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5-HT_{1B} receptor augmented 5-HT vasoconstrictor response of pulmonary artery in monocrotaline-induced pulmonary hypertensive rats[1]

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KEY WORDS serotonin; serotonin receptors; pulmonary hypertension

ABSTRACT

AIM: To study the relationship between the vasoconstrictor effect to 5-hydroxytryptamine (5-HT) and the expression of 5-HT_{1B}-and 5-HT_{1D}-receptors in pulmonary arteries (PA) from normal and pulmonary hypertensive (PHT) rats. **METHODS**: Monocrotaline (MCT)-treated rats were used as a model for chronic PHT. Concentration-response curves of 5-HT induced pulmonary vasoconstriction were established and semiquantitative RT-PCR was performed to identify mRNA expression of 5-HT_{1B}- and 5-HT_{1D}-receptors in rat PA. **RESULTS**: 5-HT induced vasoconstrictor response of PA from MCT rats was enhanced (E_{max}: 50 % ± 20 % *vs* control 38 % ± 21 %, $P < 0.05$). A significantly higher level of 5-HT_{1B} receptor mRNA expression was detected in PA from MCT rats. The ratio of the PCR products of 5-HT_{1B} receptor gene to those of β-actin gene was much higher in MCT rats than that in control rats (0.43 ± 0.14 *vs* control 0.27 ± 0.15, $P < 0.05$). **CONCLUSION**: 5-HT_{1B} receptor is involved in pulmonary vasoconstriction and the enhanced level of 5-HT_{1B} receptor mRNA expression is closely related to the augmentation of 5-HT induced pulmonary vasoconstriction in PHT rats.

INTRODUCTION

5-Hydroxytryptamine (5-HT) is one of the vasomotor agents in serum, which is mainly released from pulmonary neuroendocrine cells and activated platelets in pulmonary vessels. Large amounts of 5-HT from neuroendocrine cells along the airways in response to hypoxia directly contribute to secondary pulmonary hypertension (PHT)[1]. In the model of "inflammatory" PHT, activated platelets accumulate in pulmonary capillaries and release 5-HT leading to an increase in 5-HT levels in serum. Meanwhile, the injured endothelium in pulmonary artery (PA) decreases the functions of uptaking and degrading 5-HT[2]. The elevated plasma levels of 5-HT have also been reported in primary PHT and hypoxia-induced PHT in the newborn. Many *in vitro* studies have shown that 5-HT produced vasoconstrictor effects on PA in human and other animals, while the effects in the PA from PHT patients were more intense. There is an evidence that the 5-$HT_{1B/1D}$ receptor agonist sumatriptan causes pulmonary vasoconstriction equipotent to 5-HT[3,4]. In man, the 5-HT_{1B} receptor-mediated pulmonary vasoconstriction is likely to play a significant role in the increased pulmonary vasoconstrictor effect to 5-HT observed in PHT[5]. So, it is of importance to identify the differentiation of subtypes of 5-HT receptors and to understand whether there exist any change in the expression of these receptors sub-type genes concerned with the augmented contractile response to 5-HT in PA during PHT. The present study was designed to compare the vasoconstrictor effects of 5-HT and the expression of 5-HT_{1B}- and 5-HT_{1D}- receptor genes in PA from normal and chronic "inflammatory" PHT rats.

MATERIALS AND METHODS

Monocrotaline model of chronic "inflammatory" PHT in rats Wistar rats (70 ♂, Grade Ⅱ, weighting 154 g ± 9 g) from Animal Resource Center, China Medical University (Certificate No-Liaoning 034), were devided into two groupes. The test rats were treat-

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ed with a single, intraperitoneal injection of monocrotaline 60 mg/kg (MCT, Wako Pure Chemical Research Company, JAP), while the control rats were treated with an equal volume of saline, then these rats were fed solid food and water *ad lib* in an alternating 12 h light/dark cycle under controlled temperature (18 – 22℃) and humidity (50 % – 70 %) for 3 weeks[6].

The rats were killed by stunning on d 21 after monocrotaline or saline administration (32 and 36 rats respectively). Then the right ventricle (RV), septum and the left ventricle (S + LV) were dissected and weighed separately to evaluate the magnitude of the right ventricular hypertrophy [expressed as the right ventricular index, RV/(S + LV)].

***In vitro* studies** The main PA were dissected free from connective tissues and cut into cylindrical segments (2 – 3 *mm in width*). Then the PA rings were mounted on two stainless steel hooks suspended in 10 mL of Krebs' solution (composition in mmol/L: NaCl 119; KCl 4.7; $MgSO_4$ 0.6; KH_2PO_4 1.2; $CaCl_2$ 2.5; $NaHCO_3$ 25 and Glucose 11.1, pH 7.4) bubbled constantly with 95 % O_2 – 5 % CO_2 gas mixture at 37 ℃ and connected to a force-displacement transducer (TB-611T, Japan) for tension recording with a resting tension of 1.0 g and were equilibrated for 1 h before the beginning of each experiment, and rinsed every 15 min.

Vascular effects to acetylcholine chloride (ACh, Academy of Military Medical Sciences of China) and 5-HT (Sigma, USA) were examined by cumulative application of increasing concentrations of the drugs and concentration-response curves were obtained. Vasodilatory responses to ACh were expressed as percentage of the contractile response to 1 μmol/L phenylephrine previously, and contractile response to 5-HT were expressed as percentage of the contractile response to 50 mmol/L KCl. The maximum contractile response (E_{max}) and the potency of the ACh and 5-HT (pEC_{50}) were calculated, where appropriate concentration-response curves were fitted to the data using regression analysis.

RT-PCR PA (endothelium-denuded) were dissected free from peripheral tissues immediately and stored at −70 ℃. Total RNA was extracted with TRIzol Reagent (GIBCO, USA) as described in its directions. The concentration and purification of the total RNA was estimated with uviospectrophotometer (UV-200, JAP).

RT-PCR to identify mRNA expression of 5-HT_{1B}- and 5-HT_{1D}- receptors in rat PA was performed with Access RT-PCR kit (Promega, USA). The special primers were designed as described previously[7]. 5-HT_{1B}-, 5-HT_{1D}-, and β-actin gene were synthesized as follows: 5-HT_{1B} a: 5′-ACT ACA TTT ACC AGG ACT CCA T-3′, b: 5′-CAG TGA CCG TGT ACA TGG TGC-3′; 5-HT_{1D} a: 5′-TCA TGC CCA TGA GCA T(CA)G CC-3′, b: 5′-CTT CCC (AG)TA GAG (TG)GA GGG TG-3′; β-actin a: 5′-TGT ATG CCT CTG GTC GTA CCA C-3′, b: 5′-ACA GAG TAC TTG CGC TCA GGA G-3′.

For the RT-PCR, 1 μg of each RNA was reverse transcribed and PCR amplified in the same volume of 25 μL using 2.5 U AMV reverse transcriptase, 200 μmol/L dNTP, 2.5 U Tfl/DNA polymerase. The reaction conditions were as follows: reversed transcription to synthesized cDNA at 48 ℃ for 45 min, then in active AMV and denature RNA/cDNA/primer at 94 ℃ for 2 min. Next 35 PCR cycles (94 ℃ for 30 s, 55 ℃ for 1 min, 72 ℃ for 2 min) were performed.

The PCR products were checked with 8 % polyacrylamide gel electrophoresis (PAGE) stained with ethidium bromide (5 mg/L), then scanned and semi-quantified with a gel analyzer (UVP, GDS8000, USA). Gene expression of the subtype of 5-HT receptor was represented by the relative yield to the β-actin gene.

Statistical methods All the data in the text and illustrations are presented as $\bar{x} \pm s$. Statistically significant differences were determined with non-paired *t*-test. $P < 0.05$ was considered significant.

RESULTS

MCT model of chronic "inflammatory" PHT in rats and *in vitro* studies Chronic PHT model in rats induced by MCT was successfully established at the end of 3 weeks and confirmed by a significant increase of right ventricular hypertrophy index (0.44 ± 0.08, $n = 32$ *vs* control 0.26 ± 0.06, $n = 36$, $P < 0.01$) and the decrease of vasodilation to ACh in PA.

Tab 1 summarizes the pEC_{50} and E_{max} values of 5-HT and ACh in PA from control and MCT rats. The vasodilatory responses to ACh in PAs from MCT rats were incomplete, and the E_{max} to ACh decreased by 29.8 % compared with control rats (Fig 1). There were significant differences in the responsiveness to 5-HT in rat PA between control and MCT rats. The E_{max} of 5-HT induced contraction in PA from MCT rats was increased by 34 % (Fig 2).

RT-PCR To detect low levels of subtype mRNA of 5-HT receptor, we performed RT-PCR using primers

Tab 1. Response to ACh and 5-HT in PA from control and MCT rats.

		n	pEC_{50}	E_{max} (%)
ACh	Control	12	7.18 (6.83 – 7.53)	60 ± 24
	MCT	14	6.38 (6.16 – 6.59)^c	41.3 ± 1.0^b
5-HT	Control	20	4.59 (4.42 – 4.76)	38 ± 21
	MCT	30	5.06 (4.85 – 5.26)^c	50 ± 20^b

The numbers in the parentheses represent the 95 percent confidence intervals of pEC_{50}. $^bP < 0.05$, $^cP < 0.01$ *vs* control. pEC_{50}: negative logarithm of the molar concentration of drugs inducing half maximum effects. E_{max}: maximum effects (ACh: as percent of 1 μmol/L phenylephrine induced contraction; 5-HT: as percent of 50 mmol/L KCl induced contraction). n: number of PA rings.

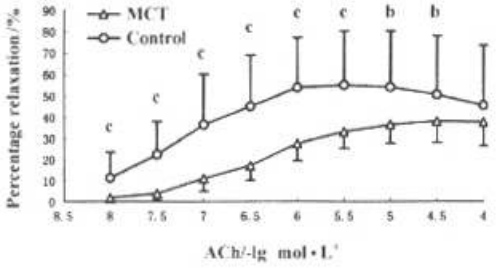

Fig 1. The relationship response to ACh in PA of control ($n = 12$) and MCT ($n = 14$) rats. The results are expressed as percentage of the 0.1 mmol/L phenylephrine induced contraction. $x \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

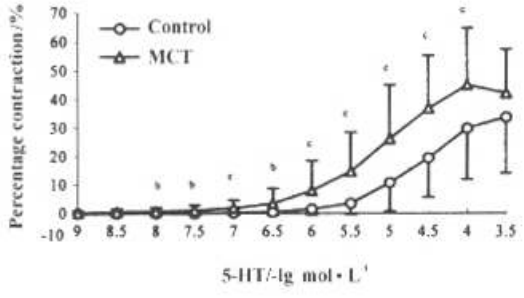

Fig 2. The contractile response to 5-HT in PA of control ($n = 20$) and MCT ($n = 30$) rats. The results are expressed as percentage of the 50 mmol/L KCl induced contraction. $x \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

specific for the 5-HT_{1B} and 5-HT_{1D} receptor. β-Actin gene, used as a control, was detected in all preparations. PCR products of the expected size for 5-HT_{1B}, 5-HT_{1D} receptor, and β-actin were 228, 441, and 592 bp, respectively. Three PCR products obtained were consistent with the estimated sizes. The typical results of 8 % PAGE are shown in Fig 3,4. Only 5-HT_{1B} receptor gene was detected in PA examined. The levels of 5-HT_{1B} receptor gene expressed in PA from MCT and control rats were of significant difference. The ratio of the PCR products of 5-HT_{1B} receptor gene to those of β-actin gene was much higher in MCT rats than that in control rats (0.43 ± 0.14, $n = 10$ *vs* control 0.27 ± 0.15, $n = 14$, $P < 0.05$) (Fig 5).

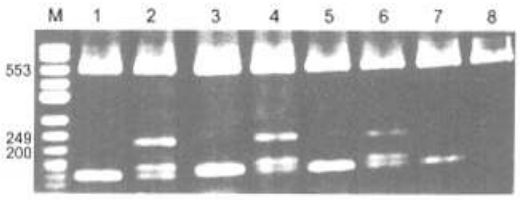

Fig 3. Eight percent PAGE of PCR-amplified products derived from cDNA of normal rat pulmonary arteries. Lane 2, 4, 6, 8 show 5-HT_{1B} receptor mRNA expression (228 bp). Lane 1, 3, 5, 7 show no 5-HT_{1D} receptor mRNA expression.

Fig 4. Eight percent PAGE of PCR-amplified products derived from cDNA of pulmonary hypertensive rat pulmonary arteries. Lane 2, 4, 6, 8 show 5-HT_{1B} receptor mRNA expression (228 bp). Lane 1, 3, 5, 7 show no 5-HT_{1D} receptor mRNA expression.

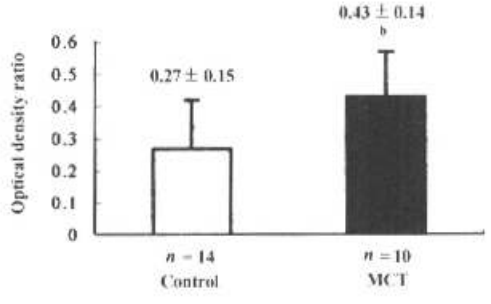

Fig 5. The ratio of the RT-PCR products of 5-HT_{1B} receptor gene to those of β-actin gene in pulmonary arteries from control and MCT rats respectively. $^bP < 0.05$ *vs* control.

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DISCUSSION

The mechanism of PHT is not quite clear, yet it is believed that several factors are involved, such as sheer stress, chronic hypoxia, chronic inflammation, thrombosis, vasomotor and growth factors (5-HT, NO, ET-1, TXA_2, PDGF, VEGF, EGF, TGF, *etc*). Most scholars are now in agreement with that 5-HT is clearly an important and pervasive factor in PHT, and 5-HT mechanisms are important in pathogenesis of PHT. It has been recently reported that 5-HT regulates the tone of PA by contracting PA directly and interacting with other vasomotor substances[8]. As a mitogen, 5-HT also causes hypertrophic and hyperplastic changes in vascular smooth muscle cells, and increases the accumulation of interstitial elastic protein and collagen around blood vessels, which leads to pulmonary vascular remodeling[9]. Experimentally, intravenous administration of 5-HT increases pulmonary vascular resistance in animals, and accelerates the progress of PHT in chronic hypoxic rat[10]. Pulmonary arterial responses to 5-HT *in vitro* are also enhanced in chronic PHT rats and in patients with PHT. Recent evidences suggest that 5-$HT_{1B/1D}$ receptors participate in mediating vasoconstriction in human PA[3].

The 5-$HT_{1B/1D}$ receptor agonist sumatriptan reportedly caused pulmonary vasoconstriction[4], and a recent report that in man, the 5-HT_{1B} receptor-mediated pulmonary vasoconstriction likely played a significant role in the increased pulmonary vasoconstrictor effect to 5-HT was observed in PHT[5]. So the present study concentrated on the 5-$HT_{1B/1D}$ receptors, and confirmed that substantial 5-HT_{1B} but not 5-HT_{1D} receptor genes were expressed in the PA. This finding is consistent with others from the cultured smooth muscle cells of human large PA, and suggests that it is 5-HT_{1B} receptor that participates in mediating the vasoconstrictor effect to 5-HT in PA. Furthermore, an increased 5-HT_{1B} receptor mRNA was detected in the PA from MCT-induced PHT rats.

As the results of Longmore's experiment that agonist response depended partly on the number or density of receptor[11], we proposed that 5-HT_{1B} receptor-mediated vasoconstriction to 5-HT was significantly important in PA from MCT-induced chronic PHT rat, because: (ⅰ) 5-HT, 5-carboxytryptamine (5-CT) and sumatriptan could produce equipotent vasopressor responses in pulmonary circulation and in isolated PA, confirming the presence of 5-$HT_{1B/1D}$ receptors, which maybe involved in the vasoconstriction effect[4,5]; (ⅱ) The responsiveness to 5-HT, 5-CT, and sumatriptan were enhanced in PA from patients and rats with PHT[3]; (ⅲ) Responses to 5-HT at physiologic and pathologic concentrations were inhibited by a selective 5-$HT_{1B/1D}$ antagonist GR55562 but were resistant to the 5-HT_{2A} antagonist ketanserin, indicating that 5-$HT_{1B/1D}$ but not 5-HT_{2A} receptors were involved[5]; (ⅳ) In the present study, large amounts of mRNA for 5-HT_{1B} but not 5-HT_{1D} receptors was detected, so the 5-HT_{1B} receptor is likely to be responsible for the vasoconstrictor effect to 5-HT; (ⅴ) An increased expression of 5-HT_{1B} receptor gene was detected in the PA from MCT-treated rats and this is closely related to the increase in PA vascular contractility, suggesting that 5-HT_{1B} receptor is one of the important mechanisms mediating PHT in MCT-treated rats.

It has been shown that 5-HT_{1B}, 5-HT_{2B}, and 5-HT_4 receptor mRNA are expressed in cultured endothelial cells and mediate nitric oxide-dependent vasodilation, so a study in endothelium-denuded vessels seemed pertinent[6]. In *in vitro* pharmacologic study, however, we did not remove the vascular endothelium of the PA rings because mechanical disruption would significantly damage the smooth muscle layer and reduce the responsiveness of PA. This is due to the relative fragility of the vascular smooth muscle cells in the pulmonary circulation. We carefully dissected PA rings and investigated their vasodilator response to ACh to detect the degree of the damage of intima. Affected by the intima, the E_{max} and sensitivity of 5-HT in this study were lower than that in other experiments *in vitro*[3].

The study provided molecular evidence that only 5-HT_{1B} receptor gene were expressed in PA, and participated in mediating contractile response to 5-HT, and the higher level of expression of 5-HT_{1B} receptor gene was associated with the higher vasoconstrictor response to 5-HT in PA from MCT-treated rats. Therefore, we concluded that 5-HT_{1B} receptor mediated pulmonary vasoconstriction and the enhanced expression of 5-HT_{1B} receptor mRNA was closely related to the augmentation of 5-HT induced pulmonary vasoconstriction in PHT, implicating that 5-HT_{1B} receptor is one of the important mechanisms of PHT and this may provide a novel and potential therapeutic target for PHT.

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5-HT_{1B}受体增强野百合碱诱导的肺性高血压大鼠的肺血管对5-HT收缩反应[1]

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关键词 血清素；血清素受体；肺性高血压

目的：研究肺动脉高压大鼠5-羟色胺引起的肺血管收缩反应及其与5-HT_{1B}-，5HT_{1D}-受体的关系．**方法**：应用野百合碱(MCT)诱导的大鼠慢性肺动脉高压模型，通过离体肺动脉环实验建立5-HT的浓度-反应曲线，采用RT-PCR技术检测大鼠肺血管5-HT_{1B}-，5HT_{1D}-受体mRNA表达．**结果**：MCT大鼠对5-羟色胺引起的肺血管收缩反应明显增强（E_{max}由38 % ± 21 %增加到50 % ± 20 %，$P < 0.05$），肺动脉高压大鼠肺血管的5-HT_{1B}-受体mRNA表达明显明显增多，MCT大鼠肺动脉5-HT_{1B}受体mRNA相对表达比从0.43 ± 0.14增加到0.27 ± 0.15（$P < 0.05$）．**结论**：肺血管收缩与5-HT_{1B}受体有关，MCT大鼠肺动脉5-HT_{1B}-受体mRNA表达水平增多与其对5-HT引起的收缩反应增强有关，提示5-HT_{1B}受体有可能成为治疗肺动脉高压的新靶点．

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