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DIFFERENTIAL ACTIVATION OF JNK IN RAT HIPPOCAMPUS FOLLOWING ACUTE AND/OR CHRONIC STRESSORS

D. Filipović, J. Zlatković and S.B. Pajović

Laboratory of Molecular Biology and Endocrinology, VINČA Institute of Nuclear Sciences, P.O. Box 522, Belgrade, Serbia

Abstract

c-Jun N-terminal kinase (JNK) is activated by phosporylation in response to cellular stressors and extracellular signals and plays a role in the activation of glucocorticoid receptor (GR) and contributes to the stress-induced apoptosis. Therefore, the expression protein pattern of the active forms of cytosolic phospho-JNK (P-JNK) and inactive JNK in hippocampus of rats exposed to 21daily isolation as chronic stressor, sole and in combination with 2hrs acute stressors of immobilization (IM) or cold (4^{o}C), were followed by Western blot. Concentration of serum corticosterone was monitored. We found significant increase in the levels of P-JNK following acute IM. Decreased levels of P-JNK following chronic isolation and when isolation preceded the application of acute IM were found, compared to its level after acute IM. Data suggest that diminished expresion of P-JNK level following chronic isolation in hippocampus, might be involved in deregulation of intracellular GR negative feedback control as well as stress-induced apoptosis.

Introduction

c-Jun N-terminal kinase (JNK) phosphorylates glucocorticoid receptor (GR), modulates its subcellular localization and its function [1]. Also, recent evidences have shown a role for JNK signaling in stress-induced apoptosis. Previous study indicated that chronic isolation stress deregulates intracellular GR negative feedback control in limbic-hypothalamo-pituitary adrenal (LHPA) axis [2]. Since phosphorylation and dephosphorylation of intracellular effectors molecules are important regulatory mechanisms in signal transduction a variety of cellular events, including apoptosis, it is supposed that chronic isolation stress may alters JNK phosphorylation status which could be critical for stress-induced apoptosis in rat brain.

Experimental

Adult Wistar male rats, aged three months, were divided at random into two main groups, a control (unstressed) group, where the rats were housed of four individuals *per* cage and chronic isolation stress group *i.e.* rats were individually housed for 21 day (isolates). The control group consisted of three subgroups: unstressed control alone and unstressed controls which were exposed to the 2hrs of acute stressor either immobilization (IM) or cold (4°C). The chronic isolates group also consisted of three subgroups: isolation stress alone, and isolates which were subsequently

exposed to IM or cold for 2hrs representing combined stressors. Separation of hippocampal cytosolic proteins was examined by SDS-PAGE and quantification of levels p-JNK and JNK by Western blot. Concentration of serum corticosterone (CORT) was monitored by ELISA. Data were analyzed by two-way ANOVA followed by Tukey post-hoc test.

Results and Discussion

Two immunoreactive bands of 46kDa and 54kDa corresponding to the predicted molecular mass of (P) JNK1 and (P) JNK2/3 protein were detected (Fig.1 A and B). A significant increase in the level of cytosolic P-JNK was found following acute IM. At the same time, serum CORT concentration was significantly increased by acute IM (Fig.2, left panel), indicating IM-related activation of adrenocortical system. Since the CORT signaling transduction is regulated by down regulation of cytosolic GR and GR phosphorylation by P-JNK is essential for GR activation, the elevated cytosolic P-JNK protein expression following acute IM (Fig.1A, left panel) represents physiological signal transduction process providing adaptive and protective response for survival [3].

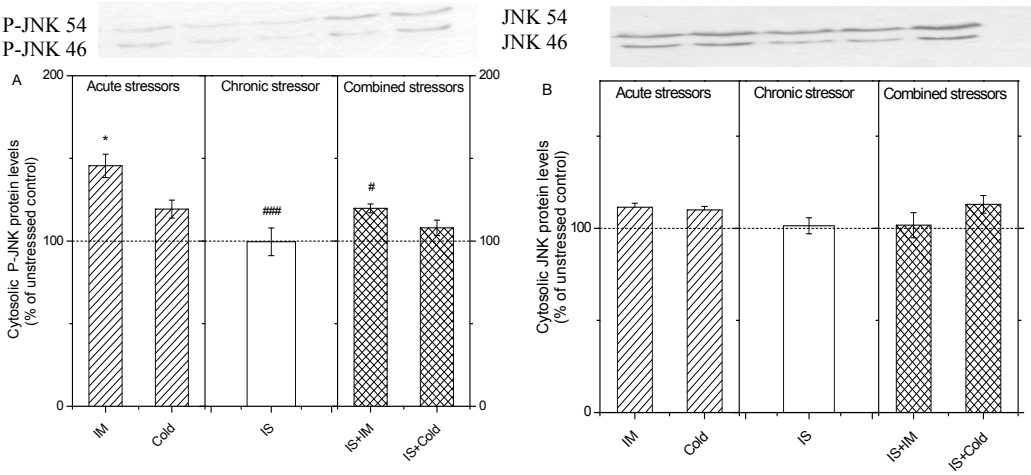

Fig.1. Protein content of cytosolic JNKs in the hippocampus of rats exposed to acute stressors immobilization (IM) or Cold, chronic isolation (IS) or their combination. (A) Upper panel: representative P-JNK Western blots for each stressor. Lower panel: cytosolic P-JNK protein expression; (B) Upper panel: representative JNK Western blots for each stressor. Lower panel: cytosolic JNK protein expression; Symbols indicate a significant difference between: the control and acute IM, $^{*}p<0.05$; combined stress or IS and acute IM, $^{\#}p<0.05$, $^{\#\#\#}p<0.001$.

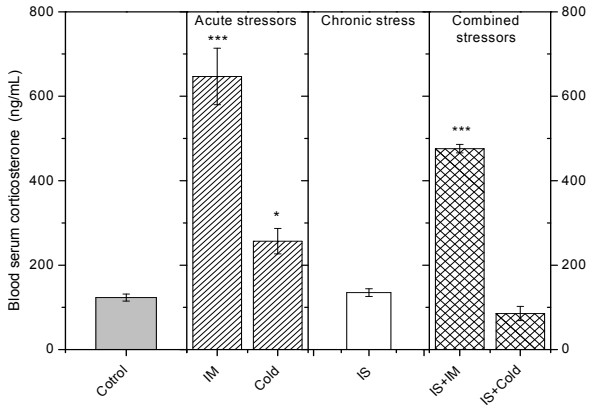

Fig.2. Changes in blood serum CORT concentrations (ng/mL) of adult Wistar male rats in control and following acute stressors immobilization (IM) or Cold, chronic isolation (IS) or combined stressors, as indicated. Asterisks indicate a significant difference between the respective stress group and unstressed control $^{*}p<0.05$, $^{***}p<0.001$.

Cytosolic P-JNK level was significantly decreased following chronic isolation, compared to its level after acute IM, while levels of JNK remained essentially unchanged (Fig.1B). The ratio between cytosolic P-JNK and JNK was decreased. Results indicate that chronic isolation impaired the hippocampal JNK signaling pathway. The two possible explanations are direct or indirect inhibition of cytosolic JNK activation in response to chronic stress and activation of putative phosphatase(s). Since, the chronic isolation compromises intracellular GR regulation [3], decreased protein expressions of cytosolic P-JNK might be included in GR protein modification and its deregulation. Decrease of P-JNK was noticed when isolation preceded the application of acute IM (Fig.1A, right panel), compared to its level after acute IM. Due to deregulated LHPA axis by chronic isolation [3], prolonged elevation of CORT level in combined stress (Fig.2, right panel) and reduction of P-JNK protein expression is most likely start up stress-induced apoptosis in hippocampus (data not shown).

Conclusion

Results show that decreased phosphorylation (activation) of JNK by chronic isolation stress in hippocampus might be included in deregulation of intracellular GR feedback system and may have a potential role in stress-induced apoptosis Nevertheless, the precise molecular mechanisms of proapoptotic JNK signaling pathway with other factors acting for apoptosis, such as p53, remains yet to be elucidated.

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