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Biological variations in adrenal gland response to immobilization and glucoprivation stressors in rats

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Objectives. The aim of the present study was to investigate the potential variation in adrenal gland response to two different types of acute stressors, immobilization and glucoprivation.

Methods. Twenty-four adult male albino rats were randomly divided into three main groups (8 rats/group): a) control, i.e. non-stressed group, b) immobilized group (IS), and c) glucoprivated (GS) group. Plasma catecholamines (CAs), including epinephrine (E), norepinephrine (NE), dopamine (DA), adrenocorticotrophic hormone (ACTH), corticosterone (CORT), sodium, potassium, and glucose were measured. Adrenals weight, CAs levels, and nitric oxide (NO) content were also determined.

Results. Immobilized group of rats showed significantly higher plasma NE and DA levels along with a significantly lower adrenal NE content than GS group. On the other hand, GS group was associated with significantly higher plasma E, ACTH, CORT, glucose, and Na^+ levels as well as higher adrenal DA and NO levels along with significantly lower plasma K^+ levels and adrenal E content in comparison with IS group.

Conclusion. Stress response is unique according to the nature of the stressor. Adrenal glands play a key role in this stress-induced differentiated response probably via modulation of its adrenomedullary and/or adrenocortical hormone levels in order to assign the body cope with different types of stress challenges during the life.

Key words: adrenal gland, immobilization stress, glucoprivation stress

The adrenal gland is the primary peripheral endocrine gland in the sympathetic stress response (Petrovic-Kosanovic and Koko 2012). It is also the common downstream organ of the hypothalamic-pituitary-adrenal (HPA) axis and locus coeruleus-norepinephrine/sympathetic-adrenal medulla (LC/NE) axis, which are critical for the stress response (Zeng et al. 2013).

The major endocrine functions of adrenal gland are to produce catecholamines (CAs) and steroids. CAs synthesized by the adrenal medulla are responsible for blood pressure and blood flow regulation whereas steroids, produced by the cortex, control energy and water homeostasis as well as immune responses (Huang et al. 2012).

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 (Joels et al. 2007). Appropriate physiological responses to stress are important for survival. The HPA axis and the sympatho-adrenomedullary system (SAS) are the primary systems that are responsible for the maintenance of homeostasis during stress (Nostramo et al. 2012). The adrenal gland is an essential organ that is common to both systems (Ulrich-Lai et al. 2006; Rabasa et al. 2011).

Stressful stimuli evoke complex endocrine, autonomic, and behavioral responses that are extremely variable and

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specific depending on the type and nature of the stressors and duration of exposure. There are also different patterns of sympatho-neural, sympatho-adrenomedullary (SA) and HPA responses to different stressors. Some studies have demonstrated that even two branches of SAS can be independently activated by different stressors. Data suggest that gene expression could be specifically affected by different stressors. The observed dissociation in responses of plasma E and NE levels as well as tyrosine hydroxylase (TH) and phenylethanolamine-N-methyltranferease (PNMT) gene expression during various stressors demonstrates that different pathways control specifically the responses of sympathetic nervous, adrenomedullary, and brain norepinephrinergic systems during stress (Kvetnansky and Sabban 2006). However, the role of adrenal gland in this differential response to stress is not fully understood and needs further clarification.

Therefore, the aim of the present study was to investigate the potential variation in adrenal gland response to different models of stress, using two types of stressors, immobilization stress (an example of psychological stress) and glucoprivation stress (a model of metabolic stress) in adult male albino rats.

Materials and Methods

Animals. Adult male albino (Sprague-Dawley strain) rats weighing 250-300 g were used in the present study. Rats were purchased from the National Research Center, Cairo, Egypt. Animals were housed in staleness steel cages (2 rats/cage) at room temperature with normal hour's dark: light cycles for two weeks acclimatization to laboratory conditions (Kondo et al. 2013). Rats were fed a standard diet of commercial rat chow and tap water *ad libitum* throughout the time of the experiment (Popovic and Pajovic 2010). All experimental procedures were in accordance with our institutional guidelines. The ethics protocol was approved by The Laboratory Animals Maintenance and Usage Committee of Faculty of Medicine at Minia University.

Experimental design. Rats were randomly divided into the following groups (8 rats in each) designated as: 1) Control non-stressed group (in which rats freely moved in their home cages); 2) Immobilization-stress (IS) group (in which each rat was immobilized on a wooden board by fixing the four limbs with surgical tapes to a specially prepared metal mounts for 3 h (Nostramo et al. 2012) and then immediately sacrificed); and 3) Glucoprivation stress (GS) group (in which each rat received a single injection of 2-DG (500 mg/kg, intraperitoneally) (Sigma, St. Louis, USA). Following injection, food and water were removed from the cage. One hour later, animals were sacrificed (Bobrovskaya et al. 2010).

Blood sample collection and storage. At the end of the experiment and just before sacrification, all rats were anesthetized by light ether anesthesia. Blood samples were obtained by cardiac puncture collected in tubes containing heparin as anticoagulant and then centrifuged at 3000 rpm for 15 min in a cooling centrifuge (Hettich centrifuge). The supernatant plasma was collected in labeled Eppendorf tubes and stored at -20°C for further analysis.

Removal and storage of adrenal glands (Sfikakis et al. 2008). The rats were sacrificed immediately after collecting the blood samples. Both suprarenal glands were removed, trimmed of surrounding fat and tissues, and weighed. To prevent possible dehydration, cleaning of the gland was performed on filter paper saturated in 0.9% NaCl solution and stored at -80°C for determination of CAs and nitric oxide (NO) contents.

Determination of plasma E, NE, and DA. Plasma CAs were determined spectro-photoflurometrically as previously described by Ciarlone (1978) using spectroflurometer (Shimadzu RF-5000, Japan), based on the oxidation of CAs in the sample by addition of 0.1 ml normal iodine followed by stoppage of the reaction by addition of alkaline sulfite to produce certain fluorescence. The induced fluorescence was measured at specific emission wavelength after excitation at another specific wavelength that differs according to the type of CAs. The intensity of the fluorescence produced is directly proportional to the concentration of CAs in the sample (Ciarlone 1978).

Determination of plasma CORT. Plasma CORT level was determined as previously described by Mattingly (1962). This method measures the free 11-hydroxycorticosteroids in plasma, mainly cortisol and CORT, as the principal circulating glucocorticoid (GC) in rats is CORT (Bush and Sandberg 1953). Briefly, the procedure essentially entails an extraction of free 11-hydroxycorticosteroids from plasma by methylene chloride followed by their condensation with an acidic fluorescence reagent. Careful timing is necessary to keep the interference of nonspecific fluorescence at low and uniform level. The induced fluorescence is measured at 510 nm, after excitation at 450 nm.

Determination of plasma ACTH. Plasma ACTH level was determined by enzyme linked immunosorbant assay (ELISA) technique (Ganong et al. 1974) using ACTH kit (DRG international Inc., USA) following manufacturer's instructions.

Determination of plasma Na^+ level. Plasma Na^+ level was measured using commercial sodium kit (Bio-diagnostic,

Egypt). It depended on the reaction of sodium ions with excess uranyl acetate and magnesium acetate forming residual uranyl acetate. The later reacts with potassium ferrocyanide producing a colored complex, which can be measured colorimetrically and the absorbance varies inversely with Na^+ concentration in the sample (Trinder 1951).

Determination of plasma K^+ level. Plasma K^+ level was measured using commercial potassium kit (Bio-diagnostic, Egypt). Potassium ions in protein-free filtrate react with sodium tetraphenyl boron forming colloidal solution, which can be measured colorimetrically. The procedure compares well with flame photometric analysis (Sunderman and Sunderman 1958).

Determination of plasma glucose level. Plasma glucose level was determined by enzymatic colorimetric method using commercial kits (Biodiagnostic, Egypt). This method is based on the enzymatic oxidation of glucose in the presence of glucose oxidase (GOD). The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminoantipyrine to form a red violet quinoneimine dye, the absorbance of which can be measured spectrophotometrically at 546 nm (Trinder 1969; Tietz 1995).

Determination of adrenal content of CAs. Equal samples from both adrenals of each rat were weighed and homogenized together in a conical glass homogenizer tube containing 5 ml of an acidified solution of n-butanol and submerged in ice. Then, the crude homogenate was centrifuged at 1000 rpm in a cooling centrifuge (Hettich centrifuge) for 5 min. 2.5 ml of the supernatant was transferred to a glass tube containing 1.6 ml of 0.2 N acetic acid and 5 ml n-heptane. The content of the tube was thoroughly mixed for 30 s using vortex mixer (Vortex-Genie Scientific Industries Inc., USA) and then centrifuged in a cooling centrifuge (Hettich centrifuge) at 2000 rpm for exactly 5 min. The supernatant organic layer was discarded and one ml of the remaining fluid was transferred to another tube for determination of adrenal CAs contents (E, NE, and DA) spectrophotoflurometrically as previously described (Ciarlone 1978).

Determination of NO in the adrenal gland. Adrenal NO content was determined by using the colorimetric assay kit (Bio-diagnostic, Egypt) for determination of total nitrite (NO_2) as an indicator of endogenous NO production. It depends on the addition of Griess reagent, which converts nitrite into a deep purple azo compound. Photometric measurement of the absorbance due to this azo chromophore accurately determines NO_2 concentration (Montgomery and Dymock 1961).

Equal samples from both adrenals were weighed and then homogenized in 1 ml cold phosphate buffered saline (PBS), centrifuged at 4000 rpm for 15 min at 4°C. The supernatants were taken immediately for NO assay.

Statistical analysis. All the data were expressed as means ± standard errors (SEM). Data were analyzed using one-way analysis of variance (ANOVA) with repeated measurements. All the statistical analyses were performed using general linear model procedure (SAS Institute Inc., NC, USA, 2003). Significant differences among groups were detected using Duncan's multiple rang test (1955) with a value of $p \leq 0.05$ considered statistically significant.

Results

Effect of different types of stressors on plasma CAs levels. Data presented in Table 1 show that plasma E was significantly ($p<0.001$) higher in GS group than in both C and IS groups. On the other hand, plasma levels of NE and DA were significantly higher in IS group than both C and GS groups (Table 1).

Table 1
Effect of different types of stressors on plasma catecholamines

Parameter	C	IS	GS	p
E (ng/ml)	55.24±0.87[c]	145.52±1.18[b]	153.32±0.88[a]	
% from C	-	+163.43	+177.55	***
NE (ng/ml)	114.84±0.62[c]	135.29±0.89[a]	125.37±0.86[b]	
% from C	-	+17.81	+9.17	***
DA (ng/ml)	35.63±0.56[c]	54.43±1.04[a]	45.55±0.98[b]	
% from C	-	+52.76	+27.84	***

Data are expressed as mean ± SEM of 8 rats in each group. Means in the same horizontal row with different superscripts ([a, b, c]) are significantly different ($p<0.05$). ***$p<0.001$

C – control; IS – immobilization stress; GS – glucoprivation stress; E – epinephrine; NE – norepinephrine; DA – dopamine

Table 2
Effect of different types of stressors on plasma adrenocorticotrophic hormone and corticosterone

Parameter	C	IS	GS	p
ACTH (pg/ml)	63.75±0.81[c]	85.43±0.86[b]	95.37±1.06[a]	
% from C	-	+34.01	+49.60	***
CORT (µg/ml)	13.96±0.65[c]	36.48±0.89[b]	45.38±0.85[a]	
% from C	-	+161.32	+225.10	***

Data are expressed as mean ± SEM of 8 rats in each group. Means in the same horizontal row with different superscripts ([a, b, c]) are significantly different ($p<0.05$). ***$p<0.001$
C – control; IS – immobilization stress; GS – glucoprivation stress; ACTH – adrenocorticotrophic hormone; CORT – corticosterone

Table 3
Effect of different stressors on plasma glucose, Na+ and K+ levels

Parameter	C	IS	GC	p
Glucose (mg/dl)	94.50±0.98[c]	133.42±0.85[b]	254.05±1.10[a]	
% from C	-	+41.19	+168.84	***
NA^+ (mmol/l)	139.55±0.85[c]	154±1.03[b]	166.25±0.75[a]	
% from C	-	+10.35	+19.13	***
K^+ (mmol/l)	4.30±0.06[a]	3.47±0.07[b]	3.19±0.02[c]	
% from C	-	-19.30	-25.81	***

Data are expressed as mean ± SEM of 8 rats in each group. Means in the same horizontal row with different superscripts ([a, b, c]) are significantly different ($p<0.05$). ***$p<0.001$
C – control; IS – immobilization stress; GS – glucoprivation stress

Table 4
Effect of different stressors on adrenal weight and catecholamines content

Parameter	C	IS	GS	p
Weight (mg)	59.06±2.26[a]	62.27±2.36[a]	57.57±1.96[a]	
% from C	-	+5.44	-2.52	-
E (µg/g)	459.34±0.61[a]	436.44±0.87[b]	420.1±0.87[c]	
% from C	-	-4.99	-8.54	***
NE (µg/g)	159.47±0.60[a]	149.88±0.87[b]	159.68±0.88[a]	
% from C	-	-6.01	-0.13	***
DA (µg/g)	49.59±0.59[c]	60.49±0.88[b]	68.99±0.86[a]	
% from C	-	+21.98	+39.12	***

Data are expressed as mean ± SEM of 8 rats in each group. Means in the same horizontal row with different superscripts ([a, b, c]) are significantly different ($p<0.05$). ***$p<0.001$
C – control; IS – immobilization stress; GS – glucoprivation stress; E – epinephrine; NE – norepinephrine; DA – dopamine

Effect of different types of stressors on plasma ACTH and CORT levels. As shown in Table 2, a plasma level of ACTH and CORT were significantly higher in GS group than in both C and IS groups (Table 2).

Effect of different types of stressors on plasma glucose, Na^+ and K^+ levels. Table 3 shows that GS group had significantly higher plasma glucose and Na^+ along with significantly lower K^+ levels than in both C and IS groups (Table 3).

Effect of different stressors on adrenal weight and CAs contents. Data presented in Table 4 show the effect of IS and GS on total adrenal weight and CAs content in

Table 5
Effect of different types of stressors on adrenal nitric oxide content

Parameter	C	IS	GS	p
NO (nmol/g)	74.08±0.83[c]	85.61±0.86[b]	95.11±0.84[a]	
% from C	-	+15.56	+28.39	***

Data are expressed as mean ± SEM of 8 rats in each group. Means in the same horizontal row with different superscripts ([a, b, c]) are significantly different ($p<0.05$). ***$p<0.001$
C – control; IS – immobilization stress; GS – glucoprivation stress; NO – nitric oxide

comparison with the C group (Table 4). Both IS and GS groups showed no change in adrenal weight as compared with that of the C group (Table 4).

Regarding the adrenal CAs content, GS group showed significantly lower adrenal E along with significantly higher DA contents/g tissue than in both C and IS groups. On the other hand, adrenal NE content/g tissue was significantly lower in IS group than in both C and GS groups (Table 4).

Effect of different stressors adrenal NO content. Data presented in Table 5 show that adrenal NO content was significantly ($p<0.001$) higher in GS group than in both C and IS groups (Table 5).

Discussion

The results of the present study prove the existence of biological variation in adrenal gland response according to the type of stressor. Some stressors are associated with preferential activation of the SNS and release of NE such as emotional stressors, as in case of IS, while other stressors preferentially stimulate the adrenal medulla to release E, as in case of GS. The stress-induced consequences including metabolic and electrolyte disturbances also varied in each type of stressor tested, as it was more evident in GS than IS group.

It is quite evident that not all stressors provoke the identical package of responses. Some stressors provoke the strongest reaction of adrenocortical activity than that of adrenomedullary, while others do the opposite (Cvijic and Dordevic 2003). Each type of stressor has its own central neurochemical and peripheral neuroendocrine signature with quantitative and qualitative distinct mechanisms and the adrenal gland seems to play a key role in these distinctive responses to different stressors (Kvetnansky 2004; Kvetnansky et al. 2009).

In the present study, IS produced insignificant change in the total adrenal glands weights as compared with that of the control group. This is in agreement with Rostamkhani et al. (2012) who have shown that acute stress has no significant effect on adrenal weight as the increase in adrenal weight represents one of the adaptive mechanisms, which occur more frequently in chronic stress, which was not the case in the present study.

As regards biochemical changes, IS produced a significant rise in plasma E, NE, DA, CORT, ACTH, glucose and Na^+ along with significantly lowered plasma K^+ levels. This was accompanied by significantly lower adrenal E and NE contents, while adrenal DA and NO contents were significantly higher in comparison with the control group. These results could be attributed to the mixed nature of IS as physical and psychological stressor and thus it 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 (Kondo et al. 2013) as well as activation of HPA axis which in turn results in ACTH secretion from adenohypophysis and finally CORT release from the adrenal cortex (Rostamkhani et al. 2012; Garcia-Iglesias et al. 2013), findings which were all confirmed by the results of the present study.

It is well known that the PNMT gene is glucocorticoid-regulated and the adrenal medulla is the first tissue to be exposed to GCs newly synthesized in the adrenal cortex. Therefore, with the ability of IS to activate HPA and increase the production of GCs, the activity of this enzyme would be increased and subsequently CAs release (Stroth and Eiden 2010).

The ACTH-induced increase in CORT, observed in IS group, may be mediated via an increase in NO production, which activates cyclooxygenase enzyme resulting in prostaglandin E_2 (PGE_2) production. In turn, PGE_2 was hypothesized to facilitate release of CORT from the adrenal cortex (Mancuso et al. 2010; Mohn et al. 2011), findings which were confirmed by the concurrent increase in adrenal NO content and plasma CORT level in IS group, suggesting a regulatory role for NO in adrenal hormone synthesis and release in conditions of stress exposure.

In the present study, the observed increase in plasma glucose level in IS group could be explained because of the concurrent rise in plasma CAs and CORT levels. CAs secretion is a part of the "fight or flight" response that stimulates hepatic glycogenolysis and increases the basal metabolic rate and production of glucose (Teague et al. 2007; Rostamkhani et al. 2012). In addition, GCs, mainly CORT, are one of the hyperglycemic hormones that induce liver gluconeogenesis resulting in elevation of blood glucose level (Popovic and Pajovic 2010; Rather et al. 2013).

As regards the plasma electrolytes (Na^+ and K^+), the IS-induced increase in Na^+ may be secondary to the sodium-retaining effect of GCs (namely CORT) on renal tubules. Possible mechanisms may involve increased activity of the basolateral membrane Na^+-K^+ ATPase, sodium-hydrogen exchange by Na^+/H^+ exchanger in the proximal tubule, furosemide-sensitive cotransport by Na^+-K^+-Cl^- cotransporter in the thick limb of Henle, thiazide-sensitive cotransport by Na-Cl cotransporter in the distal tubule and the epithelial sodium channel in the connecting tubule and collecting ducts (Bailey et al. 2009). On the other hand, the concurrent reduction in plasma K^+ level may be secondary to E release, which has been reported to increase the uptake of potassium from the bloodstream, probably via stimulation of β-adrenoceptors, causing hypokalemia (Fisher et al. 1991; Christensen et al. 2009).

The results of the present study also showed that IS produced a significant decrease in adrenal E and NE contents as compared with the C group. This could be explained by the possibility that 3-h IS, used in this study, is considered to be of a relatively long duration or due to an increase of the activity of the uptake systems and/or stress-induced turnover of CAs. Sanchez et al. (2003) and Pajovic et al. (2006) have reported that E and NE contents in adrenal gland increased significantly after the exposure to IS for 30 min. However, the results showed that a maximum increase is reached at 30 min of IS and then a decrement of CAs levels starts at 50 min of the experimental design, findings which is in agreement with the observed depletion of adrenal CAs in IS group. On the other hand, the detected rise in adrenal DA content, after IS exposure, seems to provide sufficient precursor to both replenish medullary pools of E and also to sustain the elevated circulating E levels elicited by and critical to the stress response (Tai et al. 2007).

Glucoprivation (GS), as an example of metabolic stress, was the second model of stress tested in the present study using 2-DG. 2-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 (Ishihara et al. 2009). Its peripheral infusion produces neuroglucopenia, 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 (Bobrovskaya et al. 2010).

The dose of 2-DG, used in this study, was based on previous studies showing its maximal effect on CA release and activation of CA biosynthetic enzymes (Vietor et al. 1996; Wang and Whim 2013).

As the case in IS group, acute GS caused insignificant change in total adrenal glands weights as compared with that of the control group. It could be stated that only strong stimulation of adrenal glands during prolonged stress situations is known to cause adrenal hyperplasia and hypertrophy. In such conditions, the hyperactivity of adrenals could be due to the stress-induced adrenomedullary response leading to increased production of corticotrophic hormone that leads to increased adrenals weight (Rather et al. 2013). However, this is different from the case of our study as the models of stress used were acute not chronic.

In the present work, GS group showed significantly higher plasma E, NE, DA, CORT, ACTH, glucose and Na^+ along with significantly lower plasma K^+ levels in comparison with the C group. This was accompanied with significantly lower adrenal E content, higher adrenal DA and NO contents with insignificant change in NE content as compared with the control group.

The concurrent rise in plasma E level along with its depletion in adrenal tissue, observed in GS group, is in agreement with Parker et al. (2013) who have indicated that acute 2-DG administration results in activation of sympathetic preganglionic neurons (SPN) which activate the adrenergic chromaffin cells secreting E. On the other hand, the observed rise in plasma NE along with unchanged adrenal NE content following 2-DG administration suggests that noradrenergic chromaffin cells were either not activated or may be stimulated but not to the same extent as adrenergic chromaffin cells, so that the rate of release of NE was balanced by the increased rate of synthesis (Bobrovskaya et al. 2010). Accordingly, the adrenal NE content did not change significantly in GS group as compared with the control group.

Acute 2-DG administration also caused significantly higher plasma ACTH and CORT levels than that of the C group. This could be attributed to the nature of GS as

a metabolic stressor and its activation of HPA resulting in significant increase in plasma ACTH and GCs, which play a key role in regulation of body metabolism during stress exposure (Brunelin et al. 2008).

In the GS group, the observed rise in adrenal NO contents can be interpreted by the presence of a causal relationship between the increase in adrenal NO level and the release of adrenal gland hormones in cases of acute stress. Fioramonti et al. (2010) have reported that inhibition of NO signaling impairs E secretion in response to hypoglycemia. Other studies have shown that stressor exposure triggered an increased E release in wild type (WT) animals, but did not significantly alter plasma E levels in neuronal NO synthase (nNOS) knock-out (KO) mice. Therefore, these findings suggest that a constitutive lack of NO of nNOS origin affects the capability of the adrenal glands to mount an adequate E response to acute stressor exposure (Orlando et al. 2008). Finally, Ahren et al. (1995) and Da Cunha et al. (2005) showed that NOS inhibitors reduced plasma levels of E and NE after 2-DG administration. These data suggest that NO may play a regulatory role in the release of both E from the adrenals and NE from sympathetic nerve terminals during stress response.

Our results also showed that in GS group, plasma glucose and Na^+ levels were significantly higher, along with significantly lower plasma K^+ level in comparison with the C group. Glucoprivation induces a range of counter regulatory mechanisms including the release of E 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. 2013). Similarly, Thompson et al. (1981) and Elman et al. (2003) have reported that the GS-induced increase in plasma E level was associated with increases in plasma glucose, Na^+ and renin activity and decreases in plasma K^+, findings that are in agreement with the results of the present study.

Comparing the effect of both stressors on adrenal gland response, GS was associated with significantly higher plasma E, ACTH, CORT, glucose and Na^+ levels as well as higher adrenal DA and NO contents along with significantly lower plasma K^+ level and adrenal E content in comparison with IS group. On the other hand, IS group showed significantly higher plasma NE and DA levels along with significantly lower adrenal NE content than that of GS group.

The results obtained in this study could be attributed, in part, to the specific nature of each type of stressor. GS is a metabolic stressor, so it activates mainly the adrenomedullary system, which is the main source of plasma E that promotes glycogenolysis in order to restore glucose homeostasis. On the other hand, IS, as a type of psychological stressors, has been observed to be associated with hyperactivity of noradrenergic and dopaminergic neurons in the brain (Lee et al. 2012) in addition to its activation of the sympathoadrenomedullary system (SAS) which is the main source of plasma NE, DA and E (Kvetnansky et al. 2009; Stroth et al. 2013).

The greater increase in plasma ACTH and CORT levels observed in the GS than that of the IS group may also represent an additional factor contributing to the preferential elevation in plasma E level following GS. Similar results have been reported by Dronjak et al. (2004) and Kvetnansky et al. (2009) who demonstrated a greater elevation of plasma E and CORT when 2-DG was injected into previously immobilized rats, and they explained this by the ability of 2-DG to activate both the adrenomedullary and adrenocortical systems.

One of the suggested mechanisms explaining the greater increase in plasma ACTH and CORT levels in GS than IS group could be obtained from the corresponding higher adrenal NO content in the GS group than that of IS group indicating the essential role played by NO in their release in case of acute stress exposure (Orlando et al. 2008).

As a consequence of the detected higher rise in plasma E and CORT levels in GS group, so did plasma glucose and Na^+ levels which were significantly higher, while plasma K^+ level was significantly lower in GS group than that of IS group. Both hormones are well known for their role in glucose homeostasis (Bobrovskaya et al. 2010) in addition to the mineralocorticoid-like action of CORT (Frindt and Palmer 2012).

In conclusion, the response to stress varies according to the type of stressor. This variation seems to be mediated mainly by the adrenal gland probably via modulation of its adrenomedullary and/or adrenocortical hormone levels and their subsequent effects overall body in order to maintain homeostasis, in face of stress exposure, which is critical for life.

The exact mechanism of CAs, selectivity in both types of stressors tested, is still not proved and needs further investigation. Proposed mechanisms may include unequal stimulatory response from both SNS and adrenal cortex or selective response of adrenomedullary chromaffine cells to each type of stressor or it may be due to different stimulatory effect of the stressor on either side with predominance of CAs released from each of them.

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