5-HT\textsubscript{1B} receptor augmented 5-HT vasoconstrictor response of pulmonary artery in monocrotaline-induced pulmonary hypertensive rats\textsuperscript{1}

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KEY WORDS serotonin; serotonin receptors; pulmonary hypertension

ABSTRACT

AIM: To study the relationship between the vasoconstrictor effect on 5-hydroxytryptamine (5-HT) and the expression of 5-HT\textsubscript{1B} and 5-HT\textsubscript{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\textsubscript{1B} and 5-HT\textsubscript{1D} receptors in rat PA. RESULTS: 5-HT induced vasoconstrictor response of PA from MCT rats was enhanced (\(E_{\text{max}}\): 50 \% \pm 20 \% vs control 38 \% \pm 21 \%, \(P < 0.05\)). A significantly higher level of 5-HT\textsubscript{1B} receptor mRNA expression was detected in PA from MCT rats. The ratio of the PCR products of 5-HT\textsubscript{1B} receptor gene to those of \(\beta\)-actin gene was much higher in MCT rats than that in control rats (0.43 \pm 0.14 vs control 0.27 \pm 0.15, \(P < 0.05\)). CONCLUSION: 5-HT\textsubscript{1B} receptor is involved in pulmonary vasoconstriction and the enhanced level of 5-HT\textsubscript{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 vasoconstrictor 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)\textsuperscript{[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\textsuperscript{[2]}. The elevated plasma levels of 5-HT have also been reported in primary PHT and hypoxia-induced PHT in the newborn. Many \textit{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\textsubscript{1B/1D} receptor agonist sumatriptan causes pulmonary vasoconstriction equivalent to 5-HT\textsuperscript{[3,4]}. In man, the 5-HT\textsubscript{1B} receptor-mediated pulmonary vasoconstriction is likely to play a significant role in the increased pulmonary vasoconstrictor effect to 5-HT observed in PHT\textsuperscript{[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\textsubscript{1B} and 5-HT\textsubscript{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 g, Grade II, weighting 154 g \pm 9 g) from Animal Resource Center, China Medical University (Certificate No-Liaoning 034), were devided into two groups. The test rats were treat-
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 °C) and humidity (50% - 70%) for 3 weeks.

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; MgSO4 0.6; KH2PO4 1.2; CaCl2 2.5; NaHCO3 25 and Glucose 11.1, pH 7.4) bubbled constantly with 95% O2 - 5% CO2 gas mixture at 37 °C 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, 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 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 °C. 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-2000, 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.

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.

<table>
<thead>
<tr>
<th></th>
<th>pEC50</th>
<th>Emax (%)</th>
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<tbody>
<tr>
<td>ACh</td>
<td>Control 12</td>
<td>7.13 (6.83 - 7.53)</td>
</tr>
<tr>
<td></td>
<td>MCT    14</td>
<td>6.86 (6.16 - 6.29)</td>
</tr>
<tr>
<td>5-HT</td>
<td>Control 20</td>
<td>4.59 (4.12 - 4.76)</td>
</tr>
<tr>
<td></td>
<td>MCT    30</td>
<td>5.06 (4.85 - 5.26)</td>
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The numbers in the parentheses represent the 95 percent confidence intervals of pEC50. *P < 0.05, **P < 0.01 vs control. pEC50: negative logarithm of the molar concentration of drugs inducing half maximum effects. Emax: 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 ± s. *P < 0.05, **P < 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 ± s. *P < 0.05, **P < 0.01 vs control.

Fig 3. Eight percent PAGE of PCR-amplified products derived from cDNA of normal rat pulmonary arteries. Lane 2, 4, 6, 8 show 5-HT1B receptor mRNA expression (228 bp). Lane 1, 3, 5, 7 show no 5-HT1B 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-HT1B receptor mRNA expression (228 bp). Lane 1, 3, 5, 7 show no 5-HT1B receptor mRNA expression.

Fig 5. The ratio of the RT-PCR products of 5-HT1B receptor gene to those of β-actin gene in pulmonary arteries from control and MCT rats respectively. *P < 0.05 vs control.

The ratio of the RT-PCR products of 5-HT1B receptor gene was detected in PA examined. The levels of 5-HT1B receptor gene expressed in PA from MCT and control rats were of significant difference. The ratio of the PCR products of 5-HT1B 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).

PAGE are shown in Fig 3, 4. Only 5-HT1B receptor gene was detected in PA examined. The levels of 5-HT1B receptor gene expressed in PA from MCT and control rats were of significant difference. The ratio of the PCR products of 5-HT1B receptor gene to those of β-actin gene was much higher in MCT rats than that in control rats (0.43 ± 0.14, n = 14 vs control 0.27 ± 0.15, n = 14, P < 0.05) (Fig 5).
DISCUSSION

The mechanism of PHT is not quite clear, yet it is believed that several factors are involved, such as shear stress, chronic hypoxia, chronic inflammation, thrombosis, vasomotor and growth factors (5-HT, NO, ET-1, TXA₂, 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 substances⁶. 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 remodeling⁷. Experimentally, intravenous administration of 5-HT increases pulmonary vascular resistance in animals, and accelerates the progress of PHT in chronic hypoxic rats⁸. 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-HT₁B/₁D receptors participate in mediating vasoconstriction in human PA⁹.

The 5-HT₁B/₁D receptor agonist sumatriptan reportedly caused pulmonary vasoconstriction⁴, and a recent report that in man, the 5-HT₁B receptor-mediated pulmonary vasoconstriction likely played a significant role in the increased pulmonary vasoconstrictor effect to 5-HT was observed in PHT⁵. So the present study concentrated on the 5-HT₁B/₁D receptors, and confirmed that substantial 5-HT₁B but not 5-HT₁D 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-HT₁B receptor that participates in mediating the vasoconstrictor effect to 5-HT in PA. Furthermore, an increased 5-HT₁B 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 receptor¹¹, we proposed that 5-HT₁B receptor-mediated vasoconstriction to 5-HT was significantly important in PA from MCT-induced chronic PHT rat, because; (1) 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-HT₁B/₁D receptors, which may be involved in the vasoconstriction effect⁴-⁵; (II) The responsiveness to 5-HT, 5-CT, and sumatriptan were enhanced in PA from patients and rats with PHT⁴; (III) Responses to 5-HT at physiologic and pathologic concentrations were inhibited by a selective 5-HT₁B antagonist GR55562 but were resistant to the 5-HT₂A antagonist ketanserin, indicating that 5-HT₁B/₁D but not 5-HT₂A receptors were involved⁵; (IV) In the present study, large amounts of mRNA for 5-HT₁B but not 5-HT₁D receptors was detected, so the 5-HT₁B receptor is likely to be responsible for the vasoconstrictor effect to 5-HT; (V) An increased expression of 5-HT₁B 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-HT₁B receptor is one of the important mechanisms mediating PHT in MCT-treated rats.

It has been shown that 5-HT₁B, 5-HT₂B, and 5-HT₄ receptor mRNA are expressed in cultured endothelial cells and mediate nitric oxide-dependent vasodilation, so a study in endothelium-denuded vessels seemed pertinent⁶. 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 Eₘₐₓ and sensitivity of 5-HT in this study were lower than that in other experiments in vitro³.

The study provided molecular evidence that only 5-HT₁B receptor gene were expressed in PA, and participated in mediating contractile response to 5-HT, and the higher level of expression of 5-HT₁B receptor gene was associated with the higher vasoconstrictor response to 5-HT in PA from MCT-treated rats. Therefore, we concluded that 5-HT₁B receptor mediated pulmonary vasoconstriction and the enhanced expression of 5-HT₁B receptor mRNA was closely related to the augmentation of 5-HT induced pulmonary vasoconstriction in PHT, implicating that 5-HT₁B receptor is one of the important mechanisms of PHT and this may provide a novel and potential therapeutic target for PHT.
REFERENCES


5-HT1B 受体增强野百合碱诱导的肺高压血压大鼠的肺血管对 5-HT 收缩反应

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关键词 血清素；血清素受体；肺高压血压

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-HT1B，5HT1D 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-HT1B，5HT1D 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（5-HT1B，5HT1D 受体 mRNA 表达明显增多，5-HT1B 受体 mRNA 相对表达比从 0.43 ± 0.14 增加到 0.27 ± 0.15（P < 0.05），肺动脉高压大鼠肺血管的 5-HT1B 受体 mRNA 表达明显增多，MCT 大鼠肺血管 5-HT1B 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-HT1B 受体有可能成为治疗肺动脉高压的新靶点。

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