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Heme oxygenase-1 dependant pathway contributes to protection by tetramethylpyrazine against chronic hypoxic injury on medulla oblongata in rats

Yan Ding [a], Xuefei Hou [b], Li Chen [b], Hua Zhou [b], Yanju Gong [a], Liqun Dai [a], Yu Zheng [b,*]

[a] Department of Histology, Embryology and Neurobiology, Sichuan University, Chengdu, Sichuan 610041, PR China
[b] Department of Physiology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, Sichuan 610041, PR China

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ABSTRACT

Tetramethylpyrazine (TMP), one of the active ingredients of the Chinese herb *Lingusticum Wallichii* (Chuan Xiong) has been proved to protect the medulla oblongata from chronic hypoxia injury. However, the underlying mechanism remains unclear. The purpose of this study was to determine whether the protective effects of TMP are associated with the heme oxygenase-1 (HO-1) dependant pathway in adult rats. The morphological changes of neurons in the hypoglossal nucleus (12N), the nucleus ambiguus (Amb), the nucleus tractus solitarius (NTS), and the pre-Bötzinger complex (pre-BötC) were investigated by Nissl staining; the malondialdehyde (MDA) content and superoxide dismutase (SOD) activity were measured to evaluate the anti-oxidant effect; some apoptosis parameters, Bax mRNA and Bcl-2 mRNA, were tested; and the double immunochemistry staining of active caspase-3/NeuN was performed. Meanwhile, the HO-1 protein expression and heme oxygenase (HO) activity were examined. Tin-protoporphyrin (SnPP), a potent inhibitor of HO, was used to further confirm the effect of HO-1. We found that TMP ameliorated the neuron loss in 12N, Amb and NTS, the decrease in SOD activity and the increase in MDA content, the decrease in Bcl-2 mRNA of medulla oblongata ($P < 0.05$), and the increase in percentage of apoptotic neurons in Amb ($P < 0.05$) induced by chronic hypoxia. Co-administration with SnPP abolished the beneficial effects above of TMP to some extent ($P < 0.05$). Moreover, TMP significantly increased HO activity and HO-1 protein expression, which was most likely enhanced in the neurons ($P < 0.05$), and co-administration of SnPP reduced these up-regulated effects ($P < 0.05$). This study demonstrated that HO-1 dependant pathway may be involved in the protective action of TMP against chronic hypoxic damage on medulla oblongata in the rats.

1. Introduction

Chronic hypoxia is involved in many pathological conditions, such as cardiac arrest, chronic obstructive pulmonary diseases, and obstructive sleep apnea. Following chronic hypoxia injury, reactive oxygen species are generated and oxidative stress occurs in neuronal cells [1,2]. Increased oxidative stress may be associated with chronic hypoxia-mediated neuronal cell apoptosis [1]. Chronic hypoxia may cause functional damage of nervous system, for example, the oxidative stress induced by chronic intermittent hypoxia could cause the spatial learning defects [3].

As we all know, the medulla oblongata is the kernel of control of cardiovascular and respiratory activity in mammals. For example, the pre-Bötzinger complex (pre-BötC), a limited region of ventrolateral medulla, is considered to be the primarily neuronal center of respiratory rhythmogenesis in mammals [4]. Bilateral lesion of pre-BötC eliminated the breathing rhythm activity in rats [5]. The nucleus tractus solitarius (NTS), located in the dorsomedial medulla, is the main site of cardiorespiratory reflex integration. Small lesion of commissural NTS neurons in

Table 1
Comparison of blood gas analysis between different groups of rats.

	pH	PO_2 (mm Hg)	PCO_2 (mm Hg)	HCO_3^- (mmol/L)
C	7.39 ± 0.06	86.03 ± 3.61	44.21 ± 1.80	27.40 ± 0.70
T	7.38 ± 0.04	83.30 ± 3.06	46.10 ± 0.53	27.52 ± 0.76
H	7.38 ± 0.04	51.03 ± 5.29*	40.63 ± 3.94	22.71 ± 1.28*
HT	7.40 ± 0.08	52.30 ± 6.65*	40.07 ± 2.77	24.37 ± 0.57*
HST	7.37 ± 0.02	53.00 ± 1.15*	42.01 ± 1.11	22.20 ± 1.82*
HS	7.41 ± 0.03	52.02 ± 1.73*	39.03 ± 3.95	22.92 ± 0.60*

C: control group, T: TMP group, H: chronic hypoxia group, HT: chronic hypoxia + TMP group, HST: chronic hypoxia + SnPP + TMP, HS: chronic hypoxia + SnPP, n = 5.
* $P < 0.05$ vs C.

* Corresponding author at: Department of Physiology, West China School of Preclinical and Forensic Medicine, Sichuan University, 3-17 Renmin South Road, Chengdu, Sichuan 610041, PR China.

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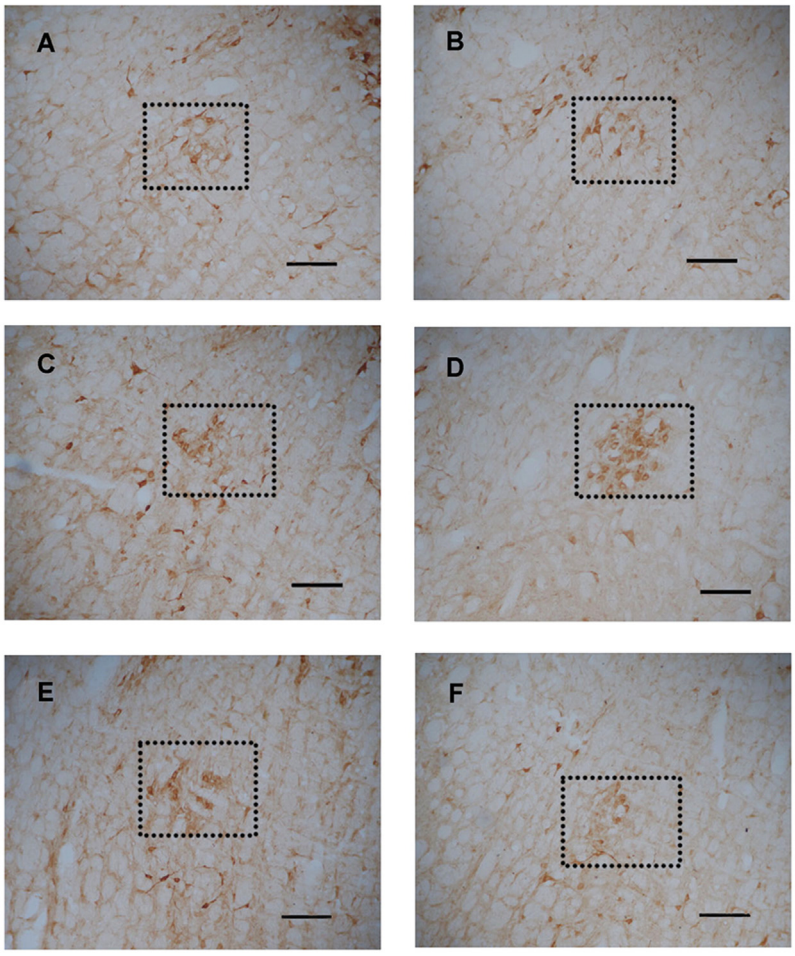
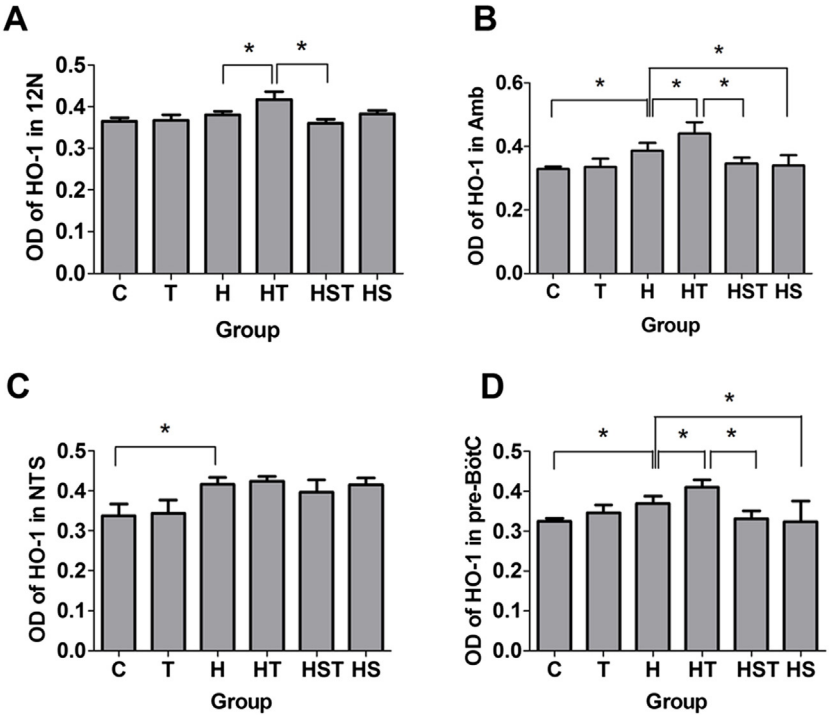
(1)


A
B
C
D
E
F

(2)


A
OD of HO-1 in 12N
0.0
0.1
0.2
0.3
0.4
0.5
C T H HT HST HS
Group
B
OD of HO-1 in Amb
0.0
0.2
0.4
0.6
C T H HT HST HS
Group
C
OD of HO-1 in NTS
0.0
0.1
0.2
0.3
0.4
0.5
C T H HT HST HS
Group
D
OD of HO-1 in pre-BötC
0.0
0.1
0.2
0.3
0.4
0.5
C T H HT HST HS
Group