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REDOX-SENSITIVE TRANSCRIPTION FACTOR NFkB IN BRAIN OF STRESSED WISTAR RATS

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Abstract

Neuroendocrine stress (NES) causes stress-hormones increase and alters balance of intracellular reactive oxygen species (ROS) leading to vulnerability of brain neurons. Prolonged alterations of cell redox *milieux* may also lead to altered expression of redox sensitive transcription factors, such as NFκB, resposible for brain cell protection. In the presented study NFκB p65 (NFκB) levels were determined in hippocampus and brain cortex of Wistar male rats.exposed to stress of different duration The cytosolic NFκB level was unchanged in acute stress (A, 2h), but significantly down-regulated in either chronic (C,21-day) or combined (C+A) stress. The depletion of NFκB from cell cytosol probably reflected its nuclear translocation and decoupling from the repressive action of glucocorticoid receptor. Through initially protective, NFkB overactivity may lead to accumulation of toxic H_2O_2 and compromise brain cell capacity to restore homeostasis.

Introduction

Neuroendocrine stress (NES) is followed by the marked increase in the concentration of stress hormones: catecholamines (CAM) and glucocorticoids (GCs). Such conditions are known to increase the basal production of reactive oxygen species (ROS) in stress-responsive brain structures, leading to increased vulnerability of brain neurons [1]. Neurotoxicity is brought about by the oxidative metabolic pathways of catecholamines (CAM) which generate highly reactive quinones, superoxide radical (O_2^-) and H_2O_2, and by the GCs induced alterations in calcium metabolism [1]. Intracellular changes in ROS equilibrium are sensed by redox-sensitive transcription factors, such as nuclear factor κB (NF-κB) and glucocorticoid receptor (GR), which alter expression of antioxidant enzymes (AOE) and other stress responsive cellular enzymes. The extent of NES-triggered effects and the resulting neurotoxicity is related to the NES duration. Our previous results indicated that chronic social isolation is highly potent stressor which causes pulsed elevation of CAM and prolonged elevation of GCs concentration in Wistar male rat brain [2]. It is supposed that these conditions may alter redox *milieux* and alter expression of NFκB and GR in hippocampus and brain cortex [3] which is tested in this preliminary study.

Experimental

Adult Wistar rat males (2-3 months old, body mass 330-400g) were divided into four groups: Group I consisted of unstressed animals (control=Ctrl) kept four *per* cage; Group II was exposed to 4 °C for 2h as acute stressor (A); Group III was exposed to chronic isolation by individual housing for 21 day (C); Group IV was exposed to combined stress of chronic isolation plus acute cold (C+A). Hippocampus and cortex were homogenized in 20mM Na-phosphate buffer pH 7.0, 0.1mM EDTA-Na_2, 0.1mM EGTA-Na_2, 10% glycerol, 50mM NaCl, 1mM DTT, 1mM Na_2MoO_4, 1.5mM spermin, 1mM PMSF and 500μg/mL apro-

tinin. Cell nuclei and mitochondria were pelleted by centrifugation and supernatant was taken as cytosol. Cytosolic proteins were separated by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose and incubated with rabbit anti-NFκB (NFκB p65) or rabbit anti-actin antibody. A secondary goat anti-rabbit IgG-HRP conjugate was used for visualization of specific protein bands quantified by PC Imager. The data were presented as mean±S.D. (n=4). Analysis of variance (one-way ANOVA) and Tukey's post-hoc test were used to determine statistically significant differences (P<0.05).

Results and Discussion

Western blot (WB) quantification of redox-sensitive transcription factor NFκB in hippocampus and brain cortex of Wistar male rats revealed significant down-regulation of its p65 subunit after either chronic social isolation (C) or combined stress (C+A) *(Figure 1)*. The expression level of p65 was unchanged by the acute stress (A).

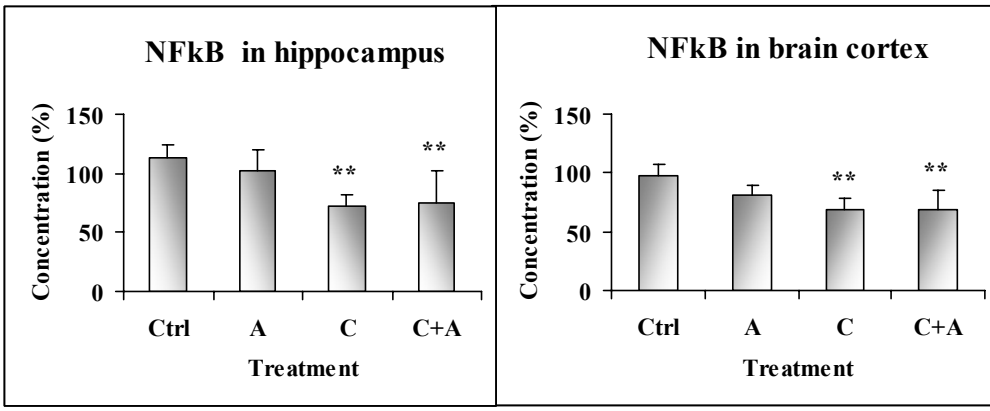

Fig. 1. NFκB in control (Ctrl) acute (A), chronic (C) and combined (C+A) stress. Results are presented as mean ± S.D (n=4)

Table 1. The expression level of NFkB and GR proteins in brain cell cytosol
Results are presented as mean ± S.D (n_{NFkB} =4, and n_{GR} =6)

Structure	HIPPOCAMPUS				BRAIN CORTEX			
Stress	*Ctr (%)*	*A (%)*	*C (%)*	*C+A (%)*	*Ctr (%)*	*A (%)*	*C (%)*	*C+A (%)*
NFκB	100	102±17	72±9**	74±27**	100	81±8	69±9**	69±17**
GR[3]	100	60±7***	89±5**	71±3***	100	60±5***	83±7	70±7

As both *in vitro* and *in vivo* NFκB p65 was found in association with GR, which led to their mutual transcriptional antagonism, the NES-triggered alterations in the expression of hippocampal and cortical NFκB may be better understood if compared with the respective expression of GR determined in the previous study *(Table 1)* [3]. The unaltered level of NFκB (p65) expression in the cytosol compartment after acute stress (A) is most probably

due to the GC-triggered nuclear translocation of GR and the repression of NFκB promoter. The cytosolic expression pattern of both NFκB and GR most probably reflects typical adaptive response to acute NES, resulting in successful restoration of cell homeostasis. Contrary to that the depletion of cytosolic NFkB after chronic isolation (C) may be linked to its translocation to the nucleus and activation of cellular defence mechanisms, particularly those related to oxidative stress response. One of the defence mechanisms involves CuZn-superoxide dismutase (CuZnSOD), the major cytosolic antioxidant enzyme whose expression is under NFkB control. CuZnSOD expression is shown to be up-regulated in chronic isolation [5]. Although CuZnSOD activity is primarily protective, if not coupled to the respective peroxidase activity, it may lead to accumulation of toxic H_2O_2. Moreover, H_2O_2 is a stimulator of NFkB activity, thus that it may trigger a positive feed-forward cycle with NFkB and enhance oxidative stress leading to neuronal cell death. In this way the price of cell accommodation to chronic stress may be paradoxal neurotoxicity. The incomplete nuclear translocation of cytosolic GR in chronic or combined NES *(Table 1)* may add to NFkB nuclear overactivity [3]. Taken together the results on NES exposed Wistar rats show that deregulation of the LHPA in chronic stress, affects also NFkB p65 expression level in cytosolic compartment. The lack of NFkB repression by GR might indicate that the brain cells will be continually experiencing NES-linked oxidative stress without resting, which, in a prolong period, may compromise their capacity to restore homeostasis and lead to cell energy exhaustion and cell death.

Conclusion

The cytosolic depletion of NFkB p65 found under chronic- and combined neuroendorine stress (NES) conditions most probably reflects its nuclear translocation leading to transactivation of oxidative stress protective enzymes, such as CuZnSOD. However, due to deregulated GR and its cytosolic retention in chronic and combined stress, nuclear NFkB may be uncoupled from its GR repression at more levels. The overactivity of NFkB may lead to disproportional CuZnSOD enhancement and cell accumulation of toxic H_2O_2 In that way, *via* NFkB derepression, NES may be converted to oxidative stress, and instead of cell protection NFkB may compromise cell capacity to restore homeostasis.

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