Research article

Study on the Early Neurological biomarkers in Adult Stressed Male Albino Rats

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Abstract
Blood brain barrier (BBB), as crucial gate of brain-blood molecular exchange, seems to be affected by oxidative stress inducers in early stages of different brain diseases. Therefore, the present study was done to investigate the effect of different types of stressors as immobilization restraint (IS), water immersion restraint (WIRS) with different timing (٣hours and ٦hours) and glucoprivation (GS) on the BBB permeability through evaluation of the serum and brain levels of specific permeability biomarkers such as S١٠٠B protein and Neuron Specific Enolase (NSE) with other stress parameters. Our results revealed that different stressors significantly increased the serum and brain levels of S١٠٠B protein and NSE. In addition, there were significant higher levels of catecholamines, corticosterone, glucose, nitric oxide and malondialdehyde (MDA). WIRS seems to produce more oxidative damage (more BBB disruption) than IS. This was evidenced by significant increase of the serum and brain levels of the different parameters; with WIRS as compared with corresponding IS groups. In addition, the metabolic disturbance including hyperglycemia was more evident in GS than IS and WIRS groups. In conclusion and according to our results, different stressors can disrupt BBB permeability and this disrupted permeability can be early evaluated by measuring specific neurological markers. Furthermore, the response to stress is time dependent.
Key words: S١٠٠B protein, Neuron Specific Enolase, immobilization restraint stress, water immersion restraint and glycoprivation.

Introduction
Specific brain regions can recognize stressors and then initiate many changes known as stress response. Stress responses are alterations in the behavioral, autonomic function and in the secretion of hormones. These appropriate physiological responses are important for survival. Maintenance of homeostasis during stress is the primary role of the hypophysial pituitary axis (HPA axis) and the sympatho-adrenomedullary system (SAS) (Nostramo et al., ٢٠١٢).

Blood–brain barrier (BBB) is the interface between the periphery of circulatory system and the central nervous system. The BBB forms a protective regulatory barrier that aims for neuronal microenvironment protection. BBB integrity is maintained by microvascular endothelial cells through their tight junctions (TJ) and basal lamina. TJ proteins are key structures that ensure the integrity of the BBB (Enciu et al., ٢٠١٣).

In addition to the protective BBB, cerebral endothelial cells are equipped with a defense system against oxidative stress including increased reduced glutathione (GSH), GSH peroxidase, GSH reductase and catalase compared to the rest of the brain. GSH in particular has been shown to play an important role in maintenance of BBB integrity (Freeman and Keller, ٢٠١١).

In addition, all areas of the healthy brain also consist of antioxidants such as superoxide dismutase (SOD) in order to provide a balance against the high concentration of reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced. Without this system, peroxy-nitrite can rapidly form due to high concentrations of nitric oxide (NO), parti-

cularly at the endothelial cells (Forstermann, ٢٠١٠).

On the other hand, there are a number of factors inherent to the brain and cerebral

endothelial cells that increase opportunities for oxidative stress. The high brain levels of peroxidizable polyun-saturated fatty acids and transition minerals (reduced form) that induced lipid peroxidation. Additionally, the brain's oxygen demand is particularly high and the presence of antioxidant defense mechanisms is relatively limited. It is known that lipid peroxide (LPO) level in the brain of rats is the highest among main nine tissues, i.e., brain, adrenal, liver, kidney, heart, lung, skeletal muscle, spleen, and testis, while the levels of GSH in the brain are relatively low among the nine tissues. A more recent and novel hypothesis is that brain injury could be caused by disruption of the BBB and altering the BBB permeability leads to an influx of inflammatory cells as well as toxic compounds, resulting in potential damage to several brain areas (Falcone et al., ٢٠١٤).

Many neurological biomarkers, synthesized by astraglial cells, including S١٠٠B protein, Neuron Specific Enolase (NSE) and Glial Fibrillary Acidic Protein (GFAP) are useful diagnostic tools in the diagnosis of functional brain disorders (Tomaszewski, ٢٠١٥). S١٠٠B protein, a calcium binding protein, is involved in glial-to-neuron signaling as a part of response of glial cell to stress. S١٠٠B expression and function is affected by glucocorticoids (GCs) and intense stress (Jauregui-Huerta et al., ٢٠١٠). Also, S١٠٠B is present in other tissues but in lower concentrations. S١٠٠B level increases in serum immediately after BBB disruption or brain (or neuronal) damage, but this increase is related to BBB disruption more than brain damage, so S١٠٠B is a potential early marker and increase in serum independent of brain damage (Bargerstock et al., ٢٠١٤).

Neuron-specific enolase (NSE) is acidic soluble protein of ٧٨-kDa. It is located predominately in the cytoplasm of neurons, which participates in slow axoplasmic transport, and in neuroectodermal cells. In a small amount, NSE is present in erythrocytes, blood platelets, plasmatic cells, lymphocytes, capillary walls, and myoepithelial cells, which explains its physiologically low concentrations in blood. NSE is not secreted normally, but when axons are damaged, NSE is up-regulated to maintain homeostasis. Therefore, NSE is the only marker that directly assesses functional damage to neurons (Cheng et al., ٢٠١٤). After BBB disruption or damage, NSE moves from plasma to CSF but this occurs more in case of damage more than with disruption, So conditions that cause opening of BBB without neuronal damage would expected to leave NSE level unchanged (Mrozek et al., ٢٠١٤).

Therefore, this study is an attempt to investigate the effects of different types of stressors as immbolization restraint stress (IS), water immersion restraint stress (WIRS) and glucoprivation stress (GS) on the BBB permeability neurological biomarkers and on the different stress parameters. In addition, an important question to this discussion is: If the timing of stress has obvious different effects on these parameters? To answer this question we selected ٣ and ٦ hours exposure timing for IS and WIRS.

Materials and Methods

***I*- Animals:**

Sixty adult male albino (Sprague dawley strain) rats, of average weight ٢٤٠-٣٤٠ gm, about ٤ months old were used in the present study. Rats were purchased from the National Research Center, Cairo, Egypt. Rats were housed in stainless steel cages at room temperature. They were left freely wandering in their cages for two weeks with normal hour's dark: light cycle for acclimatization before starting the experiment. They were allowed free access to tap water and normal rats' diet (El-Nile Company, Egypt). All experimental protocols were approved by the animal care committee of Minia University that coincides with international guidelines.

Rats were randomly classified into the following six groups:

١. Control (C) group: In which rats were left freely wandering in their cages at room temperature
٢. Three hours Immobilization stress (٣h-IS) group: In which each rat was immobilized on a wooden board by taping the four limbs with surgical tapes to a specially prepared metal mounts (Nostramo et al., ٢٠١٢) for ٣ hours (Stroth et al., ٢٠١٣) then immediately decapitated after stress was relieved.
٣. Six hours Immobilization stress (٦h-IS) group: In which each rat was immobilized for ٦ hours (Mona, ٢٠١٤) then immediately decapitated after stress was relieved.
٤. Three hours Water Immersion restraint stress (٣h-WIRS) group: In which each rat was immobilized on a wooden board by taping the four limbs with surgical tapes to a specially prepared metal mounts and immersed up to the depth of the xiphoid process in water bath at ٢٣°C for ٣ hours (Ohta et al., ٢٠١٢) then immediately decapitated after stress was relieved.
٥. Six hours Water Immersion restraint stress (٦h-WIRS) group: In which each rat was immobilized as in the above group but for ٦ hours (Ohta et al., ٢٠١٢) then immediately decapitated after stress was relieved.
٦. Glucoprivation Stress (GS) group: In which ٢-deoxy-d-glucose (٢-DG, ٥٠٠mg/kg) (Sigma, St. Louis, USA) was administered intraperitoneally (Wang and Whim, ٢٠١٣). Following injection of ٢-DG, food and water were removed from the cage. Animals were sacrificed by decapitation ١ hour later (Bobrovskaya et al., ٢٠١٠).

***II*- Biochemical analysis:**

At the end of all experiments and after an overnight fast, all rats were sacrificed by decapitation and blood samples were obtained from jugular vein. Blood samples were allowed to clot and then centrifuged and its supernatant serum was then withdrawn into labeled eppendorf tubes and stored at - ٢٠ °C till the time of assay of:

١. S١٠٠B and Neuron specific enolase (NSE) were measured using enzyme-linked immunosorbent assay (ELISA) kits (Wuhan El Arab Science co. Ltd) following the instructions of the manufacturer.

٢. Serum catecholamines, CAs (epinephrine, norepinephrine and dopamine) were determined using spectrophotoflurometric method. Oxidation of CAs in plasma is performed by addition of ٠.١ normal iodine followed by stoppage of oxidation by addition of alkaline sulfite to produce certain fluorescence. The induced fluorescence is measured at specific emission wave length after excitation at another specific wave length that differs according to the type of CAs; epinephrine, norepinephrine or dopamine. The intensity of the fluorescence produced is directly proportional to the concentration of CAs in the serum sample (Ciarlone, ١٩٧٨).

٣. Serum corticosterone was determined using spectrophotoflurometric method; the procedure essentially entails an extraction of free ١١-hydroxycorticosteroids from serum, mainly corisol and corticosterone, by methylene chloride followed by their condensation with an acidic fluorescence reagent. The induced fluorescence is measured at ٥١٠ nm after excitation at ٤٥٠ nm (Mattingly, ١٩٦٢).

٤. Serum Glucose and malon-dialdehyde (MDA) were determined using colorimetric assay kits according to the recommendations of the manufacturer (Biodiagnostic, Egypt).

***III*- Analysis of brain tissues:**

The skulls were opened carefully to take the brain dissecting it at different regions (hypothalamus and hippocampus) and it was then stored at -٢٠^{O}C until analyzed for measurement of PS١٠٠ and NSE using enzyme-linked immunosorbent assay (ELISA) kits (Wuhan El Arab Science co. Ltd), while Nitric oxide, and MDA were determined using colorimetric assay kits (Biodiagnostic, Egypt). In addition, brain CAs was determined spectrophoto-metrically (Ciarlone, ١٩٧٨).

Statistical Analysis

Data were represented as means ± standard errors of the mean (SEM). Statistical analysis was performed using Graph pad Prism ٥ software and significant difference between groups was done by one-way ANOVA followed by Tukey-Kramar post hoc test for multiple comparisons with a value of $P \leq ٠.٠٥$ considered statistically significant.

Results

The results clearly demonstrated that exposure of the rats to IS (٣h and ٦h) produced a significant higher levels of the serum (fig ١) and brain (tab ١, ٢) S١٠٠B and NSE as compared to that of corresponding C group. It was also found that the exposure to IS significantly increased the serum (tab ٣) and brain (tab ١, ٢) levels of CAs and MDA. In addition, there was a significant higher level of brain NO (tab ١, ٢) and serum corticosterone and glucose (tab ٣). Our results showed that with ٦h-IS group, the increased serum and brain levels of all parameters were significant higher as compared to the corresponding ٣h-IS group.

The results of the present study revealed that exposure the rats to WIRS (٣h and ٦h) caused significant higher levels of the serum (fig ١) and brain (tab ١, ٢) S١٠٠B protein and NSE as compared to that of corresponding C group. In addition, we were found that WIRS significantly increased the serum (tab ٣) and brain (tab ١, ٢) levels of CAs and MDA. There was also a significant increase in the brain level of NO (tab ١, ٢) and serum levels of corticosterone and glucose (tab ٣). Exposure of rats to ٦h-WIRS induced a remarkably higher serum and brain levels of all parameters were significant as compared to the corresponding ٣h-WIRS.

As regard effect of ٣h-WIRS vs. ٣h-IS as well as ٦h-WIRS vs. ٦h-IS, exposure the rats to WIRS caused a significant increase in the serum and brain levels of all parameters as compared with its corresponding time of IS. On the other hand, serum and brain levels of MDA and NO with ٣-h and ٦-h WIRS showed insignificant difference as compared with its corresponding time of IS.

In comparison to the control groups, GS group showed significant higher levels of serum (fig ١) and brain (tab ١, ٢) S١٠٠B protein, NSE. . In addition, it was found that GS significantly increased the serum (tab ٣) and brain (tab ١, ٢) levels of CAs and MDA. There was also a significant higher levels of brain NO (tab ١, ٢) and serum corticosterone and glucose (tab ٣).

Table (١): shows the effect of different types of stressors on the hypothalamic neurological biomarkers, catecholamines and oxidative markers:

Groups / Parameters		C	IS		WIRS		GS
			٣h-IS	٦h-IS	٣h-WIRS	٦h-WIRS	
Neurological biomarkers	S١٠٠B (pg/mg)	٦٩.٣±١.٥	٣٣٨.٧±١.٢^{a}	٣٤٧.١±١.٣^{ab}	٣٥٨.٦±٠.٧^{a*}	٣٦٦.٤±٠.٣^{ab*}	٣٣٥.٠±١.٩٨^{a}
	NSE (pg/mg)	٦٤±٠.٠٢	٥٤٧±٠.٩^{a}	٥٥١.٨±٠.٨^{ab}	٥٦٩.١±٠.٦^{a*}	٥٧٤±٠.٧^{ab*}	٥٥٤.٢±٠.٦^{a}
Cate-cholamines	E (ng/mg)	٢٠.٤±٠.٤	١٠٥.٨±٢.٣^{a}	١٢٧.٩±٠.٧^{ab}	١٣٦.٧±٠.٨^{a*}	١٦٥.٤±١.٤^{ab*}	١٠٤.٢±٠.٣٦^{a}
	NE (ng/mg)	١٣.٩±٠.٦	٧٨±١.٣^{a}	٩٧.٦±٠.٣^{ab}	٩٨.١±٠.٧^{a*}	١٠٦.٩±١.٧^{ab*}	٨٨.٢±٠.٥٠^{a}
	D (ng/mg)	٤.٣±٠.١٢	٥٧.٢±٠.٦^{a}	٧٤.٦±٢.٠٣^{ab}	٨١±٠.٥^{a*}	٨٤.٢±١.٩٧^{a*}	٦٧.١±١.٥^{a}
Oxidative markers	MDA (nmol/mg)	١.٦±٠.٠٠٢	٣.٧±٠.٠٠١^{a}	٤.١±٠.٠٠٢^{ab}	٣.٧±٠.٠٠١^{a}	٤.١±٠.٠٠١^{ab}	٣.٦±٠.٠٠١^{a}
	NO (µmol/mg)	٠.٧±٠.٠١	١.٧±٠.٠٢^{a}	٢.٦±٠.٠٢^{ab}	١.٩±٠.٠٢^{a}	٢.٩±٠.٠٠٢^{ab}	١.٧±٠.٠٢^{a}

Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group (C), b: Significant from the corresponding ٣hour group,*: Significant of WIRS from the corresponding period of IS group, P < ٠.٠٥. IS: Immobilization stress; WIRS: Water Immersion restraint stress; GS: Glucoprivation stress; NSE: Neuron-specific enolase, E: Epinephrine; NE: Norepinephrine; DA: Dopamine; MDA: Malondialdehyde; NO: Nitric oxide.

Table (٢) shows the effect of different types of stressors on the hippocampal neurological biomarkers, catecholamines and oxidative markers:

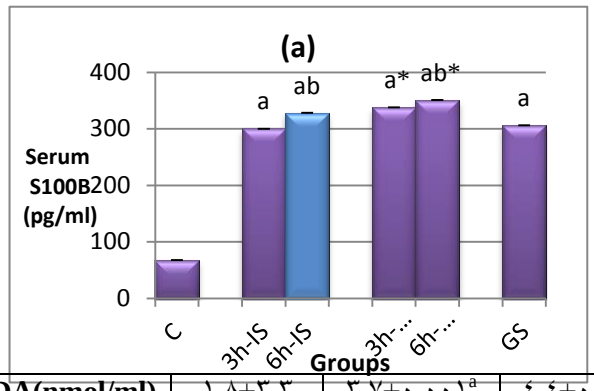
Groups / Parameters		C	IS		WIRS		GS
			٣h-IS	٦h-IS	٣h-WIRS	٦h-WIRS	
Neurological biomarkers	S١٠٠B (pg/mg)	٨٠.٤±٠.٤	٣٤٩.٤±٠.٦^{a}	٣٥٧.٢±١.١^{a} b	٣٨٩.٤±٠.٥^{a} *	٣٩٩ ±٢.٥^{ab*}	٣٤٤.٢±١.٤ a
	NSE (pg/mg)	٥٥.١±٠.٠ ١	٥٤٢.٧±١.٠٤ a	٥٤٩.٥±٠.٧^{a} b	٥٦٧.٩±٠.٩^{a} *	٥٧٠±١.٠٢^{ab*}	٥٤٧.٧±٠.٩ a
Catecholamin	E(ng/mg)	٣٩.٨±٠.٧	١١٧±٠.٧^{a}	١٣٢.٤±١.٢^{a} b	١٤٣.٧±٠.٨^{a} *	١٧٥±١.٥^{ab*}	١١٠.٨±٠.٦ a
	NE (ng/mg)	٢٣.٧±١.٦	٨٥.٤±١.٩^{a}	٨٨.٤±٠.٧^{a}	٩٧.٤±٠.٣^{a*}	١١٨.٥±٠.٥^{ab} *	٨٧.٣±٠.٧^{a}
	D (ng/mg)	١١.٩±٠.٨	٦٨.٧±٠.٣^{a}	٧٦.٥±٠.٩^{ab}	٨٠.٥±٠.٤^{a*}	٩٢.٦±٠.٦^{ab*}	٦٣.٦±١.٥١ a
Oxidative markers	MDA (nmol/mg)	١.٤±٠.٠٠ ٢	٣.٩±٠.٠٠١^{a}	٤.١±٠.٠٠١^{a} b	٣.٩±٠.٠٠١^{a}	٤.١±٠.٠٠١^{ab}	٣.٥±٠.٠٠٢ a

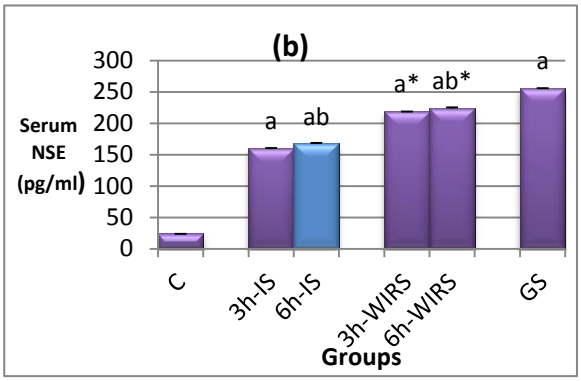
	NO (µmol/mg)	٠.٧±٠.٠٠١	١.٨±٠.٠٠٢[a]	٢.٨±٠.٠٢[ab]	١.٩±٠.٠١[a]	٢.٩±٠.٠١[ab]	١.٧±٠.٠٠٢[a]

Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group (C), b: Significant from the corresponding ٣hour group,*: Significant of WIRS from the corresponding period of IS group, P < ٠.٠٥. IS: Immobilization stress; WIRS: Water Immersion restraint stress; GS: Glucoprivation stress; NSE: Neuron-specific enolase, E: Epinephrine; NE: Norepinephrine; DA: Dopamine; MDA: Malondialdehyde; NO: Nitric oxide.

Table (٣): shows the effect of different types of stressors on serum catecholamines, corticosterone, glucose and Malondialdehyde:

Groups / Parameters	C	IS		WIRS		GS
		٣h-IS	٦h-IS	٣h-WIRS	٦h-WIRS	
E (ng/ml)	٧٩.٢±٠.٧٥	١٩٩.٥±٠.٦٤[a]	٢١٢.٥±٢.٠٢[ab]	٢١١±١.٤١[a*]	٢٢٢.٥±١.٠٤[ab*]	٢١٠.٨±٠.٨٥[a]
NE (ng/ml)	٦٨.٧±١.١	١٨٧.٥±١.١٩[a]	١٩٦.٠±٠.٤٠[ab]	١٩٨.٣±٠.٦٢[a*]	٢١٤.٥±١.٩٣[ab*]	١٧٤±٠.٤٠[a]
DA (ng/ml)	٥٩.٢±١.٣٧	١١١±١.٥٨[a]	١٢٧±١.٧٨[ab]	١٢٥.٣±٢.١٧[a*]	١٣٢.٨±١.٦٥[ab*]	٩٧±٢.١٩[a]
Corticosterone (µg/ml)	٤٠.٨±٠.٨٣	١٣٨.٩±٠.٣[a]	١٤٢.٧±٠.٣[ab]	١٤٤.١±٠.٢[a*]	١٥١.٤±٠.٣[ab*]	١٥٠.٧±٠.٥[a]
Glucose (mg/dl)	٩٦±٦.٥٩	٢٢٣.٢±١.٩[a]	٢٥٧.٧±٢.٥[ab]	٢٦٢.٣±١.٦[a*]	٢٧٦.٩±٠.٥[a*]	٣١٦.١±١٩.٧٥[a]
MDA(nmol/ml)	١.٨±٣.٣	٣.٧±٠.٠٠١[a]	٤.٤±٠.٠٠١[ab]	٣.٨±٠.٠١[a]	٤.٥±٠.٠٠١[ab]	٣.٣±٠.٠٨[a]





Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group (C), b: Significant from the corresponding ٣hour group,*: Significant of WIRS from the corresponding period of IS group, P < ٠.٠٥. IS: Immobilization stress; WIRS: Water Immersion restraint stress; GS: Glucoprivation stress; E: Epinephrine; NE: Norepinephrine; DA: Dopamine; MDA: Malondialdehyde.

Figure (١) shows the effect of different types of stressors on the serum level of S١٠٠B protein (a) and NSE (b). Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group (C), b: Significant from the corresponding ٣hour group,*: Significant of WIRS from the corresponding period of IS group, P < ٠.٠٥. IS: Immobilization stress; WIRS: Water Immersion restraint stress; GS: Glucoprivation stress; NSE: Neuron-specific enolase.

Discussion

Stress is a highly adaptive response to a state of disturbed homeostasis due to internal or external sources such as physical, metabolic or psychological stimuli which are known as stressors (Loi et al., ٢٠١٤).

The BBB excludes from the brain about ١٠٠٪ of large-molecule neurotherapeutics and more than ٩٨٪ of all small-molecule drugs. The difficulty of delivering therapeutic agents to specific regions of the brain presents a major challenge to treatment of most brain disorders. In its neuroprotective role, the BBB functions to hinder the delivery of many potentially important diagnostic and therapeutic agents to the brain. Therapeutic molecules and antibodies that might otherwise be effective in diagnosis and therapy do not cross the BBB in adequate amounts (Xiong et al., ٢٠١٥).

The main functions of BBB are determined by its specialized multi-cellular structure. But if one member of the BBB fails, and as a result the barrier breaks down, there can be loss of integrity of the BBB, dramatic consequences, neuro-inflammation and neuro-degeneration. This was referred as BBB disruption (Obermeier et al., ٢٠١٣). BBB integrity or disruption can be evaluated by many markers as S١٠٠B protein that have been reported to rise prior to any detectable changes in intra-cerebral pressure, neuro-imaging, and neurological examination findings (Bargerstock et al., ٢٠١٤).

These markers of brain damage are ideal because they have the following characteristics: (١) highly specific; (٢) highly sensitive; (٣) released in only cases of irreversible damage to cerebral neurons; (٤) detectable in the blood and/or cerebrospinal fluid within a short period of time after the injury; (٥) age- and sex-independent; and (٦) its concentration is easily measureable in laboratory tests. These biomarkers are released due to altered BBB permeability so that elevated serum and brain concentrations of these markers indicate a neuronal and/or glial injury (Tomaszewski, ٢٠١٤).

These data mentioned above is in accordance with our present study that different types of stressors can disrupt BBB permeability through opening of TJs and increase the paracellular permeability of the BBB. In addition, different stressors disrupt "enzymatic blood brain barrier" resulting in increased brain and serum levels of neurotransmitters, biomarkers and other stress related parameters. The results of the present study provide an evidence for the possible role of oxidative stress, through NO and ROS, in disrupted BBB permeability. This was evidenced by increased brain NO levels as well as increased serum and brain levels of MDA, with different stressors, as compared with corresponding control groups.

Between the perception of threat and the autonomic response to it, a complex intervening circuitry sets the sensitivity and gain of the stress response. Neuropeptides are employed as transmitters in this circuitry. A new picture of stress circuitry has emerged, in which catecholamine and neuropeptide systems are intimately intercalated, both centrally and peripherally, during response to both systemic and psychogenic stress. This neurochemical and anatomical integration allows responses to acute stressors to be translated into long-term changes. Stressors are perceived as threats to homeostasis by limbic and hypothalamic circuits whose final output is activation of the HPA and hormonal

sympathetic adrenal axes, or the sympathetic nervous system (SNS) (Eiden, ٢٠١٣).

WIRS consists of immobilization/restraint stress and a kind of cold stress in which the exposed temperature is usually around ٢٣°C (Kaida et al., ٢٠١٠). Ohta et al., (٢٠١٢) reported that exposure of rats to WIRS for ٣ to ٦ h causes oxidative damage associated with disruption of non-enzymatic antioxidant defense systems and enhanced lipid peroxidation (LPO) and NO generation. These data were in accordance with findings of the present data.

IS is one of stress models that represents an easy and convenient method to induce both psychological (escape reaction) and physical stress (muscle work) resulting in restricted mobility and aggression. Acute IS induces oxidative stress in the brain of rats, which might be involved in stress-induced neuro-behavioural alterations. Data reported that exposure of rats to ٣ and ٦ h of IS causes a decrease in GSH level and an increase in LPO level and NO level in the brain (Méndez-Cuesta et al., ٢٠١١). These results come in line with our findings.

The IS has the ability to stimulate the SAS which is the main source of circulating CAs, in addition to its direct stimulatory effect on CAs synthesizing enzymes (Renquist et al., ٢٠١٢) as well as activation of HPA axis which in turn results in ACTH secretion from corticotropes and finally CORT release from the adrenal cortex (Rostamkhani et al., ٢٠١٢; Garcia-Iglesias et al., ٢٠١٣); findings which were confirmed by the results of the present study.

Different Stressors activate HPA and SAS so altering neurological functions at both central and peripheral level. Acute stress influences significantly behavioral functions and oxidative damage in the many areas of the brain. Activation of the SAS leads to CAs release from nerves and the adrenal medulla. Secretion of the CAs is a part of the “fight or flight” response. Activation of HPA axis in turn results in corticotrophin secretion from adeno-hypophysis and finally corticosterone, as the main GCs in rodents, is released from the adrenal cortex. Secretion of CAs stimulates hepatic glycogenolysis and increases the basal metabolic rates and productions of glucose (Rostamkhani et al., ٢٠١٢). Also, GCs are one of the hyperglycemic hormones that induce liver gluconeogenesis resulting in elevation of blood glucose level (Popovic and Pajovic, ٢٠١٠). These findings were confirmed by the results of the present study.

Most neurotransmitters present in the blood do not enter the brain because of their low lipid solubility and lack of specific transport carriers in the capillary endothelial cell. Capillary endothelial monoamine oxidase may also play a role in the inactivation of neurotransmitters released by neuronal activity. The brain endothelial capillary also contains a variety of other neurotransmitter-metabolizing enzymes such as cholinesterases, GABA transaminases, aminopeptidase and endopeptidases which form "enzymatic blood brain barrier" which protects the brain not only from circulating neurotransmitters but also from many toxins. Stress can disrupt this "enzymatic blood brain barrier" leading to increased brain levels of many transmitters. In addition, glucocorticoids amplify the effects of early catecholamine release by slowing their clearance from synaptic space (Raioa and Phelps, ٢٠١٤).

In the current study, ٢- DG was used to evoke Glucoprivation. ٢-DG is a non-metabolisable glucose analogue that is transported into cells but does not undergo glycolysis providing an environment of low effective concentrations of glucose. Its peripheral infusion produces neuro-glucopenia, which induces activation of hypothalamic glucoreceptors with a consequent rise in the sympathetic output to the liver, pancreas, adrenal medulla and adipose tissue resulting in increased hepatic production of glucose, inhibition of insulin secretion and mobilization of free fatty acids from adipose tissue (Rowley and Patel, ٢٠١٣).

The dose of ٢-DG, used in this study, was based on previous studies showing its maximal effect on catecholamine release

and activation of catecholamine biosynthetic enzymes. In the present work, GS group showed significantly higher serum CAs, coticosterone and glucose levels in comparison with C group. This could be attributed to the nature of GS as a metabolic stressor and its activation of HPA and this is in agreement with the finding of Wang and Whim (٢٠١٣).

In GS group, serum glucose level was significantly higher in comparison with the control group. GS induces a range of counter regulatory mechanisms including the release of epinephrine and glucagon to promote hepatic glycogenolysis in order to restore glucose homeostasis. These responses may be mediated in part via the sympathetic nervous system and CAs release (Parker et al., ٢٠١٣).

In conclusion, not all stressors provoke the same response. In addition to increased oxidative stress, some stressors are associated with preferential activation of SNS and HPA axis such as IS and WIRS which was evidenced by increased serum and brain levels of noradrenaline, while other stressors preferentially stimulate the adrenal medulla to release CAs such as GS which was evidenced by increased serum and brain adrenaline levels. The response to stress is time dependent. This was evident by significant increased levels of different parameters with ٦h-IS and ٦h-WIRS as compared with their corresponding ٣h-IS and ٣h-WIRS. WIRS seems to produce more oxidative damage (more BBB disruption) and more activation of SNS and HPA axis than IS. This was evidenced by significant increase of serum and brain levels of different parameters, with WIRS; as compared with corresponding IS groups. Metabolic disturbance including hyperglycemia was more evident in GS than IS and WIRS groups, suggesting biological variations in the adrenal gland response under different stress conditions.

The positive correlation observed between plasma CAs (specifically epinephrine) and coticosterone levels indicates the presence of cross talks between adrenal medulla and cortex which may play a critical role in the regulation of biological activity of adrenal gland under different conditions. In addition, increased serum and brain levels of CAs indicate increase their passage through disrupted BBB as well as presence of central source of CAs.

Despite our findings, this study has several limitations. Firstly, administration of drugs or substances that can reverse the oxidative damage and studying their effect on serum and brain levels of BBB permeability biomarker and if these drugs can protect brain against oxidative stress destructive effects. Another limitation of the current study was that the focus on the effect of acute vs. chronic stress exposure on BBB permeability biomarkers and studies the relation between peripheral and central CAs. This will open the door for further future studies to complete this study.

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