Int J Clin Exp Med 2015;8(5):6947-6956
www.ijcem.com /ISSN:1940-5901/IJCEM0007124

Original Article

The 5-hydroxytryptamine transporter is functional in human coronary artery smooth muscle cells proliferation and is regulated by Interleukin-1 beta

Qing-Jie Wang, Dong Wang, Cheng-Chun Tang

Department of Cardiology, Zhongda Hospital Southeast University, Medical School of Southeast University, Nanjing 210009, Jiangsu, China

Received February 15, 2015; Accepted April 23, 2015; Epub May 15, 2015; Published May 30, 2015

Abstract: Abnormal human coronary artery smooth muscle cells (hCASMCs) proliferation and migration are key factors in coronary artery restenosis after percutaneous coronary intervention. Platelets release 5-hydroxytryptamine (5-HT), which is a strong mitogen for pulmonary artery smooth muscle cells proliferation and migration. Here, we investigated the effects of 5-HT and role of 5-HT transporter (5-HTT) on hCASMCs proliferation and migration. The 5-HT (10^{-6}-10^{-5} mol/l) significantly increased hCASMCs proliferation and migration, and these effects were inhibited by fluoxetine (10^{-5} mol/l) and citalopram (10^{-6} mol/l), two 5-HTT blocker. Overexpression in hCASMCs enhanced 5-HT induced cells proliferation and migration. The 5-HTT and interleukin-1 beta (IL-1β) expression were increased in rat balloon injury carotid arteries. Treatment with IL-1β (10 ng/ml, 3d) upregulates 5-HTT expression in hCASMCs and increased 5-HT induced currents in Human Embryonic Kidney 293-5-HTT cells.

Keywords: Human coronary artery smooth muscle cells, 5-hydroxytryptamine transporter, interleukin-1 beta

Introduction

Restenosis limits the long-term efficacy of coronary angioplasty. Proliferation and migration of activated medial smooth muscle cells (SMCs) are important mechanisms in development of restenosis after percutaneous coronary intervention (PCI) [1]. PCI inevitably leads to endothelial and medial injury during angioplasty. Platelet aggregation at the site of vascular damage and release mediators, such as 5-hydroxytryptamine (5-HT), a strong mitogen, which is actively transported by 5-hydroxytryptamine transporter (5-HTT) [2]. Lines of evidence indicate that 5-HT is a mitogen for pulmonary arterial smooth muscle cells (PASMCs) [3] and inhibition of 5-HTT can attenuate pulmonary artery remodeling via inhibiting PASMCs proliferation [4-7]. However, little is known about the effects on human coronary artery smooth muscle cells (hCASMCs).

Inflammation and cytokines have been shown to contribute to neointima formation [8]. In balloon injured porcine coronary arteries [9], the inflammatory infiltrate up to 3 days and neointimal cells at 7 days were IL-1β positive, with no IL-1β detected in non-PTCA control arteries, indicating a critical role for IL-1 in the coronary artery. However, IL-1 is a potent smooth muscle cells mitogen, dependent upon autocrine platelet derived growth factor production. It is reported that IL-1β is an activator of 5-HTT in choriocarcinoma cells [10] and RN46A cells [11]. Monocrotaline induced rat pulmonary hypertension is associated with increased expression of both 5-HTT and IL-1β [12]. Thus, it is hypothesize that IL-1β may act as a mitogen at least partially through regulation of 5-HTT in HCASMCs.

In this study, we report the first time of a study that 5-HT promote hCASMCs proliferation through 5-HTT. IL-1β contributes to human coronary artery smooth muscle cells proliferation through upregulation of 5-HTT, which gain a new insight into the mechanism of proinflammatory cytokines in restenosis.

Materials and methods

Materials, animal and ethics statement

Fluoxetine and cell proliferation reagent MTT [3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide], GR127935, ketanserin (+) -tartrate salt and citalopram hydrobromide were purchased from sigma Systems Inc. Recombinant human IL-1β, Recombinant human interleukin 1 receptor antagonist (IL-1ra), 0.25% trypsin-EDTA, smooth muscle culture medium cell-2 with containing supplement-mix were purchased from Promocell (Heidelberg, Germany). All animals were purchased from the Animal Center of Southeast University. Animal experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (DHWE publication No. 96-01, revised in 2002) and were approved by the Ethics Review Board for Animal Studies of Institute of Southeast University, Nanjing, China. All animals were fed a standard diet of rat chow and were settled in the laboratory animal room with 21°C-26°C temperature, 40%-70% humidity and 15-20 Lux lighting.

Cell culture

Human coronary artery smooth muscle cells were purchased from Promocell (Heidelberg, Germany) and cultured in Smooth Muscle Growth Medium Cell-2 containing 10% supplement-mix (PromoCell, Heidelberg, Germany). Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2. At the end of this period, the medium was removed, and cells were subjected to growth arrest in medium containing 0.1% supplement-mix before each experiment. HCASMCs between passages 3-5 were used for experiments and all explants stained positive for smooth muscle cell α-actin (Sigma, USA).

Cell proliferation and migration assay

Proliferation was measured using MTT assay as described previously [8]. HCASMCs (4×10^3 cells per well) were planted onto a 96-well culture plate, and then starved with 0.1% mix in culture media for 24 h. Cells were treated with 5-HT at concentrations of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} mol/l, respectively. The effect of 5-HT was also examined in the presence of fluoxetine (10^{-5} mol/l) or citalopram (10^{-6} mol/l), two specific 5-HTT inhibitors, GR127935 (10^{-6} mol/l), a 5-$HT_{1B/1D}$ receptor antagonist, or ketanserin (10^{-7} mol/l), a 5-HT2 receptor antagonist, added 20 minutes before the 5-HT. After 24 h, 20 mL MTT (5 mg/mL) was added into each well and cells were incubated for 4 h. The accumulative formazan crystals were dissolved with 150 μL DMSO. The optical density of the solution was measured at a wavelength of 570 nm with a microplate reader (Thermo Scientific). For cell counts: Cells were seeded onto 96-well plates (4×10^3 cells per well) and pretreated. All the experiments were performed in octuplicate, and each experiment was repeated a minimum of three times. The migration assay was performed by using Transwell system (a 6.5-mmpolycarbonate membrane with 8.0-μm pores; Corning, NY) as described 19. Briefly, 5×10^4 of cells suspensions, containing fresh serum free media, were seeded on the upper chamber. 5-HT (10^{-6} mol/l) with or without fluoxetine (10^{-5} mol/l) was added into the bottom chamber as the chemoattractant. The cells were allowed to migrate through the membrane to the lower surface for 6 h. Cells on the upper surface of the membrane that had not migrated were scraped off with cotton swabs. Cells that had migrated to the lower surface were fixed by 4% paraformaldehyde and stained with 0.1% crystal violet, and then dissolved with 30% acetic acid. Absorbance was measured under 490 nm using a microplate reader (Thermo Scientific).

LC-MS/MS measurement

48 hours prior to each experiment, 2.5×10^5 cells were seeded in 6 cm dishes in culture media. 5-HT (10^{-6} mol/l) with or without fluoxetine (10^{-5} mol/l) or citalopram (10^{-6} mol/l) were added into culture media from 10-minute to 120-minute. After that, media were aspirated and metabolites were extracted with 1.5 ml of 4:1 v/v MeOH/H_2O equilibrated at -80°C. The extract and cells were scraped and collected into conical tubes and centrifuged for 5 minutes. Supernatant was evaporated using a speed-vac. Samples were re-suspended in 20 uL HPLC-grade water for mass spectrometry. 8 μL were injected and analyzed using a 5500 QTRAP triple quadrupole mass spectrometer (AB/Sciex) coupled to a Prominence UFLC system (Shimadzu) via selected reaction monitoring (SRM) of 249, in total, endogenous water-soluble metabolites. Nozzle Voltage was +500 V and -1500 V. The dwell time was 20 ms. Data was shown as percentage of control.

5-HTT cDNA cloning and hCASMCs transfection

The human 5-HTT pcDNA3, a gift from Randy Blakely [9] (Addgene plasmid # 15483), was digested with *Not I* and *Xho I* to get 5-HTT gene coding sequence. The 5-HTT gene coding sequence was cloned into pShuttle-CMV to get pShuttle-CMV-5HTT, which would be transformed into competent E.Coli BJ5183 carried backbone plasmid pAdeasy-1 already. The identified recombinant adenovirus containing the 5-HTT gene (rAd-5-HTT) was amplified in Human Embryonic Kidney 293 (HEK293) cells and was used to transfect 5-HTT into hCASMCs. The 5-HTT expression was examined by western blot. Proliferation and migration of hCASMCs were also examined in hCASMCs with 5-HTT overexpression.

Western blot analysis

Western blotting was carried as described previously [10]. In brief, total protein extraction from rat carotid arteries was performed according to a commercially available kit (KGP250; Nanjing Keygen Biotech Co. Ltd., Nanjing, China). For hCASMCs, cells were cultured in a 9-cm-diameter dish, grown to 70-80% confluence, and then starved in 0.1% supplement-mix medium for 24 h. The cells were then transfected with rAd-5-HTT or treated with or without IL-1β. Cells were lysed in RIPA (Radio Immunoprecipitation Assay) buffer with protease and phosphatase cocktails. Equal amounts of protein (60-100 μg) were separated by 10% SDS-PAGE and electro-transferred to a PVDF membrane (Millipore). Membranes were blocked, and then incubated with antibodies overnight, such as, anti-IL-1β (1:500, Santa Cruz), anti-5-HTT (1:2000, Merck Millipore), anti-GAPDH (1:2500, Cell Signaling Technology), and then with the horseradish peroxidase-conjugated secondary antibody (Beijing TDY biotech company, Ltd) (1:5000) for 2 h. Specific protein expression levels were normalized to GAPDH for total protein analyses. The blot was detected with the ChemiDoc™ MP System detection system (Bio-Rad Laboratories, Inc). The experiments were replicated three times. The membranes were scanned and the sum optical density was quantitatively analyzed by Quantity one software (Bio-Rad, Richmond, CA, USA).

Rat carotid balloon injury

Balloon denudation of the left common carotid artery of male Sprague-Dawley rats was performed, as described [10]. Sprague-Dawley rats (300-350 g) were anesthetized using chloral hydrate (350 mg/kg, i.p.), and the left common and external carotid arteries were exposed and isolated. A 1.5 F Fogarty catheter (Edwards Lifesciences, Irvine, CA, USA) was introduced into the common carotid artery through an arteriotomy in the external carotid artery and inflated to 2.0-3.0 atm and withdrawn repeatedly for three times. Then the external carotid artery was then ligated, and the blood flow was restored. For preparing sham operation rats, the left common carotid artery and external carotid artery were exposed and ligated as above but did not insert the catheter into the vessels. The arteries were collected at day 3, 7, 14 and 21 after balloon injury.

Electrophysiological recordings in 5-HTT-expressing HEK-293

The two-electrode voltage clamp technique was used to examine response currents in 5-HTT-expressing HEK-293. HEK-293 were treated with or without IL-1β 24 hours before experiment. Cells were placed in a chamber and perfused with bath solution (120 mmol/l NaCl, 5 mmol/l KCl, 1.5 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 10 mmol/l glucose and 10 mmol/l Hepes, pH 7.4). Patch electrodes were pulled from a horizontal micropipette puller (P-1000, Sutter Instruments) and fire polished to final tip resistance of 4-6 MΩ when filled with internal solutions. the pipette solution contained: 140 mmol/l KCl, 2 mmol/l $MgCl_2$, 10 mmol/l HEPES, 0.1 mmol/l EGTA, 4 mmol/l K-2-ATP. Two electrodes were inserted into the HEK-293 and voltage clamping applied using a Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA), at a holding potential of -40 mV and stepped to -100 mV with 5 second rest interval. Current signals were filtered at 20 Hz with a low-pass Bessel filter and digitized at 50 Hz. Data acquisition and analysis were carried out using pClamp 9.0 (Axon Instruments) software.

Statistical analysis

Data are presented as means ± SEM and means ± SD. One-way analysis of variance (ANOVA) and paired or unpaired t-test were performed for statistical analysis as appropriate

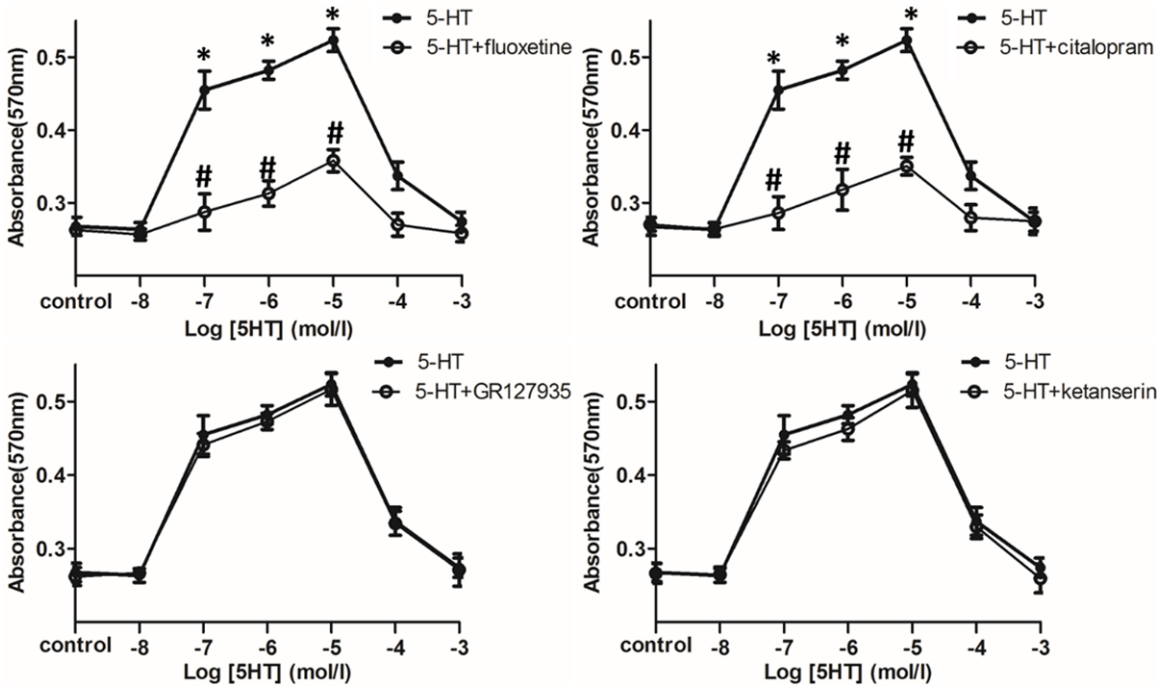

Figure 1. Effects of 5-HT on human coronary artery smooth muscle cells (HCASMCs) proliferation in presence of fluoxetine (10^{-5} mol/l), citalopram (10^{-6} mol/l), GR127935 (10^{-6} mol/l) or ketanserin (10^{-7} mol/l). HCASMCs viability was detected by the MTT assay. Results are presented as absorbance in 570 nm. Data are mean ± SD (n = 8, **P* < 0.05 vs. control; and #P < 0.05 vs. 5-HT+fluoxetine group).

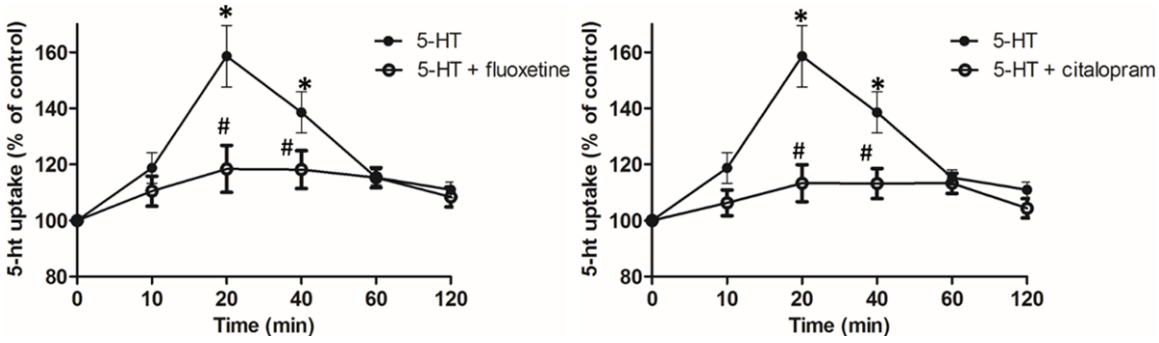

Figure 2. Effect of 5-HTT blocker on 5-HT transportation in HCASMCs. Intracellular 5-HT was measured by LC-MS/MS. Intracellular 5-HT was increased when 5-HT (10^{-6} mol/l) was added into culture media and both fluoxetine (10^{-5} mol/l) and citalopram (10^{-6} mol/l) blocked this effect. Both Data was shown as percentage of control (0 min). (n = 3, **P* < 0.05 vs. 0min; and #*P* < 0.05 vs. 5-HT group).

by SPSS 19.0 (SPSS Inc, Chicago, USA). P < 0.05 was considered statistically significant.

Results

Effects of 5-HT on HCASMCs proliferation

The effect of increasing concentrations of 5-HT on HCASMCs proliferation is shown in **Figure 1**. The 5-HT at an added concentration of 10^{-7} mol/l induced proliferation of HCASMCs, and the effect peaked at a concentration of 10^{-5} mol/l. There was a reduction in the absorbance at concentrations of 5-HT > 10^{-5} M, indicating that high dose 5-HT is cytotoxic to the HCASMCs.

Effects of 5-HTT and 5-HT receptor antagonists on the proliferative response of HCASMCs to 5-HT

Pretreatment of the cells with fluoxetine (10^{-5} mol/l) or citalopram (10^{-5} mol/l), two 5-HTT inhibitors [11], significantly abolished the 5-HT-induced increase in absorbance, while GR127935 (10^{-6} mol/l), a 5-HT1B/1D receptor

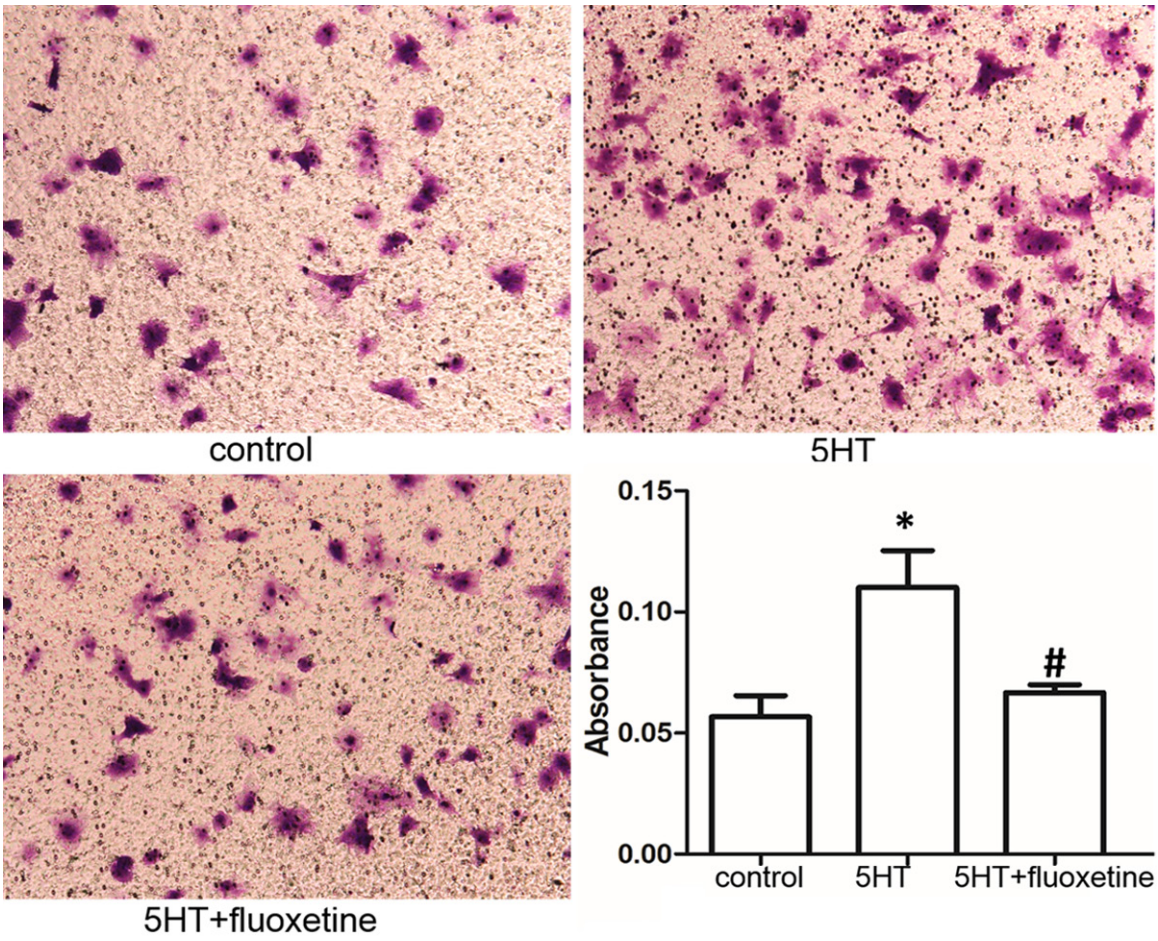

Figure 3. Effects of fluoxetine on 5-HT-induced human coronary artery smooth muscle cells (hCASMCs) migration. The transwell assay was used to evaluate hCASMCs migration. Photographs of migration were taken under a microscope (magnification, 100 ×). Absorbance of migrated cells was measured under 490 nm with a microplate reader. The results are mean ± standard error (SE) (n = 3, **P* < 0.05 vs. control group; and #*P* < 0.05 vs. 5-HT alone group).

antagonist [12], or ketanserin (10^{-7} mol/l) [13], a 5-HT2 receptor antagonist, had little effect on 5-HT induced proliferation (**Figure 1**).

Effect of 5-HTT blocker on 5-HTT transportation in HCASMCs

As shown in **Figure 2**, intracellular 5-HT was increased when 5-HT (10^{-6} mol/l) was added into culture media and the effect peaked at 20 minutes. Both fluoxetine (10^{-5} mol/l) and citalopram (10^{-6} mol/l) significantly diminished intracellular 5-HT concentration.

Effect of 5-HT and fluoxetine on hCASMCs migration

As shown in **Figure 3**, 5-HT at 10^{-6} mol/l increased hCASMCs migration. Fluoxetine (10^{-5} mol/l) treatment 20 minutes before 5-HT decreased absorbance compared to the 5-HT stimulated group.

Effect of 5-HTT overexpression on hCASMCs migration and proliferation

Effect of 5-HTT overexpression by rAd-5-HTT transfection was examined by western blot (**Figure 4A**). As shown in **Figure 4B** and **4C**, 5-HTT overexpression significantly increased hCASMCs migration. 5-HTT overexpression also increased 5-HT induced hCASMCs proliferation at concentrations of 10^{-7} and 10^{-6} mol/l compared with control group. However, 5-HTT overexpression did not affect hCASMCs proliferation when cultured with 5-HT over 10^{-5} mol/l.

IL-1β and 5-HTT increased after rat carotid balloon injury

We used balloon catheter-injured rat carotid artery for studying smooth muscle cells (SMCs) proliferation in vivo. Effects of balloon injury

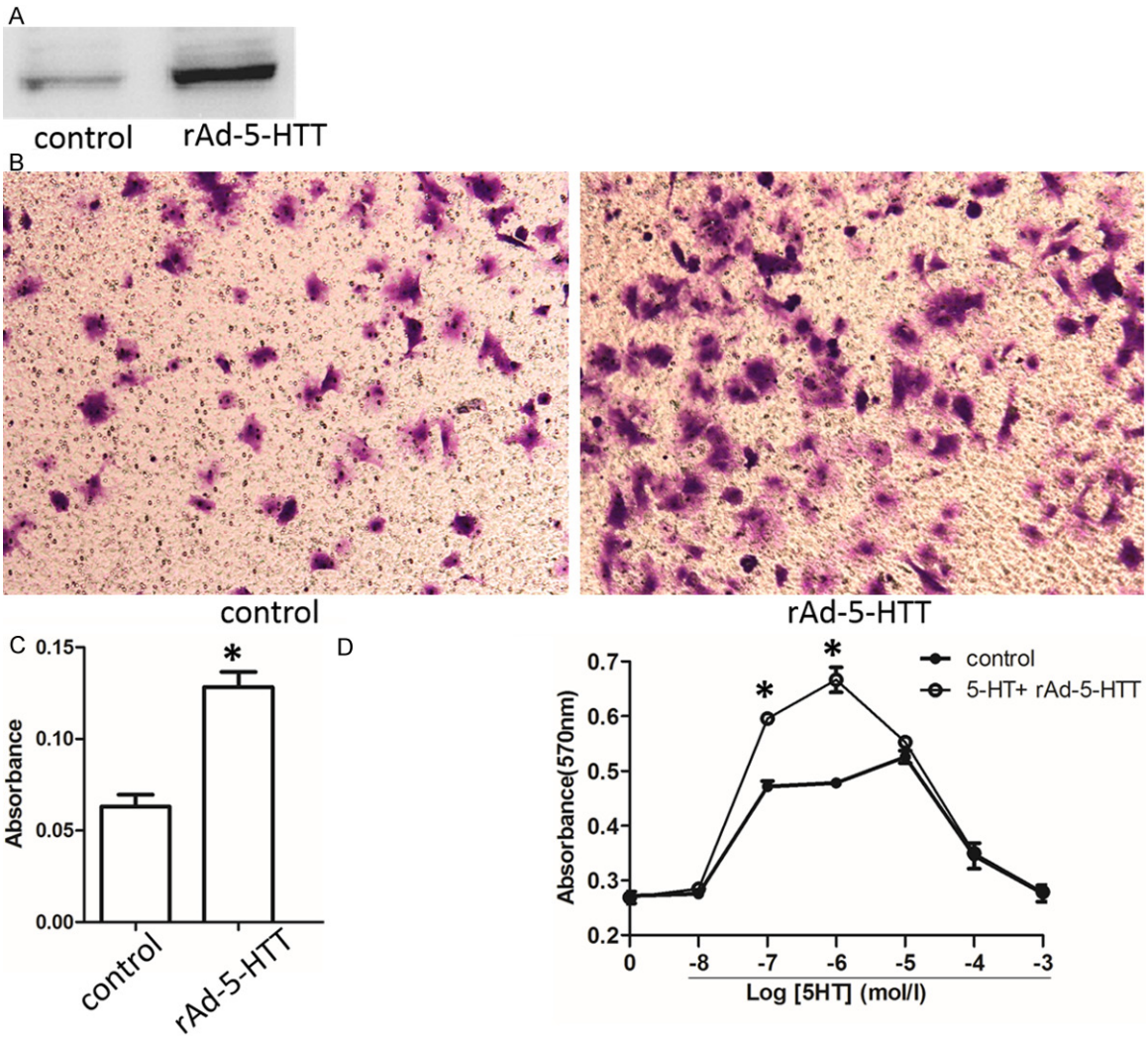

Figure 4. Effect of 5-HTT overexpression on human coronary artery smooth muscle cells (hCASMCs) migration and proliferation. A. Expression of 5-HTT in hCASMCs transfected with rAd-5-HTT. B. Photographs of 5-HT (10^{-7} mol/l)-induced migration were taken under a microscope (magnification, 100 ×) in control group and 5-HTT overexpression group (rAd-5-HTT). C. Absorbance of migrated cells was measured under 490 nm with a microplate reader. The results are mean ± SD (n = 3, *P < 0.05 vs. control group). D. Effects of 5-HTT overexpression (rAd-5-HTT) on HCASMCs proliferation compared with control group (5-HT+rAd). HCASMCs viability was detected by the MTT assay. Results are presented as absorbance in 570 nm. Data are mean ± SD (n = 8, *P < 0.05 vs. control).

catheter-injured rat carotid artery models were confirmed by hematoxylin-eosin (HE) staining (**Figure 5A**). IL-1β and 5-HTT expression in injured rat carotid arteries was measured by Western blot (**Figure 5B**). We found that IL-1β protein expression increased significantly 3-day after injury, peaked at 7 days, and then began to decrease; 5-HTT protein expression increased significantly 3 days after injury, peaked at 14 days, and then began to decrease compared with the sham group (**Figure 5B**, P < 0.05, n = 3/group).

IL-1β upregulated 5-HTT in HCASMCs

In cultured HCASMCs, IL-1β at 10 ng/ml, increased 5-HTT expression in 3-day. IL-1ra (10 ng/ml) partially blocked IL-1*β*-induced 5-HTT overexpression (**Figure 6A**, **6B**).

IL-1β increased 5-HT induced current in HEK-293-5-HTT

Human 5-HTT was expressed in HEK-293 cells. As shown is **Figure 6C**, 5-HT (10^{-6} mol/l) induced

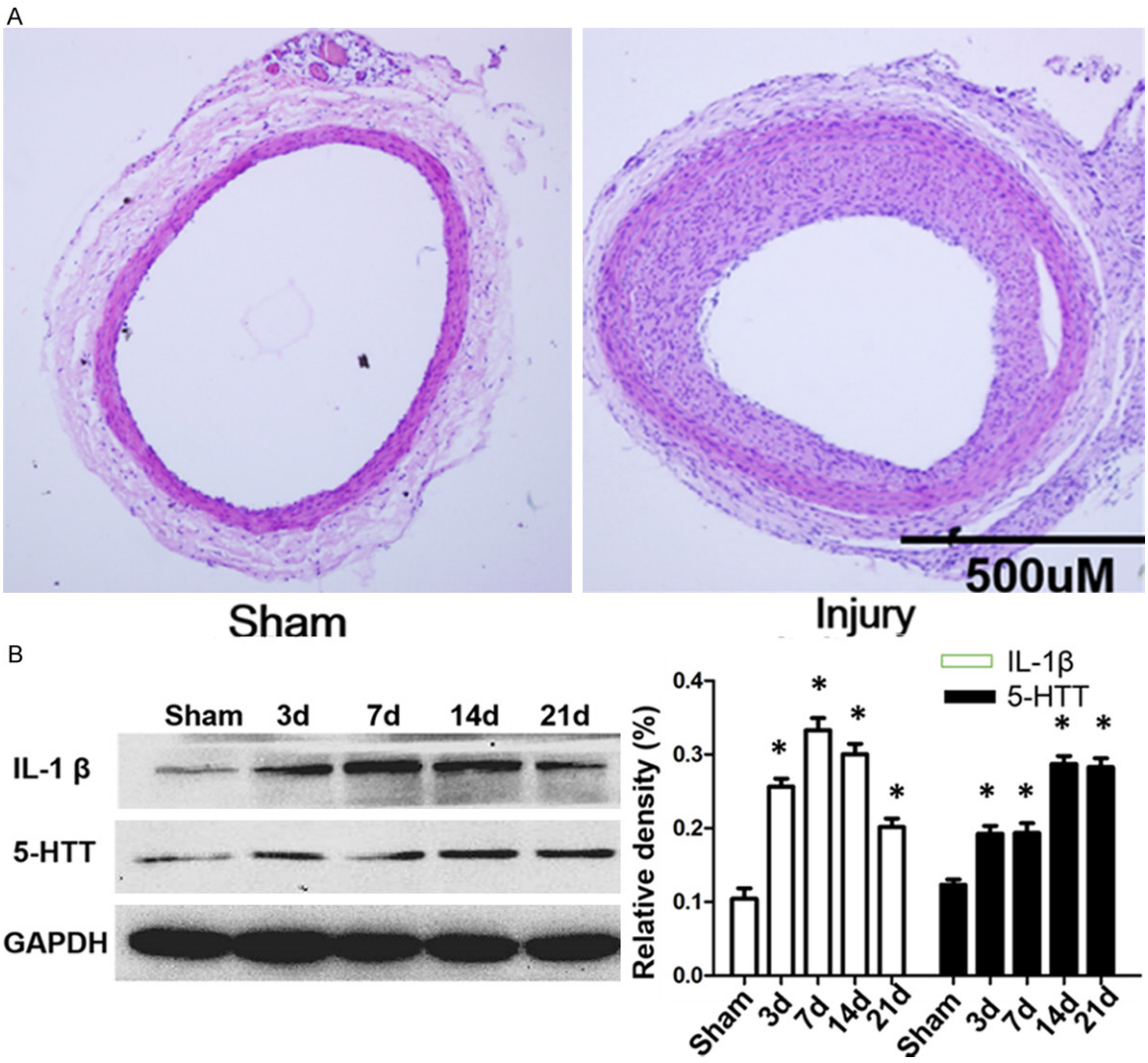

Figure 5. A. Balloon injury model was confirmed by HE staining. B. Levels of IL-1β and 5-HTT were measured by western blotting after 3, 7, 14, 21 days after carotid balloon injury surgery (n = 3/group). d: days; Data are presented as mean ± SEM. *P < 0.05 compared with sham group.

an inward current at hyperpolarized membrane potentials in HEK-293-5-HTT, and this current can be blocked by pretreatment with fluoxetine (10^{-5} mol/l, 5 minutes). The 5-HT-induced current was significantly increased as HEK-293-5-HTT cells were cultured in presence of IL-1β (10^{-6} mol/l, 24 h) (**Figure 6D**).

Discussion

Platelet adhesion and aggregation at the site of vascular injury is an initial events after PCI [14]. There is high concentration of 5-HT in platelet [15], thus 5-HT concentrations is high in coronary sinus blood samples after PCI in human [16]. It is believed that 5-HT is a strong mitogen that stimulate PASMCs proliferation and migration [17]. The present study showed that 5-HT also induced hCASMCs proliferation and migration through 5-HTT, and 5-HTT was upregulated in balloon injured rat carotid artery *in vivo*. IL-1β is a potent activator of 5-HTT expression and function.

The 5-HT induced HCASMCs proliferation was examined by MTT. 5-HT induced HCASMCs proliferation peaked at concentration of 10^{-7}-10^{-5} mol/l and then began to decrease, indicating that high concentration of 5-HT is toxic to hCASMCs in *vitro*. Effects of 5-HT-induced proliferation rely on 5-HTT activities, as both fluoxetine and citalopram, two 5-HTT blockers [12, 13], inhibited 5-HT-induced HCASMCs proliferation, whereas GR127935, a 5-HT1B/1D

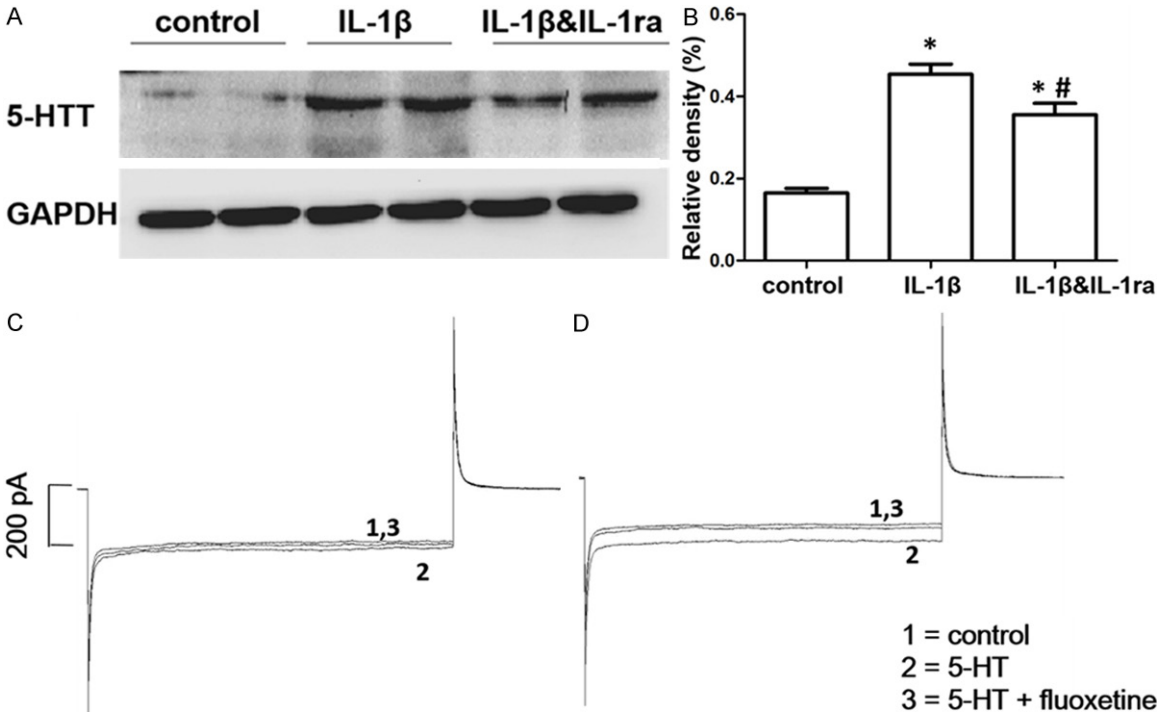

Figure 6. A. Effect of IL-1β on 5-HTT protein Levels in HCASMC. Treatment with IL-1β (10 ng/ml, 3 d) upregulates 5-HTT. This effect was diminished by recombinant IL-1β receptor antagonist (IL-1βra, 10 ng/mL). B. Relative density of 5-HTT protein level. Data are presented as mean ± SEM. *P < 0.05 compared with control group. #P < 0.05 compared with IL-1β group. C. 5-HT-induced currents in HEK-293-5-HTT cells. HEK-293-5-HTT cells were held at -40 mV and stepped to -100 mV with 5 second rest interval. The current increased by adding 5-HT (10^{-6} mol/l), and this effect was blocked by adding fluoxetine (10^{-5} mol/l) 5 minute before 5-HT. D. 5-HT-induced currents in HEK-293-5-HTT cells pretreated by IL-1β (10 ng/ml, 24 h). The current increased by adding 5-HT (10^{-6} mol/l), and this effect was blocked by adding fluoxetine (10^{-5} mol/l) 5 minute before 5-HT.

receptor antagonist [12], or ketanserin (10^{-7} mol/l) [13], a 5-HT2 receptor antagonist, do not affect 5-HT induced proliferation. Both fluoxetine and citalopram blocked 5-HT flux in hCASMCs, suggested that intracellular 5-HT is important to stimulate cells proliferation. Overexpression 5-HTT enhanced the effects of 5-HT induced hCASMCs proliferation and migration, further verified the role of 5-HTT on 5-HT mitogen effects on hCASMCs.

Previous studies indicated that 5-HTT is overexpressed in PASMCs during PAH, so we examined the 5-HTT expression of injured arteries *in vivo* by using balloon catheter-injured rat carotid artery. In balloon catheter-injured rat carotid artery, 5-HTT significantly increased, indicating that more 5-HT was transported into smooth muscle cells and endothelial cells and leading to neointima formation.

IL-1β is a potent proinflammatory cytokines on SMCs [18] and is reported to be increased in injured porcine coronary arteries [19]. In this study, we also found increased IL-1β expression in rat carotid artery. Moreover, IL-1β activates 5-HTT in choriocarcinoma cells [20] and RN46A cells [21], indicating that IL-1β might regulates 5-HT induced hCASMCs proliferation via 5-HTT. Subsequently, hCASMCs were treated with IL-1β (10 ng/ml, 72 h) *in vitro*, the 5-HTT expression was upregulated. This effect can be blocked by IL-1 receptor antagonist, implying a functional role in IL-1β induced 5-HTT upregulating. We also examined whether IL-1β regulates the function of 5-HTT in HEK-293-5-HTT cells. Treatment with IL-1β (10 ng/ml, 24 h) increased the 5-HT induced inward current in HEK-293-5-HTT cells, confirmed that IL-1β regulates 5-HT flux. The protein kinase C (PKC) activating is part of IL-1 signaling mechanism [22], wherevers β-PMA, a PKC activator, caused reduction in 5-HT uptake [23]. Therefore, it appears that the stimulatory effect of IL-1β on the 5-HTT in hCASMCs relays on PKC-independent kinases and should be investigated in our further research.

In summary, our data suggest that 5-HT is an important mitogen for hCASMCs proliferation and migration. Both in vitro and in vivo data support a model whereby high IL-1β level after catheter-injury induces the expression of 5-HTT, which, in turn, potentiates hCASMCs to the mitogenic action of 5-HT.

Acknowledgements

This study was supported grants to Cheng-Chun Tang from the Natural Science Foundation of China (research Grant#81170105) and Natural Science Foundation of China (research Grant#81370225).

Disclosure of conflict of interest

None.

Address correspondence to: Cheng-Chun Tang, Department of Cardiology, Zhongda Hospital Southeast University, Medical School of Southeast University, Nanjing 210009, Jiangsu, China. Tel: +8613914705570; Fax: +862583272038; E-mail: tangchengchun@medmail.com.cn

References

[1] Casscells W. Migration of smooth muscle and endothelial cells. Critical events in restenosis. Circulation 1992; 86: 723-729.

[2] Seuwen K, Magnaldo I and Pouyssegur J. Serotonin stimulates DNA synthesis in fibroblasts acting through 5-HT1B receptors coupled to a Gi-protein. Nature 1988; 335: 254-256.

[3] Lee SL, Wang WW, Lanzillo JJ and Fanburg BL. Serotonin produces both hyperplasia and hypertrophy of bovine pulmonary artery smooth muscle cells in culture. Am J Physiol 1994; 266: L46-52.

[4] Zhai FG, Zhang XH and Wang HL. Fluoxetine protects against monocrotaline-induced pulmonary arterial hypertension: potential roles of induction of apoptosis and upregulation of Kv1.5 channels in rats. Clin Exp Pharmacol Physiol 2009; 36: 850-856.

[5] Han DD, Wang Y, Zhang XH, Liu JR and Wang HL. Fluoxetine protects against monocrotaline-induced pulmonary arterial remodeling by inhibition of hypoxia-inducible factor-1alpha and vascular endothelial growth factor. Can J Physiol Pharmacol 2012; 90: 445-454.

[6] Ren W, Watts SW and Fanburg BL. Serotonin transporter interacts with the PDGFbeta receptor in PDGF-BB-induced signaling and mitogenesis in pulmonary artery smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2011; 300: L486-497.

[7] MacLean MR, Deuchar GA, Hicks MN, Morecroft I, Shen S, Sheward J, Colston J, Loughlin L, Nilsen M, Dempsie Y and Harmar A. Overexpression of the 5-hydroxytryptamine transporter gene: effect on pulmonary hemodynamics and hypoxia-induced pulmonary hypertension. Circulation 2004; 109: 2150-2155.

[8] Kwon JS, Joung H, Kim YS, Shim YS, Ahn Y, Jeong MH and Kee HJ. Sulforaphane inhibits restenosis by suppressing inflammation and the proliferation of vascular smooth muscle cells. Atherosclerosis 2012; 225: 41-49.

[9] Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, Ganapathy V and Blakely RD. Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. Proc Natl Acad Sci U S A 1993; 90: 2542-2546.

[10] Wang L, Zheng J, Du Y, Huang Y, Li J, Liu B, Liu CJ, Zhu Y, Gao Y, Xu Q, Kong W and Wang X. Cartilage oligomeric matrix protein maintains the contractile phenotype of vascular smooth muscle cells by interacting with alpha(7) beta(1) integrin. Circ Res 2010; 106: 514-525.

[11] Eddahibi S, Humbert M, Fadel E, Raffestin B, Darmon M, Capron F, Simonneau G, Dartevelle P, Hamon M and Adnot S. Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. J Clin Invest 2001; 108: 1141-1150.

[12] De Vries P, Apaydin S, Villalon CM, Heiligers JP and Saxena PR. Interactions of GR127935, a 5-HT(1B/D) receptor ligand, with functional 5-HT receptors. Naunyn Schmiedebergs Arch Pharmacol 1997; 355: 423-430.

[13] Muller T, Durk T, Blumenthal B, Grimm M, Cicko S, Panther E, Sorichter S, Herouy Y, Di Virgilio F, Ferrari D, Norgauer J and Idzko M. 5-hydroxytryptamine modulates migration, cytokine and chemokine release and T-cell priming capacity of dendritic cells in vitro and in vivo. PLoS One 2009; 4: e6453.

[14] Harrington RA, Stone GW, McNulty S, White HD, Lincoff AM, Gibson CM, Pollack CV Jr, Montalescot G, Mahaffey KW, Kleiman NS, Goodman SG, Amine M, Angiolillo DJ, Becker RC, Chew DP, French WJ, Leisch F, Parikh KH, Skerjanec S and Bhatt DL. Platelet inhibition with cangrelor in patients undergoing PCI. N Engl J Med 2009; 361: 2318-2329.

[15] Brenner B, Harney JT, Ahmed BA, Jeffus BC, Unal R, Mehta JL and Kilic F. Plasma serotonin levels and the platelet serotonin transporter. J Neurochem 2007; 102: 206-215.

[16] Golino P, Piscione F, Benedict CR, Anderson HV, Cappelli-Bigazzi M, Indolfi C, Condorelli M, Chiariello M and Willerson JT. Local effect of

serotonin released during coronary angioplasty. N Engl J Med 1994; 330: 523-528.
[17] Liu Y and Fanburg BL. Serotonin-induced growth of pulmonary artery smooth muscle requires activation of phosphatidylinositol 3-kinase/serine-threonine protein kinase B/mammalian target of rapamycin/p70 ribosomal S6 kinase 1. Am J Respir Cell Mol Biol 2006; 34: 182-191.
[18] Dzau VJ, Braun-Dullaeus RC and Sedding DG. Vascular proliferation and atherosclerosis: new perspectives and therapeutic strategies. Nat Med 2002; 8: 1249-1256.
[19] Chamberlain J, Gunn J, Francis S, Holt C and Crossman D. Temporal and spatial distribution of interleukin-1 beta in balloon injured porcine coronary arteries. Cardiovasc Res 1999; 44: 156-165.
[20] Ramamoorthy S, Ramamoorthy JD, Prasad PD, Bhat GK, Mahesh VB, Leibach FH and Ganapathy V. Regulation of the human serotonin transporter by interleukin-1 beta. Biochem Biophys Res Commun 1995; 216: 560-567.
[21] Zhu CB, Blakely RD and Hewlett WA. The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. Neuropsychopharmacology 2006; 31: 2121-2131.
[22] Dinarello CA. The interleukin-1 family: 10 years of discovery. FASEB J 1994; 8: 1314-1325.
[23] Qian Y, Galli A, Ramamoorthy S, Risso S, DeFelice LJ and Blakely RD. Protein kinase C activation regulates human serotonin transporters in HEK-293 cells via altered cell surface expression. J Neurosci 1997; 17: 45-57.