Journal of the American Society of Hypertension 12(8) (2018) 605–613

Research Article

Short-term exposure to dexamethasone promotes autonomic imbalance to the heart before hypertension

Francine Duchatsch, BS[a,b], Paula B. Constantino, MS[a,b], Naiara A. Herrera, MS[a,b], Mayara F. Fabrício, MS[a,b], Lidieli P. Tardelli, MS[a,b], Aline M. Martuscelli, MS[b], Thiago J. Dionísio, PhD[c], Carlos F. Santos, PhD[c], and Sandra L. Amaral, PhD[a,b,*]

[a]*Joint Graduate Program in Physiological Sciences, PIPGCF UFSCar/UNESP, São Carlos, SP, Brazil;*
[b]*Department of Physical Education, Science Faculty, UNESP – São Paulo State University, Bauru, SP, Brazil; and*
[c]*Department of Biological Sciences, Bauru School of Dentistry, USP – University of São Paulo, Bauru, SP, Brazil*

Manuscript received February 7, 2018 and accepted June 5, 2018

Abstract

Hypertension is one of the chronic side effects of dexamethasone (DEX) treatment; however, almost nothing is known about its acute effects. Therefore, the aim of this study was to investigate the possible mechanisms involved in blood pressure control after acute or short-term DEX treatment in adult animals. Eighty Wistar rats were divided into four groups: C1 and C5, for rats treated with saline for 1 or 5 days, respectively; D1 and D5, for rats treated with DEX for 1 or 5 days, respectively (decadron, 1 mg/kg, *i.p.*). Heart rate was increased in DEX treatment, but arterial pressure and cardiac muscle mass were not altered. Only few and isolated changes on gene expression and protein level of renin-angiotensin system components were observed. Five days of DEX treatment, but not one day, determined an increase in sympathetic component of spectral analysis (+75.93%, $P < .05$) and a significant reduction of parasympathetic component (–18.02%, $P < .05$), which contributed to the autonomic imbalance to the heart (LF/HF, +863.69%). The results of this present study demonstrated, for the first time, that short-term exposure to DEX treatment impairs the autonomic balance to the heart before hypertension, which was independent of renin-angiotensin system. J Am Soc Hypertens 2018;12(8):605–613.

Keywords: Autonomic nervous system; blood pressure; glucocorticoids.

Introduction

Dexamethasone (DEX)-induced hypertension is a well-known side effect that occurs after its chronic use in both animals[1–3] and humans.[4,5] In addition to hypertension, this widely used synthetic glucocorticoid may cause body weight (BW) loss (in animals), muscle atrophy, hyperglycemia, peripheral insulin resistance, dyslipidemia, and liver steatosis.[3,6–12]

The mechanisms responsible for DEX-induced hypertension are not well established, but some studies have suggested the role of nitric oxide (NO),[13–16] oxidative stress followed by endothelial dysfunction,[16,17] as well as autonomic nervous system[3,18–20] and renin-angiotensin system (RAS) alterations.[21,22] Recently, our group has demonstrated that chronic DEX treatment determined hypertension associated with decreases in baroreflex activity, without any significant tissue RAS changes.[23]

Grant Support: This study was supported by São Paulo Research Foundation (FAPESP #2014/23229–6 grant to S.L.A. and 2015/03965-2 to CFS). F.D., N.A.H., and P.B.C. were recipients of scholarships from FAPESP (#2014/23050–6, #2014–18177–7 and #2012/19722–3, respectively). M.F.F. and A.M.M. were recipients of scholarships from the Coordination for the Improvement of Higher Education Personnel (CAPES). T.J.D. was a recipient of a scholarship from the National Council for Scientific and Technological Development (CNPq, process # 140206/2011–6).

Conflict of interest: The authors have no conflicts of interest to disclose.

*Corresponding author: Sandra L. Amaral, PhD, Department of Physical Education, Science Faculty, UNESP– São Paulo State University, Bauru, Joint Graduate Program in Physiological Sciences, UFSCar/UNESP, SP, Brazil. Tel: +55 14 3103–6082x9609; Fax: +55 14 3103–6082.

E-mail: slamaral@fc.unesp.br

1933-1711/$ - see front matter
https://doi.org/10.1016/j.jash.2018.06.004

Over the last years, most of the studies have analyzed the effects of a prenatal exposure to short-term DEX treatment (or other glucocorticoid, such as betamethasone) to understand the role of glucocorticoids on hypertension. RAS involvement on this type of programmed hypertension is still controversial. Although DEX increased vasoconstriction induced by angiotensin II (Ang II) in sheep's offspring coronary rings,[24] other studies have shown that treatment with AT1 receptor antagonist, in lambs whose mothers were treated with dexamethasone[25] or betamethasone,[26] failed to decrease mean arterial pressure. In addition, it has been shown that treatment of pregnant ewes with DEX or betamethasone throughout gestation provokes hypertension in offspring lambs, which may be associated with hemodynamic and autonomic dysfunctions.[26,27] In addition, in this model of programmed hypertension induced by antenatal treatment with glucocorticoids, sometimes baroreflex alterations may appear before hypertension, which may be associated with cardiac hypertrophy.[27–29]

Our group has shown that acute DEX treatment increases glucose and insulin levels[30] and determines body and muscle weight loss.[11,30] Nevertheless, the autonomic balance to the heart and RAS components expression in the left ventricle (LV) muscle have not been evaluated after acute or short-term DEX treatment in adult rats. Therefore, the aim of this study was to investigate the possible mechanisms involved in blood pressure control after acute or short-term DEX treatment in adult animals. The hypothesis of this study was that acute or short-term DEX treatment may impair autonomic balance to the heart and increase gene/protein level of RAS components in myocardium, which may contribute to the establishment of hypertension.

Methods

Animal Care

For this study, 80 rats (Wistar, 200–250 g) from the Center for Research and Production Facilities of UNESP (Botucatu, SP, Brazil) were used. All rats were kept in cages (five in each) at the Animal Facility Maintenance from Faculty of Science, UNESP at Bauru. Water and food (Biobase, Águas Frias, SC, Brazil) were given *ad libitum*. Rats were maintained in dark-light cycle (12–12 hours) with controlled temperature (22°C). All procedures were approved by the Committee for Ethical Use of Animals of UNESP—São Paulo State University, campus at Bauru (approved protocol # 1434–2014).

Groups and Pharmacological Treatment

Rats were randomly divided into four groups: 1/C1, 20 animals that received saline injection for 1 day *(i.p.)*; 2/D1, 20 animals that received DEX injection for 1 day (Decadron, 1 mg/kg of BW, *i.p.*, at 9 AM); 3/C5, 20 animals that received daily saline injections during 5 days *(i.p.)*; 4/D5, 20 animals that received daily DEX injections (Decadron, 1 mg/kg of BW, *i.p.*, at 9 AM) during 5 days.

Cardiovascular Parameters

On the last day of DEX or saline treatment, rats were anesthetized with tribromoethanol (250 mg/kg, *i.p.*) and a small incision on carotid artery was done to insert a polyethylene catheter. After 24 hours, arterial pressure (AP) and heart rate (HR) were continuously recorded for 30 minutes, in a quiet room, using a pressure transducer (DPT100, Utah Medical Products Inc, Midvale, UT, USA) connected to the artery cannula that sent the signal to an amplifier (Quad Bridge Amp, ADInstruments, NSW, Australia) and then to an acquisition board (Powerlab 4/35, ADInstruments, NSW, Australia) as published.[3] Mean arterial pressure, systolic arterial pressure, diastolic arterial pressure, and HR were derived from pulsatile AP recordings.

Spectral Analysis

Cardiac pulse interval (PI) from long recordings (15–30 minutes) was processed by a computer software (Labchart v7.0, ADInstruments, NSW, Australia) as previously published,[3] which uses an algorithm that detects cycle-to-cycle inflection points in the pulsatile AP signal. Thus, HR variability analysis within frequency domain was processed using DIAS software (DPM, from University of São Paulo, Brazil, CardioSeries V2.4, http://www.danielpenteado.com) by a non-parametric fast Fourier transform algorithm. From these data, it was obtained a low frequency band power (LF, 0.20–0.75 Hz) and high frequency band power (HF, 0.75–3.0 Hz), which are related to sympathetic and parasympathetic activity, respectively. Results were expressed as normalized units (nu) and, to assess the sympathovagal balance, the LF/HF ratio of PI variability was calculated.[31,32]

Tissue Harvesting

After cardiovascular parameters measurements, all animals were euthanized by an overload of xylazine hydrochloride (20 mg/kg, *i.p.*; Anasedan, Paulínia, SP, Brazil) and ketamine hydrochloride (160 mg/kg, *i.p.;* Dopalen, Paulínia, SP, Brazil). LV muscle was removed, cleaned, and immediately weighed. One sample of the heart was stored at –80°C for protein analysis and another portion was kept in RNAlater tissue storage reagent (Qiagen, 21 Strasse, Germany) and maintained at −80°C until RNA extraction, as previously published.[12]

Total RNA Extraction

RNeasy mini kit (Qiagen, Hilden, Germany) was used for total RNA extraction according to the manufacturer's instructions. Once reconstituted, total RNA sample was measured and qualified in a spectrophotometer (NanoDrop 1000, Thermo Scientific, Waltham, MA, USA). Two microliters of each sample were used to obtain the readings at wavelengths of 260 nm (A260) and 280 nm (A280), which provided information about the quantity and quality of RNA. Samples with values between 1.9 and 2.1 on the A260/A280 were transcribed. All total RNA samples were treated with DNase (gDNA wipeout—Qiagen, Hilden, Germany) and immediately subjected to reverse transcription process with Quantitect Reverse Transcription kit (Qiagen, Hilden, Germany). Relative quantification of RAS components was analyzed by means of real-time PCR reactions using the TaqMan system (Applied Biosystems, Foster City, CA, USA) in a thermocycler (ViiA 7; Applied Biosystems). PCR reactions were performed in duplicate and water was used as a negative control. The quantitative mRNA expression was analyzed for AT1a (Rn01775763_g1), AT2 (Rn00560677_sl), ACE (Rn00561094_m1), ACE–2 (Rn01416293_m1), MAS receptor (Rn00562673_sl), renin (Rn00561847_m1), and angiotensinogen (Rn00593114_m1). mRNA expression data were calculated for values of the threshold cycle (Ct) using the $\Delta\Delta$Ct method with the $2^{-\Delta\Delta Ct}$ formula to calculate the relative quantification and normalized by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Rn01775763_g1). Therefore, data are presented as mRNA expression relative to the control group.

Western Blotting Procedures

LV muscle samples were homogenized (IKA T18, Staufen, Germany) in RIPA solution (Cell Signaling Technology, Danvers, MA, USA); 0.1% protease inhibitor cocktail (Pic, Sigma Aldrich, SLM, USA) and 1% phenylmethanesulfonyl fluoride (PMSF, Sigma Life Science, USA) were added to the samples just before the homogenization, as previously published[9] and centrifuged for 10 minutes at 4°C with 11,200 × *g*. The supernatant was transferred to a microcentrifuge tube and stored at –20°C. Bradford assay was used to determine protein concentration of the samples (Bio-Rad Kit, Protein Assay Standard II, Hercules, CA, USA) with albumin as standard.[33] Absorbance values were analyzed in a microplate reader (BMG LABTECH, SPECTROstar Nano, Ortenberg, Germany). Western blotting was performed according to previously reported procedures.[9] In summary, 50–80 μg of protein were electrophoretically separated by size using a gel system with two layers of polyacrylamide, at different concentrations: 5% in the upper layer and from 8% to 12% in the lower layer, depending on the molecular weight of the protein. These gels were then transferred to a nitrocellulose membrane for 2 hours. Membranes were then stained with Ponceau for protein bands verification and washed in Tris-buffered saline solution with tween–20 (TBS–T). In the next step, the membranes were incubated with blocking solution diluted in 5% bovine serum albumin in TBS–T solution for 2 minutes, using the SNAP i.d. 2.0 Protein Detection System (Merck Millipore, Darmstadt, Germany). Then, the membrane was incubated for 10 minutes with the following primary antibodies (in 3% bovine serum albumin): polyclonal renin antibody (Cell Signaling Technology, #5250, 1:1,000), monoclonal rabbit antiangiotensinogen (clone EPR 2931, Merck Millipore, #MABC123, Germany, 1:1,000), polyclonal rabbit anti-AT1 receptor (Merck Millipore, #AB15552 - 50 μL, Germany, 1:500), polyclonal rabbit anti-AT2 receptor (Merck Millipore, #AB15554 - 50 μL, Germany, 1:500), polyclonal antiangiotensin 1-7 anti-MAS receptor (Alomone Labs, #AAR-013, Israel 1:200), monoclonal mouse antihuman CD 143 (ACE, AbD Serotec, #MCA2056, UK) and ACE-2 rabbit antihuman monoclonal antibody (EPR4435(2), Lifespan Biosciences, #LS-B6324, 1:1,000) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, R&D System, # AF5718, 1: 1,000, Minneapolis, MN, USA) for normalization. Then, membranes were washed 3 × 10 minutes with TBS–T and incubated for 10 minutes with the respective secondary antibody: antimouse or antirabbit depending on the source of each primary antibody. Thereafter, antibody was detected using a chemiluminescence reaction kit (SuperSignal Pico, Pierce, Rockford, IL, USA), and the blots were visualized on x–ray film. The bands were analyzed by a computer program (Scion Image, Corporation, Beta 4.02).

Statistical Analysis

All data are expressed as mean ± standard error of the mean. Unpaired Student's t test was used to compare the groups. The significance level considered was $P < .05$.

Results

BW and Muscle Weight

One day of DEX treatment did not cause any change in BW when compared with control group (347 ± 6 g vs. 349 ± 7 g, respectively) ($P = .871$). However, when the animals were treated for 5 days with DEX, a significant reduction of 13.2% on BW was observed (296 ± 4 g vs. 342 ± 6 g, for D5 vs. C5, $P < .001$). DEX treatment did not change heart, right ventricle or LV weight, normalized by tibia bone size, as shown in Table 1.

Hemodynamic Responses

As shown in Figure 1, neither 1 day nor 5 days of DEX treatment changed values of systolic arterial pressure, diastolic arterial pressure, and mean arterial pressure.

Table 1
Values of body and heart weight in all groups analyzed

	C1	D1	C5	D5
Body Weight (g)	349 ± 6.55	347 ± 5.69	342 ± 6.35	296 ± 4.00*
Tibia (cm)	4.1 ± 0.03	4.1 ± 0.03	4.0 ± 0.02	3.9 ± 0.02
Heart (g)	1.01 ± 0.04	1.07 ± 0.03	0.91 ± 0.02	0.94 ± 0.02
Heart/Tibia(mg/cm)	246.38 ± 8.73	263.65 ± 7.30	230.65 ± 4.46	239.84 ± 5.04
RV (g)	0.22 ± 0.00	0.23 ± 0.001	0.15 ± 0.00	0.14 ± 0.001
RV/Tibia (mg/cm)	53.93 ± 1.34	56.81 ± 1.80	37.25 ± 1.00	36.81 ± 2.09
LF (g)	0.74 ± 0.03	0.75 ± 0.02	0.68 ± 0.02	0.70 ± 0.01
LV/Tibia (mg/cm)	180.14 ± 6.90	185.58 ± 6.03	173.27 ± 4.54	177.22 ± 2.98

C1 and D1, control and DEX-treated groups for 1 day; C5 and D5, control and DEX-treated groups for 5 days; RV, right ventricle; LV, left ventricle; LF, low frequency.

Values of heart weight, right ventricle, and left ventricle were normalized by tibia bone length.

Significance: * versus respective control; $P < .05$.

However, HR was significantly increased (8%, $P < .05$) after 5 days of DEX treatment.

Spectral Analysis

Through spectral analysis of the AP records, it was possible to verify that 1 day of DEX treatment did not cause any significant alteration, as illustrated in Figure 2. On the other hand, 5 days of DEX treatment significantly increased LF (+75.93%, $P < .003$), reduced HF (–18.02%, $P < .003$) and consequently promoted an autonomic imbalance to the heart (+863.69%, $P = .003$). DEX treatment for 5 days was not able to significantly change the sympathetic component value to the vessels ($P = .674$), as shown in Figure 2.

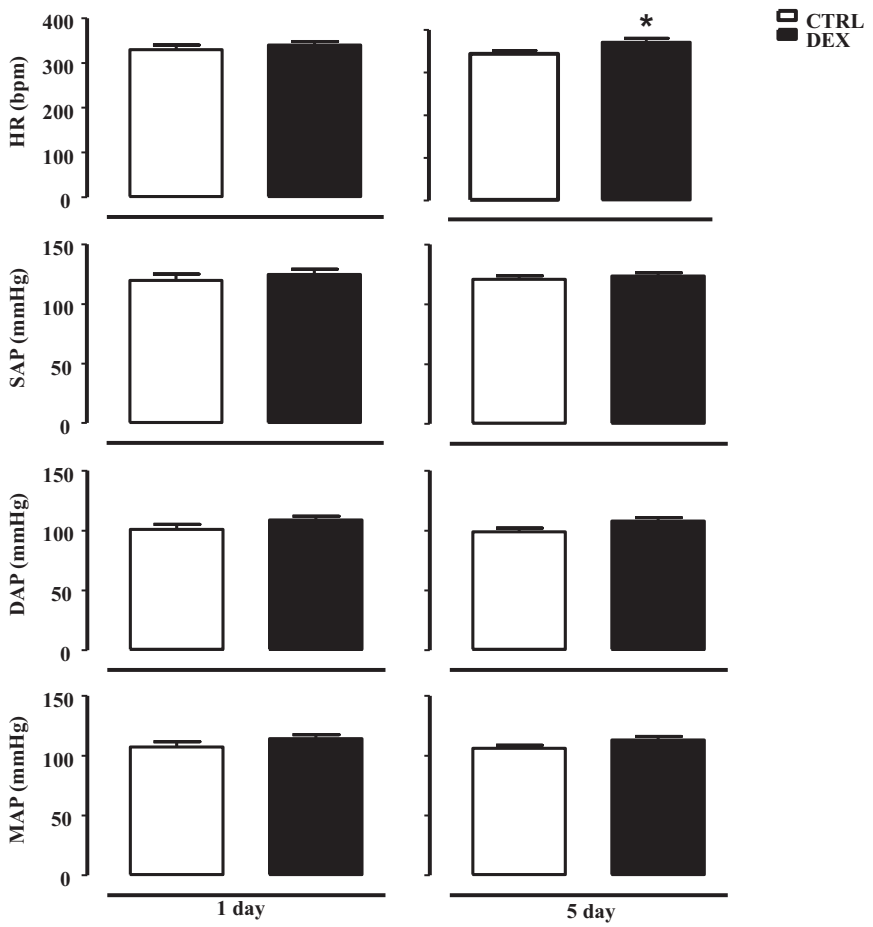

Figure 1. Values of heart rate (HR), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) in those groups treated with DEX for 1 day (left panel) or 5 days (right panel). (C1, n = 15; D1, n = 17; C5, n = 15; D5, n = 22). Significance: * versus respective control; $P < .05$.

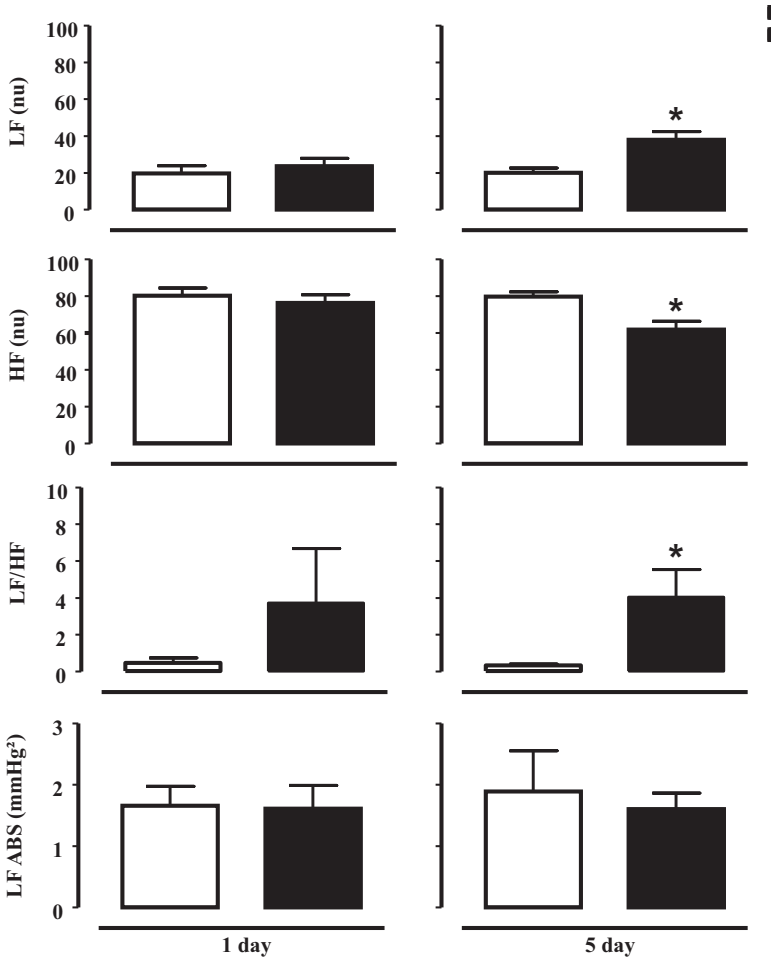

Figure 2. Analysis of autonomic balance to the heart. Low frequency in pulse interval (LF–PI), high frequency in pulse interval (HF–PI), low frequency divided by high frequency in pulse interval (LF/HF), and low frequency in 20 systolic arterial pressure (LF–SAP) in all groups: treated with saline for 1 day (C1, n = 14), treated with dexamethasone (DEX) for 1 day (D1, n = 14), treated with saline for 5 days (C5, n = 13), and treated with DEX for 5 days (D5, n = 14). Significance: * versus respective control, $P < .05$.

Gene Expression

DEX treatment promoted only isolated changes in RAS components in the LV muscle. One day of DEX treatment significantly reduced AGT (–43.42%, $P < .005$) and AT1 (–1.18%, $P < .043$) gene expression, compared with C1 group. These results were further decreased after 5 days of DEX treatment (–80.55%, $P < .015$ and –70.75%, $P < .024$, for AGT and AT1, respectively). In addition, ACE2 was decreased after 5 days of DEX treatment (–80.70%, $P < .010$), as shown in Figure 3.

Protein Level

Figure 4 shows that 1 day of DEX treatment did not alter protein level of any RAS components in the LV muscle; however, 5 days of DEX treatment significantly increased ACE (+106.57%, $P < .039$) and MAS (+91.12%, $P < .027$) protein level, compared with control (C5 group; Figure 4).

Discussion

The most interesting finding in this study was that short-term, but not acute, DEX treatment provoked autonomic balance alterations to the heart without any change on arterial pressure. In addition, both treatments were not able to promote significant alterations in myocardium expression of RAS components.

Our group has shown that chronic treatment with DEX promotes muscle atrophy and food intake decrease, which may be associated with BW decrease in rats.[7–11,30,34] In this present study, BW loss was observed only after 5 days of DEX treatment. The mechanisms responsible for this response were not investigated in this present study, but it is possible that food intake decrease could be contributing because this response is normally observed since the first day of DEX treatment.[10,11]

DEX-induced hypertension is an unwanted effect observed by several groups, using different protocols, dosages, and time of administration.[3,16,25,27,35–37] Similarly, arterial pressure increase is also observed after systemic or local betamethasone, cortisol or corticosterone

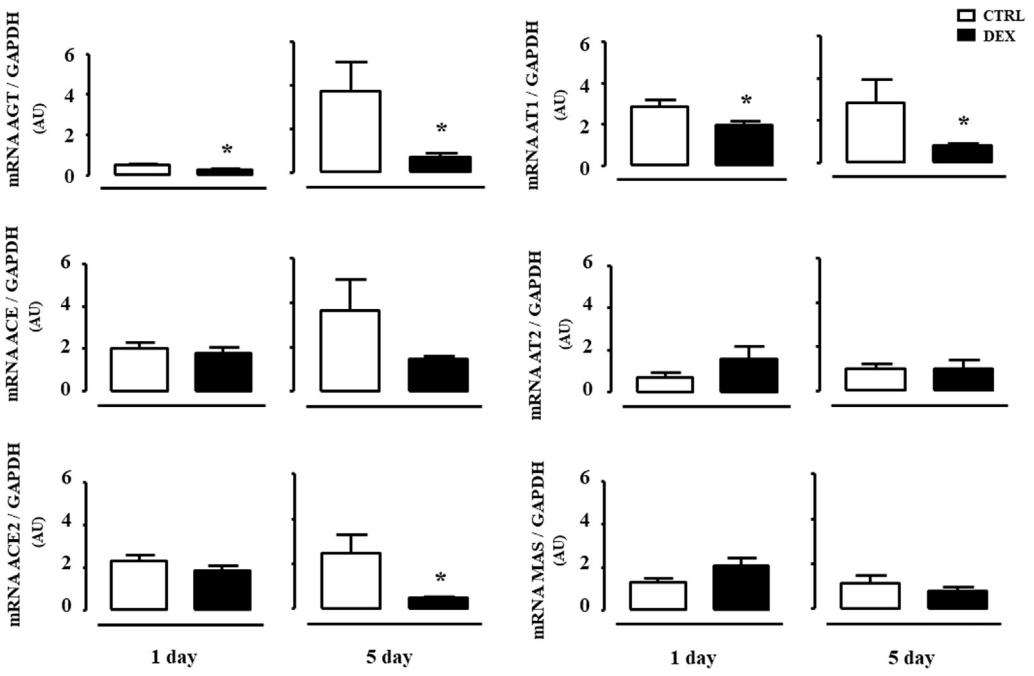

Figure 3. Relative expression of mRNA of the renin-angiotensin system (RAS) components in myocardium, in all groups analyzed: treated with saline for 1 day (C1, n = 14), treated with dexamethasone (DEX) for 1 day (D1, n = 14), treated with saline for 5 days (C5, n = 13), and treated with DEX for 5 days (D5, n = 14). Angiotensinogen (AGT), angiotensin II receptor type 1 (AT1a); angiotensin II receptor type 2 (AT2); angiotensin converting enzyme (ACE); angiotensin converting enzyme 2 (ACE2) and angiotensin$^{1–7}$ receptor (MAS). Significance: * versus respective control, $P < .05$.

treatment.[26,38–41] Although DEX is known to cause hypertension, as well as other glucocorticoids, little is known about the mechanisms involved in this effect.

The role of hemodynamic and autonomic control for etiology of hypertension induced by glucocorticoids has been studied for the last 2 decades, but the results are still unclear. While some authors have shown that hypertension in offspring sheep exposed prenatally to cortisol was associated with increased total peripheral resistance, due to sympathetic nerve activity increases,[40] others have postulated that programmed hypertension after DEX treatment during gestation may be associated with increased cardiac output and stroke volume, due to cardiac hypertrophy, without changes in total peripheral resistance.[27,36] In addition, it has been shown that DEX treatment (or betamethasone) provokes alterations in baroreflex curve,[26,27,41] but, in these studies, it is not possible to determine if baroreflex alterations contributed to the establishment of hypertension or increased blood pressure impaired baroreflex function. Accordingly, our group has recently demonstrated that chronic treatment with DEX provokes hypertension associated with baroreflex alterations[23] and autonomic unbalance to the heart, as well as increased sympathetic nerve activity to the periphery,[3] but the effects of short-term DEX treatment on autonomic functions and blood pressure control remain poorly understood.

In the present study, acute and short-term DEX treatment was not able to provoke increase in arterial pressure. However, short-term but not acute DEX treatment provoked an increase in heart rate may be due to an increase in sympathetic nervous activity and a decrease in parasympathetic nerve activity to the heart (which reflects an autonomic imbalance to the heart - higher LF/HF), suggesting that even though arterial pressure did not change, some neural alterations were already occurring, which may have important clinical relevance. Neural changes before any blood pressure alteration have been shown after antenatal treatment with DEX[27,28] and betamethasone.[29] These authors have demonstrated decreases of baroreflex gain or shifts of baroreflex curve toward higher pressure. These autonomic changes, even before hypertension, suggest that these effects may precede hypertension.

It has been proposed that these autonomic changes may be due to cardiac hypertrophy, which could reduce cardiac functional reserve[28,36]; however, the results of the present study do not confirm cardiac hypertrophy. It is important to note that those previous studies were performed using antenatal DEX treatment and, in the present study, the rats were treated in adult phase. DEX-induced autonomic imbalance to the heart, demonstrated for the first time in this present study, could be explained by the action of DEX on receptors localized in the dorsal hind brain because it has been shown that local treatment with corticosterone pellets on dorsal hind brain, in adult rats,

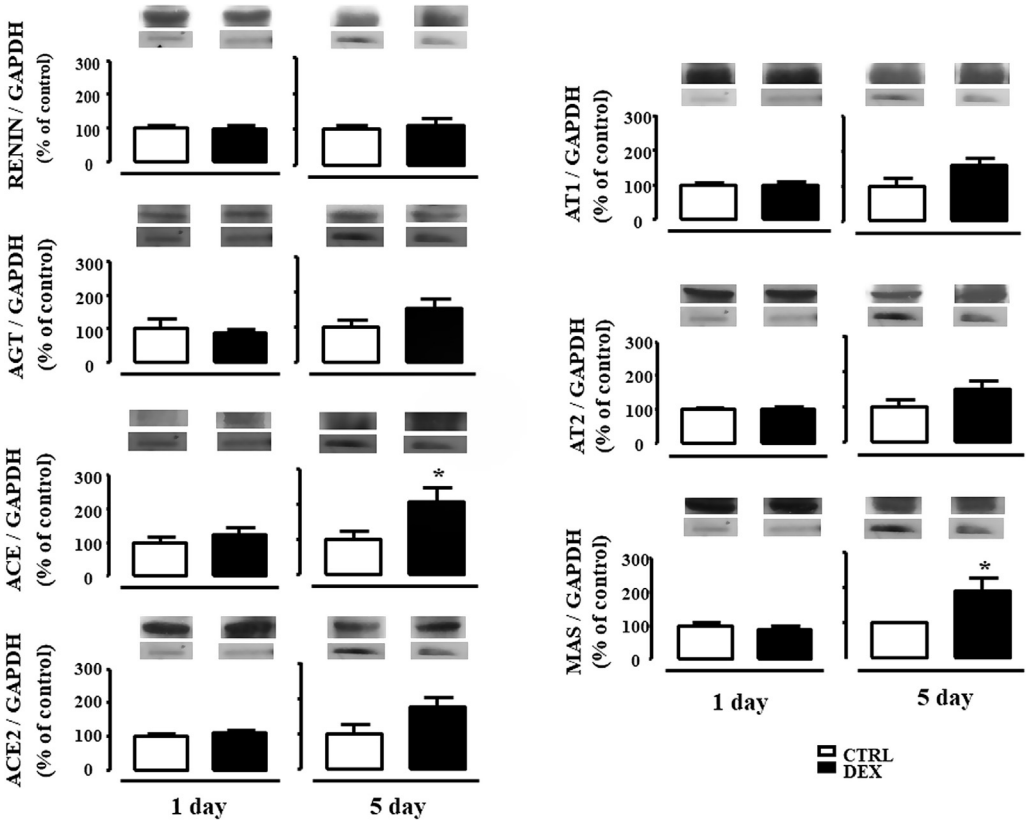

Figure 4. Protein level of the renin-angiotensin system (RAS) components in myocardium, in all groups analyzed: treated with saline for 1 day (C1, n = 14), treated with dexamethasone for 1 day (D1, n = 14), treated with saline for 5 days (C5, n = 13), and treated with DEX for 5 days (D5, n = 14). Renin, angiotensinogen (AGT), angiotensin II receptor type 1 (AT1a); Angiotensin II receptor type 2 (AT2); angiotensin converting enzyme (ACE); angiotensin converting enzyme 2 (ACE2), and angiotensin[1–7] receptor (MAS). Significance: * versus respective control, $P < .05$.

provokes autonomic changes such as decreases of baroreflex gain.[39]

The known role of RAS on autonomic functions and the overexpression of the AT1 receptor on selected and important cardiovascular regions of the brain after DEX treatment[37] suggest that RAS could be contributing to the autonomic changes observed after DEX treatment, in the presence or absence of hypertension, but the results are still inconclusive. Studies with programmed hypertension have shown that treatment with AT1 receptor blocker attenuates arterial pressure and brings back to normal the baroreflex decreased gain and the reduced heart rate variability induced by antenatal betamethasone treatment.[29] On the other hand, other studies using blockade of AT1 receptor fail to attenuate the changes observed on arterial pressure or baroreflex function induced by betamethasone[26] or DEX.[25] Besides increases in AT1 receptor expression on medulla oblongata that may be observed after antenatal DEX treatment,[37] plasma levels of RAS components were not altered.[25] In this present study, plasma levels of RAS were not observed, which is a limitation of this study. On the other hand, this study investigated the role of DEX treatment on cardiac RAS components. After acute or short-term DEX treatment, there was a decrease in gene expression of AGT and AT1 receptors, but the protein levels of AGT or AT1 receptor were not altered. Even though ACE protein level was higher after 5 days of DEX treatment, MAS protein level was higher also, which could help to explain the lack of cardiac or pressure alterations. These results are in agreement with our previous observation that heart RAS components are not altered in rats treated for 10 days with DEX, even though hypertension was present.[23]

In summary, the results of this present study demonstrated, for the first time, that short-term exposure to DEX impairs the autonomic balance to the heart before hypertension, which was independent of RAS. These responses improve our understanding regarding the mechanisms promptly induced by DEX treatment on central control of autonomic function, which may have important clinical relevance and could contribute to the important cardiovascular risk imposed by elevated glucocorticoid levels.

Acknowledgments

We gratefully acknowledge Dr Daniel Penteado Martins Dias for allowing us to use CardioSeries V2.4 (http://www.danielpenteado.com) program to perform spectral analysis.

Authors' contributions: F.D. and P.B.C. contributed to the conception and design of the work; acquisition, analysis, and interpretation of data for the work and drafting the work or revising it critically for important intellectual content. N.A.H., M.F.F., L.P.T., A.M.M., and T.J.D. participated in the acquisition, analysis, and interpretation of data for the work and drafting the work or revising it critically for important intellectual content. C.F.S. and S.L.A. contributed to the conception and design of the work; analysis and interpretation of data for the work and revising it critically for important intellectual content.

All authors approved the final version of the article and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

References

1. Ong SL, Vohra H, Zhang Y, Sutton M, Whitworth JA. The effect of alpha-lipoic acid on mitochondrial superoxide and glucocorticoid-induced hypertension. Oxid Med Cell Longev 2013;2013:517045.
2. Safaeian L, Zabolian H. Antioxidant effects of bovine lactoferrin on dexamethasone-induced hypertension in rat. ISRN Pharmacol 2014;2014:943523.
3. Herrera NA, Jesus I, Shinohara AL, Dionisio TJ, Santos CF, Amaral SL. Exercise training attenuates dexamethasone-induced hypertension by improving autonomic balance to the heart, sympathetic vascular modulation and skeletal muscle microcirculation. J Hypertens 2016;34(10):1967–76.
4. Pirpiris M, Sudhir K, Yeung S, Jennings G, Whitworth JA. Pressor responsiveness in corticosteroid-induced hypertension in humans. Hypertension 1992;19(6 Pt 1):567–74.
5. Fardet L, Nazareth I, Petersen I. Synthetic glucocorticoids and early variations of blood pressure: a Population-Based Cohort study. J Clin Endocrinol Metab 2015;100(7):2777–83.
6. Pinheiro CH, Sousa Filho WM, Oliveira Neto J, Marinho MJ, Motta Neto R, Smith MM, et al. Exercise prevents cardiometabolic alterations induced by chronic use of glucocorticoids. Arq Bras Cardiol 2009;93(4). 400–408, 392-400.
7. Barel M, Perez OA, Giozzet VA, Rafacho A, Bosqueiro JR, do Amaral SL. Exercise training prevents hyperinsulinemia, muscular glycogen loss and muscle atrophy induced by dexamethasone treatment. Eur J Appl Physiol 2010;108(5):999–1007.
8. Dionisio TJ, Louzada JC, Viscelli BA, Dionisio EJ, Martuscelli AM, Barel M, et al. Aerobic training prevents dexamethasone-induced peripheral insulin resistance. Horm Metab Res 2014;46(7):484–9.
9. Macedo AG, Krug AL, Herrera NA, Zago AS, Rush JW, Amaral SL. Low-intensity resistance training attenuates dexamethasone-induced atrophy in the flexor hallucis longus muscle. J Steroid Biochem Mol Biol 2014;143:357–64.
10. Krug AL, Macedo AG, Zago AS, Rush JW, Santos CF, Amaral SL. High-intensity resistance training attenuates dexamethasone-induced muscle atrophy. Muscle & Nerve 2016;53(5):779–88.
11. Macedo AG, Krug AL, Souza LM, Martuscelli AM, Constantino PB, Zago AS, et al. Time-course changes of catabolic proteins following muscle atrophy induced by dexamethasone. Steroids 2016;107:30–6.
12. Herrera NA, Jesus I, Dionisio EJ, Dionisio TJ, Santos CF, Amaral SL. Exercise training prevents dexamethasone-induced rarefaction. J Cardiovasc Pharmacol 2017;70(3):194–201.
13. Wallerath T, Witte K, Schafer SC, Schwarz PM, Prellwitz W, Wohlfart P, et al. Down-regulation of the expression of endothelial NO synthase is likely to contribute to glucocorticoid-mediated hypertension. Proc Natl Acad Sci U S A 1999;96(23):13357–62.
14. Severino C, Brizzi P, Solinas A, Secchi G, Maioli M, Tonolo G. Low-dose dexamethasone in the rat: a model to study insulin resistance. Am J Physiol Endocrinol Metab 2002;283(2):E367–73.
15. Schafer SC, Wallerath T, Closs EI, Schmidt C, Schwarz PM, Forstermann U, et al. Dexamethasone suppresses eNOS and CAT-1 and induces oxidative stress in mouse resistance arterioles. Am J Physiol Heart Circ Physiol 2005;288(1):H436–44.
16. Mondo CK, Yang WS, Zhang N, Huang TG. Antioxidant effects of atorvastatin in dexamethasone-induced hypertension in the rat. Clin Exp Pharmacol Physiol 2006;33(11):1029–34.
17. Ong SL, Whitworth JA. How do glucocorticoids cause hypertension: role of nitric oxide deficiency, oxidative stress, and eicosanoids. Endocrinol Metab Clin North Am 2011;40(2). 393–407, ix.
18. Watanabe T, Noshiro T, Akama H, Kusakari T, Shibukawa S, Miura W, et al. Effect of dexamethasone on plasma free dopamine: dopaminergic modulation in hypertensive patients. Hypertens Res 1995;18(Suppl 1):S197–8.
19. Silvan G, Martinez-Mateos MM, Blass A, Camacho L, Gonzalez–Gil A, Garcia–Partida P, et al. The effect of long-term exposure to combinations of growth promoters in Long Evans rats: part 1: Endocrine adrenal function. Anal Chim Acta 2007;586(1-2):246–51.

20. Sakaue M, Hoffman BB. Glucocorticoids induce transcription and expression of the alpha 1B adrenergic receptor gene in DTT1 MF-2 smooth muscle cells. J Clin Invest 1991;88(2):385–9.
21. Suzuki H, Handa M, Kondo K, Saruta T. Role of renin-angiotensin system in glucocorticoid hypertension in rats. Am J Physiol 1982;243(1):E48–51.
22. Sato A, Suzuki H, Murakami M, Nakazato Y, Iwaita Y, Saruta T. Glucocorticoid increases angiotensin II type 1 receptor and its gene expression. Hypertension 1994; 23(1):25–30.
23. Constantino PB, Dionisio TJ, Duchatsch F, Herrera NA, Duarte JO, Santos CF, et al. Exercise attenuates dexamethasone-induced hypertension through an improvement of baroreflex activity independently of the renin-angiotensin system. Steroids 2017;128: 147–54.
24. Roghair RD, Miller FJ Jr, Scholz TD, Lamb FS, Segar JL. Coronary constriction to angiotensin II is enhanced by endothelial superoxide production in sheep programmed by dexamethasone. Pediatr Res 2008;63(4):370–4.
25. Peers A, Campbell DJ, Wintour EM, Dodic M. The peripheral renin-angiotensin system is not involved in the hypertension of sheep exposed to prenatal dexamethasone. Clin Exp Pharmacol Physiol 2001;28(4):306–11.
26. Segar JL, Bedell KA, Smith OJ. Glucocorticoid modulation of cardiovascular and autonomic function in preterm lambs: role of ANG II. Am J Physiol Regul Int Comp Physiol 2001;280(3):R646–54.
27. Dodic M, Peers A, Coghlan JP, May CN, Lumbers E, Yu Z, et al. Altered cardiovascular haemodynamics and baroreceptor-heart rate reflex in adult sheep after prenatal exposure to dexamethasone. Clin Sci (Lond) 1999;97(1):103–9.
28. Segar JL, Roghair RD, Segar EM, Bailey MC, Scholz TD, Lamb FS. Early gestation dexamethasone alters baroreflex and vascular responses in newborn lambs before hypertension. Am J Physiol Regul Integr Comp Physiol 2006;291(2):R481–8.
29. Shaltout HA, Chappell MC, Rose JC, Diz DI. Exaggerated sympathetic mediated responses to behavioral or pharmacological challenges following antenatal betamethasone exposure. Am J Physiol Endocrinol Metab 2011;300(6):E979–85.
30. Rafacho A, Roma LP, Taboga SR, Boschero AC, Bosqueiro JR. Dexamethasone-induced insulin resistance is associated with increased connexin 36 mRNA and protein expression in pancreatic rat islets. Can J Physiol Pharmacol 2007;85(5):536–45.
31. Ishise H, Asanoi H, Ishizaka S, Joho S, Kameyama T, Umeno K, et al. Time course of sympathovagal imbalance and left ventricular dysfunction in conscious dogs with heart failure. J Appl Physiol (1985) 1998;84(4): 1234–41.
32. Sanches IC, Conti FF, Bernardes N, Brito JO, Galdini EG, Cavaglieri CR, et al. Impact of combined exercise training on cardiovascular autonomic control and mortality in diabetic ovariectomized rats. J Appl Physiol (1985) 2015;119(6):656–62.
33. Amaral SL, Linderman JR, Morse MM, Greene AS. Angiogenesis induced by electrical stimulation is mediated by angiotensin II and VEGF. Microcirculation 2001;8(1):57–67.
34. Gilson H, Schakman O, Combaret L, Lause P, Grobet L, Attaix D, et al. Myostatin gene deletion prevents glucocorticoid-induced muscle atrophy. Endocrinology 2007;148(1):452–60.
35. Dodic M, May CN, Wintour EM, Coghlan JP. An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. Clin Sci (Lond) 1998; 94(2):149–55.
36. Dodic M, Samuel C, Moritz K, Wintour EM, Morgan J, Grigg L, et al. Impaired cardiac functional reserve and left ventricular hypertrophy in adult sheep after prenatal dexamethasone exposure. Circ Res 2001;89(7):623–9.
37. Dodic M, Abouantoun T, O'Connor A, Wintour EM, Moritz KM. Programming effects of short prenatal exposure to dexamethasone in sheep. Hypertension 2002;40(5):729–34.
38. Dodic M, McAlinden AT, Jefferies AJ, Wintour EM, Cock ML, May CN, et al. Differential effects of prenatal exposure to dexamethasone or cortisol on circulatory control mechanisms mediated by angiotensin II in the central nervous system of adult sheep. J Physiol 2006;571(Pt 3):651–60.
39. Bechtold AG, Scheuer DA. Glucocorticoids act in the dorsal hindbrain to modulate baroreflex control of heart rate. Am J Physiol Regul Integr Comp Physiol 2006; 290(4):R1003–11.
40. Moritz KM, Dodic M, Jefferies AJ, Wintour EM, DeMatteo R, Singh RR, et al. Haemodynamic characteristics of hypertension induced by prenatal cortisol exposure in sheep. Clin Exp Pharmacol Physiol 2009; 36(10):981–7.
41. Shaltout HA, Rose JC, Figueroa JP, Chappell MC, Diz DI, Averill DB. Acute AT(1)-receptor blockade reverses the hemodynamic and baroreflex impairment in adult sheep exposed to antenatal betamethasone. Am J Physiol Heart Circ Physiol 2010;299(2):H541–7.