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LITHIUM MODULATES THE CHRONIC STRESS-INDUCED EFFECT ON BLOOD GLUCOSE LEVEL OF MALE RATS

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Abstract - In the present study we examined gross changes in the mass of whole adrenal glands and that of the adrenal cortex, as well as the serum corticosterone and glucose level of mature male Wistar rats subjected to three different treatments: animals subjected to chronic restraint-stress, animals injected with lithium (Li) and chronically stressed rats treated with Li. Under all three conditions we observed hypertrophy of whole adrenals, as well as the adrenal cortices. Chronic restraint stress, solely or in combination with Li treatment, significantly elevated the corticosterone level, but did not change the blood glucose level. Animals treated only with Li exhibited an elevated serum corticosterone level and blood glucose level. The aim of our study was to investigate the modulation of the chronic stress-induced effect on the blood glucose level by lithium, as a possible mechanism of avoiding the damage caused by chronic stress. Our results showed that lithium is an agent of choice which may help to reduce stress-elevated corticosterone and replenish exhausted glucose storages in an organism.

Key words: Wistar rats, lithium, restraint stress, adrenal glands, corticosterone, glucose

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INTRODUCTION

Stress is a state of disturbed homeostasis due to internal or external sources such as physical or psychological stimuli known as stressors. The main physiological response to a stressor is the activation of the sympatho-adrenomedullary (SAS) and hypothalamic-pituitary-adrenal (HPA) systems, which can lead to an enhanced release of catecholamines (CATs) and glucocorticoids (GCs) (Joels et al., 2007; Herman et al., 2003). The adaptive response of an organism is triggered by the stress-induced elevation of serum GCs which regulate glucose metabolism including the stimulation of liver gluconeogenesis. The elevated blood glucose level provides the energy necessary for stress response (McKay and Cidlowski, 2000). Prolonged exposure to a stressor could compromise the adaptive response to stress and such conditions could exacerbate many mood disorders like anxiety, depression and bipolar disorders. In recent years, animal models have been developed that use a different chronic stress paradigm to induce neuro-endocrine and central nervous system changes similar to those occurring in the course of the development of the mentioned pathologies (De Kloet et al., 2005; Machado-Vieira et al., 2004). One such model is chronic restraint stress, shown to induce the activation of the HPA axis and increase plasma corticosterone in certain rat strains (Uchida et al., 2008). Similar prolonged GCs elevation as a result of HPA axis hyperactivity in humans may have a central role in the pathophysiology of mood disorders (Watson and Mackin, 2006) and such conditions are successfully treated with lithium. In addition to that, lithium, an effective drug for both the treatment and prophylaxis of bipolar disorder, is also suggested to stimulate both the adrenomedullary and adrenocortical functions in rats (Chaudhuri-Sengupta et al., 2003; Ghosh et al., 1990). Although lithium acts through the hypothalamic pathway (Spencer et al., 2005), its mechanism of action at the level of secretion of GCs has not been well defined. Since in normal rats lithium administration causes a rise in plasma glucose, which is a major biomarker of GCs action

(Fontela et al., 1986), it is also of interest to determine lithium action regarding this parameter under chronic stress.

Based on these presumptions it was of interest to investigate the potential effects of lithium administration regarding both GCs and glucose in naive and Wistar male rats subjected to chronic restraint as a long-term stress model.

MATERIALS AND METHODS

Materials

The OCTEIA Corticosterone EIA kit was purchased from Immunodiagnostic Systems Inc. (IDS Inc.) Fountain Hills, AZ, USA. Accutrend strips for determination of blood glucose were purchased from Roche Diagnostics GmbH, Mannheim, Germany. Lithium chloride (LiCl) was purchased from Sigma Chemical Company, St. Louis, USA.

Animals and experimental protocol

Male Wistar rats (3 months old) weighing 330-400 g were housed under standard laboratory conditions at a temperature of (20 ± 2°C) with a natural 12-h light/dark cycle and free access to standard pellet chow and drinking water *ad libitum*. All experimental procedures were carried out within the light period of the light/dark cycle. The experimental protocol was in strict accordance with regulations and prescribed animal ethical procedures outlined by the Ethical Committee for the Use of Laboratory Animals of the Vinča Institute of Nuclear Sciences in accordance with the guidelines of the EU FELASA-registered Serbian Laboratory Animal Science Association (SLASA).

All animals were habituated to maintenance conditions for a week prior to the experimental manipulations. During this time, they were randomly assigned to weight-matched groups of 12 animals each. The following groups were constituted: I - control (untreated) group (Ctrl); II - animals subjected to chronic restraint stress (Res); III - animals injected with LiCl (Li) and IV - chronically stressed rats with Li given each day immediately prior to daily restraint (Res+Li).

Restraint stress was performed by placing each animal in a 25 x 7 cm plastic bottle as described previously (Gamaro et al., 1999). Animals in these groups were exposed to 2h of restraint stress every day at random times during the light period of the light/dark cycle to avoid habituation during the experimental procedure of 14 days (Kim and Han, 2006).

Lithium was administered intraperitoneally (IP) to the animals, once a day for 14 days as described previously (Nonaka and Chuang, 1998). The initial lithium dose was 1.5 mEq/kg for 2 days, and was then increased to 2.3 mEq/kg for 7 days followed by 3 mEq/kg for 5 days. This lithium administration protocol maintained the plasma lithium concentration above the minimal therapeutic concentration (*i.e.* 0.4 mM) for the treatment of bipolar disorder throughout the treatment period.

To reduce variance in the physiological parameters due to daily rhythms, all animals were sacrificed at the same time point in the circadian cycle, between 9:00 and 11:00 am, *i.e.*, one day after the last treatments. Animals were sacrificed under no-stress conditions by rapid decapitation.

Preparation of serum and determination of corticosterone and glucose concentration

Blood from each animal was collected at the time of sacrifice. Serum was prepared by 15-min centrifugation at 3000 rpm. The corticosterone (CORT) level was determined by using the OCTEIA Corticosterone EIA kit according to the manufacturer's instructions (Immunodiagnostic Systems Inc.). Calibrators, controls and diluted samples were loaded in duplicates on a 96-well plate coated with a polyclonal anti-CORT antibody, along with HRP-labeled CORT. The plate was incubated overnight at 4°C, washed, and color was developed using a chromogenic substrate. The reaction was stopped by adding HCl and the absorbance at 450 nm (reference 650 nm) was

determined with a microplate reader (Wallac, VICTOR2 1420, PerkinElmer). The concentration of CORT (ng/ml) was determined using a standard curve. For determination of glucose (GLU) concentration, a drop of fresh blood from each animal was applied to an Accutrend strip and assayed colorimetrically with an Accutrend GCT reader (Roche, Mennheim, Germany).

Preparation of adrenal tissues and determination of their mass

After sacrifice, the adrenal glands were carefully excised *in situ* and placed on an ice bath. The mass of the right or left gland was determined by weighing with a Mettler AE 50 electronic analytical balance having a precision of 0.1 mg (Mettler, Toledo, OH, USA). The following procedures were all carried on ice-cold Petri dishes placed in the ice-bath. The adrenal cortices were carefully peeled off and completely removed and the remaining medullas were weighed using the Mettler electronic analytical balance. The mass of each adrenal cortex was calculated by subtracting the mass of the respective medulla from the total mass of the adrenal gland.

Statistical analysis of data

Data are presented as means ± SEM from 12 animals per group. In order to establish significant differences between the control and treated animals, data were analyzed by one-way ANOVA. Values were considered statistically significant if the p value was less than 0.05.

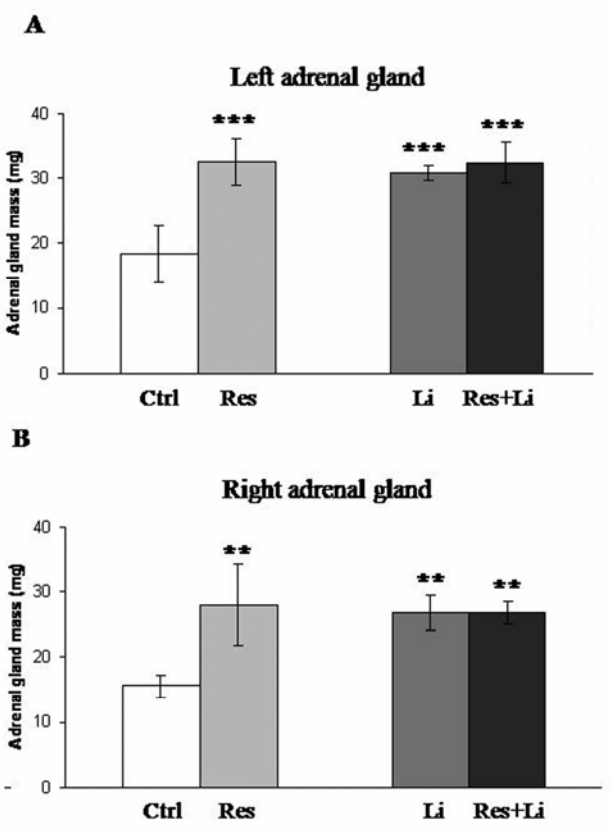

Figure 1. Changes in the adrenal gland mass induced by chronic-stress and lithium treatment in male Wistar rats. The mass of the left (A) and right (B) adrenal gland are presented as means ± SEM; n = 12 in each experimental group [control (Ctrl), subjected to restraint stress (Res), injected with LiCl (Li) and stressed rats with Li given prior to restraint (Res+Li)]. Differences are statistically significant at *p<0.05, **p<0.01, and ***p<0.001 (*treatment *vs.* control).

RESULTS

Chronic-restraint stress and lithium treatment increase the adrenal gland mass in male Wistar rats. Adrenal gland masses were determined in 4 experimental groups of 3-month-old Wistar male rats: I - control (untreated) group; II - animals subjected to chronic restraint stress; III - animals injected with Li and IV - chronically stressed rats treated with Li (see Methods section). As shown in Fig. 1 (A and B) the mass of the adrenal glands (both left and right) were significantly increased in the treated animals (II, III and IV) compared to controls (**p<0.01; ***p<0.001).

The effects of both chronic-restraint stress and lithium treatment on the adrenal cortex mass. Changes in the adrenal cortex mass, the main source of serum corticosterone, were determined for both the left and right gland. The results presented in Fig. 2 (A and B) revealed a significant increment in the mass of both the left and right

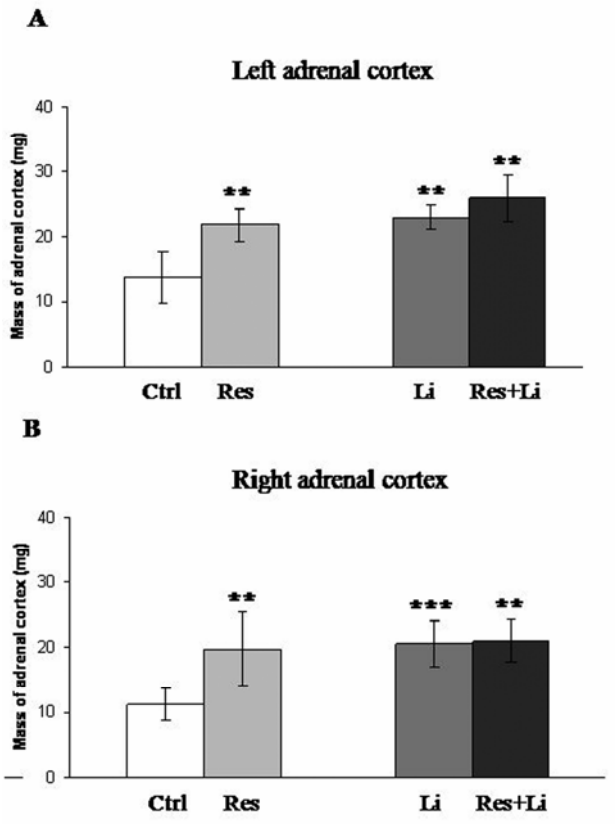

Figure 2. Changes in the mass of adrenal cortex induced by chronic-stress and lithium treatment in male Wistar rats. The cortical mass of the left (A) and right (B) adrenal gland are presented as means ± SEM; n = 12 in each experimental group [control (Ctrl), subjected to restraint stress (Res), injected with LiCl (Li) and stressed rats with Li given prior to restraint (Res+Li)]. Differences are statistically significant at *p<0.05, **p<0.01, and ***p<0.001 (*treatment *vs.* control).

adrenal cortex in all treated groups in respect to the control (**p<0.01; ***p<0.001).

Changes in serum corticosterone levels were not followed by changes in blood glucose in chronically stressed rats. Chronic restraint stress, solely or in combination with Li treatment, significantly elevated the corticosterone level (**p<0.01; *p<0.05), but did not change the blood glucose level (Fig. 3 A and B). Animals treated only with Li exhibited an elevated serum corticosterone level, as well as blood glucose level (*p<0.05) (Fig. 3 A and B).

DISCUSSION

Stress produces an endocrine and metabolic response leading to a quick adaptation of the animal to a novel situation. A strong feature of the endocrine response to stress is the increase of the corticosterone (CORT) level. Long-term exposure to glucocorticoids has been associated with several metabolic disorders, including fasting hyperglycemia and impaired glucose tolerance (Munck, 1971; Doyle et al., 1994; Horner et al., 1990). Therefore, it was of interest to analyze the potential effects of lithium administration regarding both GCs and glucose in naive and Wistar male rats subjected to chronic restraint as a long-term stress model.

Our experimental data indicated that animals subjected to chronic restraint stress exhibited increased CORT level. This was not unexpected since it has been previously reported that this type of stress induces CORT secretion (Kim and Han, 2006; Uchida et al., 2008). This result is in accordance with enlarged adrenal glands (Tuli et al., 1995), especially the cortex, found under this condition (Figs. 1 and 2). In previous reports from other authors (Adzic et al., 2009) the difference in size between the left and right glands was reported under a different type of chronic stress, but under restraint stress this difference was observed only as a trend, without statistical significance. Although it is well known that GCs induce liver gluconeogenesis resulting in elevated blood glucose (McKay and Cidlowski, 2000), the results obtained from our model system indicated that prolonged exposure to high GCs was not accompanied with the expected increase in blood glucose (GLU) level. The plasma glucose concentration increased significantly during acute restraint stress in male Wistar rats, but after exposure to repeated restraint, there was no difference between the control and stressed groups when the plasma glucose level was measured 24 h after the last restraint session (Torres et al., 2001). This observation may perhaps be explained to be due to exhaustion of metabolic pools serving to generate glucose.

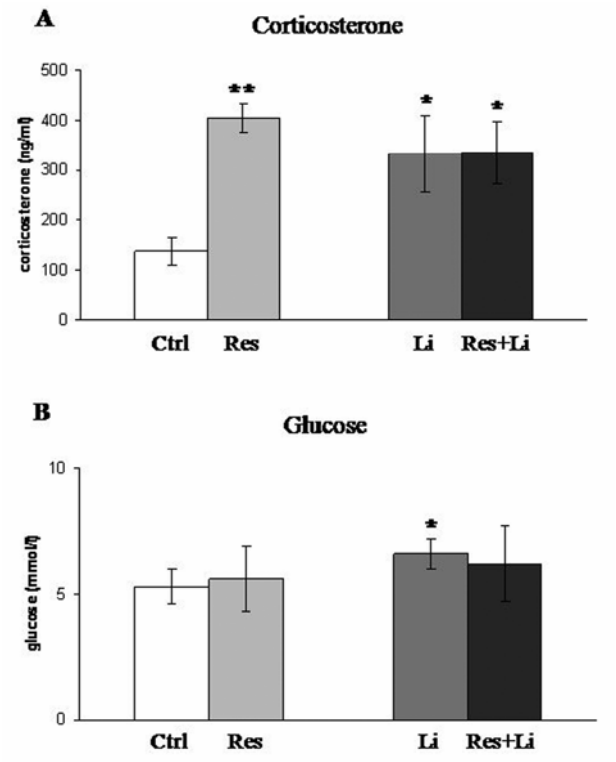

Figure 3. Changes in corticosterone and glucose levels induced by chronic-stress and lithium treatment in male Wistar rats. Serum levels of corticosterone and blood levels of glucose are presented as means ± SEM; n = 12 in each experimental group [control (Ctrl), subjected to restraint stress (Res), injected with LiCl (Li) and stressed rats with Li given prior to restraint (Res+Li)]. Differences are statistically significant at *p<0.05, **p<0.01, and ***p<0.001 (*treatment *vs.* control).

When lithium was administered to the control animals, it induced an increase in the adrenal glands mass (Fig. 1), serum CORT and blood GLU levels (Fig. 3). Taking into account that lithium is implicated in the regulation of the adrenocortical functions in rats through the stimulation of adrenocortical activity by both synthesis and release of CORT (Chaudhuri-Sengupta et al., 2003), the obtained result in our model with a high CORT and an increment in the adrenal cortex mass was as expected. It has been reported previously that lithium treatment increases the amount of blood GLU, but depletes the store of glycogen (Ghosh, 2007), suggesting that lithium probably affects the function of insulin (Fontela et al., 1986) which is unable to convert more glycogen from glucose. Moreover, the actions of lithium could also be explained to be a direct effect on the phosphorylation state of some key enzymes (like glycogen synthase kinase 3) (Rodriguez-Gil et al., 2000; Bosch et al., 1986).

Finally, we analyzed the effect of lithium administered immediately prior to the daily restraint procedure, regarding both serum CORT and blood GLU. Even though in previously described combined treatment the CORT level was elevated in respect to control, it seemed that lithium attenuated HPA axis hyperactivity evoked solely by the restraint stress. Also, at the level of blood GLU, exposure to lithium disabled the effect of chronic restraint stress.

Taken together the results of our study suggested that Li treatment counteracted at least in part the effects of chronic restraint regarding CORT elevation. The observed decrease in CORT caused by Li treatment of chronically stressed animals may also help to replenish exhausted glucose generating pools. Thus we concluded that Li, a widely used substance for the treatment of manic-depression, is an agent of choice which may help in the treatment of HPA hyperactivity.

This could be just one of the possible explanations for lithium's beneficial effects on a disturbed HPA system. On the other hand, among the numerous effects of lithium on intracellular targets, its stimulatory action on impaired energy generation by mitochondrial oxidative phosphorylation seems to be crucial for its therapeutic efficacy (Maurer et al., 2009; Shalbuyeva et al., 2007). However, the precise mechanism of lithium action demands further investigations.

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ЛИТИЈУМ МОДУЛИШЕ ОДГОВОР НА ХРОНИЧНИ СТРЕС УЗРОКУЈУЋИ ПРОМЕНУ НИВОА ГЛУКОЗЕ У КРВИ МУЖЈАКА ПАЦОВА

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У приказаној студији праћена је промена масе адреналних жлезда и адреналних кортекса, као и нивоа кортикостерона у серуму и глукозе у крви мужјака Wistar пацова који су излагани различитим третманима: хроничном (restraint) стресу, деловању литијума, као и хроничном стресу уз претходно третирање литијумом. Под деловањем сва три третмана запажена је хипертрофија адреналних жлезда и њихових кортекса. Код хроничног стреса, било да је примењен без или са претходним давањем литијума, значајно је повећан ниво кортикостерона, али није уочена промена нивоа глукозе у односу на нетретиране животиње. Код животиња третираних само литијумом примећено је поред пораста нивоа кортикостерона и повећање нивоа глукозе у крви. Циљ рада био је испитивање потенцијала литијума да модулише одговор на хронични стрес преко промене нивоа глукозе у крви, што би могло да умањи штетне последице стреса на организам. На основу добијених резултата може се закључити да литијум снижава повишени ниво кортикостерона и спречава прекомерно исцрпљивање организма током стреса.