activities in blood vessels have been characterized as follows: 5-HT induces (i) contraction via SMC 5-HT_{2A} receptors and/or SMC 5-$HT_{1B/1D}$ receptors (Hoyer *et al.*, 1994; Alexander *et al.*, 2011) and (ii) relaxation via SMC 5-HT_7 receptors (Itoh and Kajikuri, 2011) and/or two types of endothelial receptors (5-$HT_{1B/1D}$ and 5-HT_{2B}) that are coupled to the release of endothelial NO (Gupta, 1992; Ellis *et al.*, 1995; Grayson and Gupta, 1995; Glusa and Roos, 1996). However, as these receptor subtypes co-exist in certain types of arteries and veins, the net effect of 5-HT on mechanical activities in a given vessel might depend upon the relative expressions of the various receptor subtypes. For example, we recently found that in rabbit normal jugular vein, 5-HT activates both SMC 5-HT_7 receptors (to induce relaxation) and 5-HT_{2A} receptors (to induce contraction), with the net effect being relaxation (Itoh and Kajikuri, 2011). Our conclusion was that the SMC 5-HT_{2A}-receptor-mediated depolarization and contraction may be masked by the 5-HT_7-receptor-mediated hyperpolarization and relaxation in that particular vein. Furthermore, factors such as changes in endothelial function could affect the 5-HT-induced mechanical activities in arteries and veins.

The internal thoracic artery is the first-choice arterial graft for coronary artery bypass graft surgery (Okies *et al.*, 1984; Loop *et al.*, 1986). However, implantation of an autologous vein into the arterial side of the circulation continues to be the most commonly used option for coronary artery bypass grafting and for the surgical treatment of peripheral arterial disease, its main advantages being ready availability and suppleness (Kodama *et al.*, 2009a; Lopes *et al.*, 2009). Such venous implants are subject to increased shear stress, loss of endothelial cells, migration and invasion of inflammatory cells, and migration and proliferation of SMCs (Itoh *et al.*, 1994; Ishida *et al.*, 2001). These changes can lead to intimal hyperplasia and subsequently to accelerated atherogenesis, effects that may be responsible for vein graft occlusion (Davies and Hagen, 1995; Komori *et al.*, 1997; Motwani and Topol, 1998; Jiang *et al.*, 2004).

The SMCs in human and rabbit arteries contain at least three types of myosin heavy chain (MHC) isoform: SM1, SM2 and SMemb. The first two are specific to smooth muscle but SMemb is a non-muscle type of MHC that is abundantly expressed in the embryonic aorta and down-regulated during vascular development (Nagai *et al.*, 1989; Kuro-o *et al.*, 1991; Aikawa *et al.*, 1993). It has been found that SMC proliferation is coupled to the expression of SMemb and that dedifferentiation of SMCs towards the embryonic phenotype is involved in the mechanisms underlying atherosclerosis (Kuro-o *et al.*, 1991). These findings suggest that alterations in MHC-isoform expressions may be linked in some way to changes in contractile properties in vein grafts. It has been suggested that 5-HT plays a role in the pathogenesis of vein graft spasms (Makhoul *et al.*, 1987; Radic *et al.*, 1991) and indeed this agonist induces an enhanced contraction in rabbit autologous jugular vein grafts (Furuyama *et al.*, 2006). Moreover, increased expression and activity of the 5-HT transporter, together with an enhanced mitogenic response to 5-HT, are found in conjunction with SMC proliferation in the pulmonary artery in patients with pulmonary arterial hypertension (Marcos *et al.*, 2004). Although roles have been suggested for Rho/Rho-kinase in enhanced 5-HT-induced contractions (Somlyo and Somlyo, 1994; Kimura *et al.*, 1996; Furuyama *et al.*, 2006), the actions by which 5-HT affects electrical and mechanical activities in vein grafts, and the underlying mechanisms, remain to be fully clarified.

In this study, we set out to remedy this deficiency, using specimens isolated from rabbit autologous jugular vein grafts 4 weeks after implantation into carotid arteries. To clarify the role played by the endothelium, 5-HT-induced responses were examined both in endothelium-intact preparations, treated or not treated with the NO-synthase inhibitor N^{ω}-nitro-L-arginine (L-NNA), and in endothelium-denuded preparations. The receptor subtypes mediating these actions of 5-HT were characterized pharmacologically. The changes in the expressions of the MHC isoforms and 5-HT-receptor subtypes in the vascular cells were examined by immunohistochemistry and by Western blot analysis. Finally, the roles played by L-type Ca^{2+} channels and Rho-kinase were examined by observing the effects of nifedipine, with or without either Y-27632 or hydroxyfasudil, on 5-HT-induced contractions in the grafts.

Methods

Animals and vein graft implantation

All animal care and experimental procedures performed in this study conformed to Guidelines on the Conduct of Animal Experiments issued by the Nagoya University Graduate School of Medicine and the Graduate School of Medical Sciences in Nagoya City University and were approved by the Committee on the Ethics of Animal Experiments in those institutions. Male Japan albino rabbits (supplied by Kitayama Labes, Ina, or Nippon SLC, Hamamatsu, Japan), each weighing 2.5–3.0 kg, were used in this study (total number used 28). The rabbits were fed commercial rabbit chow (CR3; CLEA Japan Inc, Tokyo, Japan) and were housed individually in a temperature- and light-controlled room (around 22 ± 2 °C, 12-h light/dark cycles) with free access to water.

Carotid interposition-reversed, autologous jugular vein grafting was performed, as previously described (Jiang *et al.*,

2004; Yamanouchi *et al.*, 2005; Banno *et al.*, 2006; Kodama *et al.*, 2009a,b). Anaesthesia was induced with ketamine hydrochloride (50 mg·kg^{-1}, i.m.) plus xylazine (10 mg·kg^{-1}, i.m.) and maintained by i.v. administration of ketamine hydrochloride (10 mg·kg^{-1}) plus xylazine (10 mg·kg^{-1}) whenever required. The level of anaesthesia was monitored by cessation of reflex responses to paw or tail pinch or corneal touching.

After a longitudinal neck incision, the right jugular vein and the right common carotid artery were exposed. The branches of the jugular vein were carefully ligated, using 8-0 polypropylene sutures, and then divided. An approximately 2.5-cm-long segment of the jugular vein was taken for autologous reversed-vein grafting. Graft harvesting was performed with meticulous care to avoid injuring the graft wall, and the harvested graft was kept moist with heparin-containing saline (5 IU·mL^{-1}) at room temperature. The animals were systemically treated with heparin (200 IU·kg^{-1}), and the internal carotid artery and two of the three branches of the external carotid artery were then ligated. The most inferior branch of the external carotid artery served as the only outflow for the poor run-off conditions. The common carotid artery was clamped distally and proximally, and a graft was anastomosed in an end-to-end fashion into the divided artery, using interrupted 8-0 polypropylene sutures, under a surgical microscope. The wound was closed in a layer-to-layer fashion (Kodama *et al.*, 2009a,b).

Tissue preparation

At 4 weeks after the above implantation, rabbits were killed with an overdose of pentobarbital (50 mg·kg^{-1} i.v.). Normal rabbits (i.e. with no vein graft) were killed in the same way. The right jugular vein (termed 'Normal vein') and right carotid artery were obtained from each normal rabbit. A carotid jugular vein graft ('Vein-graft') was obtained from each vein-grafted rabbit. In some vein-grafted rabbits, the *in situ* left jugular vein (to be used as 'Control vein') and the 'Vein graft' were both obtained. Immediately after excision, vessels were placed in Krebs solution (Itoh *et al.*, 1992; Kodama *et al.*, 2009a,b).

The mid-portion of the harvested graft was used in the present experiments. In some preparations, the endothelium was removed as described elsewhere (Itoh *et al.*, 1992). Guanethidine (5 μM, to prevent effects due to release of sympathetic transmitters) and diclofenac (3 μM, to inhibit the production of COX products) were present throughout the experiments.

Electrophysiological study

Membrane potentials were measured in SMCs using a conventional microelectrode technique (Watanabe *et al.*, 1996; Kajikuri *et al.*, 2009; Itoh and Kajikuri, 2011). To obtain reproducible electrical responses in vein grafts, 5-HT (0.1 μM) was applied for 1.5 min at 25 min intervals (Itoh and Kajikuri, 2011). The effects on these responses of the agents of interest (5-HT receptor-subtype antagonists) were then examined.

Isometric tension measurement

Rings (~1 mm wide) cut from the isolated vessels were suspended for measurement of isometric tension (calculated per mm width of ring) in an organ chamber between two stainless-steel wires inserted into the lumen of the ring. One wire was connected to a force transducer (UL-2GR; Minebea Co, Nagano, Japan) and then a carrier amplifier (AS2101; NEC-San-ei Instruments, Tokyo, Japan). The output signal was fed into an IBM/AT-compatible PC through an analogue-digital converter (PowerLab; ADInstruments Pty Ltd, Bella Vista, Australia). The organ chamber was filled with 3 mL Krebs solution at 37°C and gassed with 95% oxygen and 5% carbon dioxide. Resting tension was adjusted to obtain maximum contraction in high K^+ solution (128 mM). To observe its concentration-dependent effects, 5-HT (0.01–10 μM) was cumulatively applied in an ascending order of concentrations to endothelium-intact or -denuded strips. This protocol was repeated to obtain reproducible responses. The roles played by endothelium-derived NO in 5-HT-induced responses were examined by observing the effect of the inhibitor of endothelial NO synthase, L-NNA (0.1 mM), which was applied as pretreatment for 60 min and was present thereafter.

Assessment of vascular wall thickness

Segments of vessels were immersion fixed in 4% paraformaldehyde in 10 mM phosphate buffer and embedded in OCT compound (Tissue Tek; SAKURA Finetechnical, Tokyo, Japan), then frozen and stored at –80°C. A few frozen sections, cut at 5 μm thickness, were obtained from each vein using a cryostat (Microtome Cryostat HM 550; MICROM International GmbH, Walldorf, Germany), then mounted on Matsunami adhesive silane-coated glass slides (Matsunami Glass, Kishiwada, Japan) for immunohistochemistry. For morphometrical analysis, each section was counterstained with haematoxylin and eosin. Vascular wall thickness was taken as the average of measurements made at eight randomly selected places per section. Four or five sections were examined in the same way and the values obtained from them were averaged to represent the wall thickness of the vein graft.

Immunohistochemical staining

To examine phenotypic alterations in the SMCs of vein grafts, MHC isoforms were immunostained using specific monoclonal antibodies against rabbit SM1, SMemb and SM2 (1:1000, 1:750 and 1:200 dilutions, respectively; Yamasa Corp., Tokyo, Japan) and against α-smooth muscle actin (1:200 dilution; Sigma Chemical Co., St. Louis, MO, USA). To examine the expressions of 5-HT receptor subtypes, staining was performed using polyclonal antibodies against 5-HT_{2A} and 5-HT_7 (each 1:100 dilution; ImmunoStar Incorporated, Hudson, WI, USA) and against $5HT_{1B/1D}$ (1:100 dilution; MBL International Corporation, Woburn, MA, USA). The sections were incubated overnight at 4°C with the appropriate primary antibody, and then incubated for 1 h at room temperature with the second antibody (Alexa Fluor 488 anti-mouse or anti-rabbit IgG; 1:1500–5000 dilution; Molecular Probes, Eugene, OR, USA) as described previously (Kajikuri *et al.*, 2009; Itoh and Kajikuri, 2011). The fluorescence of Alexa Fluor 488 was then detected by confocal laser-scanning microscopy (LSM5 PASCAL; Carl Zeiss, Jena, Germany), under identical conditions in each case. Negative control (background staining) data were obtained by application of second

antibody only (i.e. without application of primary antibody). The acquisition of images from different groups of rabbits was performed under identical conditions. Fluorescent 12 bit images were acquired and then analysed using commercial software (LSM5 PASCAL), as previously reported (Kodama *et al.*, 2009a,b; Itoh and Kajikuri, 2011).

Western blot analysis

A 'Vein-graft' and a 'Control vein' were obtained from one and the same rabbit, and each was homogenized in sample buffer [62.5 mM Tris-HCl (pH 6.8), 10% glycerol and 2% SDS]. Western blotting was performed by a method described previously (Kajikuri *et al.*, 2009). A monoclonal antibody against rabbit SM1, SMemb or SM2 (each at 1:20 000 dilution) or against α-smooth muscle actin (1:50 000 dilution; Sigma Chemical Co.) or a goat polyclonal antibody against either 5-HT_{2A} (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or 5-HT_{1B} (1:200 dilution; Abcam, Tokyo, Japan) was used as the primary antibody. The signals from the immunoreactive bands were detected by means of an enhanced chemiluminescence-detection system (SuperSignal West Pico; Pierce, Rockford, IL, USA). The density of the protein was measured by densitometric scanning, as described previously (Kajikuri *et al.*, 2009).

Solutions

The composition of the Krebs solution was as follows (mM): NaCl, 122; KCl, 4.7; $MgCl_2$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 15.5; KH_2PO_4, 1.2; glucose, 11.5. It was bubbled with 95% oxygen and 5% carbon dioxide (pH 7.3–7.4).

Drugs

The drugs used were as follows: L-NNA (Peptides Institute Inc., Osaka, Japan); 5-HT hydrochloride and diclofenac sodium (Sigma Chemical Co.); hydroxyfasudil-HCl (Calbiochem, Darmstadt, Germany); SB269970 hydrochloride, SB200646 hydrochloride, TCB-2 and AS 19 (Tocris Bioscience, Ellisville, MO, USA); guanethidine (Tokyo Kasei, Tokyo, Japan). Sarpogrelate hydrochloride was kindly provided by Mitsubishi Tanabe Pharma (Osaka, Japan) and both Y-26763 and Y-27632 by Yoshitomi Pharmaceutical Ind. (Osaka, Japan).

Stock solutions were made of SB200646, AS 19, Y-26763 and Y-27632 (each at 0.1 M, in dimethylsulfoxide), and $PGF_{2\alpha}$ (10 mM, in ethanol). All other drugs were dissolved in ultrapure Milli-Q water (Japan Millipore Corp., Tokyo, Japan). The above stock solutions were stored at –80°C and diluted in Krebs solution just before use.

Statistical analysis

All results are expressed as mean ± SEM, with n values representing the number of rabbits used (each rabbit provided only one segment for a given experiment). The negative log of the EC_{50} value (pD_2 value) was determined for each curve using iterative curve-fitting software fitting an asymmetric sigmoidal function (using a non-linear least-square fitter supplied by Origin®, OriginLab Corporation, Northampton, MA, USA). A one-way or two-way repeated measures ANOVA, with *post hoc* comparisons made using the Scheffé procedure or Student's unpaired t-test, was used for the statistical analysis. The level of significance was set at $P < 0.05$.

Results

Differences in contractile properties between normal and grafted veins

In normal jugular vein preparations with intact endothelium, high K^+ (128 mM) induced a phasic, followed by a tonic contraction and the NO-synthase inhibitor L-NNA (0.1 mM) significantly increased the contraction (n = 6, Figure 1Aa1 and B). In grafted jugular vein preparations with intact endothelium, 128 mM K^+ induced a phasic, followed by a tonic contraction and L-NNA significantly increased these contractions (n = 6, Figure 1Aa2 and B).

5-HT (0.03–10 μM) did not induce a contraction in either the absence or presence of L-NNA in endothelium-intact 'Normal vein' preparations from normal rabbits (Figure 1Aa1 and B). Similarly, 5-HT (1–10 μM) did not induce a contraction in endothelium-intact 'Control vein' preparations from vein-grafted rabbits (n = 3, data not shown). In contrast, 5-HT (0.01–10 μM) induced a concentration-dependent contraction in endothelium-intact vein graft preparations and L-NNA significantly enhanced this contraction (n = 5; $P < 0.05$ by two-way repeated ANOVA; Figure 1C). The pD_2 values were 6.84 ± 0.15 and 6.91 ± 0.06 before and after application of L-NNA, respectively (n = 5; $P > 0.5$). The 5-$HT_{2B/2C}$-receptor antagonist SB200646 (1 μM) did not significantly alter the 5-HT (0.01–10 μM)-induced contraction in endothelium-intact vein graft preparations (n = 9; P = 0.987 by two-way repeated ANOVA; not shown in Figures).

Histological characterization of grafted veins

In haematoxylin-eosin-stained preparations, the thickness of the intima/media was 16.1 ± 1.0 μm (n = 4) in 'Normal vein' but 224.3 ± 19.0 μm in 'Vein-graft' (n = 4; $P < 0.001$). The number of nuclei across the intima/media was 3.1 ± 0.1 in 'Normal vein' (n = 4) and 16.3 ± 1.6 in 'Vein-graft' (n = 4; $P < 0.001$; Figure 2).

Figure 3 shows immunohistochemical staining against α-smooth muscle actin and the MHC isoforms SM1, SMemb and SM2 in rabbit carotid artery (upper row) and jugular veins (middle row for 'Normal vein' and lower row for 'Vein-graft'). Expression of α-smooth muscle actin was detected in all three vessel types, while the expression of MHC isoforms varied among the vessels. All three of the MHC isoforms were diffusely expressed in 'Vein-graft', with SMemb being more abundant in 'Vein-graft' than in 'Normal vein'. In the Western blot analysis, the expression level of SM1 was similar between 'Control vein' and 'Vein-graft' (n = 5; $P > 0.5$; Figure 4A). The expression level of SM2 was lower in 'Vein-graft' than in 'Control vein' (n = 5; $P < 0.01$; Figure 4B), while that of SMemb was higher in 'Vein-graft' than in 'Control vein' (n = 5; $P < 0.01$; Figure 4C).

Receptor subtypes mediating 5-HT-induced changes in SMC membrane potential

The resting membrane potential in SMCs was –49.7 ± 0.4 mV (n = 8) in 'Normal vein' from normal rabbits. The values obtained from vein-grafted rabbits were –50.3 ± 0.5 mV for 'Control vein' (n = 3; P = 1.0 vs. 'Normal vein') and –49.9 ± 1.5 mV for 'Vein-graft' (n = 8, P = 0.437 vs. 'Normal vein'). The SMC hyperpolarization induced by the ATP-sensitive

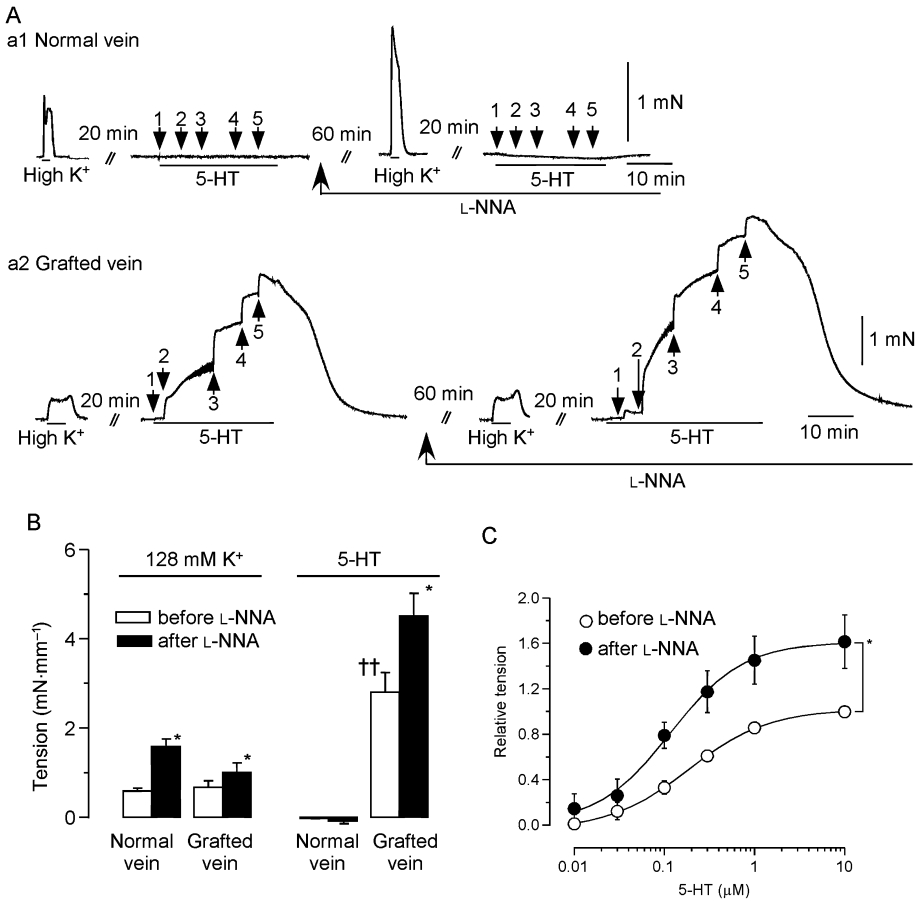

Figure 1

5-HT-induced mechanical responses in endothelium-intact strips of normal vein and grafted vein. (A) Effects of L-NNA on the mechanical responses induced by high K^+ (128 mM) and 5-HT (0.03–10 μM) in normal vein (a1) and grafted vein (a2). After recording control responses to high K^+ and 5-HT (left column), L-NNA (0.1 mM) was applied for 60 min and high K^+ and 5-HT were applied in the presence of L-NNA. Concentrations of 5-HT: (1) 0.03 μM; (2) 0.1 μM; (3) 0.3 μM; (4) 1 μM; (5) 10 μM. (B) Absolute maximum tension induced by 128 mM K^+ or 10 μM 5-HT in the absence and presence of L-NNA in normal vein ('Normal vein') and vein graft ('Grafted vein') ($n = 6$ in each case). Data are shown as mean ± SEM. *$P < 0.05$ versus before L-NNA, ††$P < 0.01$ versus 'Normal vein'. (C) Effects of L-NNA on 5-HT-induced contractions in vein graft. Data are shown as mean ± SEM. *$P < 0.05$ after versus before L-NNA.

K^+-channel activator Y-26763 (3 and 10 μM; Watanabe *et al.*, 1996) was similar among 'Vein-graft' and 'Control vein' from vein-grafted rabbits and 'Normal vein' from normal rabbits (for 3 μM, $n = 3$, $P = 0.087$; for 10 μM, $n = 8$, $P = 0.44$; Figure 5).

In grafted veins, 5-HT (0.1 μM) induced SMC depolarization (20.4 ± 1.1 mV, $n = 6$). The 5-HT_{2A}-receptor antagonist sarpogrelate (1 μM) did not alter the resting membrane potential but it greatly attenuated the 5-HT-induced depolarization ($n = 6$, Figure 6Bb1 and Bb2). In the presence of sarpogrelate, the 5-$HT_{1B/1D}$-receptor antagonist GR55562 (1 μM) did not alter the resting membrane potential but it further attenuated the 5-HT-induced depolarization ($n = 6$, Figure 6Bb1 and Bb2).

The selective 5-HT_7-receptor agonist AS 19 (10 μM) did not alter the resting SMC membrane potential in grafted veins (–46.7 ± 1.9 mV and –46.7 ± 2.0 mV in the absence and presence of AS 19, respectively, $n = 3$; $P > 0.5$).

Receptor subtypes mediating 5-HT-induced smooth muscle contraction

In endothelium-denuded vein graft preparations, 5-HT (0.01–10 μM) induced a concentration-dependent contraction. Sarpogrelate (1 μM) significantly attenuated this contraction ($n = 6$; $P < 0.001$ by two-way repeated ANOVA; Figure 7A). GR55562 (1 μM) attenuated the 5-HT-induced contraction in both the presence ($n = 6$; $P < 0.05$ vs. 'Sarpogrelate'; Figure 7A) and absence ($n = 6$; $P < 0.05$ vs. 'Control'; Figure 7B) of sarpogrelate. The pD_2 values for 5-HT were 7.46 ± 0.12 ($n = 6$) for 'Control' and 6.01 ± 0.16 ($n = 6$; $P < 0.05$ vs. 'Control') and 5.71 ± 0.12 ($n = 6$; $P < 0.05$ vs. 'Control') for 'Sarpogrelate' and 'Sarpogrelate+GR55562', respectively. The pD_2 values for 5-HT were 6.89 ± 0.15 ($n = 6$) and 6.06 ± 0.22 ($n = 6$) for 'Control' and 'GR55562' ($P < 0.05$ vs. 'Control'), respectively.

The selective 5-HT_{2A}-receptor agonist TCB-2 (0.1–10 μM, $n = 9$) and the selective 5-$HT_{1B/1D}$-receptor agonist sumatriptan

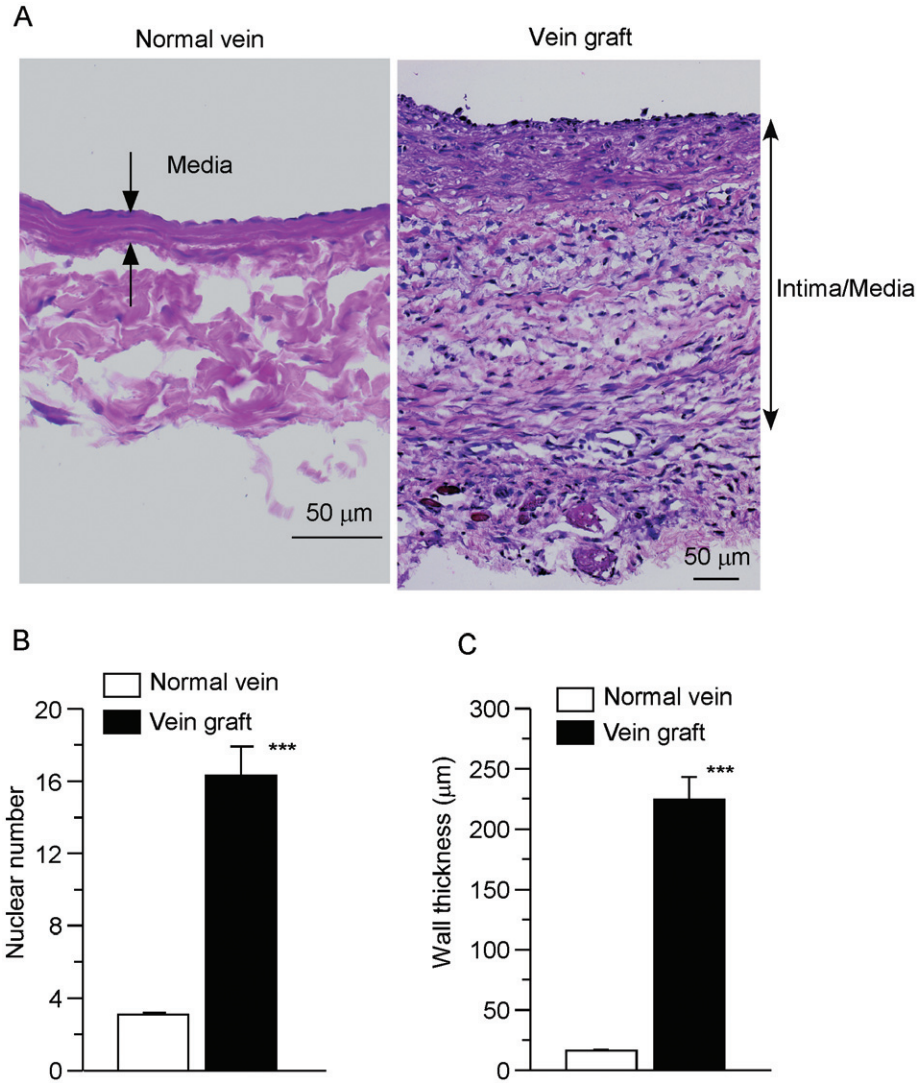

Figure 2

Haematoxylin-eosin staining in vascular wall of vein grafts. (A) Haematoxylin-eosin staining in 'Normal vein' (left panel) and 'Vein-graft' (right panel). (B) Number of nuclei in the media region of 'Normal vein' ($n = 4$) and in the intima/media region of 'Vein-graft' ($n = 4$). (C) Wall thickness of media region in 'Normal vein' ($n = 4$) and of intima/media region in 'Vein-graft' ($n = 4$). Data are shown as mean ± SEM. ***$P < 0.001$ versus 'Normal vein'.

(0.1–10 μM, $n = 7$) each induced a concentration-dependent contraction in endothelium-denuded vein graft preparations, with the contraction induced by TCB-2 being significantly larger than that induced by sumatriptan ($P < 0.001$, by two-way repeated ANOVA; Figure 7C).

The 5-HT_7-receptor antagonist SB269970 (1 μM) did not modify the contraction induced by 5-HT (0.01–10 μM) in endothelium-denuded vein graft preparations ($n = 5$; $P = 0.562$; Figure 8A). Furthermore, during the contraction induced by $PGF_{2\alpha}$ (1 μM) in the presence of sarpogrelate plus GR55562: (i) 5-HT (0.1–1 μM) induced a contraction (rather than a relaxation) and (ii) the selective 5-HT_7-receptor agonist AS 19 (10 μM) did not induce a relaxation (Figure 8B).

Changes in the immunoreactive expressions of receptor subtypes (5-HT_{2A}, 5-$HT_{1B/1D}$ and 5-HT_7) were examined in normal and grafted jugular veins. The 5-HT_{2A} and 5-HT_7 receptors were each distributed in the medial region of 'Normal vein' (upper row in Figure 9A), and immunoreactive responses against 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors (but not against the 5-HT_7 receptor) were detected in the intima/media region of vein grafts (lower row in Figure 9A). The protein expressions of 5-HT_{2A} and 5-HT_{1B} receptors were higher in 'Vein-graft' than in 'Control vein' ($n = 4–5$, $P < 0.05$ in each case, Figure 9B).

Effects of nifedipine, with or without either Y-27632 or hydroxyfasudil, on 5-HT-induced contraction in vein grafts

High K^+ (128 mM) and 5-HT (10 μM) each induced a phasic and tonic contraction in the present vein grafts. The L-type Ca^{2+}-channel inhibitor nifedipine (1 μM) virtually abolished each phase of the high K^+-induced contraction and attenu-

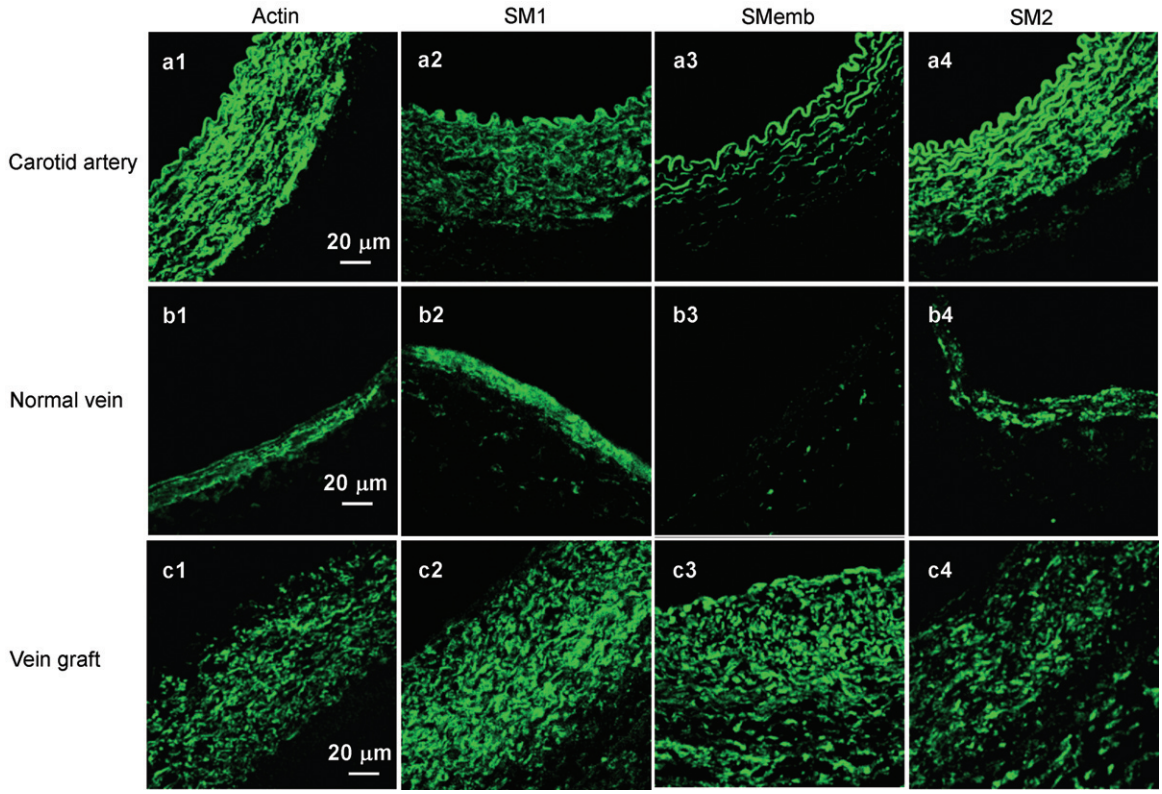

Figure 3

Immunohistochemical staining for α-smooth muscle actin and myosin heavy chain isoforms (SM1, SM2 and SMemb) in vascular wall of carotid artery ('Carotid artery'), jugular vein from normal rabbit ('Normal vein') and jugular vein graft from vein-grafted rabbit ('Vein-graft'). Carotid artery, a1–a4; Normal vein, b1–b4; Vein graft, c1–c4. Immunohistochemistry was performed using antibodies against α-smooth muscle actin (a1, b1, c1), SM1 (a2, b2, c2), SMemb (a3, b3, c3) and SM2 (a4, b4, c4). Note that strong green fluorescence indicates elastic lamina in carotid artery. Similar observations were made in three other preparations.

ated each phase of the 5-HT-induced contraction. In the presence of nifedipine, the Rho-kinase inhibitor Y-27632 (10 μM; Uehata *et al.*, 1997) greatly attenuated the residual 5-HT-induced phasic and tonic contractions in the vein grafts (n = 6, Figure 10Bb2). Similarly, in the presence of nifedipine another type of Rho-kinase inhibitor, hydroxyfasudil (10 μM; Furuyama *et al.*, 2006), also strongly attenuated the 5-HT-induced phasic and tonic contractions in vein grafts (n = 3, Figure 10C).

Discussion and conclusions

Although 5-HT did not induce a contraction in the rabbit normal jugular vein, it induced a large contraction in an autologous jugular vein graft isolated at 4 weeks after its transplantation into the carotid artery. The number of cells expressing α-smooth muscle actin as well as the MHC isoforms SM1 and SM2 was greatly increased in such a vein graft. Further, we suggest that in the vein graft, the number of SMCs expressing immunological responses against 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors (each of which leads to a contraction) is increased. We also found that immunological protein expressions against 5-HT_{2A} and 5-$HT_{1B/1D}$ receptor mg^{-1} vascular wall proteins were both increased in the vein graft, while that of the $5HT_7$ receptor (which leads to relaxation) may be decreased. These changes in expression were presumably causally related to 5-HT inducing a large contraction in the jugular vein graft.

The phenotypic characteristics of the SMCs in arteries have been studied using antibodies against MHC isoforms (Nagai *et al.*, 1989; Kim *et al.*, 1993; Takahashi *et al.*, 2000). SM1 and SM2 are SMC-specific MHC isoforms, and whereas the former is constitutively positive from the fetal stage to adulthood in the media of the aorta, the latter is recognized only in well-differentiated smooth muscle. SMemb, a non-muscle type of MHC, is abundantly expressed in the embryonic aorta and in phenotypically modulated, dedifferentiated SMCs in the arteriosclerotic neointima in the aorta (Kuro-O *et al.*, 1991). We found in the present vein grafts that: (i) the number of cells positive for α-smooth muscle actin across the vascular wall increased as the wall thickness increased; (ii) the immunoreactive responses against SM1 and SM2 were diffusely distributed in the vein grafts; (iii) although Western blot experiments revealed a decrease in SM2 expression and/or no change in SM1 expression mg^{-1} vascular wall protein in 'Vein graft' versus 'Control vein', the number of SMCs expressing SM1 and SM2 in the vascular wall (i.e. abso-

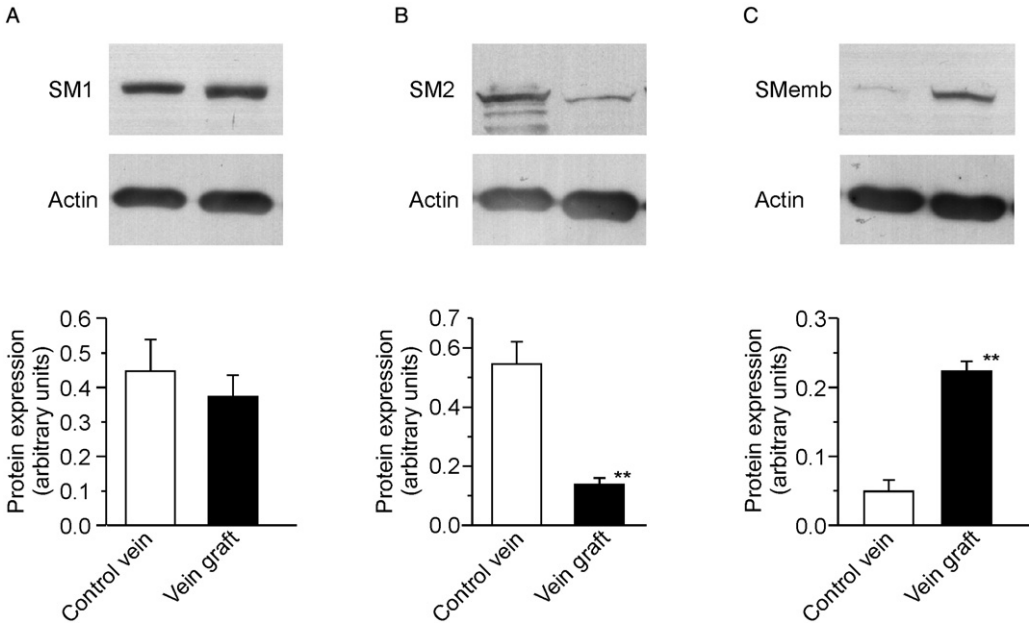

Figure 4

Expressions of SM1, SM2 and SMemb in 'Control vein' and 'Vein-graft'. Protein expressions of SM1 (A), SM2 (B) and SMemb (C) were measured by Western blot analysis in 'Control vein' and 'Vein-graft'. Each column represents the mean of data from five different preparations (each from a different animal) with SEM. $^{**}P < 0.01$ versus 'Control vein'.

lute amount of SM1 and SM2 expression in the vascular wall) seemed to be increased; and (iv) 5-HT induced a very large contraction (five times the tension induced by 128 mM K^+). These findings are in part consistent with previous findings (Kim *et al.*, 1993; Itoh *et al.*, 1994; Zhang *et al.*, 1999; Ishida *et al.*, 2001; Kodama *et al.*, 2009a,b). Taken together, the above results indicate that in such vein grafts, many SMCs retain SM1 and/or SM2, which presumably enables them to generate a powerful 5-HT-induced contraction (which would allow the graft to resist the higher pressure present on the arterial side of the circulation).

Receptor subtypes mediating 5-HT-induced contraction in vein grafts

An immunological response against 5-HT_{2A} receptors, but not against 5-$HT_{1B/1D}$ receptors, was present in the media region of the rabbit normal jugular vein, while both 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors were present in the intima/media region of the jugular vein graft. Furthermore, Western blot analysis of immunoreactive proteins indicated that the expressions of the 5-HT_{2A} and 5-HT_{1B} receptors were each higher in 'Vein-graft' than in 'Control vein'. This suggests that in the rabbit grafted jugular vein, there are increases in the expressions of the 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in the vascular wall.

Membrane depolarization plays a role in 5-HT-induced contraction in some arteries (Harder and Waters, 1983; Garland, 1985; 1987). We found that the resting membrane potential and the membrane hyperpolarization induced by the ATP-sensitive K^+-channel opener Y-26763 in SMCs were each similar between rabbit normal and grafted jugular veins. 5-HT (1–10 μM) induces a small membrane hyperpolarization in the SMCs of the rabbit normal jugular vein (Itoh and Kajikuri, 2011), but here a 5-HT concentration as low as 0.1 μM induced a large SMC membrane depolarization in the grafted jugular vein. The 5-HT_{2A}-receptor antagonist sarpogrelate (1 μM), when used alone, partly attenuated that depolarization, while its combined application with the 5-$HT_{1B/1D}$-receptor antagonist GR55562 abolished it. In the present vein grafts, high K^+ (128 mM) and 5-HT (10 μM) each induced a phasic, followed by a tonic contraction. The L-type Ca^{2+}-channel inhibitor nifedipine blocked the high K^+-induced contraction but only partly attenuated the 5-HT-induced contraction. These results indicate that 5-HT induces a SMC membrane depolarization through activation of 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in the rabbit jugular vein graft, and thereby induces a large contraction.

In endothelium-denuded vein grafts, sarpogrelate and GR55562 each attenuated the 5-HT (0.01–10 μM)-induced contractions, while combined application of these two antagonists inhibited the 5-HT contractions more strongly than either one did when applied alone. Furthermore, the selective 5-HT_{2A}-receptor agonist TCB-2 and the 5-$HT_{1B/1D}$-receptor agonist sumatriptan each induced a contraction in endothelium-denuded preparations of the grafted vein, although the effect of TCB-2 was significantly stronger than that of sumatriptan. These results indicate that 5-HT induces a contraction through activation of SMC 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in the present rabbit jugular vein graft.

We recently found that in rabbit normal jugular vein: (i) immunological responses against 5HT_{2A} and 5-HT_7 receptors are both present in the media and (ii) 5-HT and the selective 5-HT_7-receptor agonist AS 19 each induce a SMC hyperpolarization and a relaxation, which are blocked by the 5-HT_7-

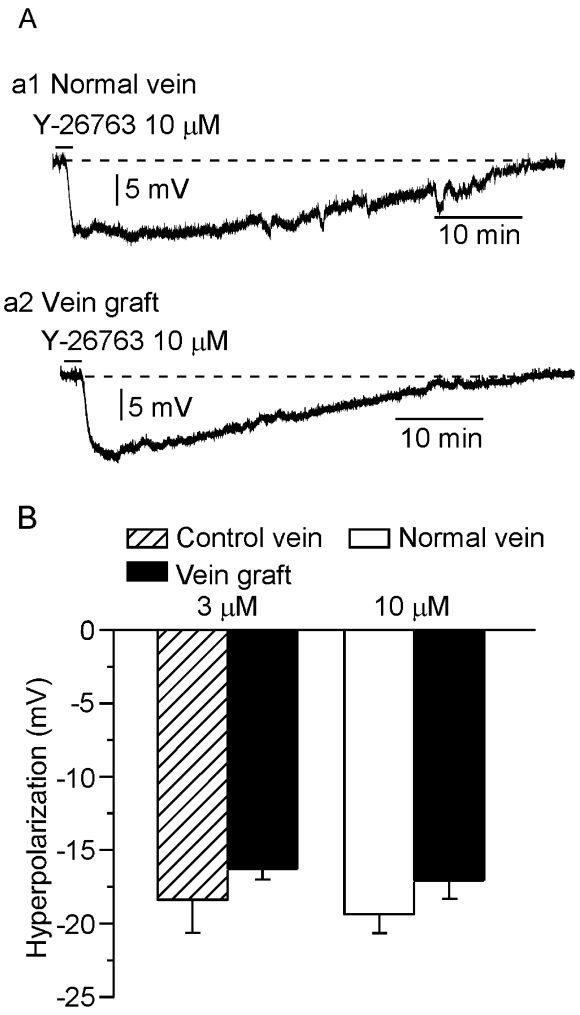

Figure 5

Effects of the ATP-sensitive K^+-channel opener Y-26763 on membrane potential in smooth muscle cells of non-grafted vein and grafted vein. (A) Actual traces of Y-26763 (10 μM)-induced membrane potential changes in normal vein (a1) and vein graft (a2). (B) Summary of the effects of Y-26763 on SMC membrane potential (n = 3–8). Data are shown as mean ± SEM.

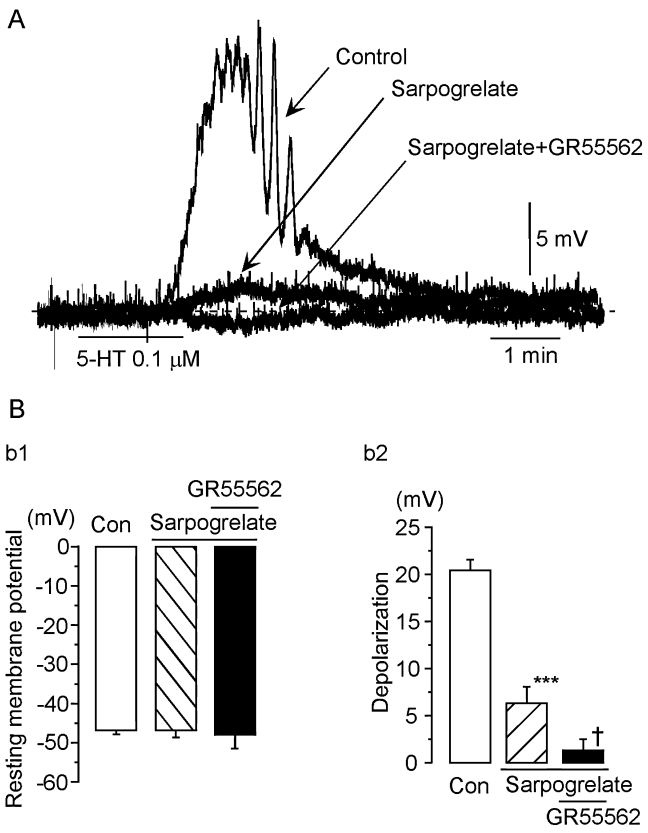

Figure 6

5-HT-induced changes in membrane potential in smooth muscle cells of vein graft. (A) Actual traces of 5-HT (0.1 μM)-induced membrane potential changes. After recording the control 5-HT response (control), sarpogrelate (1 μM) was pretreated for 20 min and 5-HT was then applied in the presence of sarpogrelate. Next, GR55562 (1 μM) was added for 20 min in the presence of sarpogrelate. Finally, 5-HT was applied in the presence of sarpogrelate+GR55562. (B) b1, Effects of sarpogrelate and sarpogrelate+GR55562 on resting membrane potential in smooth muscle cells (n = 6). b2, Effect of sarpogrelate on the 5-HT-induced membrane depolarization in smooth muscle cells and effect of GR55562 on that response (n = 6). Data are shown as mean ± SEM. ***P < 0.001 versus control, $^{\dagger}P$ < 0.05 versus sarpogrelate.

receptor antagonist SB269970 (Itoh and Kajikuri, 2011). Thus, we suggest that under physiological conditions, the function of 5-HT_{2A} receptors in SMCs (to produce a contraction) is masked by the 5-HT_7-receptor-mediated relaxation response in rabbit normal jugular vein. By contrast, we found here that in a rabbit jugular vein graft: (i) a smaller immunological response against 5-HT_7 receptors was detected (vs. 'Normal vein'); (ii) AS 19 did not alter the SMC membrane potential; (iii) neither AS 19 nor 5-HT (0.1–1 μM), when applied in the presence of sarpogrelate+GR55562, induced a relaxation during the $PGF_{2\alpha}$-induced contraction; and (iv) the selective 5-HT_7-receptor antagonist SB269970 did not significantly modify the 5-HT (0.01–10 μM)-induced contraction. Taken together, these results suggest that the 5-HT_7 receptor plays the important role of down-regulating 5-HT-induced contraction in rabbit normal jugular vein and that a loss of the function of this receptor contributes to 5-HT inducing the enhanced contraction seen in the vein graft.

Roles played by endothelium-derived NO in regulating contraction in a grafted vein

We found here that the NO-synthase inhibitor L-NNA significantly enhanced the contraction induced by 5-HT (0.01–10 μM) in endothelium-intact preparations of grafted veins, indicating that endothelium-derived NO serves to regulate 5-HT-induced contraction in the present vein graft. It was found some years ago that 5-HT enhances NO production via activation of endothelial 5-HT_{1B}-subtype receptors in pig and dog isolated coronary arteries (Cocks and Angus, 1983; Schoeffter and Hoyer, 1990) but via endothelial 5-HT_{2B}-subtype receptors in pig pulmonary artery (Glusa and Pertz, 2000). We reported recently that activation of 5-HT_{2A},

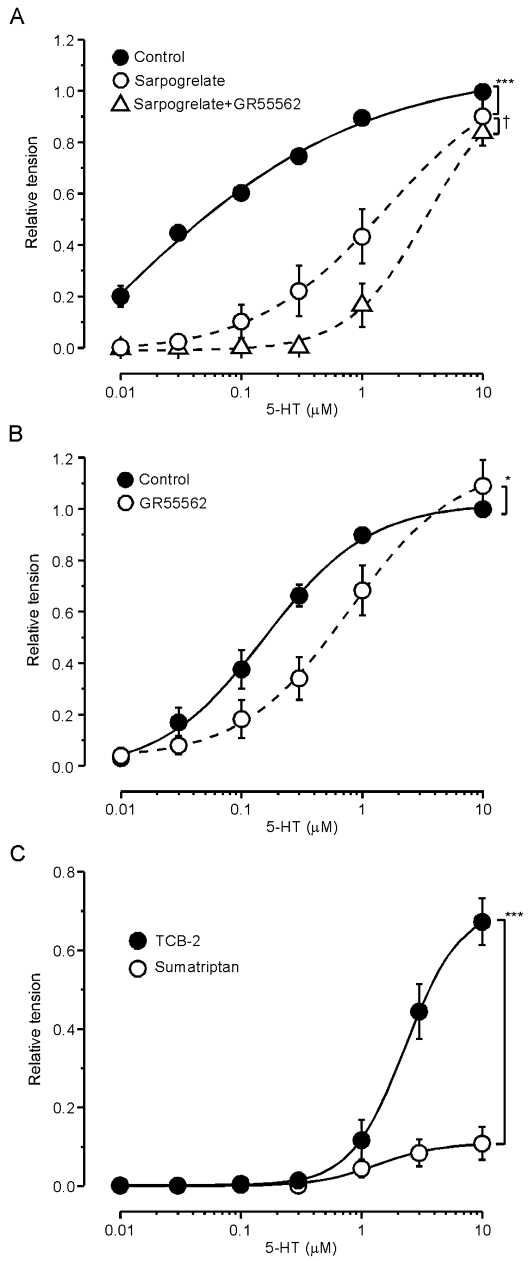

Figure 7

Effects of sarpogrelate and/or GR55562 on the contraction induced by 5-HT, together with the effects of TCB-2 and sumatriptan on mechanical activities in endothelium-denuded vein grafts. (A) Effects of sarpogrelate (1 μM) with or without GR55562 (1 μM) on 5-HT-induced contraction. Data are shown as mean ± SEM. ***$P < 0.001$ versus 'Control' ($n = 6$). †$P < 0.05$ versus sarpogrelate ($n = 6$). (B) Effects of GR-55562 alone. *$P < 0.05$ versus control ($n = 6$). (C) Effects of TCB-2 ($n = 9$) and sumatriptan ($n = 7$). The maximum tension induced by 10 μM 5-HT was normalized as a relative tension of 1.0 in any given strip. ***$P < 0.001$ for TCB-2 versus sumatriptan.

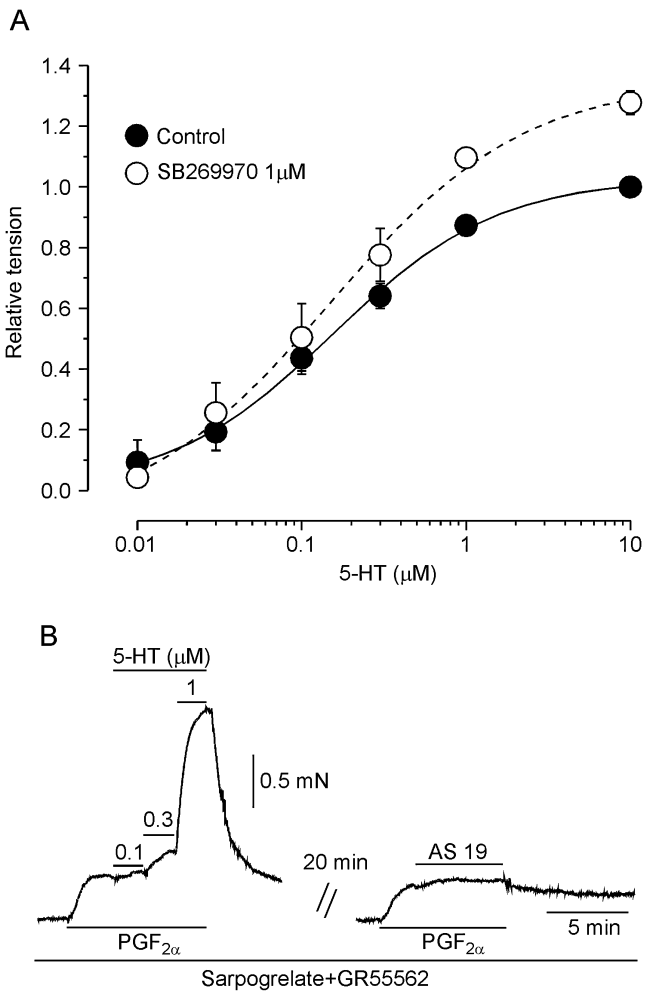

Figure 8

Effects of SB269970 on 5-HT-induced contraction in endothelium-denuded vein grafts. (A) SB269970 (1 μM) did not modify the contraction induced by 5-HT (0.01–10 μM; $n = 4$). (B) Effects of 5-HT (0.1–1 μM) and AS 19 (10 μM) during the contraction induced by 1 μM $PGF_{2\alpha}$ in the presence of sarpogrelate+GR55562. 5-HT was cumulatively applied in the presence of $PGF_{2\alpha}$. Following a 20 min washout with Krebs solution, $PGF_{2\alpha}$ (1 μM) was again applied and AS 19 (10 μM) was added during the $PGF_{2\alpha}$ contraction.

$5\text{-HT}_{1B/1D}$ and 5-HT_7 receptors do not induce endothelium-dependent relaxation in rabbit normal jugular vein (Itoh and Kajikuri, 2011). Two years before, we noted that in endothelium-intact rabbit jugular vein grafts, the $5\text{-HT}_{1B/1D}$-receptor blocker GR55562 did not enhance (rather, it inhibited) the 5-HT-induced contraction (Kodama *et al.*, 2009b). In addition, we found here that the $5\text{-HT}_{2B/2C}$-receptor antagonist SB200646 failed to modify the contraction induced by 5-HT in endothelium-intact vein grafts. These results suggest that neither the $5\text{-HT}_{1B/1D}$ receptors nor the $5\text{-HT}_{2B/2C}$-receptors in endothelial cells serve to modulate 5-HT-induced contraction in the present rabbit jugular vein graft. Thus, it is suggested that NO spontaneously released from the endothelium serves to down-regulate the 5-HT-induced contraction in such a graft.

Roles of Rho-kinase in 5-HT-induced contraction in the vein graft

It has been suggested that Rho-kinase plays a role in the pathogenesis of the hypercontraction seen in arteriosclerotic

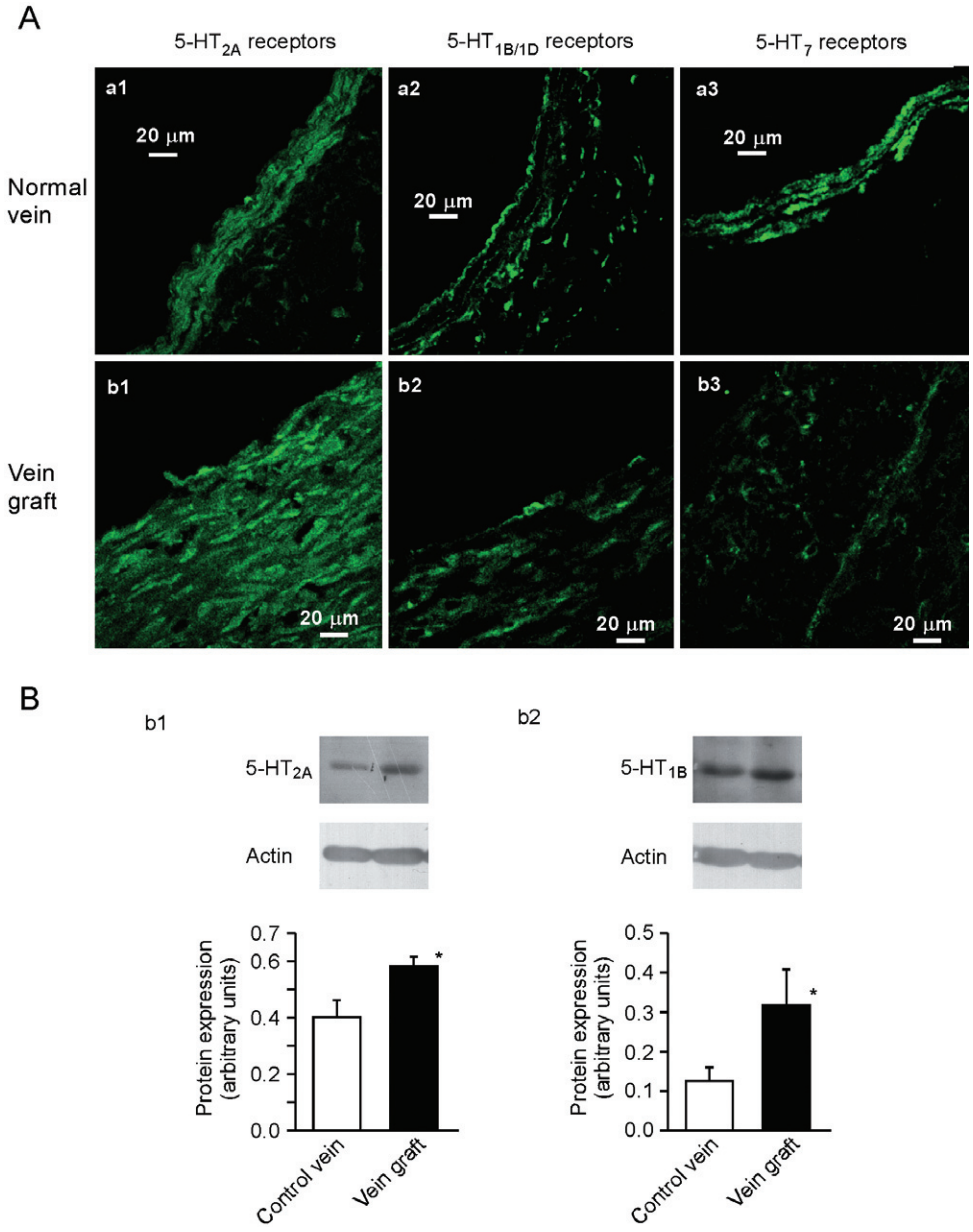

Figure 9

Expressions of 5-HT_{2A}, 5-$HT_{1B/1D}$ and 5-HT_7 receptors in vascular wall of normal vein and vein graft. (A) Normal vein: a1–a3. Vein graft: b1–b3. Immunofluorescence images showing localization of antibodies against the 5-HT_{2A} (a1, b1), 5-$HT_{1B/1D}$ (a2, b2) or 5-HT_7 (a3, b3) receptor in cross-sections of jugular vein. Similar observations were made in three other preparations. (B) Western blot analysis of 5-HT_{2A} receptors (b1) and 5-HT_{1B} receptors (b2) in 'Control vein' and 'Vein-graft'. Each column represents the mean of data from four to five different preparations (each from a different animal) with SEM. *$P < 0.05$ versus 'Control vein'.

arteries from both animals and humans (Kandabashi *et al.*, 2002; Yamanouchi *et al.*, 2005; Furuyama *et al.*, 2006). This kinase phosphorylates myosin phosphatase targeting subunit MYPT1, and inhibits myosin light chain phosphatase, thus enhancing SMC myofilament Ca^{2+}-sensitivity (Somlyo and Somlyo, 1994; Kimura *et al.*, 1996). Here, we found in the rabbit jugular vein graft that hydroxyfasudil and Y-27632, another type of Rho-kinase inhibitor, each abolished the contraction induced by 5-HT in the presence of nifedipine. These results, which are partly consistent with previous findings (Furuyama *et al.*, 2006), suggest that activation of Rho-kinase is causally involved in the enhancement of the 5-HT-induced contraction seen in the present rabbit jugular vein graft.

Vein graft adaptation and increase in 5-HT-induced contraction

Venous adaptation to the arterial environment is characterized by thickening of the intima, media and adventitia. These modifications, which result from deposition of SMCs and extracellular matrix components, stimulate remodelling and

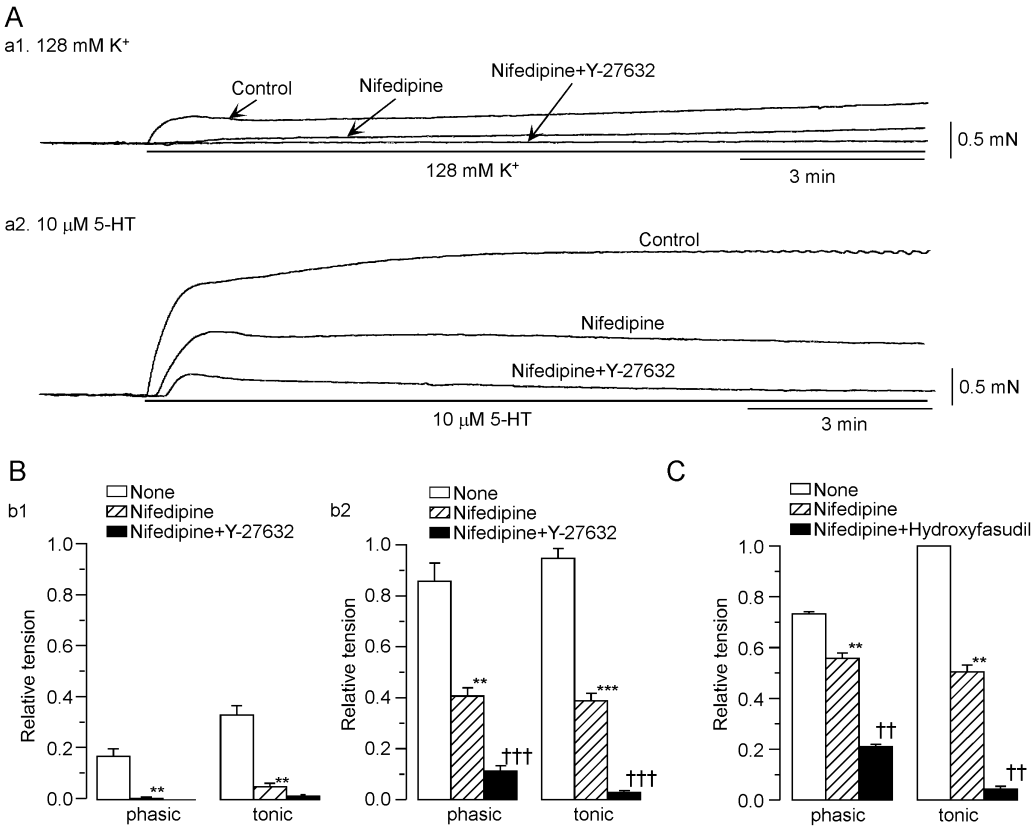

Figure 10

Effects of nifedipine, with or without Y-27632 or hydroxyfasudil, on contractions induced by high K^+ and 5-HT in endothelium-denuded vein grafts. (A) Actual traces showing the effects of nifedipine (1 μM), in the absence or presence of Y-27632 (10 μM), on the contractions induced by 128 mM K^+ (a1) and 10 μM 5-HT (a2). After the control response induced by either high K^+ or 5-HT had been recorded, nifedipine was applied as a pretreatment for 30 min and the same stimulant was applied in the presence of nifedipine. Under these conditions, Y-27632 was applied as a pretreatment and the same stimulant was finally applied in the presence of nifedipine+Y-27632. (B) Summary of the results obtained for the effects of nifedipine with or without Y-27632 on the contractions induced by 128 mM K^+ (b1) and 10 μM 5-HT (b2). The maximum tension induced by 10 μM 5-HT was normalized to 1.0 in each preparation. (C) Summary of the results obtained (using a similar protocol to that described above) for the effects of nifedipine with or without hydroxyfasudil on the contractions induced by 10 μM 5-HT. Data are shown as mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ versus 'None', $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ versus 'Nifedipine'.

reduce compliance (Abeles *et al.*, 2006; Owens, 2010; Muto *et al.*, 2011). Such vein graft remodelling appears to involve at least two distinct temporal phases: lumen outward remodelling at an earlier phase and wall stiffness changes at a later phase (Owens, 2010). The former is thought to be important for successful adaptation to increased diameter and wall thickness in human vein grafts. We found here that characteristic morphological and mechanical changes had occurred in rabbit autologous jugular vein grafts examined at 4 weeks after transplantation into the carotid artery. We suggest that these changes may be responsible for 'physiological adaptation' in the vein graft and may help it to resist the higher pressure present on the arterial side of the circulation.

By the use of intravascular ultrasound technique, it has been found that morphological modifications leading to observable differences between a native 'in situ radial artery' (muscular artery) and a 'coronary-radial artery graft' (elastomuscular artery) result in an inhibition of the former's spastic response to 5-HT (Gaudino *et al.*, 2005). These changes contrast with those found in the present vein grafts, suggesting that changes in 5-HT-induced mechanical responsiveness in grafted vessels may differ between arteries and veins. The mechanism underlying these differences remains to be fully clarified.

In genetically modified mice, the neointimal SMCs in vein grafts may be recruited from various anatomical locations (viz. graft-extrinsic sources and graft-intrinsic sources). The former include migrating medial SMCs from the adjacent artery as well as bone-marrow- and non-bone-marrow-derived vascular progenitor cells that infiltrate the graft having arrived either via the blood (through the vasa vasorum or through transendothelial diapedesis) or via post-surgical adventitial adhesions (Hu *et al.*, 2002; Cooley, 2004; Zhang *et al.*, 2004; Diao *et al.*, 2008). The latter (graft-intrinsic) sources include adventitial myofibroblast-localized vascular progenitor cells residing in the media and adventitia. However, the main sources of SMCs and whether SMCs from different sources differ in the way in which they modulate 5-HT-induced contraction in rabbit vein grafts remains to be clarified.

Acknowledgements

We thank Dr R. J. Timms for a critical reading of the manuscript. We also thank T. Goto, Y. Mizuno and K. Uno, medical students at Nagoya City University, for their technical assistance with the present experiments. This work was partly supported by a Grant-In-Aid for Scientific Research from the Japan Society for the Promotion of Science.

Conflict of interest

None.

References

Abeles D, Kwei S, Stavrakis G, Zhang Y, Wang ET, García-Cardeña G (2006). Gene expression changes evoked in a venous segment exposed to arterial flow. J Vasc Surg 44: 863–870.

Aikawa M, Sivam PN, Kuro-o M, Kimura K, Nakahara K, Takewaki S *et al.* (1993). Human smooth muscle myosin heavy chain isoforms as molecular markers for vascular development and atherosclerosis. Circ Res 73: 1000–1012.

Alexander SPH, Mathie A, Peters JA (2011). Guide to Receptors and Channels (GRAC), 5th Edition. Br J Pharmacol 164 (Suppl. 1): S1–S324.

Banno H, Takei Y, Muramatsu T, Komori K, Kadomatsu K (2006). Controlled release of small interfering RNA targeting midkine attenuates intimal hyperplasia in vein grafts. J Vasc Surg 44: 633–641.

Cocks TM, Angus JA (1983). Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature 305: 627–630.

Cooley BC (2004). Murine model of neointimal formation and stenosis in vein grafts. Arterioscler Thromb Vasc Biol 24: 1180–1185.

Davies MG, Hagen PO (1995). Pathophysiology of vein graft failure: a review. Eur J Vasc Endovasc Surg 9: 7–18.

Diao Y, Guthrie S, Xia SL, Ouyang X, Zhang L, Xue J *et al.* (2008). Long-term engraftment of bone marrow-derived cells in the intimal hyperplasia lesion of autologous vein grafts. Am J Pathol 172: 839–848.

Ellis ES, Byrne C, Murphy OE, Tilford NS, Baxter GS (1995). Mediation by 5-hydroxytryptamine$_{2B}$ receptors of endothelium-dependent relaxation in rat jugular vein. Br J Pharmacol 114: 400–404.

Frieden M, Bény JL (1995). Effect of 5-hydroxytryptamine on the membrane potential of endothelial and smooth muscle cells in the pig coronary artery. Br J Pharmacol 115: 95–100.

Furuyama T, Komori K, Shimokawa H, Matsumoto Y, Uwatoku T, Hirano K *et al.* (2006). Long-term inhibition of Rho kinase suppresses intimal thickening in autologous vein grafts in rabbits. J Vasc Surg 43: 1249–1256.

Garland CJ (1985). Endothelial cells and the electrical and mechanical responses of the rabbit coronary artery to 5-hydroxytryptamine. J Pharmacol Exp Ther 233: 158–162.

Garland CJ (1987). The role of membrane depolarization in the contractile response of the rabbit basilar artery to 5-hydroxytryptamine. J Physiol 392: 333–348.

Gaudino M, Prati F, Caradonna E, Trani C, Burzotta F, Schiavoni G *et al.* (2005). Implantation in coronary circulation induces morphofunctional transformation of radial grafts from muscular to elastomuscular. Circulation 112 (9 Suppl.): I208–I211.

Glusa E, Pertz HH (2000). Further evidence that 5-HT-induced relaxation of pig pulmonary artery is mediated by endothelial 5-HT$_{2B}$ receptors. Br J Pharmacol 130: 692–698.

Glusa E, Roos A (1996). Endothelial 5-HT receptors mediate relaxation of porcine pulmonary arteries in response to ergotamine and dihydroergotamine. Br J Pharmacol 119: 330–334.

Grayson KL, Gupta P (1995). Preliminary characterization of an endothelial 5-HT receptor which mediates relaxation in a preparation of dog isolated vena cava. Br J Pharmacol 116: 409P.

Gupta P (1992). An endothelial 5-HT receptor that mediates relaxation in guinea-pig isolated jugular vein resembles the 5-HT$_{1D}$ subtype. Br J Pharmacol 106: 703–709.

Harder DR, Waters A (1983). Electromechanical coupling in feline basilar artery in response to serotonin. Eur J Pharmacol 93: 95–100.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ *et al.* (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 46: 157–203.

Hu Y, Mayr M, Metzler B, Erdel M, Davison F, Xu Q (2002). Both donor and recipient origins of smooth muscle cells in vein graft atherosclerotic lesions. Circ Res 91: e13–e20.

Ishida M, Komori K, Yonemitsu Y, Taguchi K, Onohara T, Sugimachi K (2001). Immunohistochemical phenotypic alterations of rabbit autologous vein grafts implanted under arterial circulation with or without poor distal runoff-implications of vein graft remodeling. Atherosclerosis 154: 345–354.

Itoh H, Komori K, Funahashi S, Okadome K, Sugimachi K (1994). Intimal hyperplasia of experimental autologous vein graft in hyperlipidemic rabbits with poor distal runoff. Atherosclerosis 110: 259–270.

Itoh T, Kajikuri J (2011). Characteristics of the actions by which 5-hydroxytryptamine affects electrical and mechanical activities in rabbit jugular vein. Br J Pharmacol 164: 979–991.

Itoh T, Seki N, Suzuki S, Ito S, Kajikuri J, Kuriyama H (1992). Membrane hyperpolarization inhibits agonist-induced synthesis of inositol 1,4,5-trisphosphate in rabbit mesenteric artery. J Physiol 451: 307–328.

Jiang Z, Wu L, Miller BL, Goldman DR, Fernandez CM, Abouhamze ZS *et al.* (2004). A novel vein graft model: adaptation to differential flow environments. Am J Physiol Heart Circ Physiol 286: H240–H245.

Kajikuri J, Watanabe Y, Ito Y, Ito R, Yamamoto T, Itoh T (2009). Characteristic changes in coronary artery at the early hyperglycaemic stage in a rat type 2 diabetes model and the effects of pravastatin. Br J Pharmacol 158: 621–632.

Kandabashi T, Shimokawa H, Mukai Y, Matoba T, Kunihiro I, Morikawa K *et al.* (2002). Involvement of Rho-kinase in agonist-induced contractions of arteriosclerotic human arteries. Arterioscler Thromb Vasc Biol 22: 243–248.

Kim HS, Aikawa M, Kimura K, Kuro-o M, Nakahara K, Suzuki T *et al.* (1993). Ductus arteriosus. Advanced differentiation of smooth muscle cells demonstrated by myosin heavy chain isoform expression in rabbits. Circulation 88: 1804–1810.

Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M *et al.* (1996). Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science 273: 245–248.

Kodama A, Komori K, Hattori K, Yamanouchi D, Kajikuri J, Itoh T (2009a). Sarpogrelate hydrochloride reduced intimal hyperplasia in experimental rabbit vein graft. J Vasc Surg 49: 1272–1281.

Kodama A, Komori K, Kajikuri J, Itoh T (2009b). Chronic treatment of hydroxytryptamine type 2A receptor antagonist sarpogrelate hydrochloride modulates the vasoreactivity of serotonin in experimental rabbit vein grafts. J Vasc Surg 50: 617–625.

Komori K, Yamamura S, Ishida M, Matsumoto T, Kuma S, Eguchi D *et al.* (1997). Acceleration of impairment of endothelium-dependent response under poor runoff conditions in canine autogenous vein grafts. Eur J Vasc Endovasc Surg 14: 475–481.

Kuro-o M, Nagai R, Nakahara K, Katoh H, Tsai RC, Tsuchimochi H *et al.* (1991). cDNA cloning of a myosin heavy chain isoform in embryonic smooth muscle and its expression during vascular development and in arteriosclerosis. J Biol Chem 266: 3768–3773.

Loop FD, Lytle BW, Cosgrove DM, Stewart RW, Goormastic M, Williams GW *et al.* (1986). Influence of the internal-mammary-artery graft on 10-year survival and other cardiac events. N Engl J Med 314: 1–6.

Lopes RD, Hafley GE, Allen KB, Ferguson TB, Peterson ED, Harrington RA *et al.* (2009). Endoscopic versus open vein-graft harvesting in coronary-artery bypass surgery. N Engl J Med 361: 235–244.

Makhoul RG, Davis WS, Mikat EM, McCann RL, Hagen PO (1987). Responsiveness of vein bypass grafts to stimulation with norepinephrine and 5-hydroxytryptamine. J Vasc Surg 6: 32–38.

Marcos E, Fadel E, Sanchez O, Humbert M, Dartevelle P, Simonneau G *et al.* (2004). Serotonin-induced smooth muscle hyperplasia in various forms of human pulmonary hypertension. Circ Res 94: 1263–1270.

Motwani JG, Topol EJ (1998). Aortocoronary saphenous vein graft disease: pathogenesis, predisposition, and prevention. Circulation 97: 916–931.

Muto A, Yi T, Harrison KD, Dávalos A, Fancher TT, Ziegler KR *et al.* (2011). Eph-B4 prevents venous adaptive remodeling in the adult arterial environment. J Exp Med 208: 561–575.

Nagai R, Kuro-o M, Babij P, Periasamy M (1989). Identification of two types of smooth muscle myosin heavy chain isoforms by cDNA cloning and immunoblot analysis. J Biol Chem 264: 9734–9737.

Okies JE, Page US, Bigelow JC, Krause AH, Salomon NW (1984). The left internal mammary artery: the graft of choice. Circulation 70 (3 Pt 2): I213–I221.

Owens CD (2010). Adaptive changes in autogenous vein grafts for arterial reconstruction: clinical implications. J Vasc Surg 51: 736–746.

Radic ZS, O'Donohoe MK, Schwartz LB, Stein AD, Mikat EM, McCann RL *et al.* (1991). Alterations in serotonergic receptor expression in experimental vein grafts. J Vasc Surg 14: 40–47.

Schoeffter P, Hoyer D (1990). 5-hydroxytryptamine (5-HT)-induced endothelium-dependent relaxation of pig coronary arteries is mediated by 5-HT receptors similar to the 5-HT_{1D} receptor subtype. J Pharmacol Exp Ther 252: 387–395.

Somlyo AP, Somlyo AV (1994). Signal transduction and regulation in smooth muscle. Nature 372: 231–236.

Takahashi K, Ino T, Ohkubo M, Akimoto K, Kishirou M (2000). Restenosis after balloon angioplasty of coarctation: relationship with ductus arteriosus. Pediatr Int 42: 658–667.

Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T *et al.* (1997). Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 389: 990–994.

Watanabe Y, Suzuki A, Suzuki H, Itoh T (1996). Effect of membrane hyperpolarization induced by a K^+ channel opener on histamine-induced Ca^{2+} mobilization in rabbit arterial smooth muscle. Br J Pharmacol 117: 1302–1308.

Yamanouchi D, Banno H, Nakayama M, Sugimoto M, Fujita H, Kobayashi M *et al.* (2005). Hydrophilic statin suppresses vein graft intimal hyperplasia via endothelial cell-tropic Rho-kinase inhibition. J Vasc Surg 42: 757–764.

Zhang L, Freedman NJ, Brian L, Peppel K (2004). Graft-extrinsic cells predominate in vein graft arterialization. Arterioscler Thromb Vasc Biol 24: 470–476.

Zhang WD, Bai HZ, Sawa Y, Yamakawa T, Kadoba K, Taniguchi K *et al.* (1999). Association of smooth muscle cell phenotypic modulation with extracellular matrix alterations during neointima formation in rabbit vein grafts. J Vasc Surg 30: 169–183.