Original Research

p38 MAPK mediates cardiovascular and behavioral responses induced by central IL-1β and footshock in conscious rats[1]

Rui-mao ZHENG, Chang-jiang ZOU, Shi-gong ZHU[2]

Department of Physiology and Pathophysiology, Health Science Center, Peking University, Beijing 100083, China

KEY WORDS mitogen-activated protein kinase p38; interleukin-1; blood pressure; motor activity

ABSTRACT

AIM: To investigate the roles of p38 mitogen-activated protein kinase (p38 MAPK) in the cardiovascular and behavioral responses induced by intracerebral ventricular injection (icv) of interleukin-1β (IL-1β) or footshock. **METHODS:** We examined the effects of p38 MAPK on mean artery blood pressure (mABP), heart rate (HR), and motor activity (MA) during central administration of IL-1β, or footshock after icv SB203580 (a specific inhibitor of the p38 MAPK) with Cardiovascular and Behavior Telemetry System in conscious SD rats. **RESULTS:** (1) IL-1β (icv) or footshock remarkably rise the mABP, and the maximal changes are (7.8±1.8) and (12.3±3.5) mmHg, respectively, which was abrogated by the pretreatment with p38 inhibitor SB203580 intracerebroventricularly. (2) Compared with icv saline group, the motor activity was significantly decreased in SB203580 group with maximal changes (-7.6±1.1) counts/min after footshock. **CONCLUSION:** p38 MAPK plays an important role in the pressor response induced by central administration of IL-1β or footshock and change of motor activity after footshock in conscious rats.

INTRODUCTION

Sharing characteristics of stress mediators[1], central interleukin-1β (IL-1β) is very important in cardiovascular and behavioral responses to stressors[2-4]. It can induce similar hypertensive responses to stress via cardiovascular regulatory regions[5]. We observed previously that central IL-1β mediated cardiovascular responses to some stressful stimuli, such as conditioned fear stimuli and footshock[6]. Many stressful stimuli may increase IL-1β mRNA expression and IL-1β bioactivity in such cardiovascular regulatory areas in the brain as hypothalamus, *etc*[7]; IL-1β innervations exist in hypothalamus[5,8]. IL-1β in hypothalamus mediates the pressor effects of amygdala[9]. Central IL-1β is involved in the behavior responses to stress[10], and central administration of IL-1β can increase motor activity during conditioned fear stimuli[11]. IL-1β mediate pressor responses to footshock via IL-1β receptor (IL-1RI)[6]. p38 mitogen-activated protein kinase (p38 MAPK) is a pivotal molecule of signal pathway initiated by IL-1RI[2]. In neurons of hippocampus, inhibition of p38 MAPK activation induced by SB203580 blocks suppression of long term potentiation (LTP) evoked by IL-1β[12] and cellular stress responses to IL-1β[13]. In addition, SB203580 inhibits effects of IL-1β on AP-1[14]. Electric stimuli increase not only protein levels of IL-1β, but also the phosphorylation of p38 MAPK simultaneously in rat brain[7,15]. These suggest that effects of IL-1β are partially dependent on the activation of p38

[1] Project supported by a grant from the National Major Basic Research Program of China (G2000056908).
[2] Correspondence to Prof Shi-gong ZHU. Phn 86-10-8280-1477. Fax 86-10-8280-1443. E-mail sgzhu@bjmu.edu.cn
Received 2003-06-06 Accepted 2003-11-20

MAPK. Our previous studies have shown that icv IL-1β induced pressor responses and central IL-1β participates in the mediation of pressor responses to footshock[6]. In this study, we further investigate whether p38 MAPK is involved in the pressor responses and behavior responses induced by icv IL-1β or footshock.

MATERIALS AND METHODS

Drugs administration Recombinant rats interleukin-1β (PeproTech EC Ltd UK), SB203580 (ALEXIS, Qbiogene, Inc USA) was diluted with saline to 0.01 g/L and 0.1 g/L, respectively. IL-1β, SB203580 or saline were intracerebroventricularly (icv) administered in a volume of 10 μL over 1 min period via cerebroventricular cannula. Pretreatment with SB203580 was performed at 30 min prior to icv IL-1β or footshock. The concentration of SB203580 was determined by dose response previously (Data not shown).

Experimental animals Experiments were performed on male Sprague Dawley rats (180-230 g), purchased from center of laboratory animals of Peking University and housed individually with a 12-h light/dark cycle and had free access to food and water. The procuration of animals, the husbandry and the experiments conformed to the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Council of Europe No 123, Strasbourg 1985).

Surgery for implantation of cerebroventricular cannula After the rats were anesthetized with chloral hydrate (0.4 g/kg, ip), a stainless cannula was inserted into the right lateral cerebral ventricle with the tip located at 0.6 mm caudal to the bregma, 1.6 mm lateral to the midline and 4.5 mm below the skull. Then, the rats were placed back into the cage and allowed to recover for 2 to 3 d.

Telemetry system The telemetry system (Data Sciences Inc (DSI), USA) was used in this study. This system can measure the cardiovascular and behaviours responses of conscious rats[16]. The system consists of 5 major components: (1) Telemetric implant (TA11PA-C40) for measurement and transmit signals of artery blood pressure (mABP), heart rate (HR) and motor activity (MA) of rats. (2) Radio receiver platform (RPC-1 receivers) to receive telemetered signals, (3) Ambient pressure monitor to measure absolute atmospheric pressure, (4) Data Exchange Matrix to multiplex multiple cage signals to the computer, and (5) a PC-based Data Acquisition System (Dataquest ART System 2.0) to process signals.

Surgery for telemetric implant The rats, which recovered in surgical operation for implantation of cerebroventricular cannula, were anaesthetized with chloral hydrate (0.4 g/kg, ip) again. Telemetric implants (PA-C40, DSl, USA) were implanted as described below. Briefly, the telemetric implants were inserted in the peritoneal cavity under aseptic conditions. A nonoccluding catheter (attached to telemetric implant) with an antithrombogenic tip was inserted in the abdominal aorta with the tip just below the renal arteries; it was fixed in place with tissue adhesive (3M Vetbond USA). The body of the radiotelemetric implant was immobilized by suturing it to the ventral abdominal wall and the wounds were closed by suture. Antibiotics were administered; the animals were allowed to recover for at least 5 d prior to experiments.

Record the cardiovascular and behavioral responses by telemetry system Under conscious condition of rats, we tested the cardiovascular and behavior responses with Telemetry System. RPC-1 receivers platform (DSI, USA) were placed under each animal's cage or experimental box. When telemetry implant in abdomen of rat was initiated by a magnetic switch, the signals of mABP, HR and MA which measured and emitted by telemetry implant were received by the RPC-1 receivers platform and transmitted via Data Exchange Matrix (DSI, USA), the signals were logged, deposited and analyzed by Dataquest ART. System (DSI, USA).

Footshock The continuous footshock was conducted as described below. On the first and the second days of experiments, the animals received two habituation procedures per day. In each habituation procedure, animals were transferred to the experimental room, placed in an experimental box (25 cm×30 cm×30 cm) (RTC-20, BRS/LVE division of Tech Serv Inc USA) equipped with a grid floor made of stainless steel tubes, left there for 5 min. On the third day, rats were placed in the experimental box and footshock (14 V, 50 Hz) were given over 1 min to the rats.

Statistical analysis All data were expressed as mean±SEM and analyzed with ANOVA followed by post-hoc Fisher LSD test set at α=0.05.

RESULTS

Effects of SB203580 on cardiovascular responses and motor activity induced by icv IL-1β In IL-1β group (n=8), the responses of mABP were

observed starting at 3 min after icv IL-1β and lasting over 30 min, the maximal changes (7.8±1.8 mmHg) of mABP were significantly higher than those of saline control group (n=9) (P<0.05). In addition, compared with IL-1β group, in 3, 6, 12 min, the mABP did not increased in the rats pretreated with SB203580 at 30 min prior to icv IL-1β (n=6) (Fig 1A). There was no significant change in heart rate and motor activity (Fig 1B, Fig 1C).

Effects of SB203580 on cardiovascular responses and changes of motor activity induced by footshock As shown in Fig 2, in saline+footshock group (n=7), the responses of mABP were observed starting at 3 min after footshock and lasting over 15 min. The maximal changes of mABP (12.3±3.5 mmHg) were significantly higher than that of unshock group (n=9) (P<0.05). In SB203580+footshock group (n=6), the changes of mABP (in 3, 6 min) were significantly lower than the changes in saline+footshock group (P<0.05) (Fig 2A). Compared with unshock group, the significant increase of heart rate were observed at 3, 6, 9 min and 3, 6 min in saline+footshock group and SB203580+footshock group, respectively (P<0.05) (Fig 2B). Compared with unshock group, the motor activity in 3 min after footshock were significantly increased in saline+footshock group (P<0.05), the maximal change was 37.1±4.1 counts/min. When compared with saline+footshock group, the motor activity were significantly decreased in SB203580+footshock group in 12, 15, 21 min after footshock (P<0.05), the maximal change is -7.6±1.1 counts/min (Fig 2C).

DISCUSSION

In present study, we observed the roles of p38 MAPK in the behavior responses to footshock and in the changes of blood pressure and heart rate induced by icv IL-1β or footshock.

Footshock is a stressor which induces cardiovascular response such as hypertension, increasing heart rate and motor activity. In previous study, we observed that hypertension induced by footshock was mediated by central IL-1β via central IL-1 receptor and icv IL-1β can induce pressor response[6]. The mechanism of those responses is not clear yet. In this study, we observed that icv SB203580 (a specific inhibitor of p38 MAPK) attenuated the pressor response induced by footshock or icv IL-1β.

IL-1β is a stressor as well as a well-known activator of p38 MAPK[2], and plays important roles in cardiovascular or behavior response to stressors[2-4,6]. In the rat brain, the levels of IL-1β increased by stressful stimuli, was coupled with the IL-1β-triggered a signaling cascade which leads to the activation of the p38 MAPK[2,7,15,17]. The p38 MAPK, an intracellular stress-

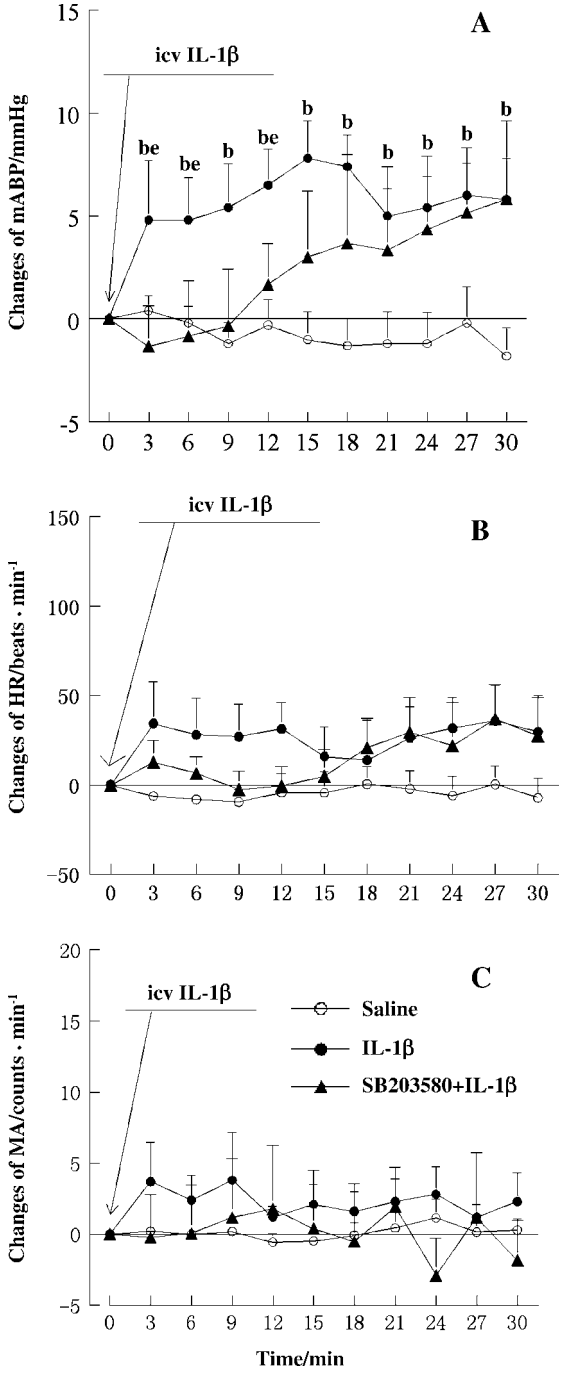

Fig 1. Effects of icv SB203580 on cardiovascular responses and motor activity (MA) induced by icv IL-1β. mABP, artery blood pressure. HR, heart rate. Saline: icv saline 10 μL (n=8); IL-1β: icv IL-1β 100 ng (n=9); SB203580+IL-1β: icv SB203580 1 μg at 30 min before icv IL-1β 100 ng (n=6). Mean±SEM. ^bP<0.05 *vs* saline. ^eP<0.05 *vs* SB203580+IL-1β.

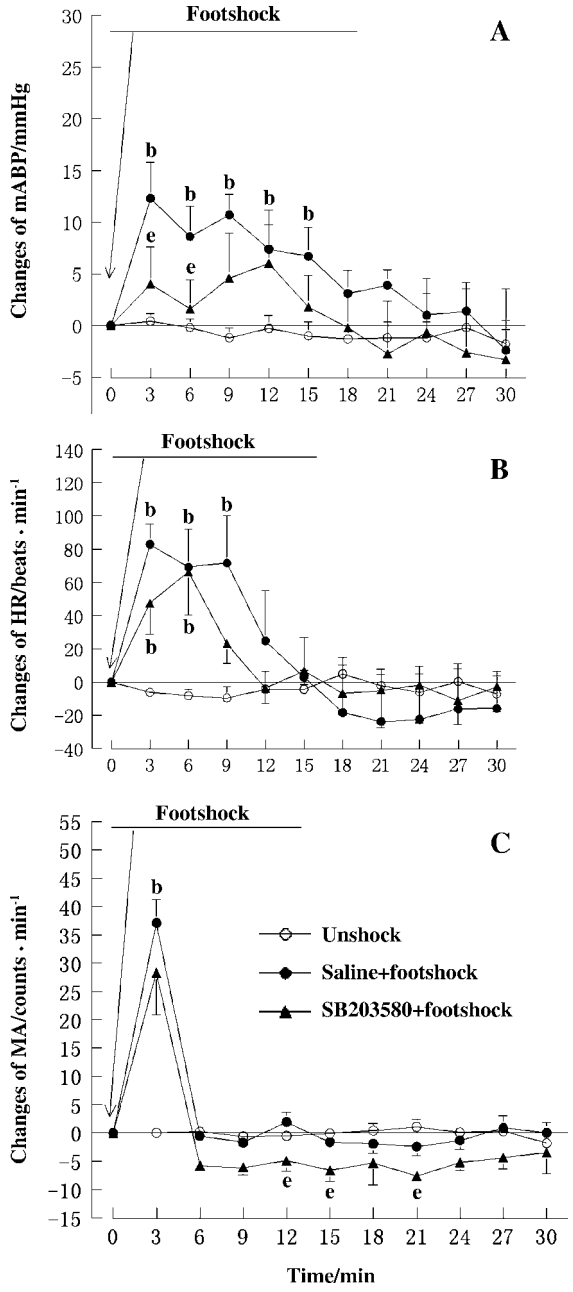

Fig 2. Effects of icv SB203580 on cardiovascular responses and motor activity induced by footshock. mABP, artery blood pressure. HR, heart rate. unshock: icv saline 10 μL only (*n*=9); footshock: icv saline 10 μL at 30 min before footshock (14 V, 50 Hz) was given to the rats (*n*=7); SB203580+footshock: icv SB203580 1 μg at 30 min before footshock (14 V, 50 Hz) was given to the rats (*n*=6). Mean±SEM. $^{b}P<0.05$ *vs* unshock. $^{e}P<0.05$ *vs* saline+footshock.

activated protein kinase (SAPK) associated with stressful stimuli, is a major signaling system by which cells transduce extracellular stressful stimuli such as IL-1β, *etc* into intracellular responses[18]. After p38 MAPK was activated by stressors, the activated p38 MAPK can phosphorylates transcription factors in the nucleus that are responsible for the regulation of cell in stress [19]. Moreover, p38 MAPK is a pivotal molecule of signal pathway initiated by IL-1 receptors[2], and that, central IL-1β mediates pathological effects on cortical neurons through the p38-MAPK pathway[20], and inhibition of p38 MAPK can attenuate the stressful effects of IL-1β[13]. Further, administration of SB203580 can block the effects of IL-1β on neurons in the rat[12]. These suggest the functions of IL-1β are partially dependent on the activation of p38 MAPK. Our results implied that p38 MAPK was involved in pressor responses induced by central administration of IL-1β or footshock.

Footshock can trigger complex stress process, which not only include the cardiovascular responses, but also the behavior responses in rats[21]. The motor activity of rats was transiently increased during footshock in present study. The changes of motor activity may be elicited by the escape behavior to footshock. Interestingly, we observed that icv SB203580 significantly decreased motor activity after footshock. Thus, these data suggest that inhibition of activation of p38 MAPK may reduce motor activity after footshock.

We have noted that central IL-1β mediates physiological and pathophysiological responses to stressors as well as psychological responses to stressors[2,6]. It has been reported that the endogenous or exogenous central IL-1β can enhance motor activity under psychological stress[5,10], and footshock can significantly increase endogenous IL-1β levels in rat brain[17], the biological functions of IL-1β may attenuated by SB203580 through inhibition of p38 MAPK activation[13]. Our results show that SB203580 can decrease motor activity after footshock. Therefore, we supposed that central endogenous IL-1β induced by footshock may act as upregulative factor which control the balance of motor activity via p38 MAPK. Although central IL-1β affect MA under the psychological stress, it did not obviously affects MA under present stressful condition. We supposed that influence of central IL-1β on MA may relate to the properties of stressors.

In this study, we observed that footshock increases mABP, HR, and MA significantly, and icv IL-1β did not affect on the HR or MA. To take account of properties of central administration of IL-1β and footshock, although IL-1β is a typical stressor, it may be one factor which was included in these complicated responses stimulated by footshock. Thus, as distinct to cardiovascular response induced by icv IL-1β, footshock increases mABP, HR, and MA significantly.

Similarly, pretreatment with SB203580 did not affect on the HR. It is possible that central IL-1β or p38 MAPK is not very important in regulation of HR.

In conclusion, p38 MAPK mediates the cardiovascular responses and behavior responses induced by central administration of IL-1β or footshock.

ACKNOWLEDGEMENTS We greatly acknowledge the support of Prof Xian WANG. We also thank Ms Ming-fen WEI, Ms Hui PAN, and Ms Ling GUO for technical help.

REFERENCES

1 Bianchi M, Sacerdote P, Locatelli L, Mantegazza P, Panerai AE. Corticotrophin releasing hormone, IL-1α, and tumor necrosis factor-α share characteristics of stress mediators. Brain Res 1991; 546: 139-42.

2 Rothwell NJ, Luheshi GN. Interleukin 1 in the brain: biology, pathology and therapeutic target. Trends Neurosci 2000; 23: 618-25.

3 Maier SF, Watkins LR. Intracerebroventricular interleukin-1 receptor antagonist blocks the enhancement of fear conditioning and interference with escape produced by inescapable shock. Brain Res 1995; 695: 279-82.

4 Merali Z, Lacosta S, Anisman H. Effects of interleukin-1beta and mild stress on alterations of norepinephrine, dopamine and serotonin neurotransmission: a regional microdialysis study. Brain Res 1997; 761: 225-35.

5 Nakamori T, Morimoto A, Murakami N. Effect of a central CRF antagonist on cardiovascular and thermoregulatory responses induced by stress or IL-1 beta. Am J Physiol 1993; 265: 834 -9.

6 Zou CJ, Liu JD, Zhou YC. Roles of central interleukin-1 on stress-induced-hypertension and footshock-induced-analgesia in rats. Neurosci Lett 2001; 311: 41-4.

7 Minami M, Kuraishi Y, Yamaguchi T, Nakai S, Hirai Y, Satoh M. Immobilization stress induces interleukin-1 beta mRNA in the rat hypothalamus. Neurosci Lett 1991; 123: 254-6.

8 Breder CD, Dinarello CA, Saper CB. Interleukin-1 immunoreactive innervation of the human hypothalamus. Science 1988; 240: 321-4.

9 Lu Y, Chen W, Zou CJ. Interleukin-1 beta is involved in the pressor response of amygdaloid neurons excited by sodium glutamate. Neuroreport 2002; 13: 559-62.

10 Pugh CR, Nguyen KT, Gonyea JL, Fleshner M, Watkins LR. Role of interleukin-1 beta in impairment of contextual fear conditioning caused by social isolation. Behav Brain Res 1999; 106: 109-18.

11 Barrientos RM, Higgins EA, Sprunger DB, Watkins LR, Rudy JW. Memory for context is impaired by a post context exposure injection of interleukin-1 beta into dorsal hippocampus. Behav Brain Res 2002; 134: 291-8.

12 Aine K, Emily V, Yvonne N, Marcella B, Claire B, Christine EL, *et al*. Activation of p38 plays a pivotal role in the inhibitory effect of lipopolysaccharide and interleukin-1β on long term potentiation in rat dentate gyrus. J Biol Chem 2003; 278: 19453-62.

13 Cuenda A, Rouse J, Doza YN, Meier R, Cohen P, Gallagher TF, *et al*. SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. FEBS Lett 1995; 364: 229-33.

14 Funakoshi M, Sonoda Y, Tago K, Tominaga S, Kasahara T. Differential involvement of p38 mitogen-activated protein kinase and phosphatidyl inositol 3-kinase in the IL-1-mediated NF-kappa B and AP-1 activation. Int Immunopharmacol 2001; 1: 595-604.

15 Oh SW, Ahn YM, Kang UG, Kim YS, Park JB. Differential activation of c-Jun N-terminal protein kinase and p38 in rat hippocampus and cerebellum after electroconvulsive shock. Neurosci Lett 1999; 271: 101-4.

16 Rekik M, El-Mas MM, Mustafa SJ, Abdel-Rahman AA. Role of endothelial adenosine receptor-mediated vasorelaxation in ethanol-induced hypotension in hypertensive rats. Eur J Pharmacol 2002; 452: 205-14.

17 Nguyen KT, Deak T, Will MJ, Hansen MK, Hunsaker BN, Fleshner M, *et al*. Timecourse and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1beta protein response to acute stress. Brain Res 2000; 859: 193-1.

18 Nebreda AR, Porras A. p38 MAP kinases: beyond the stress response. Trends Biochem Sci 2000; 25: 257-60.

19 Obata T, Brown GE, Yaffe MB. MAP kinase pathways activated by stress: the p38 MAPK pathway. Crit Care Med 2000; 28: 67-77.

20 Li YK, Liu L, Steven WB. Interleukin-1 mediates pathological effects of microglia on tau phosphorylation and on synaptophysin synthesis in cortical neurons through a p38-MAPK pathway. J Neurosci 2003; 23: 1605-11.

21 McCarty R, Chiueh CC, Kopin IJ. Behavioral and cardiovascular responses of spontaneously hypertensive and normotensive rats to inescapable footshock. Behav Biol 1978; 22: 405-10.