Research Article
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Effect of prenatal stress and forced swimming acute stress on adult rat's skeletal muscle and liver MDA levels

Abstract

The study of stress physiological consequences has gained importance. Accordingly, prenatal stress is a contributing factor on mammal gestation, causing adaptive disturbances during offspring adult life. The aim of this study was to analyze the physiological effects of prenatal stress rats on some skeletal muscle and liver parameters after forced swimming (FS) exposition. Pregnant Wistar rats were stressed by immobilization (IMO) during the two last week of pregnancy and male offspring raised in bioterium standard conditions until 90 days old. Corticosterone (COR), Glycaemia and malondialdehyde (MDA) levels were studied in all experimental group: Control animals, forced swimming stressed rats and prenatal stressed animals rats with and without FS stress. The results showed that in basal conditions COR and Glycaemia levels were increased in prenatal stressed animals. After an acute exposition to FS, COR significantly increased in prenatal stress and control groups (without prenatal stress). Glycaemia levels had similar values in all experimental conditions; however, hepatic MDA levels showed a significant increase in prenatal stressed rats and the effect was maintained in FS animals. MDA values analyzed in grooved muscle were higher in prenatal stressed groups, but after FS acute stress, MDA levels increased in control animals too. In conclusion, IMO prenatal stress causes a change in the regulation of hypothalamic-pituitary-adrenal axis (HPA), reflected in high basal levels of plasmatic COR and hyperglycaemia. Moreover, it generates a hyper sensibility to acute stressors caused in post birth life. Also, prenatal stress produces oxidative stress in liver cells while FS stimulates this process in grooved voluntary muscle.

Keywords: physiological consequences, mammal gestation, voluntary muscle

Volume 6 Issue 6 - 2019

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Received: December 05, 2019 | **Published:** December 16, 2019

Abbreviations: FS, forced swimming; IMO, immobilization; COR, corticosterone; MDA, malondialdehyde

Introduction

Numerous authors have shown the influence of prenatal stress (PS) and its effects in intrauterine medium. Studies realized in human revealed that children of mothers suffering difficult conditions during the third pregnant period showed long term consequences, such as heart malformations, hearing loss, skeletal abnormalities and low weight birth.[1] In rats, PS decreases weight, alters the HPA axis, sex maturity in males and progeny immune development. These adverse conditions or suffered stressors in prenatal stress period determine the offspring epigenome.[2] In consequence, the stress applied to female pregnant reorganized the offspring's HPA axis and produced hyperactivity in stress system.[3]

On the other hand, PS had a direct correlation with oxidative cellular stress (OS).[4] The OS has been associated to the appearance and development of diverse illnesses and chronic processes, as diabetes, obesity and cardiovascular disorders, including arterosclerosis. Recent studies showed the multiple effects of imbalance in free radicals production and its counteraction with species ageing; male infertility caused by damage in the spermatic DNA, neoplastic cells development or any other inflammatory/degenerative pathology. The OS is harmful in biological terms when the free radicals production overcomes the antioxidative endogenous mechanisms.[5]

Physical exercise represents a model of psychophysical stress due to the effect on organism homeostasis. Exercise consequences depend on intensity, duration, type of exercise, stimulus application (acute exercise vs. chronic exercise) and physiological subject conditions.[6–9] Numerous studies showed that intense physical exercise like swimming[10] or races in moving walkways[11] modified neurotransmisor (catecholamine's) and hormones (cortisol and opioids peptides) levels, altered modulators of immune function and other physiological parameters, like blood lipids concentration, cholesterol, blood pressure and short-term glucidic metabolism. During physical exercise, muscles produced and released high concentration of proinflammatory cytokines (TNF-α, IL-1, IL-10) which increased their levels during a session of acute exercises. Moreover, an intensification of leucocytes, neutrophils, lymphocytes (T and B) and natural killer cells, monocytes and plasma concentrations of C reactive protein were described.[12] OS alteration was also observed as a regulated factor in animals exposed to acute postnatal stress. In this way, Sachdev and Davies (2007), analyzed muscle and liver homogenates of rats with exhaustive exercise and found more than 100% rise in hepatic and muscular malondialdehyde (MDA) levels.

In this context, the aim of this work was to investigate the effects of forced swimming postnatal stress in prenatally stressed male rats

on Glycaemia and lipid peroxidation response and their relationship to the activity HPA axis.

Material and methods

Animals

Albino Wistar rats were grown in the National University of Río Cuarto. Five male and 10 female rats were used, the animals were housed in plastic cages in groups of 3 individuals under the following conditions: 12h light/12h dark, 22°C, constant humidity, water and food ad libitum. Male-female (1:2) were mated overnight and the following morning a vaginal smear was performed and microscopically examined. The first day of pregnancy was determined by the presence of sperm in the smear and pregnant females were located in separate home cages. All animals (pregnant females and their offspring) were maintained according to the Guide for Care and Use of Laboratory Animals and the experiments were approved by the local Institutional Animal Care Committee.

Prenatal stress

Chronic stress by immobilization (IMO) was applied to the experimental group of pregnant females. Experimental rats were stressed during the two last weeks of pregnancy by placement into immobilization bags. Each female was subjected to 30 min stress by IMO three times each week at different times in the mornings of different days (PS). Control female rats were left undisturbed in their home cages (PC). The offspring were weaned 21 days after birth and housed in groups of four males by litter and left undisturbed until testing. Only adult male (90 days-old) from PS and PC groups were used for subsequent experimentation. Experimental design is described in Figure 1.

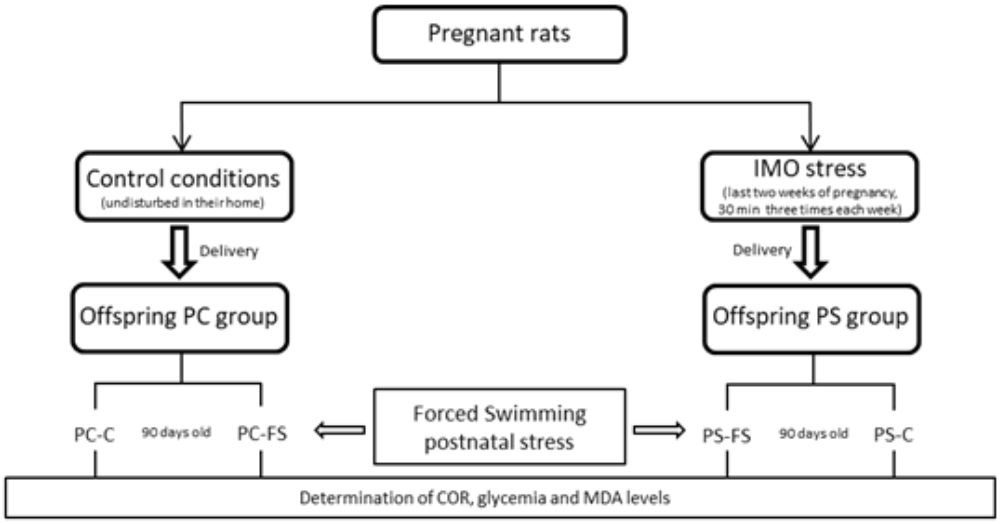

Figure 1 Schematic diagram of the experimental groups used in this research. Pregnant rats were distributed in two treatments: one was housed in control conditions and the other was subjected to immobilization stress, 30 min during the last two weeks of pregnancy, and three times each week. When male offspring became adults (90 days old) they were postnatal stressed by FS during 30 min and COR, Glycemia and MDA levels were analyzed.

Postnatal stress

Half of litters of stressed and control mothers (n: 4 per group) were postnatal stressed. The acute postnatal stress by FS is a single session of swimming during 20 min, without previous experience, in a pool of 50 cm long, 30 cm wide and 60 cm deep. Water temperature was 35±2ºC. After 20 min in water they were removed and experimental samples were obtained for parameters analysis. This test is based on the original method of Porsolt et al.[13] Clean water was used for each test, due to the behavior rats could be altered if another animal previously had swum into the pool.[14]

Blood sample

All the determinations were performed in male offspring at 90 days of age. Blood samples (heparinized tube) were taken between 9 and 12 a.m. immediately after removal from the home cage (C) or postnatal stress (FS) session. Animals were sacrificed by decapitation, and COR and Glycaemia levels were determined in heparin plasma samples.

Corticosterone levels

Blood samples (200-300µl) were collected from control and stressed litters using the tail clip method. Samples were taken at 90

postnatal days after acute postnatal stress by FS. COR levels in plasma were measured by radioimmunoassay (RIA)[15] using high specificity rabbit antiserum to COR from Bioclin (Cardiff, UK). Assay sensitivity was 10pg of COR and inter and intra assay coefficients of variability were <10 per cent.

Glycaemia levels

Glycaemia levels were carried out through the use of commercial enzymatic equipment (Wiener Lab, Rosario, Argentina). The colorimetric method is based on chemical reaction where blood glucose is oxidized to gluconic acid by the enzyme glucose oxidase. The hydrogen peroxide produced, in the presence of the peroxidase 4-aminophenazone and phenol, forms a quinoneimine with an absorption peak at 505 nm. The intensity of the color is proportional to the concentration of the glucose in the sample.

Malondialdehyde levels

As a marker of lipid peroxidation, the concentration of MDA in liver or skeletal muscle homogenates was determined by the thiobarbituric acid reactive substances (TBARS) technique. This parameter was measured using the original method of Buege & Aust[16] modified for tissues by Marcincak et al.[17]

Six hundred to 30% (w/v) of homogenate tissue was mixed with butylatedhydroxytoluene (BTH) as an antioxidant and trichloroacetic acid at 15%, stirred and incubated at 90°C for 30min. After cooling and centrifugation at 2500 rpm (10min), 600µl of supernatant was removed and reacted with an equal volume of 0.25N chlorhydric acid and 0.375% thiobarbituric acid (TBA). The reaction mixture was incubated again at 90°C for 30min and then reacted with two volumes of butanol. The colored layer was measured at 535nm using 1, 1, 3, 3-tetraethoxypropane (Sigma, St. Louis, MO, USA) as the standard.

Statistical analysis

Data were analyzed using Statistical Analysis Software (Cary, USA). Differences between prenatally stressed offspring that were postnatal stressed or not stressed were analyzed using two-way 2 x 2 ANOVA: the factors were prenatal stress and postnatal forced swimming. Post-hoc comparisons were performed using Duncan's test. The values are expressed as means ± SEM and $p<0.05$ was considered statistically significant.

Results

Effect of prenatal stress on plasmatic and corticosterone levels after forced swimming postnatal stress (Figure 2)

COR levels were higher in PS-C and PS-FS compared to their respectively controls PC-C and PC-FS. Moreover, after postnatal acute stress by forced swimming PC-FS and PS-FS showed significant high COR levels (**p<0,005) compared to basal and prenatal stressed rats groups (PC-C, PS-C). Each column represents the media ± S.E.M.

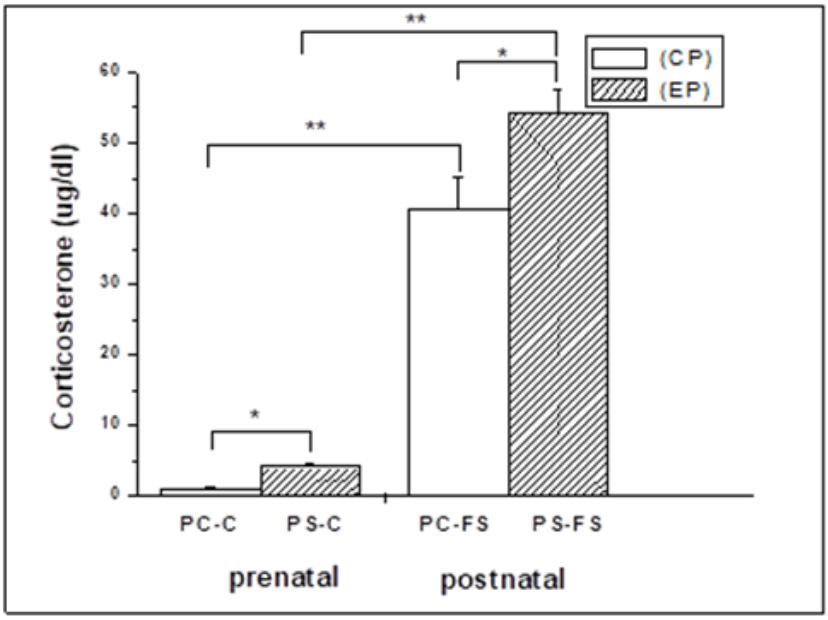

Figure 2 Effect of prenatal stress and postnatal forced swimming on adult corticosterone levels. Prenatally stressed rats without postnatal forced swimming stress (PS-C), prenatally stressed offspring with postnatal FS stress PS-FS, control adult male offspring PC-C, control adult male offspring with postnatal FS stress PC-FS. Bars represent mean ± SEM.

Effect of prenatal stress on glycaemia plasmatic levels after forced swimming postnatal stress (Figure 3)

Glycaemia levels were higher (*p<0,05) in PS-C compared to their control PC-C. Moreover, after postnatal FS stress PC-FS and PS-FS group had higher levels of plasmatic glucose (**p<0,005) compared to basal PC-C and prenatal PS-C groups. Each column represents the media ± S.E.M.

Figure 3 showed that prenatal stress PS-C caused a significant

Citation: Emiliano S, Cecilia LA, Karen B, et al. Effect of prenatal stress and forced swimming acute stress on adult rat's skeletal muscle and liver MDA levels. *MOJ Anat & Physiol.* 2019;6(6):226–231. DOI: 10.15406/mojap.2019.06.00277

increase of Glycaemia plasmatic levels (p<0,02). This difference is minimums after postnatal stress by FS (p:0,77). Besides, postnatal stress in PC and PS increased significantly Glycaemia levels compared to basal and prenatal stress respectively PC-C; PS-C. The correlation between Glycaemia (g/l) and plasmatic COR levels (ug/dl) is positive (r: 0,7559; p<0,0001).

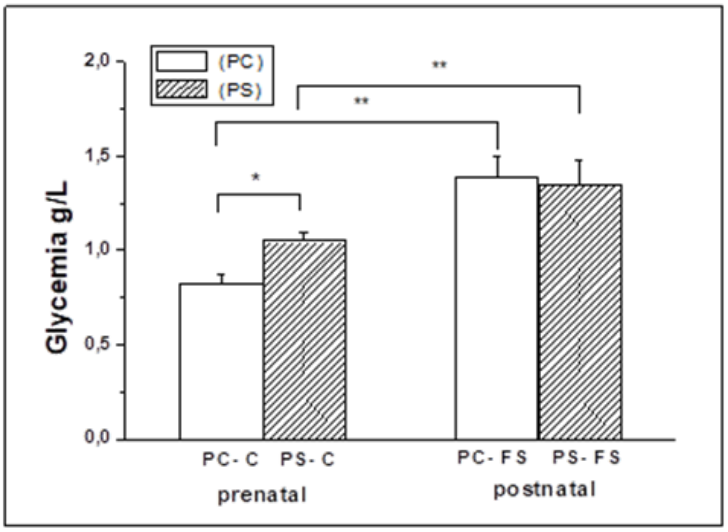

Figure 3 Effect of prenatal stress and postnatal forced swimming on adult glycaemia levels (g/l). Prenatally stressed rats without postnatal forced swimming stress (PS-C), prenatally stressed offspring with postnatal forced swimming stress PS-FS, control adult male offspring PC-C, control adult male offspring with postnatal forced swimming stress PC-FS. Bars represent mean ± SEM.

Effect of prenatal stress on malondialdehyde levels after forced swimming postnatal stress (Figure 4)

The concentration of MDA in the liver as a marker of the oxidative damage was bigger in offspring prenatal stress control (PS-C) and with postnatal stress stimuli (PS-FS) compared to their respective controls PC-C and PC-FS (Figure 4A). In muscle (Figure 4B), MDA levels were higher in prenatal stressed rats PS-C and in postnatal stressed animals compared to basal conditions PC-C and PC-FS. Furthermore, forced swimming stress showed a significant rise in MDA concentrations in striated muscle in adult males with respect to its controls without prenatal stress.

Discussion

The results of the present work confirmed that basal level of plasmatic COR is higher in prenatal stressed animals compared to control ones.[3,18–22] COR levels in postnatal stressed animals were shown to be higher than animals that had the same prenatal treatment but were not forced swimming stress. Glycaemia levels, as stress marker, showed the same behavior as COR hormone. Prenatal stress increases MDA levels in liver, values that were not modified by acute postnatal stress. The same effect was also observed in skeletal muscle. Conversely, acute postnatal stress per se increases oxidative stress in striated muscle, effect not observed in liver.

COR is a liposoluble glucocorticoid that pierces the placenta and strongly interacts with diverse cells and fetus tissue. This hormone affects fetus adrenal development and generates a bigger relationship cortex: marrow[3,23] producing long term effects in different organ systems, such as metabolism, the cardiovascular function and immune development. Some authors suggest that higher COR levels have been caused by a decrease of mineralcorticoids (MR) receptors present in offspring hippocampus,[24] altering the mechanisms of HPA feedback. The decrease of corticosteroids receptors would be accompanied by a higher GC secretion, starting resistance to negative feedback. In previous works realized in our laboratory, it was observed that the adrenal cortex suffers histological and physiological changes that were seen by an increase in the relation cortex-marrow of animals exposed to prenatal stress.[3,22]

Acute postnatal stress effects are caused by Nervous Noradrenergic System action on hippocampus because maternal GC would modify the activity of fetus HPA axis. In this sense, it is known that the PS increases norepinephrine secretion, noradregenic neurons number in the locus coeruleus, exerts direct inhibitory control over the hippocampal glucocorticoid receptor (GR) and stimulates COR secretion before any stressor to which the offspring is exposed to PS.[24–26]

Our work showed that postnatal stress by FS in rats produced HPA hyperactivity. The regulation of the abnormal feedback of the HPA axis in the offspring would be associated to a decrease of GR number, receptor involved at least in part in the answer to acute stress.[27] The increased levels of COR, produced during acute FS, would stimulate GR downstream mechanism.[28] These aspects mentioned before would allow us to understand the increase of COR peak in animal prenatal and forced swimming heterotypical stress in their adult lives.

In our experiments, PS animals showed high basal Glycaemia. These results are similar to the ones obtained in other studies[19] which demonstrate that prenatal stress by IMO during the last week of gestation produced the same effect. This could be the result of the basal hyperactivity of the HPA axis, since there was positive

correlation among the plasmatic COR levels and Glycaemia. The mechanisms by which high COR levels generate hyperglycaemia are numerous, among them there is the decrease of tissue sensibility to detect the glucose. The use of glucose at an intracellular level is also affected by the COR, causing a decrease in the oxidation of adenine dinucleotide nicotinamide reduced (NADH) to rusty adenine dinucleotide nicotinamide (NAD+). It is known that the NAD+ is necessary for the glycolysis.

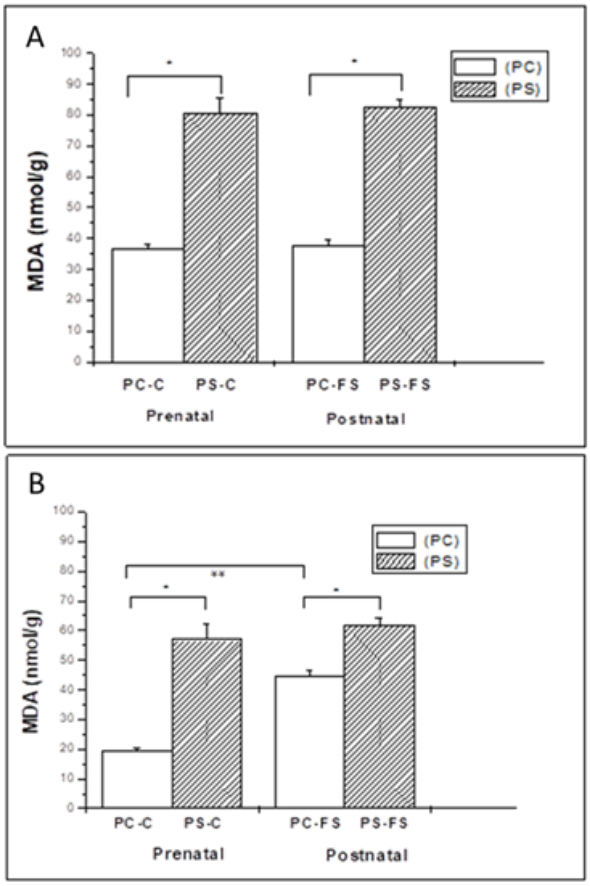

Figure 4 Effect of prenatal stress and postnatal forced swimming on adult liver (A) and muscle (B) MDA levels (nmol/l). Prenatally stressed rats without postnatal forced swimming stress (PS-C), prenatally stressed offspring with postnatal forced swimming stress PS-FS, control adult male offspring PC-C, control adult male offspring with postnatal forced swimming stress PC-FS. Bars represent mean ± SEM.

The increase of Glycaemia, observed in prenatal stressed animals as in their respective controls without prenatal stress, as response to an acute stress could be the result of the adrenal sympathetics systems activation, with the following release of adrenalin. While, in our experiments the application of acute FS generates hyperglycaemia that attenuates the basal differences among prenatal stressed and no prenatal stressed groups. This finding is due to the fact that animals CP when are exposed to acute stress session have a higher increase of basal Glycaemia than prenatal stressed animals. The correlation between COR and Glycaemia levels was positive (r=0,7559, P<0,0001). Significant evidence shows that rats which have been pushed to a determined EP assimilate the same stress with less intensity in the adult life.[29,30] Consequently, hyperglycaemia levels produced by acute stress are minor as long as the same stress is repeated. The MDA has been widely used during many years as an optimum biomarker of lipid perioxidation, in fatty omega-3 and omega-6 acids, due to its easy reaction with TBARS. TBARS is one of the most popular and trust worthy markers that determines the OS in clinic situations as heart attack or certain kinds of stroke.[31]

As our experiments showed, FS not increase significantly the values of MDA levels in liver of male adult offspring. The MDA in basal conditions, are significantly higher in prenatal stressed groups, revealing a greater oxidative stress level in hepatic cells. These results coincide with the ones described by Brun N[32] in which the authors described an increase in MDA concentration of homogenate in rats' liver exposed to prenatal stress. The production of MDA in the striated skeletal muscle and liver has been studied by other authors with comparable objectives. Similar results were obtained in determinations done in striated voluntary muscle of sural triceps (muscular group with type I isoforms myofibrils), registered a significant increase in the MDA levels in the group CP-FS compared to the CP-C, increasing from 20nmoles/g to 45nmoles/g. Furthermore, in prenatal stressed individuals, FS did not cause an increase in MDA muscle levels. MDA values in voluntary striated muscle tissue, coincide with the detected values by Cunningham P[33]; in which after an intense anaerobic type exercise (sprints of 30 seconds at 30 meters per second and an inclination of 15°), MDA concentration was raised in the external long muscle, indicating an increase in the lipidic perioxidation. In the same study, it has been suggested that acute exercise of high intensity, applied as a unique stress in time could be a type of prenatal stress due to the fact that oxidative damage caused by exercise in many tissues could be reduced by long term high intensity training.

We concluded that prenatal stress by chronic IMO produces, during the adult life, alterations in basal conditions of the HPA axis function as shown by COR serum levels. Forced swimming postnatal stress caused hyper sensibility of HPA showed by major COR secretion in prenatal stressed animals. In similar way, prenatal stress enhances Glycaemia levels independently of postnatal condition which could be associated to the high levels of MDA observed in hepatic tissue. In voluntary striated muscle, lipid peroxidation is produced by prenatal and postnatal stress treatment but an atopic postnatal stress situation does not modify MDA levels in prenatal stressed animals.

Acknowledgments

None.

Conflicts of interest

The authors declare there are no conflicts of interest.

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