BRAIN RESEARCH 1381 (2011) 141–147

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BRAIN RESEARCH

Research Report

Functional characterization of the hypothalamic–pituitary–adrenal axis of the Wistar Audiogenic Rat (WAR) strain

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ARTICLE INFO

Article history:
Accepted 12 January 2011
Available online 21 January 2011

Keywords:
HPA axis
Audiogenic seizure
Corticosterone
ACTH
Restraint stress

ABSTRACT

The Wistar Audiogenic Rat (WAR) strain is a genetic model of sound-induced reflex epilepsy which was selected starting from audiogenic seizures susceptible Wistar rats. Wistar resistant rats were used as WAR's control in this study. In the acute situation, audiogenic seizures (AS) in WARs mimic tonic–clonic seizures and, in the chronic protocol, mimic temporal lobe epilepsy. AS have been shown to evoke neuroendocrine responses; however, the hypothalamic–pituitary–adrenal activity in the WAR has not been established. The aim of this study was to evaluate the hypothalamic–pituitary–adrenal axis (HPA) responses to exogenous ACTH stimulation (8 ng/rat), fifteen minute restraint stress and circadian variation (8 am and 8 pm) under rest conditions in these animals through plasma measurements of ACTH and corticosterone concentrations. We also measured the body weight from birth to the 9th week of life and determined adrenal gland weight. We found that WARs are smaller than Wistar and presented a higher adrenal gland weight with a higher level of corticosterone release after intravenous ACTH injection. They also showed altered HPA axis circadian rhythms and responses to restraint stress. Our data indicate that, despite the lower body weight, WARs have increased adrenal gland weight associated with enhanced pituitary and adrenal responsiveness after HPA axis stimulation. Thus, we propose WARs as a model to study stress–epilepsy interactions and epilepsy–neuropsychiatry comorbidities.

1. Introduction

The Wistar Audiogenic Rat (WAR) strain is a genetic model of sound-induced reflex epilepsy that, in the acute situation, mimics tonic–clonic seizures (audiogenic seizures; AS) and, in the chronic protocol, mimics temporal lobe epilepsy (Garcia-Cairasco et al., 1996; Dutra Moraes et al., 2000; for review see Garcia-Cairasco, 2009). There is a strong relationship between

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0006-8993
doi:10.1016/j.brainres.2011.01.042

hormones and epilepsy; epileptic seizure can promote hormonal and metabolic alterations after seizures (Trimble, 1978; Meldrum et al., 1979; Wyllie et al., 1984; Pritchard et al., 1985). Some hypothalamic hormones are known to facilitate (corticotrophin releasing factor—CRF) or to inhibit (thyrotropin releasing hormone—TRH and luteinizing hormone releasing hormone—LHRH) epileptic seizures (Plotnikoff and Kastin, 1977; Bajorek et al., 1984; Delgado-Escueta et al., 1986).

Some pathophysiological aspects of seizure susceptibility are directly related to the hypothalamus–pituitary system (Amado et al., 1993), which is regulated by limbic structures, such as the hippocampus and amygdala, which play an important role in the genesis of epilepsy (Sperling and Wilson, 1986). During convulsive seizures, HPA axis activation occurs along with an increase in plasma glucocorticoid concentration.

Significant increases in plasma concentrations of cortisol, GH and PRL were observed after spontaneous generalized seizures (Culebras et al., 1987). We also observed higher postictal PRL levels, in comparison to those observed in other stress paradigms in WAR (Garcia-Cairasco et al., 1996). In addition, we found that lactation-induced hyperprolactinemia is also a strong modulator of seizure sensitivity in WARs (Doretto et al., 2003b).

The HPA axis is characterized by the presence of a circadian rhythm in basal and stress conditions, and the HPA axis response to stress has been shown to be higher during the nadir than during the daily rhythm peak (Kant et al., 1986; Bradbury et al., 1991; Dallman et al., 1992). Moreover, stress is a very common, self-reported precipitant of seizures in patients with epilepsy (Frucht et al., 2000; Spector et al., 2000; Nakken et al., 2005).

There is evidence showing the relationship among epilepsy, hormones, stress and circadian rhythms. In this sense, the aim of this study was to evaluate the HPA axis in response to different stimulations in the WAR strain, a genetic model of epilepsy.

2. Results

2.1. Body weight

At birth, there was no difference in the body weight between WAR and Wistar groups. However, after the first week until the ninth week, the body weight was significantly lower ($p<0.05$) in the WAR group compared with the Wistar group (Fig. 1A). Additionally, the adrenal gland weight of the WAR group (13.4±0.6 g/100 g) was significantly higher than the adrenal gland weight of the Wistar group (10.0±0.6 g/100 g) (Fig. 1B).

2.2. Histopathology and morphometric analysis of the adrenal gland

In the panoramic histopathological analysis, the adrenal medulla was apparently normal in both groups of rats (Fig. 2A and B). In Fig. 2C and D are illustrated the cortical layers of Wistar rats and WARs, respectively. In the fasciculate layer of the cortical adrenal gland we observed hyperplasia and intensive capillary ingurgitation associated to a marked vacuolization of the fasciculata cells in WARs (Fig. 2E and F).

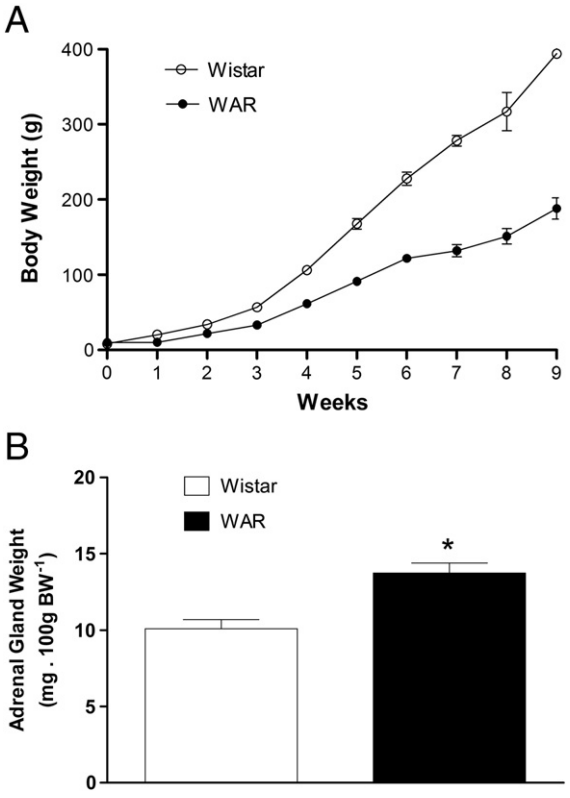

Fig. 1 – (A) Body weight curve of male Wistar rats and WARs from birthday to 9th week of life and (B) left adrenal gland weight of Wistar and WAR strains.* $p<0.05$.

Histological morphometry revealed a significant increase in adrenal medullar area in WARs when compared with Wistar rats (2.745±0.392 mm^2 vs. 1.443±0.405 mm^2), (Fig. 3A). Quantification of the cortical layers also demonstrated a significant increase in the fasciculate layer thickness in WARs when compared with Wistar rats (831.2±66.1 μm vs. 533.0±34.1 μm), with no significant difference in either reticularis or glomerulosa layers in both groups of rats (Fig. 3B)

2.3. Restraint stress

Plasma corticosterone values in basal conditions and after restraint stress were 1.4±0.4 μg/dl and 30.1±1.4 μg/dl, respectively, in WARs and 0.6±0.1 μg/dl and 32.1±1.7 μg/dl, respectively, in Wistar rats (Fig. 4A). Plasma ACTH values in basal conditions and after restraint stress were 30.9±6.1 pg/ml and 632.0±50.5 pg/ml, respectively, in WARs and 23.8±3.9 pg/ml and 468.9±33.8 pg/ml, respectively, in Wistar rats (Fig. 4B). Compared to basal conditions, there was an increase in plasma corticosterone and ACTH levels after restraint in both groups. There was no difference in corticosterone responses to stress between WARs and Wistar; however, plasma ACTH levels after stress were higher ($p<0.01$) in WARs as compared to Wistar rats.

2.4. Circadian rhythms

Plasma corticosterone values in basal conditions at 8 a.m. and 8 p.m. were 0.7±0.1 μg/dl and 6.1±1.4 μg/dl, respectively, in

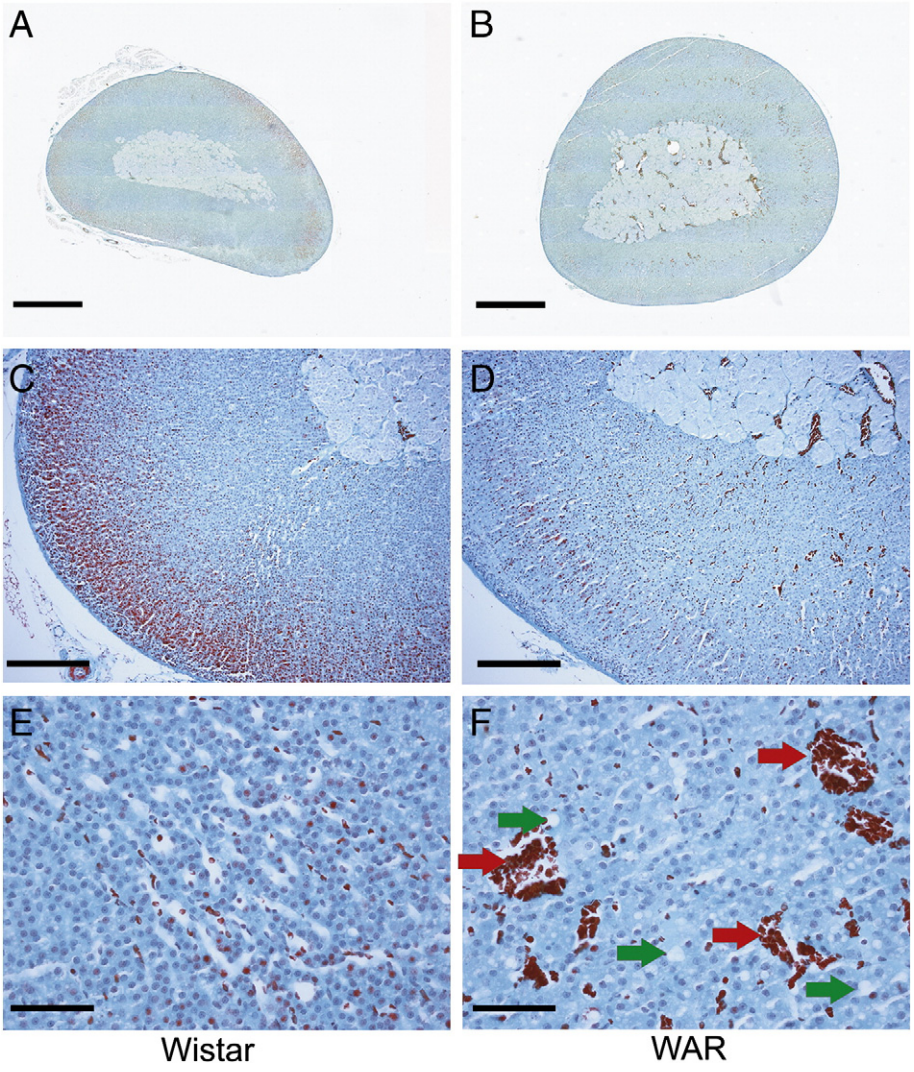

Fig. 2 – Panoramic view of adrenal gland of Wistar rats (A) and WARs (B); higher magnification of adrenal cortical layers of Wistar (C) and WARs (D); cortical fasciculate layer of Wistar (E) and WARs (F). Observe enhanced vacuolization (green arrows) and capillary ingurgitation (red arrows) in WARs. Calibration bars for A and B is 10 mm, for C and D is 400 μm and for E and F is 100 μm.

WARs and 0.7±0.1 μg/dl and 17.4±2.6 μg/dl, respectively, in Wistar rats (Fig. 5A). Plasma ACTH values in basal conditions at 8 a.m. and 8 p.m. were 20.5±4.9 pg/ml and 25.7±3.4 pg/ml, respectively, in WARs and 33.7±5.6 pg/ml and 73.4±9.9 pg/ml, respectively, in Wistar rats (Fig. 5B). There was no circadian variation of plasma ACTH levels in WARs.

Plasma corticosterone values after ACTH stimulus were significantly higher in WARs (19.0±3.6 μg/dl) as compared with Wistar rats (9.2±0.9 μg/dl) (Fig. 6).

3. Discussion

We demonstrated differences between Wistar rats and WARs in body growth and alterations in responses to activation of the HPA axis. We observed that Wistar control rats have a higher body weight than WARs. Although smaller than Wistar, WARs showed higher adrenal gland weight. Histopathology and morphometric analysis showed a significant increase in the adrenal cortical fasciculate layer in WARs, which is consistent with the functional alterations found in the HPA axis, such as higher glucocorticoid release after ACTH stimulus in WARs.

Glucocorticoids are known to promote several events such as energy mobilization, muscular and bone growth inhibition, proteolysis and lipolysis (McEwen and Stellar, 1993; Munck et al., 1984), as well as improve the restorative recovery capacity after stress and prepare the organism for the challenge (De Kloet et al., 2005). We might speculate that some of these events can be associated with the difference in the body weight curve between Wistar rats and WARs.

In order to test HPA axis activity of WARs, we verified the ACTH response after restraint stress, and we found that the plasma ACTH levels were higher in WARs than in Wistar.

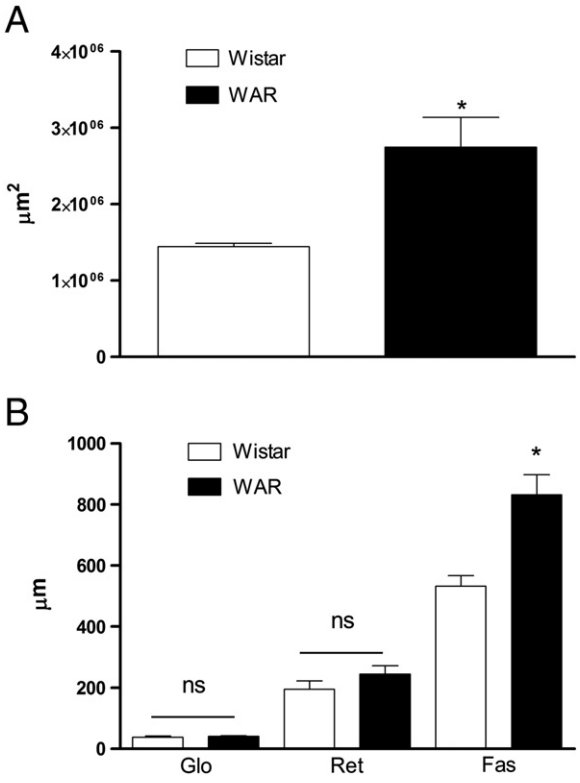

Fig. 3 – Adrenal gland medullar area (A) and thickness of glomerulosa (Glo), reticularis (Ret) and fasciculata (Fas) cortical layers (B) of Wistar rats and WARs in basal conditions obtained from morphometric analysis. *p < 0.05 vs. Wistar.

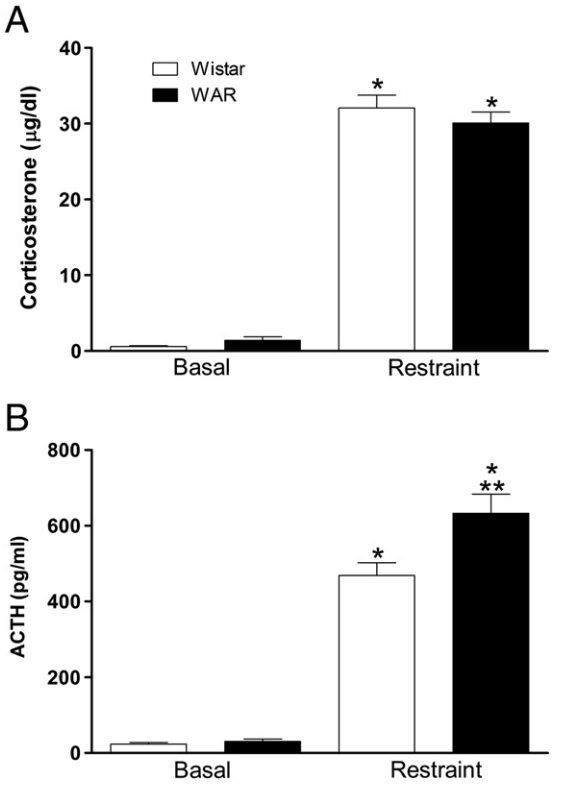

Fig. 4 – Plasma corticosterone (A) and ACTH (B) levels in Wistar rats and WARs after 15 min of restraint stress. * p < 0.05 vs. Basal and ** p < 0.05 vs. Wistar restraint.

Despite this difference in ACTH release, in the same protocol, the plasma corticosterone level did not differ between WARs and Wistar, suggesting a possible ACTH roof effect. It is important to point out that ACTH is a known anti-convulsant factor and it has long been used in clinical protocols to treat infantile spasms (IS) in West Syndrome (WS) and other syndromes that are resistant to conventional treatment (Mackay et al., 2004; Riikonen, 2004). However, there is not a well-established animal model for WS, and in several animal models of IS ACTH shows low efficacy to reduce the spasms (Chudomelova et al., 2010). Scantlebury et al. (2010), for example, showed that in a multiple-hit model of symptomatic IS cosyntropin—a synthetic derivative of ACTH—fails to suppress spasms. Therefore, ACTH is not necessarily anti-convulsant in rodent models of epilepsy, and more studies are necessary to better understand the role of ACTH in audiogenic seizures in WARs. In contrast to ACTH, corticosterone is a well-established pro-convulsant molecule in both acute and chronic animal models of epilepsy (Kling et al., 1993; Roberts and Keith, 1995; Karts et al., 1999).

The plasma levels of ACTH and corticosterone in Wistar rats after 15 min of restraint stress were similar to those found by Elias et al. (2002). These authors also showed that Wistar animals in basal conditions, when treated with exogenous CRH and ACTH between 8 a.m. and 10 a.m., had elevated values of ACTH and corticosterone. Our current experiments, however, show that WARs submitted to exogenous application of ACTH had plasma corticosterone levels that were even more elevated

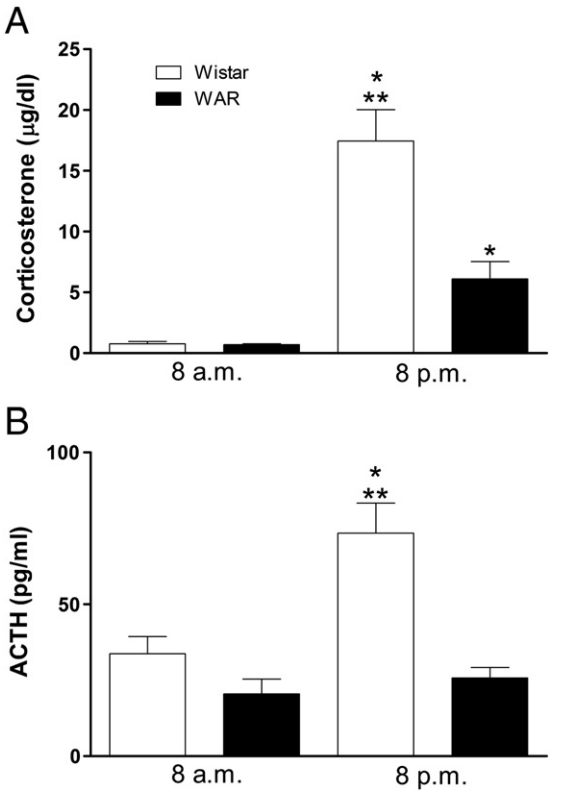

Fig. 5 – Plasma corticosterone (A) and ACTH (B) values in basal conditions at 8 a.m. and 8 p.m. in Wistar rats and WARs. * p < 0.05 vs. 8 a.m. and ** p < 0.05 vs. WARs 8 p.m.

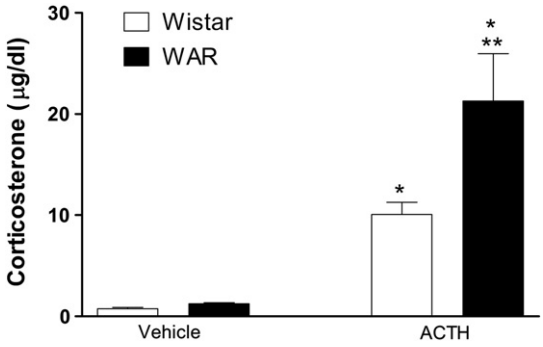

Fig. 6 – Plasma corticosterone values after ACTH stimulus in Wistar rats and WARs. * p<0.05 vs. vehicle and ** p<0.05 vs. Wistar ACTH.

than those of Wistar rats. This higher response to exogenous ACTH in WARs could be ascribed to their increased adrenal gland weight. It will be interesting to test whether this adrenal weight increase in WARs might be a phenomenon compatible with the known pro-convulsant effect of glucocorticoids (Roberts and Keith, 1995).

It is well known that glucocorticoids exert neuronal excitatory effects, which are mediated through binding to central mineralocorticoid receptor (MR) in the hippocampus. Clear evidence of excitatory effects of MR was shown by Joëls and de Kloet (1992). These authors examined, for example, the effect of the MR antagonist spironolactone on hippocampal slice electrophysiology and showed that low doses of this substance blocked the excitatory effect of corticosterone. MR also plays an important role in mediating limbic seizures (Roberts and Keith, 1995). In addition, mice were more susceptible to seizures induced by kainic acid when their plasma corticosterone levels were near their circadian peak (Roberts and Keith, 1994). In accordance with these data, there is a positive association between stress and seizure frequency in adult epileptics (Lambie et al., 1986; Temkin and Davis, 1984; Mattson, 1991). Accordingly, it was recently demonstrated that soldiers in combat units have a higher seizure incidence than soldiers that work under less stressful conditions (Moshe et al., 2008).

We also studied the circadian rhythm of the HPA axis of Wistar rats and WARs, and as expected, we observed that Wistar rats present a normal circadian rhythm, with higher plasma corticosterone and ACTH levels at 8 p.m. (lights off) as compared with 8 a.m. Moreover, WARs showed preserved daily variation of plasma corticosterone levels; however, they did not show diurnal variation in plasma ACTH levels. Disruption of circadian rhythm and of the HPA axis associated with seizures was previously reported in humans and animal experimental models. Linkowski et al. (1987) showed that the timing of the circadian rhythms of ACTH and cortisol, as well as the duration of the quiescent period of cortisol secretion, was normalized in patients after electroconvulsive therapy. Quigg et al. (1998) showed that electrically-induced seizures modify the circadian rhythm of body temperature in hippocampally kindled rats.

Thus, because WARs are endogenously susceptible to seizures and they have an altered circadian rhythm pattern of ACTH release, it will be interesting to demonstrate in a new set of experiments whether or not WARs also present alterations in circadian rhythms related to other factors (e.g., body temperature control). In light of the strong associations between stress hormones and epilepsy, additional studies are under way in order to test the impact of neuroendocrinological alterations found in WARs on ictogenesis and epileptogenesis. In this direction an example is the study by Mazarati et al. (2009) where the use of the dexamethasone/CRH test (Johnson et al., 2006) demonstrated that *status epilepticus* induced by pilocarpine/LiCl leads to hyperactivity of the HHA axis.

We observed morphologic alterations in adrenal medulla in WARs, which is compatible with endogenous hypertension, increased heart rate and increased sympathetic tonus observed in these rats (Fazan et al., 2010). Moreover, the increase in adrenal gland cortical fasciculate layer thickness helps to explain the hyper-responsivity of WARs to HPA axis stimulation, as shown here and by the stressful profile of WARs in the elevated plus maze and the open field (Garcia-Cairasco et al., 1998). It will be extremely important to further investigate whether the increased area of the adrenal medulla is associated with changes in the adrenal release of catecholamines.

In conclusion, WARs have a hyperplasic adrenal gland, do not present ACTH circadian cycles and have higher corticosterone levels in response to exogenous ACTH than Wistar controls. These HPA axis abnormalities make WARs a suitable model to study stress and epilepsy as well as epilepsy–neuropsychiatry comorbidities.

4. Experimental procedures

Male Wistar rats that were not susceptible to audiogenic seizures from the main breeding colony at the Campus of Ribeirão Preto of the University of São Paulo and males from the WAR strain susceptible to sound-induced seizures (Doretto et al., 2003a) were used in this study.

All experimental protocols used in this study were reviewed and approved by the Animal Care and Use Committee of the School of Medicine of Ribeirão Preto of the University of de São Paulo (Protocol number 203/2005).

WARs were derived from a Wistar strain of albino rats and have been selected for audiogenic seizure sensitivity (Doretto et al., 2003a) at the Vivarium of the Physiology Department of the Ribeirão Preto School of Medicine at the University of São Paulo. Wistar and WARs were age-matched (56 to 63 days) and individually housed with free access to standard rat food and water in a controlled environment with a light/dark cycle of 12/12 h (light on at 6 a.m. and light off at 6 p.m.). The animals were allowed to habituate to the room for at least 5 days prior to the studies and were handled and weighed daily in order to reduce stress during the experiments.

4.1. *Body weight curve construction*

To determine the animal's growth, both WARs and Wistar were weighed weekly, from their birth until the 9th week of age. When animals were 21 days old, they were separated from their mothers and housed in collective cages with free access to food and water.

4.2. Circadian rhythms of corticosterone and ACTH

To evaluate the circadian rhythm of corticosterone and ACTH plasma levels and adrenal gland weight, rats were decapitated under basal conditions at 8 a.m. and 8 p.m., and trunk blood samples were used for plasma corticosterone and ACTH measurements. In the morning, we also determined the left adrenal gland weight. Groups: Wistar 8 am (n=6), Wistar 8 pm (n=6), WAR 8 am (n=6) and WAR 8 pm (n=7).

4.3. Histology and morphometric analysis of adrenal glands

To perform the morphometric analysis of adrenal gland, we collected the glands of WAR and Wistar under basal conditions. Adrenal glands were fixed for 24 h in formalin, embedded in paraffin, and serially sectioned at 5 μm. Sections were stained with Gomori's trichrome by standard protocols and photographed using a Zeiss Axiostar Plus microscope fitted with an Axiovision digital camera (Zeiss, Hemel Hempstead, UK).The area of the cortex was analyzed from digital images using AxiovisionRel4.6 software. The measurement was performed on four adjacent sections from the middle portion of each individual adrenal gland to ensure a reliable comparison. The medullary area and the length of the cortical layers (reticularis, fasciculata and glomerulosa) were measured under standardized conditions. Measurements were expressed in μm (length) or mm^2 (areas).

4.4. Restraint stress

Animals were subjected to restraint stress by placing them in a metal restrainer (17×6×5 cm) for 15 min, followed by decapitation and blood collection for corticosterone and ACTH measurements. The groups were as follows: Wistar basal (n=5), Wistar restraint (n=6), WAR basal (n=4) and WAR restraint (n=5).

To determine the adrenal responsiveness to ACTH 24 h before the experiments, rats were anesthetized with 2,2, 2-tribromoethanol (25 mg/100 g bw. i.p., Aldrich, Milwaukee, WI, USA); a catheter was inserted into the right external jugular vein and advanced to the right atrium (Harms and Ojeda, 1974) for i.v. drug administration. On the day of the experiment, rats were pretreated with dexamethasone (100 μg/100 g subcutaneously) and 2 h later they received an i.v. injection of ACTH (Synacthène, Novartis—8 ng/rat) or vehicle (0.9% NaCl). Trunk blood samples for plasma corticosterone determination were collected by decapitation 15 min after the injection. Groups: Wistar vehicle (n=4), Wistar ACTH (n=5), WAR vehicle (n=4) and WAR ACTH (n=5).

4.5. Radioimmunoassays

Plasma hormone levels were determined by specific radioimmunoassay, as previously described (Elias et al., 2002). The assay sensitivity was 0.4 μg/dl for corticosterone and 16 pg/ml for ACTH. The intra- and inter-assay coefficients of variation were 4% and 8% for corticosterone and 4.3% and 16% for ACTH, respectively. All samples from a single experiment were assayed in duplicate in the same assay.

4.6. Statistical analysis

Data were expressed as means±SEM. Two-way ANOVA was used to analyze the data obtained from experiments on circadian rhythm variation, restraint stress and exogenous ACTH stimulation. To analyze adrenal gland weight, adrenal medulla area and adrenal cortex layers, the *Mann–Whitney* test was used. Significance was established at $p<0.05$.

Author's contributions

Eduardo HL Umeoka: Experimental procedures, data analysis and article preparation. Sérgio Britto Garcia: Histopathology and morphometry analysis of adrenal gland. José Antunes-Rodrigues, Lucila LK Elias and Norberto Garcia-Cairasco: Experimental design, advice on execution of experimental protocols/methods and article preparation.

Acknowledgments

This project is supported by FAPESP, FAPESP-Cinapce, CAPES, PROEX-Physiology and PROEX-Neurology, FAEPA. JAR, LLEK and NGC are recipients of CNPq-Brazil research fellowship.

We wish to thank all members of the Neuroendocrinology Laboratory and Neurophysiology and Experimental Neuroethology Laboratory, especially Rodrigo Rorato, Mauricio Benedetti, Olagide Castro, Victor Santos Rodrigues and Simone Marroni. The authors also wish to thank Maria Valci Silva, Marina Holanda and Rosangela Orlandin Lopes for their technical support.

REFERENCES

Amado, D., Cavalheiro, E.A., Bentivoglio, M., 1993. Epilepsy and hormonal regulation: the patterns of GnRH and galaninimmunoreactivity in the hypothalamus of epileptic female rats. Epilepsy Res. 14, 149–159.

Bajorek, J.G., Lee, R.J., Lomax, P., 1984. Neuropeptides: a role as endogenous mediators or modulators of epileptic phenomena. Ann. Neurol. 16, S31–S38.

Bradbury, M.J., Cascio, C.S., Scribner, K.A., Dallman, M.F., 1991. Stress-induced adrenocorticotropin secretion: diurnal responses and decreases during stress in the evening are not dependent on corticosterone. Endocrinology 128, 680–688.

Chudomelova, L., Scantlebury, M.H., Raffo, E., Coppola, A., Betancourth, D., Galanopoulou, A.S., 2010. Modeling new therapies for infantile spasms. Epilepsia 51 (Suppl 3), 27–33.

Culebras, A., Miller, M., Bertram, L., Kock, J., 1987. Differential response of growth hormone, cortisol, and prolactin to seizures and to stress. Epilepsia 28, 564–570.

Dallman, M.F., Akana, S.F., Scribner, A.K., Bradbury, M.J., Walker, C.D., Strack, A.M., Cascio, C.S., 1992. Stress, feedback and facilitation in the hypothalamo–pituitary–adrenal axis. J. Neuroendocrinol. 4, 517–526.

De Kloet, E.R., Joëls, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 463–475.

Delgado-Escueta, A.V., Ward Jr., A.A., Woodbury, D.M., Porter, R.J., 1986. New wave of research in the epilepsies. In:

Delgado-Escueta, A.V., Ward Jr., A.A., Woodbury, D.M., Porter, R.J. (Eds.), Advances in Neurology. Raven Press, New York, pp. 3–55.

Doretto, M.C., Fonceca, C.G., Lobo, R.B., Terra, V.C., Oliveira, J.A., Garcia-Cairasco, N., 2003a. Quantitative study of the response to genetic selection of the Wistar audiogenic rat strain (WAR). Behav. Genet. 33, 33–42.

Doretto, M.C., Oliveira-e-Silva, M., Ferreira-Alves, D.L., Pires, S.G., Garcia-Cairasco, N., Reis, A.M., 2003b. Effect of lactation on the expression of audiogenic seizures: association with plasma prolactin profiles. Epilepsy Res. 54, 109–121.

Dutra Moraes, M.F., Galvis-Alonso, O.Y., Garcia-Cairasco, N., 2000. Audiogenic kindling in the Wistar rat: a potential model for recruitment of limbic structures. Epilepsy Res. 39, 251–259.

Elias, L.L., Dorival-Campos, A., Moreira, A.C., 2002. The opposite effects of short- and long-term salt loading on pituitary adrenal axis activity in rats. Horm. Metab. Res. 34, 207–211.

Fazan, R., Oliveira, M., Cortes-Oliveira, J.A., Salgado, H.C., Garcia-Cairasco, N., 2010. Changes in autonomic control of the cardiovascular system in the Wistar audiogenic rat (WAR) strain, an experimental model of epilepsy. FASEB J. 24, lb558.

Frucht, M.M., Quigg, M., Schwaner, C., Fountain, N.B., 2000. Distribution of seizure precipitants among epilepsy syndromes. Epilepsia 41, 1534–1539.

Garcia-Cairasco, N., 2009. Puzzling challenges in contemporary neuroscience: insights from complexity and emergence in epileptogenic circuits. Epilepsy Behav. 1, 54–63.

Garcia-Cairasco, N., Doretto, M.C., Ramalho, M.J., Antunes-Rodrigues, J., Nonaka, K.O., 1996. Audiogenic and audiogenic-like seizures: locus of induction and seizure severity determine postictal prolactin patterns. Pharmacol. Biochem. Behav. 53, 503–510.

Garcia-Cairasco, N., Oliveira, J.A., Wakamatsu, H., Bueno, S.T., Guimarães, F.S., 1998. Reduced exploratory activity of audiogenic seizures susceptible Wistar rats. Physiol. Behav. 64, 671–674.

Harms, P.G., Ojeda, S.R., 1974. A rapid and simple procedure for chronic cannulation of the rat jugular vein. J. Appl. Physiol. 36, 391–392.

Joëls, M., de Kloet, E.R., 1992. Control of neuronal excitability by corticosteroid hormones. Trends Neurosci. 15, 5–30.

Johnson, S.A., Fournier, N.M., Kalynchuk, L.E., 2006. Effect of different doses of corticosterone on depression-like behavior and HPA axis responses to a novel stressor. Behav. Brain Res. 168, 280–288.

Kant, G.J., Mougey, E.H., Meyerhoff, J.L., 1986. Diurnal variation in neuroendocrine responses to stress in rats: plasma ACTH, β-endorphin, β-LPH, corticosterone, prolactin and pituitary cyclic AMP responses. Neuroendocrinology 43, 383–390.

Karts, H., de Kloet, E.R., Joels, M., 1999. Episodic corticosterone treatment accelerates kindling epileptogenesis and triggers long-term changes in hipocampal CA1 cells in the fully kindled state. Eur. J. Neurosci. 11, 889–898.

Kling, M.A., Smith, M.A., Glowa, J.R., Pluznikk, D., Demas, J., de Bellis, M.D., Gold, P.W., Schulkin, J., 1993. Facilitation of cocaine kindling by glucocorticoids in rats. Brain Res. 26, 163–166.

Lambie, D.G., Stanaway, L., Johnson, R.H., 1986. Factors which influence the effectiveness of treatment of epilepsy. Aust. NZ J. Med. 16, 779–784.

Linkowski, P., Mendlewicz, J., Kerhofs, M., Leclercq, R., Golstein, J., Brasseur, M., Copinschi, G., Van Cauter, E., 1987. 24-h profiles of adrenocorticotropin, cortisol, and growth hormone in major depressive illness: effect of antidepressant treatment. J. Clin. Endocrinol. Metab. 65, 141–152.

Mackay, M.T., Weiss, S.K., Adams-Webber, T., Ashwal, S., Stephens, D., Ballaban-Gill, K., Baram, T.Z., Duchowny, M., Hirtz, D., Pellock, J.M., Shields, W.D., Shinnar, S., Wyllie, E., Snead, O.C., 2004. 3rd; American Academy of Neurology; Child Neurology Society. Practice parameter: medical treatment of infantile spasms: report of the American Academy of Neurology and the Child Neurology Society. Neurology 62, 1668–1681.

Mattson, R.H., 1991. Emotional effects on seizure occurrence. Adv. Neurol. 55, 453–460.

Mazarati, A.M., Shin, D., Kwon, Y.S., Bragin, A., Pineda, E., Tio, D., Taylor, A.N., Sankar, R., 2009. Elevated plasma corticosterone level and depressive behavior in experimental temporal lobe epilepsy. Neurobiol. Dis. 34, 457–461.

McEwen, B.S., Stellar, E., 1993. Stress and the individual. Mechanisms leading to disease. Arch. Intern. Med. 153, 2093–2101.

Meldrum, B.S., Horton, R.W., Bloum, S.R., Butter, J., Keenam, J., 1979. Endocrine factors and glucose metabolism during prolonged seizures in baboons. Epilepsia 20, 527–534.

Moshe, S., Shilo, M., Chodick, G., Yagev, Y., Blatt, I., Korczyn, A.D., Neufeld, M.Y., 2008. Occurrence of seizures in association with work-related stress in young male army recruits. Epilepsia 49, 1415–1456.

Munck, A., Guyre, P.M., Holbrook, N.J., 1984. Physiological functions of glucocorticoids in stress and their relations to pharmacological actions. Endocr. Rev. 5, 25–44.

Nakken, K.O., Solaas, M.H., Kjeldsen, M.J., Friis, M.L., Pellock, J.M., Corey, L.A., 2005. Which seizure-precipitating factors do patients with epilepsy most frequently report? Epilepsy Behav. 6, 85–89.

Plotnikoff, N.P., Kastin, A.J., 1977. Neuropharmacological review of hypothalamic releasing factors. In: Miller, X., Sandman, X., Kastin, A. (Eds.), Neuropeptide Influences on the Brain and Behavior. Raven Press, New York, pp. 81–107.

Pritchard III, P.B., Wannamakes, B.B., Sagel, J., Daniel, C.M., 1985. Serum prolactin and cortisol levels in evaluation of pseudoepileptic seizures. Ann. Neurol. 18, 87–89.

Quigg, M., Clayburn, H., Straume, M., Menaker, M., Bertram, E.H., 1998. Acute seizures may induce phase shifts in the circadian rhythm of body temperature in kindled rats. Epilepsia 39, 136.

Riikonen, R., 2004. Infantile spasms: therapy and outcome. J. Child Neurol. 19, 401–404.

Roberts, A.J., Keith, L.D., 1994. Mineralocorticoid receptors mediate the enhancing effects of corticosterone on convulsion susceptibility in mice. J. Pharmacol. Exp. Ther. 270, 505–511.

Roberts, A.J., Keith, L.D., 1995. Corticosteroids enhance convulsion susceptibility via central mineralocorticoid receptor Psychoneuroendocrinology 20, 891–902.

Scantlebury, M.H., Galanopoulou, A.S., Chudomelova, L., Raffo, E., Betancourth, D., Moshé, S.L., 2010. A model of symptomatic infantile spasms syndrome. Neurobiol. Dis. 37, 604–612.

Spector, S., Cull, C., Goldstein, L.H., 2000. Seizure precipitants and perceived self-control of seizures in adults with poorly-controlled epilepsy. Epilepsy Res. 38, 207–216.

Sperling, M.R., Wilson, Ch.L., 1986. The effect of limbic and extralimbic electrical stimulations upon prolactin secretion in humans. Brain Res. 371, 293–297.

Temkin, N.R., Davis, G.R., 1984. Stress as a risk factor for seizures among adults with epilepsy. Epilepsia 25, 450–456.

Trimble, M.R., 1978. Serum prolactin in epilepsy and hysteria Br. Med. J. 2, 1682.

Wyllie, E., Luders, H., McMillan, J.P., Gupta, M., 1984. Serum prolactin levels after epileptic seizures. Neurology 34, 1601–1604.