JOURNAL of ANESTHESIA and PERIOPERATIVE MEDICINE

Original Article

Altered Expression of 5-HT Receptor Subtypes in the Spinal Cord After Cardiac Ischemia-Reperfusion Injury

Zhi-Xiao Li[1], Zhi-Gang He[1], Shun-Yuan Li[2], and Hong-Bing Xiang[1]

ABSTRACT

Background: Spinal cord plays an important role in the development and progression of cardiac ischemia-reperfusion injury (CIRI).Numerous studies have been performed to identify the specific role of cardiac 5-hydroxytryptamine (5-HT) in cardiomyocyte damage, whereas reports about the potential role of spinal 5-HT during cardiac ischemia are rare. We investigated the involvement of 5-HT receptor subtypes in the spinal cord after CIRI.

Methods: Male adult SD rats (250 ~ 300 g) were randomly divided into two groups: Control group (Sham, n = 10) and Model group (30 min ischemia followed by 2 h reperfusion, n = 10). The animals of Control group had the same operation (thoracotomy) as the Model group but without left anterior descending coronary artery ligation. By using real-time quantitative polymerase chain reaction (RT-qPCR), we drew the expression profiles of 5-HT receptor subtypes in the upper thoracic segment of the spinal cord after CIRI.

Results: We found that there was a significant decrease of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{3C}, 5-HT_{5B}, and 5-HT_{6} expression after CIRI, indicating that the specific role of 5-HT receptor subtypes in the spinal cord after CIRI.

Conclusion: Our results will be beneficial for understanding the serotonergic drug targets to decrease CIRI in future preclinical and clinical studies. (Funded by the the National Natural Science Foundation of China, National Natural Science Foundation of Hubei Province, and Medical innovation project in Fujian Province, all in China.)

From the [1]Department of Anesthesiology and Pain Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [2]Department of Anesthesiology, The First Affiliated Quanzhou Hospital of Fujian Medical University, Quanzhou, China.

Correspondence to Dr. Hong-Bing Xiang at xhbtj2004@163. com or Dr. Shun-Yuan Li at cylfj@126.com.

Citation: Zhi-Xiao Li, Zhi-Gang He, Shun-Yuan Li, Hong-Bing Xiang. Altered Expression of 5-HT Receptor Subtypes in the Spinal Cord After Cardiac Ischemia-Reperfusion Injury. *J Anesth Perioper Med* 2019; 6 : x- xx.

doi: 10.24015/JAPM.2019.0007

Cardiac ischemia-reperfusion injury (CIRI) was defined as the phenomenon that blood supply into the occluded myocardium can damage cardiomyocytes (1-3). CIRI can result in long-term morbidity and increased mortality. It is now established that the interaction of heart and spinal cord is important in the pathophysiological changes of CIRI. However, the key peptides, neuropeptides, and neurotransmitters systems within the spinal cord have yet to be delineated to coordinate endocrine, autonomic, and behavioral responses to changes in CIRI.

It is well documented that central serotonergic neurons, the prominent components of the descending modulatory system, have a key role in the control of spinal nociceptive transmission (4-7). According to a number of researches, serotoninergic neurons in the central nervous system may synthesize serotonin or 5-hydroxytryptamine (5-HT) (8-11). It has been well established that serotonin receptors are classified into seven groups (5-HT_{1-7}) including 15 subtypes. Among all 5-HT receptor subtypes, the 5-HT_3 receptor is ligand-gated non-selective cation channels, whereas the others are G-protein coupled receptors (12, 13). However, the role of the spinal 5-HT system in CIRI is less clear. In the present study, we provided CIRI model and evaluated the altered expression of 5-HT receptor subtypes in the spinal cord after cardiac ischemia-reperfusion injury.

MATERIALS AND METHODS

Animals

Experimental protocols (TJ-A20150804) were approved by Institutional Animal Care and Use Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The present study was performed in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male adult SD rats (250 ~ 300 g) were purchased from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology. Two rats were housed per cage in a temperature-controlled holding room (22 ± 1 °C) on a 12 hour light/dark cycle and given food and water ad libitum.

Surgery and Myocardial Ischemia / Reperfusion Injury Model

A well-studied myocardial ischemia / reperfusion injury model was used in this study with minor modifications and briefly described as following (14-18). Rats were maintained at a constant depth of anesthesia on the heated pad in a supine position with 1.5% isoflurane in 100% oxygen, and a tracheal intubation was set up. Then an invasive incision was made to expose the heart at the fourth intercostal space. Then the left anterior descending coronary artery was located and ligatured until myocardial ischemia occurred which was indicated by visualizing a marked epicardial cyanosis. After 30 min of myocardial ischemia, the trap of the left anterior artery was opened. Reperfusion was allowed for 2 h. Animals were randomly divided into two groups: Control group (Sham, n = 10) and Model group (30 min ischemia followed by 2 h reperfusion, n = 10). The animals of Control group had the same operation (thoracotomy) as the Model group but without LAD ligation. The electrocardiogram (ECG) was made at different stages of myocardial ischemia / reperfusion injury (Basal, Ischemia 30 min, and Reperfusion 2 h). The myocardial ischemic regions were prepared for Hematoxylin-Eosin (HE) staining and Masson trichrome staining. T1-T4 spinal cord tissues were prepared for Real-Time quantitative PCR (RT-qPCR).

RT-qPCR

Total RNA was extracted from T1-T4 spinal cord tissues by use of Trizol reagent (Aidlab, China) according to the protocol of the manufacturer. RNA samples were quantified by use of a spectrophotometer and then synthesized to cDNA using reverse transcription. Real-time quantitative PCR protocol was performed on the ABI7900 real-time detection system by use of SYBR Green Master Mix to detect amplification. The PCR reaction conditions were carried out in accordance with the manufacturer's protocol: Incubation was set at 50° C for 2 minutes and then at 95°C for 10 minutes, followed by 40 cycles at 95°C for 30 seconds and 60°C for 30 seconds. The sequences of the specific primers for RT-qPCR were designed based on the previously reported sequence of rat genes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C},

Table 1. Sequences of Primers Used for RT-qPCR.

Gene	Forward (5′-3′)	Reverse (5′-3′)
5-HT_{1A}	CCATCAGCAAGGACCACGGCTA	CCCGTAGAGAACCAGCATGAGCAA
5-HT_{1B}	GTCAAGCCAAAGCGGAGGA	GCAGGGTGGGTAAATAGAAAGC
5-HT_{1D}	CCCGAGAAAGGAAAGCCACT	GAGGACCAAGGATACCACAAAGAA
5-HT_{1F}	CTGTGACCTTTGGCTGAGTGTT	CGACTGCGTCTGTGATTGCTC
5-HT_{2A}	CTTCCAACGGTCCATCCACA	GGGCACCACATTACAACAAACAG
5-HT_{2B}	CGCCATCCCAGTCCCTATT	CAGCCAGTGACCCAAAGAGC
5-HT_{2C}	GACTGAGGGACGAAAGCAAAG	GAAGGACCCGATGAGAACGA
5-HT_{3C}	GTGACCGCCTGTAGCCTTGA	GATGCTCTTGTCCGACCTCA
5-HT_{4}	TGCCTTCCTTATCATCCTCTGC	CACCACATTCCACTGTATCCCT
5-HT_{5A}	CGCTGTGCTCCTGGGATAT	CCTGTTGAACGCCGTGTAGAT
5-HT_{5B}	CGTGGTGCTCTTCGTCTACTG	TCCTGAGGTGCTTCCTTTGC
5-HT_{6}	GCACGAACTGGGCAAAGCT	GGACGCCACGAGGACAAAA
5-HT_{7}	TTCTGTCGGTCTGGCTGCTCTC	ACCGCAGTGGAGTAGATCGTGTAG

RT-qPCR, real-time quantitative polymerase chain reaction.

$5\text{-}HT_{3C}$, $5\text{-}HT_{4}$, $5\text{-}HT_{5A}$, $5\text{-}HT_{5B}$, $5\text{-}HT_{6}$, $5\text{-}HT_{7}$, and GADPH) by Biotechnology Company and detailed in Table 1. For standardization, the housekeeping gene GAPDH was used as an internal control. Relative changes in gene expression were calculated by the use of the comparative ($2^{-\Delta\Delta CT}$) method.

Statistical Analyses

Results are expressed as the mean ± SEM. The statistical analyses and graphs were performed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). $P < 0.05$ was considered statistically significant.

RESULTS

Establishment and Evaluation of Rat Models with Myocardial Ischemia/Reperfusion Injury

A total of 20 rats were used for this study. We used the ECG to confirm the different stages of myocardial ischemia / reperfusion injury (Figure 1). Left anterior descending coronary artery (LAD) was occluded for 30 min and reperfused for 2 h before examining the degree of myocardium damage. The results from HE and Masson staining clearly exhibited that those cardiac structural disorders were observed with different degrees of vacuolar degeneration, necrosis and loose stroma in model group, whereas myocardial cells were complete and arranged in the order in control group (Figure 2). These results successfully confirmed rat models with myocardial ischemia/reperfusion injury.

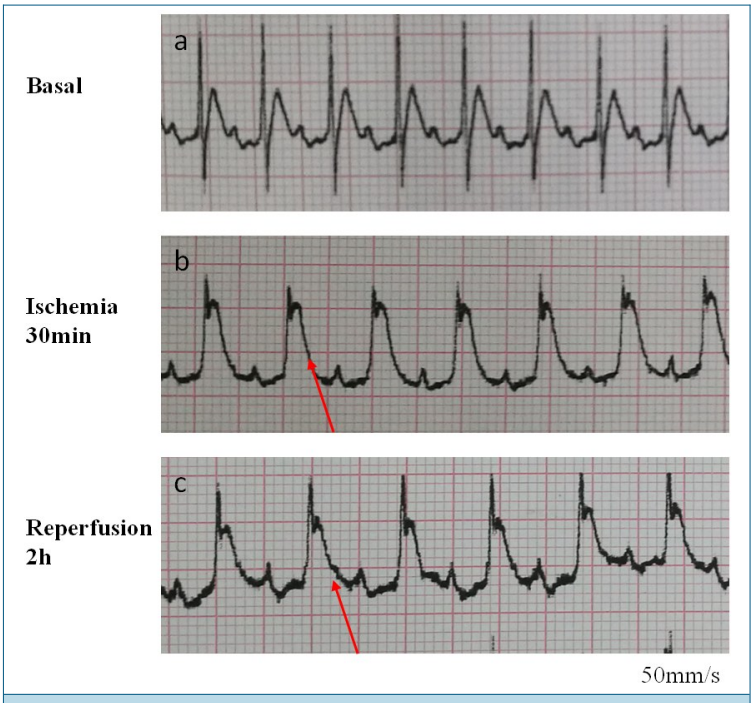

Figure 1. Variations in the ECG at Different Stages of Myocardial Ischemia-Reperfusion Injury.
(a) Normal sinus rhythm before surgery. (b and c) the ST segment was elevated after ischemia 30 min and reperfusion 2 h. The red arrow indicates the ST segment.

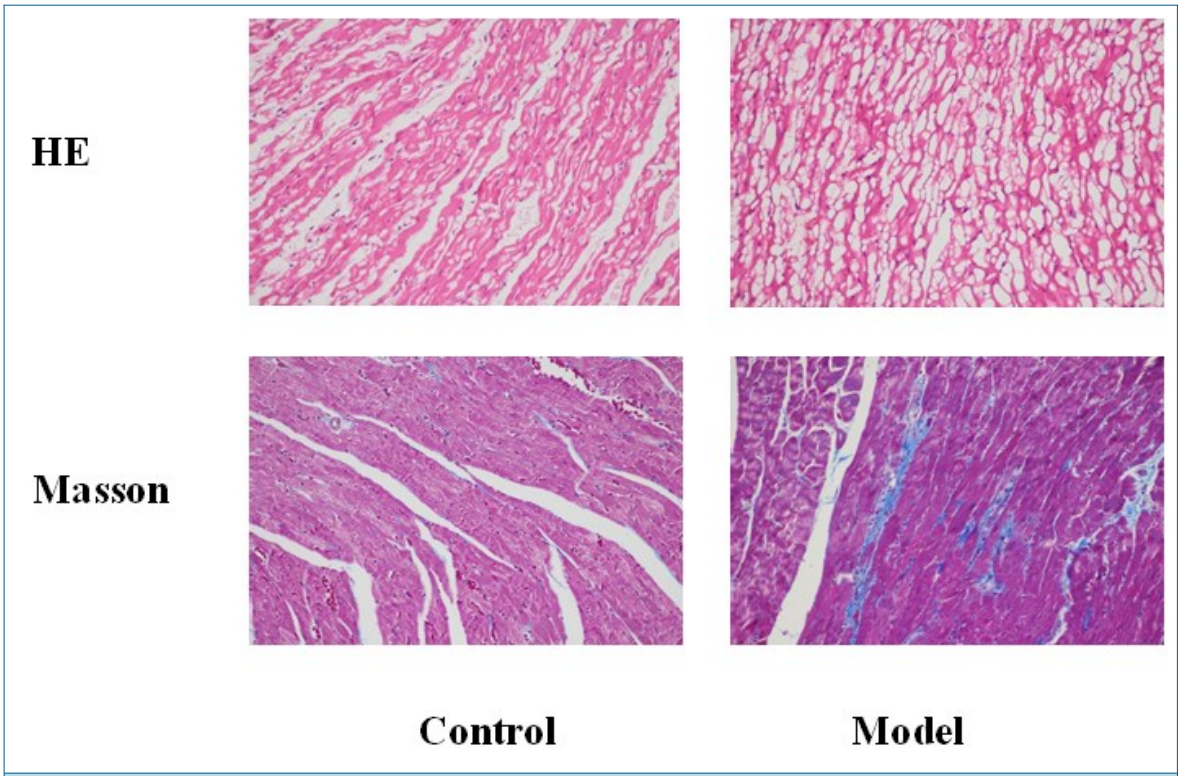

Figure 2. Evaluation of Myocardial Ischemia-Reperfusion Injury.
Representative images of hematoxylin and eosin (HE) staining and Masson trichrome staining (200×) from rat left ventricles. In the control group, the myocardiums were with normal myocardial architecture and myocardial fibers, and myocardial cells were complete and arranged in order. In the model group, the cardiac structural disorder was observed with different degrees of vacuolar degeneration, necrosis and loose stroma, and cardiomyocyte fibers were markedly increased after myocardial ischemia-reperfusion injury.

Different Expression Profiles of 5-HT Receptors in Control Group and Model Group

Fold changes of mRNA expression levels of all 5-HT receptors in the model group as compared with the control group were shown in Figure 3. There was a significant decrease in mRNA expression of 5-HT_{1A} ($P < 0.01$), 5-HT_{1B} ($P < 0.05$), 5-HT_{1D} ($P < 0.01$), 5-HT_{3C} ($P < 0.05$), 5-HT_{5B} ($P < 0.01$) and 5-HT_{6} ($P < 0.05$) in model group.

DISCUSSION

In the present study, we report the altered expression of 5-HT receptor subtypes in the spinal cord after cardiac ischemia / reperfusion injury. Our results indicate that at least six 5-HT receptor subtypes modulate the excitability of rat spinal cord. Remarkably, inhibitory 5-HT1A expression appears to decrease in the spinal cord, suggesting that spinal 5-HT receptor subtypes have important implications for the development of cardiac ischemia/reperfusion injury.

For the past half century, numerous anatomic and physiological studies of the autonomic nervous system between the heart and spinal cord have investigated this link and found it to be very complex. Cardiac ischemia is accompanied by angina pectoris pain mediated by sympathetic outflow to the spinal cord. The spinal cord contains the neurons that innervate the heart. Some studies have demonstrated that spinal cord stimulation (SCS) induces an anti-ischemic effect in patients with therapeutically refractory angina who are unsuitable for myocardial revascularization (19-21). The mechanisms by which SCS im-

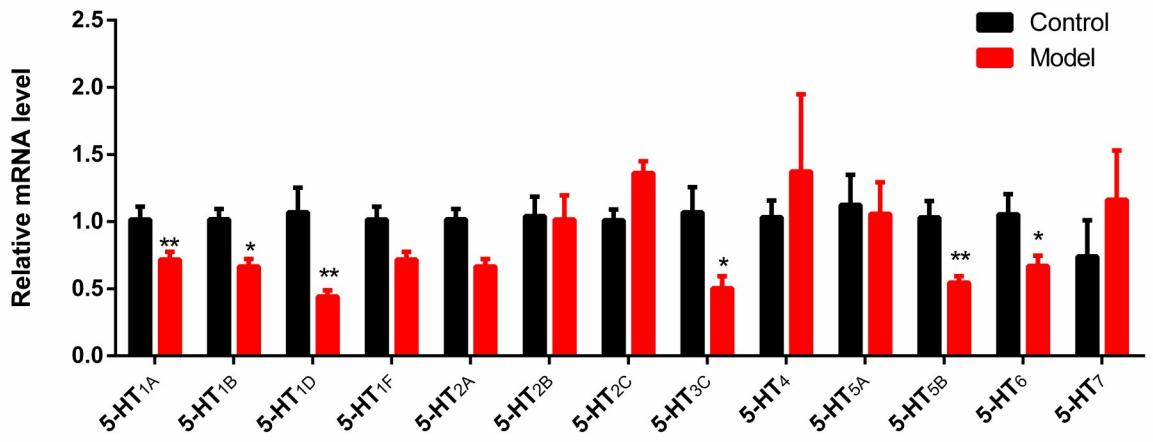

Figure 3. The mRNA Expression Changes of 5-HT Receptor Subtypes in T1-T4 Spinal Tissues between the Control Group and Model Group.
Several 5-HT receptors were down-regulated in the spinal cord from model rats, including 5-HT1A, 5-HT1B, 5-HT1D, 5-HT3c, 5-HT5B, and 5-HT6. Data are expressed as means ± SEM, *P < 0.05, **P< 0.01, unpaired t-test as compared to the control group.

proves myocardial function during acute ischemia, especially in the monoamine neurotransmitter, are not well understood. Activated platelets during cardiac ischemia release 5-HT, which is metabolized by the mitochondrial enzyme monoamine oxidase A (MAO-A) (22). Numerous studies have been performed to identify the specific role of cardiac 5-HT in cardiomyocyte death and post-ischemia-reperfusion cardiac damage, whereas reports about the potential role of spinal 5-HT during cardiac ischemia are rare.

Our previous studies suggested that spinal nociceptive transmission was implicated in the serotonergic (5-HT) pathway (8, 11). 5-HT receptor families (5-HT_1 – 5-HT_7) were identified, many of which are present in the spinal cord. Some reports show that activation of 5-HT_1 and 5-HT_5 receptor reduces cAMP, 5-HT_2 receptor activates phospholipase C to release intracellular calcium, whereas 5-HT_6 pathway increases cyclic AMP. The present data showed that there was a significant decrease of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{3C}, 5-HT_{5B}, and 5-HT_6 expression in the model group as compared with the control group. It is well known that 5-HT_{1A} receptors decrease while 5-HT_{2A} receptors increase excitability of spinal cord (23). Thus, our results suggest that spinal inhibitory 5-HT_{1A} is involved in the pathological effects of cardiac ischemia-reperfusion injury. 5-HT_{1B} receptors can function as presynaptic/postsynaptic autoreceptors that inhibit serotonergic neuron firing. The 5-HT_{1B} and 5-HT_{1D} receptor subtypes are linked to inhibition of adenylyl cyclase activity and mediate their effects at autoreceptors involved in the local inhibitory control of 5-HT release. Together, these findings are consistent with the literature indicating that the specific role of 5-HT receptor subtypes in the spinal cord after cardiac ischemia-reperfusion injury.

Overall, the gene expression profiles of 5-HT receptor subtypes in the spinal cord after cardiac ischemia/reperfusion injury were provided and differentially expressed 5-HT receptor subtypes were screened out. These results will be beneficial for understanding the serotonergic drug targets to decrease cardiac ischemia-reperfusion injury in future preclinical and clinical studies.

This work was supported by grants from the National Natural Science Foundation of China (81670240, 81873467), National Natural Science Foundation of Hubei Province (2016CFB625), and Medical Innovation Project in Fujian Province (2017-CX-48), all in China.

The authors have no other potential conflicts of interest for this work.

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