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STRESS EFFECTS ON THE PHOSPHORYLATION OF C-JUN-N-TERMINAL KINASES AND ON NUCLEAR TRANSLOCATION OF HSP70 IN RAT HIPPOCAMPUS

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Abstract

Glucocorticoids have diverse effects in cellular processes in hippocampus (HIPPO) under stress. Beside genomic pathways, their effects are also mediated by direct activation of subfamily of mitogen-activated protein kinases termed, c-Jun-N-terminal kinases (JNKs). We analysed the phosphorylation status of cytoplasmic and nuclear JNK isoforms, and expression of its inhibitor Hsp70 protein in HIPPO of rats exposed to diverse types of stress. Activity of JNK1 in cytoplasm and nucleus was decreased in all types of stress, while the activity of cytoplasmic JNK2/3 was markedly higher in acute stress, and unaltered or lowered in chronic and combined stress. Hsp70 was significantly decreased in cytoplasm and increased in nucleus under all stress conditions indicating its cytoplasmic-nuclear translocation.

Introduction

Hippocampus (HIPPO), part of limbic brain system, has a crucial role in response to stress by mediating inhibition of the hypothalamic-pituitary-adrenal (HPA) axis [1]. Stress hormones modulate brain functions by changing the structure of neurons and thus influencing neuronal damage or suppressing neurogenesis and cell survival [2]. Except for glucocortiocoid receptors, which are the main molecular regulators of stress response, MAPKs are also sensitive to stress and activated by it [3]. The JNK family belongs to the MAPKs and it is comprised of three isoforms (JNK1, 2 and 3) which have been mainly considered as degenerative signal transducers and efficient activators of apoptosis in nervous system [4]. To prevent cellular damage, cells activate the transcription of heat shock proteins. Hsp70 mediates neuroprotection and its overexpression was shown to protect HIPPO neurons from cytotoxic effects of stress [5]. We studied how different stress types (acute, chronic or combined) alter JNKs activity, expression level of Hsp70 protein and cytoplasm-nuclear translocation of both proteins in the HIPPO of Wistar rat brain.

Experimental

The adult Wistar male rats used in experiment were divided into four groups: (I) unstressed animals (controls); (II) acute immobilization, 30 min; (III) chronic isolation stress for 21 day; (IV) chronic isolation followed by 30 min immobilization. HIPPO samples were prepared by differential centrifugation and separated by SDS-electrophoresis. Western blot was performed using: anti-human JNK1/JNK2

monoclonal antibody, phospho-SAPK/JNK antibody, Hsp70 antibody and rabbit polyclonal anti-β-actin, for detection of Hsp70 and β-actin, respectively.

Results and Discussion

Effect of stress on JNKs activity: We estimated JNK1 (46kDa) and JNK2/3 (54kDa) activities in the cytoplasm and nucleus of HIPPO under stress conditions by following its phosphorylation at Thr183 and Tyr185 that are crucial for their activation (Figure 1a and b). The ratio of pJNK1 to total (tJNK1) *i.e.* pJNK1/tJNK1 indicated that cytoplasmic and nuclear JNK1 phosphorylation was low in all types of stress in respect to control. Only in the case of acute stress the phosphorylation of nuclear JNK1 was not significantly changed (Figure 1b and d). In contrast, the activation of cytoplasmic JNK2/3 was markedly higher in acute stress, while it was unaltered or lowered in other types of stress (Figure 1a and c).

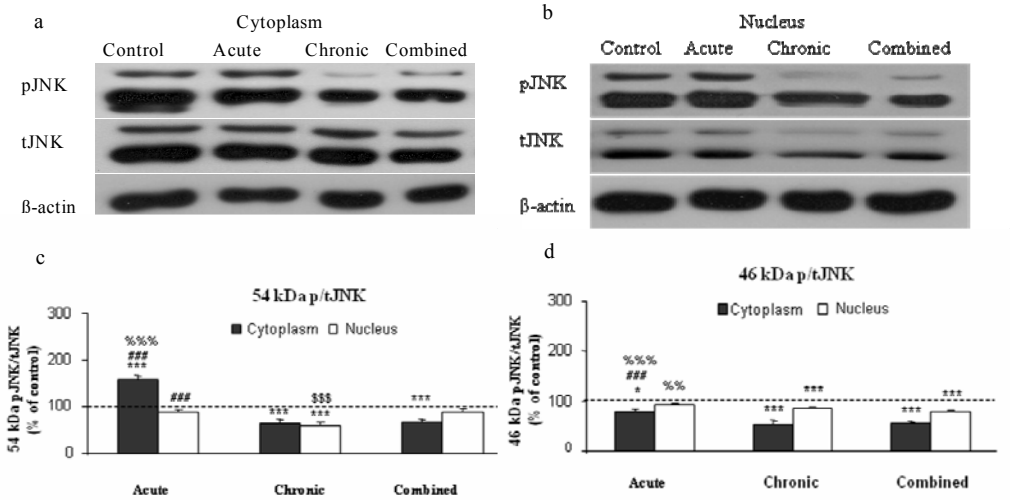
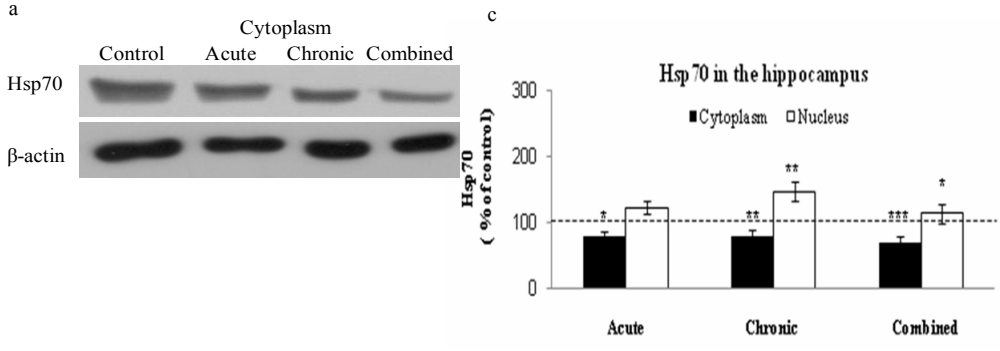


Figure 1. *Western blot demonstrating the effects of acute immobilization, chronic isolation or the combined stress on the levels of JNKs (JNK1 at 46kDa and JNK2/3 at 54kDa) and their phospoisoforms in cytoplasm (a) and nucleus (b) of hippocampus. Phospo JNK immunoreactivities represented as ratio of 54kDa pJNK/tJNK (c) or 46kDa pJNK/tJNK(d). Results are presented as mean±S.E.M (n=8) *p<0.05, **p<0.01, ***p<0.001.*

Effect of stress on the Hsp70: In parallel with JNKs activity we investigated cytoplasmic and nuclear levels of Hsp70 in acute, chronic and combined stress. The cytoplasmic level of Hsp70 was significantly decreased under all stress conditions (Figure 2a and c). Increase in nuclear Hsp70 indicated its cytoplasmic-nuclear translocation in all types of stress, with most prominent elevation under chronic stress (Figure 2b and c).



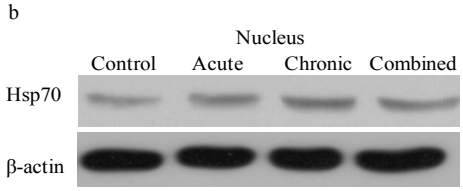


Figure 2*. Western blot demonstrating the effects of acute immobilization, chronic isolation or the combined stress on the level of Hsp70 in cytoplasm (a) and nucleus (b) of hippocampus.Hsp70 immunoreactivities (c) are represented as mean±S.E.M (n=8, *p<0.05, **p<0.01, ***p<0.001.*

Overall, the decreased activity of all JNK isoforms in both cell compartments under all stress conditions could led to interruption of JNKs signaling, which may influence neurodegeneration or neural cell remodeling (neural plasticity). Nuclear translocation of Hsp70 on the other side may represent an adaptive mechanism to stress conditions by diminishing JNKs action.

Conclusion

Since both JNK isoforms (46kDa and 54kDa) are downregulated in both cytoplasmic and nuclear compartments, and Hsp70 is simultaneously translocated to the nucleus under most of the stress conditions, the degenerative JNKs function may be influenced by Hsp70 enabling cell adaptation or remodelling particularly under severe stress conditions.

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References

[1] R.M. Sapolsky, L.C. Krey, B.S. McEwen, Endocr. Rev., 1986, **7**, 284–301.
[2] B. Czeh, T. Michaelis, T. Watanabe, et al. Proc Natl Acad Sci USA., 2001, **98**, 12796-12801.
[3] E. Meller, C. Shen, A. N. Talia, C. Jensen, Y. Tsimberg, J. Chen, R. J. Gruen, Brain Research, 2003, **979**, 57–64
[4] V. Waetzig, T. Herdegen, Neuroscience Letters, 2004, **361**, 64–67.
[5] N. Beaucamp, T. C. Harding, B. J. Geddes, J. Williams, and J. B. Uney, FEBS Lett. 1998, **441**, 215–219.