A Novel Model of Dexamethasone-Induced Hypertension: Use in Investigating the Role of Tyrosine Hydroxylase

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ABSTRACT

Our objective was to study hypertension induced by chronic administration of synthetic glucocorticoid, dexamethasone (DEX), under nonstressful conditions and examine the role of catecholamine biosynthesis. To achieve this, we did the following: 1) used radiotelemetry to record mean arterial pressure (MAP) and heart rate (HR) in freely moving rats, and 2) administered different doses of DEX in drinking water. To evaluate the involvement of tyrosine hydroxylase (TH), the rate-limiting step in catecholamine biosynthesis. We observed a dose-dependent elevation in blood pressure with a DEX dose of 0.3 mg/kg administered for 10 days, significantly increasing MAP by +15.0 ± 1.1 mm Hg, while concomitantly reducing HR. Although this DEX treatment also significantly decreased body weight, pair-fed animals that showed similar decreases in body weight due to lowered food intake were not hypertensive, suggesting that body weight changes may not account for DEX-induced hypertension. Chronic DEX treatment significantly increased the TH mRNA and protein levels in the adrenal medulla, and α-MPT administration not only reduced DEX pressor effects, but also inhibited TH (serine340) phosphorylation. Our study thus validates a novel model to study hypertension induced by chronic intake of DEX in freely moving rats not subject to the confounding factors of previous models and establishes its dependence on concomitant activation of peripheral catecholamine biosynthesis.

Introduction

Synthetic glucocorticoids are used in the chronic treatment of inflammatory diseases (Kirwan, 1995; Saag, 2002; van Everdingen et al., 2002). Dexamethasone (DEX) is one of the most commonly used synthetic glucocorticoids because of its potency (Kari et al., 1994; Kaal and Vecht, 2004; Piette et al., 2006). However, cumulative exposure to glucocorticoids increases the risk of cardiovascular outcomes, including hypertension (Souverein et al., 2004; Davis et al., 2007). Approximately 70% of the patients undergoing chronic glucocorticoid therapy become hypertensive, the severity being dependent on the dose and duration of the treatment (Souverein et al., 2004; Panoulas et al., 2008). Nevertheless, the mechanisms by which chronic glucocorticoid therapy induces hypertension are not well understood.

Cortisol, the endogenous glucocorticoid, can produce hypertension associated with increased sodium and water retention and cause renal impairment via interaction with both mineralocorticoid and glucocorticoid receptors (Whitworth et al., 1989, 2000; Mangos et al., 2003). DEX, in contrast, affects renal function (Bia et al., 1982) specifically via the glucocorticoid receptor (GR) (Reul et al., 1987). However, studies evaluating the kidney-specific homozygous deletion of GR in mice concluded that the GR in the distal nephron is not necessary for the development or maintenance of DEX-induced hypertension (Goodwin et al., 2010). A potential alternative site would be a central effect. However, DEX does not readily cross the blood brain barrier (De Kloet, 1997; Meijer et al., 1998), precluding direct activation of the central nervous system. Thus, the mechanism by which DEX raises blood pressure is most likely peripheral, possibly through activation of the peripheral sympathoadrenal system. It is long known that sympathetic hyperactivation can result in elevated cardiac output and total peripheral resistance (Uylench et al., 1968; Fletcher et al., 1976). Increased sympathetic nerve activity and elevated circulating catecholamine concentrations are common characteristics of essential hypertension (Esler et al., 1980), as well as in deoxycorticosteron salt hypertension and in spontaneously hypertensive rats (Sakaguchi et al., 1983; Lange et al., 1998). Despite this knowledge on the involvement of sympathetic nervous system...
in blood pressure development (Esler et al., 2008; DiBona and Esler, 2010), how chronic glucocorticoid exposure affects it is largely unknown.

Tyrosine hydroxylase (TH) is the first and rate-limiting enzyme in catecholamine biosynthesis and is highly expressed in the adrenal medulla and sympathetic neuronal terminals. At the molecular level, our laboratory identified an atypical glucocorticoid response element in the promoter region of rat and human TH gene that is stimulated by DEX (Rani et al., 2009; Sheela Rani et al., 2013). In the present study, we hypothesized that an underlying mechanism for DEX-induced hypertension involves increases in TH gene expression in the adrenal medulla, and perhaps in other sympathetic terminals, upon chronic DEX treatment; this in turn leads to a sustained increase in catecholamine synthesis, higher enzyme activity, and finally to hypertension. Indeed, DEX administration is known to increase plasma catecholamine levels in hypertensive patients (Watanabe et al., 1995). We chose to study adrenal medulla as a representative of peripheral sympathetic ganglia because medullary cells are, indeed, modified postganglionic cells of the autonomic nervous system that receive innervation from corresponding preganglionic fibers (Perlman and Chalfie, 1977).

Furthermore, we used α-methyl-para-tyrosine (α-MPT), an irreversible TH inhibitor (Udenfriend et al., 1965; Ankenman and Salvatore, 2007), to explore the possible role of adrenal TH in DEX-induced hypertension, because earlier studies indicated that this drug is effective in treating certain types of hypertension involving increased serum catecholamine levels (Perry et al., 1990; Chiou-Tan et al., 1994; Shimizu et al., 2001). In addition to adrenal medulla, α-MPT would also allow us to assess the role of sympathetic nerve terminals because it has been shown to cause catecholamine depletion in terminals as well (Nagatsu et al., 1964; Spector et al., 1965).

The primary aim of this study was to generate a novel in vivo model to study hypertension following chronic DEX administration without the involvement of stressors due to the methodologies used for drug administration and blood pressure recording. To accomplish this, we delivered DEX in drinking water and used a radiotelemetry approach to continuously record blood pressure. This method has the advantage of measuring blood pressure directly in freely moving/resting animals, thus avoiding handling-related changes in their arousal state (Kurtz et al., 2005). We evaluated the dose-response relationship between chronic administration of DEX in drinking water and changes in blood pressure, and also examined TH expression and phosphorylation at serine40 (pSer40) as possible mechanisms in the generation of DEX-induced hypertension by α-MPT systemic administration.

Materials and Methods

Animals. Male Fisher 344 rats, 8–10 weeks old and weighing 250–300 g, were purchased from Harlan (Houston, TX). They were maintained on a 14/10-hour light/dark cycle and fed ad libitum for the whole study. Rats were allowed to acclimate to housing conditions for 3 days and then underwent telemetric implant surgery. After the surgery, the rats were individually housed in plastic cages with continuous access to rat chow (Harlan Teklad 7912) and water. The experiments were approved and performed under the guidelines of the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Radiotelemetry Implantation and Measurement of Blood Pressure and Heart Rate. Implantation surgery was done under isoflurane anesthesia. A catheter attached to a CA11PA-C40 radiotelemetry transmitter (Data Science International, St. Paul, MN) was implanted in the peritoneal aorta and secured to the abdominal muscle. The animals were allowed to recover for 8 days. Each animal was housed in a plastic cage, placed on top of a RCP-1 receiver that was attached to a Data Exchange Matrix (Data Science International). After recovery, systolic blood pressure, diastolic blood pressure, and mean arterial pressure (MAP), as well as heart rate (HR), were monitored for 3–5 days to establish the baseline. Recordings were performed every 10 minutes; data were collected using Dataquest A.R.T. 4.1 software (Data Science International). Because systolic blood pressure and diastolic blood pressure responded similarly to MAP, we present our data only as MAP. Results of change in (Δ) MAP, ΔHR, and Δbody weight were calculated by subtracting the 24-hour mean values from the average of the mean of 3 days of baseline recording for DEX dose-response curves and pair-feeding experiments, and 5 days of baseline recording for the α-MPT experiment. Day 1 indicates the first day of recording to establish the baseline, and the numeration continues until the last day of recording/treatment on day 13 for DEX dose-response and pair-feeding experiments, and on day 17 for the α-MPT study.

Dexamethasone Treatment. To evaluate the dose of DEX that produces a significant increase in blood pressure, animals received either regular drinking water (controls) or a water-soluble form of synthetic glucocorticoid (GC), dexamethasone 21-phosphate disodium salt (Sigma Aldrich, St. Louis, MO), in drinking water. Three different groups of rats received DEX in drinking water for 10 days: low (0.03 mg/kg/d), medium (0.3 mg/kg/d), and high (3 mg/kg/d). Water intake was measured every day and to calculate daily DEX doses was adjusted to it as well. Body weight was monitored daily to determine possible catabolic DEX effects.

Pair-Feeding. To determine the role of body weight loss on the blood pressure effects of DEX, we treated a group of rats with DEX (0.16 mg/kg/d) for 10 days and performed pair feeding of a group of untreated rats with the same amount of food previously determined to be consumed by the DEX-treated group. We used this single mid-range dose (0.16 mg/kg/d) in this control study because both 0.03 and 0.3 mg/kg/d had earlier produced elevations in blood pressure. Food intake was calculated as the difference of weight of the food container between the day when the container was filled and 24 hours after that.

Measurement of TH mRNA Content in the Adrenal Medulla and Brainstem. After 10 days of DEX treatment, the animals were euthanized, and the adrenal medullae were removed, flash frozen, and stored at −80°C. Brains were removed, and the brainstem was separated from the ependymal region. A 4-mm section of the medulla oblongata was cut. The left adrenal medullae and the brainstem regions were homogenized in 1 ml Trizol reagent (Life Technologies/Invitrogen, Carlsbad, CA). Total RNA was isolated and treated with DNase and used for synthesizing cDNA using the High Capacity cDNA synthesis kit (Life Technologies/Applied Biosystems, Foster City, CA), according to manufacturer’s instructions. cDNA (10 ng) was used for quantitative real-time polymerase chain reaction, as described previously (Green et al., 2011), using a TaqMan gene expression master mix and 6-carboxyfluorescein–labeled probe for rat TH (Gene Expression Assay ID Rn00562500_m1; Life Technologies/Applied Biosystems) and 18S rRNA, which was used as the endogenous normalizer and amplified simultaneously using TaqMan Eukaryotic 18S rRNA endogenous control VIC MGB probe (primer limited, 4319413E). The assays were performed in duplicate using the ABI PRISM 7900 Sequence Detection System. The relative expression was calculated from the average difference in cycle threshold between the vehicle (VEH) control and DEX-treated samples using the ΔΔ cycle threshold method (Livak and Schmittgen, 2001).

Pharmacological Inhibition of TH. To determine the role of TH in DEX hypertension, separate groups of rats were treated with the TH inhibitor, α-methyl-DL-tyrosine methyl ester hydrochloride...
(Sigma-Aldrich), prior to induction of hypertension. After 5 days of baseline recording, one group of rats was treated with VEH (0.9% NaCl, i.p.), and the second was treated with α-MPT (50 mg/kg, i.p.) on days 6, 8, and 10 of recording (Fig. 1). Both groups were further divided into two groups, as follows: one received water and the other received DEX in drinking water (0.3 mg/kg/d) for 7 days (Fig. 1). Blood pressure and HR were recorded continuously, as described above. We computed ΔMAP and ΔHR based on the average of 5 days of baseline recording.

Measurement of Adrenal TH Protein after α-MPT by Western Blotting. Rat adrenal medulla samples kept frozen at −80°C after euthanasia were thawed on ice and homogenized in radioimmunoprecipitation assay buffer: 50 mM Tris-HCl (pH 8), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS with immunoprecipitation assay buffer: 50 mM Tris-HCl (pH 8), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS with Sigma-Aldrich protease inhibitor cocktail (P8340, 1:100 dilution) and 1 mM phenylmethylsulfonyl fluoride. Protein samples (10 μg) were heat-denatured in lithium dodecyl sulfate (LDS) buffer containing 60% formamide, 20 mM dithiothreitol reducing agent (Invitrogen) and then electrophoresed on 4–12% NuPage Bis-Tris gel and transferred to a polyvinylidene difluoride membrane using the iBlot system (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA). The membrane was probed simultaneously with monoclonal antibodies to TH (1:1000, T1299 mouse monoclonal Ab; Sigma-Aldrich) and β-actin (1:1000, ab8226; AbCam, Cambridge, MA) as well as polyclonal anti-pSer40 TH (1:500 ABS935; Millipore, Billerica, MA). The TH and β-actin signals were detected using a secondary antibody IRDye 680RD goat anti-rabbit IgG (1:10,000, 926-68071; LI-COR Biosciences). The fluorescent bands were identified at 60 kDa for TH and pSer40 TH and at 42 kDa for β-actin. Detection and densitometry quantification were performed using the Odyssey infrared imaging system (LI-COR Biosciences).

Statistical Analysis. To analyze ΔMAP, ΔHR, and Abody weight, we used two-way analysis of variance with repeated measures using SigmaStat 4.0 (Systat Software, San Jose, CA) statistical package. The Holm-Sidak test was used as post hoc analyses. TH expression analyses were performed using the Prism v7 (Graph Pad Software, La Jolla, CA). TH mRNA was analyzed by the Mann–Whitney U test, and TH protein was analyzed by one-way analysis of variance with Dunnett’s multiple comparison test; P < 0.05 (two-sided comparison) was accepted as a statistically significant level.

Results

Blood Pressure Responses to Oral DEX Treatment in Drinking Water. With administration of DEX, MAP increased significantly above baseline values in all groups (Fig. 2A). The average MAP at baseline (days 1–3) in the group with the lowest dose, 0.03 mg/kg/d (n = 3), was 104.3 ± 4.0 mmHg. After the administration of DEX, this group showed an elevation of MAP by day 11 (+9.4 ± 3.5 mmHg) with respect to the VEH control group (P < 0.01) and continued increasing to 13.5 ± 3.8 mmHg by day 13 (***P < 0.001 versus day 3 and **P < 0.001 versus VEH). The group that received the medium dose of 0.3 mg/kg/d (n = 5) showed an average MAP at baseline of 102.2 ± 2.7, and, at day 9, MAP increased 11.0 ± 1.4 mmHg, reaching earlier a high significant level (**P < 0.001 versus day 3 and **P < 0.001 versus VEH) than the group with lowest dose. This level was maintained until day 13 (+15.0 ± 1.1 mmHg, ***P < 0.001 versus day 3 and **P < 0.001 versus VEH). Animals treated with the highest dose of DEX, 3 mg/kg/d (n = 4), presented an average baseline of 101.1 ± 0.9 mmHg. Nonetheless, they did not survive beyond 7 days of DEX treatment. By day 10, there were three animals alive. They also showed significant increases in MAP from days 8 to 10 that ranged from 10.2 ± 1.8 mmHg (**P < 0.001 versus day 3) to 17.9 ± 3.8 mmHg (**P < 0.01 versus day 3 and **P < 0.01 versus VEH), respectively.

With regard to HR, there were significant dose-dependent decreases (Fig. 2B). The group with the lowest dose, 0.03 mg/kg/d, showed an average baseline of 366.9 ± 9.7 beats per minute (BPM); upon DEX treatment, there were significant decreases (**P < 0.01 versus day 3) on days 6 (−9.2 ± 4.2 BPM), 8 (−10.2 ± 2.7 BPM), 9 (−11.5 ± 4.0 BPM), and 10 (−12.0 ± 4.4 BPM). Only day 10 was different from the VEH group (**P < 0.05). The group that received the medium dose had a HR average at baseline of 368.8 ± 5.2 BPM. Specifically, at this dose, HR decreased significantly on days 8 (−22.7 ± 4.1 BPM) to 13 (−25.3 ± 3.1 BPM) with respect to day 3 of baseline recording (**P < 0.001) and from day 10 (−26.0 ± 3.2 BPM, **P < 0.05) to day 12 (−24.5 ± 1.2 BPM, **P < 0.05) with respect to the VEH group. The third group with the highest dose (3 mg/kg/d) had a baseline HR average of 373.9 ± 5.0 BPM, and it started decreasing significantly (**P < 0.01 versus day 3 and **P < 0.01 versus VEH) from day 6 (−43.8 ± 4.6 BPM) until day 10 (−54.2 ± 9.3 BPM), being that latter the last day of survival in this group.

Moreover, body weight decreased upon DEX administration in the three groups (Fig. 2C). The VEH control group showed significant increases (**P < 0.001 versus day 3) from day 4 (+18.4 ± 6.4 g) to 13 (+45.2 ± 5.2 g). Animals treated with the lowest dose had significant reductions (**P < 0.001 versus day 3) on days 12 (−13.9 ± 1.3 g) and 13 (−18.1 ± 0.8 g). It was also significantly reduced from the VEH group from day 5 to 13 (**P < 0.05). Furthermore, the group with the medium dose showed significant reductions on body weight by day 8 (−25.7 ± 1.1 g, **P < 0.001 versus day 3) and continued until day 13 (−46.3 ± 7.3 g, **P < 0.001 versus day 3). It was also significantly different from the VEH control group from day 4 to 13 (**P < 0.01). Finally, the third group receiving the highest dose of DEX showed significant decreases from day 7 (−25.9 ± 0.9 g, **P < 0.001 versus day 3) to 10 (−70.0 ± 3.2 g, **P < 0.001 versus day 3) and from day 5 to 10 with respect to the VEH group (**P < 0.01).

Reduction in Body Weight Is Not Responsible for DEX-Induced Hypertension. To isolate the effect of reduced body weight from blood pressure, we used pair feeding with reduced food intake and compared it with the effects of DEX administration (n = 3/group). In Fig. 3A, DEX-treated rats showed a significantly reduced food intake on days
hoc analysis were used to compare responses between groups with respect to day 3 of baseline. It was also significantly reduced on days 7, 8, 11, and 12 with respect to the VEH control group (***P < 0.001, **P < 0.01, and *P < 0.05) between these groups on days 12–17 (***P < 0.001 versus day 5) and 14–17 with respect to the VEH/VEH group (**P < 0.01). After the administration of α-MPT, there were no changes in MAP baseline in any of the groups. Nonetheless, the α-MPT/DEX group exhibited significantly diminished pressor effect on days 14–17 (+5.9 ± 1.6, +9.1 ± 1.4, +11.1 ± 1.2, and +11.9 ± 1.8 mmHg, respectively; **P < 0.025) compared with the responses in the group treated with DEX alone on the same days (+12.0 ± 1.2, +14.8 ± 1.4, +17.5 ± 1.3, and +18.8 ± 1.7 mmHg, respectively). Despite that, there were increases in the α-MPT/DEX group against the VEH/VEH group on days 13–17 (**P < 0.05). Furthermore, a comparison of ΔMAP of the α-MPT/DEX and VEH/DEX groups, normalized to their respective control group (Fig. 5B), was performed to discard possible interferences with baseline, and significant differences were observed (**P < 0.05) between these groups on days 12–17. Finally, the administration of α-MPT did not change HR during the baseline recording, nor did it influence DEX-induced decreases in HR (Fig. 5C). It decreased in VEH/DEX group on days 12–17 compared with day 5 of baseline (***P < 0.001) and days 13–17 compared with the VEH/VEH group (**P < 0.01). The α-MPT/DEX group had decreases on days 13–17 against day 5 of baseline (***P < 0.01) and VEH/VEH group (**P < 0.01). No significant differences were observed levels in the adrenal medulla (***P < 0.01), whereas TH mRNA in the brainstem was not modified (Fig. 4).

**Effect of α-MPT on DEX-Induced Hypertension.** In Fig. 5A, MAP increased in VEH/DEX on days 12–17 (***P < 0.001 versus day 5) and 14–17 with respect to the VEH/VEH group (**P < 0.01). After the administration of α-MPT, there were no changes in MAP baseline in any of the groups. Nonetheless, the α-MPT/DEX group exhibited significantly diminished pressor effect on days 14–17 (+5.9 ± 1.6, +9.1 ± 1.4, +11.1 ± 1.2, and +11.9 ± 1.8 mmHg, respectively; **P < 0.025) compared with the responses in the group treated with DEX alone on the same days (+12.0 ± 1.2, +14.8 ± 1.4, +17.5 ± 1.3, and +18.8 ± 1.7 mmHg, respectively). Despite that, there were increases in the α-MPT/DEX group against the VEH/VEH group on days 13–17 (**P < 0.05). Furthermore, a comparison of ΔMAP of the α-MPT/DEX and VEH/DEX groups, normalized to their respective control group (Fig. 5B), was performed to discard possible interferences with baseline, and significant differences were observed (**P < 0.05) between these groups on days 12–17. Finally, the administration of α-MPT did not change HR during the baseline recording, nor did it influence DEX-induced decreases in HR (Fig. 5C). It decreased in VEH/DEX group on days 12–17 compared with day 5 of baseline (***P < 0.001) and days 13–17 compared with the VEH/VEH group (**P < 0.01). The α-MPT/DEX group had decreases on days 13–17 against day 5 of baseline (***P < 0.01) and VEH/VEH group (**P < 0.01). No significant differences were observed

**A Novel Model of Dexamethasone-Induced Hypertension 531**

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**Fig. 2.** Dose-response curves for chronic DEX administration in drinking water. Each point represents the difference of 24-hour mean ± S.E.M. responses in ΔMAP (A), ΔHR (B), and Δbody weight (C) between the average of 3 days of baseline and each day of recording in VEH (n = 5) and three DEX doses: 0.03 mg/kg/d (n = 3), 0.3 mg/kg/d (n = 5), and 3 mg/kg/d (n = 4). Two-way analysis of variance with repeated measurements and Holm-Sidak post hoc analysis were used to compare responses between groups with respect to day 3 of baseline (***P < 0.001) and VEH group (**P < 0.05, *P < 0.01, and *P < 0.001).
when normalizing HR to the respective VEH control groups (Fig. 5D).

At the end of telemetric recordings, animals were euthanized and the adrenal medullae were isolated to measure TH protein. There was a significant increase in TH, 1.3-fold (**P < 0.01), after 7 days of DEX treatment compared with the VEH-treated group. Moreover, α-MPT pretreatment inhibited DEX-induced increase in TH protein (Fig. 6A). TH phosphorylation also increased in the VEH/DEX group, as shown by an elevation in the level of pSer40-TH (*P < 0.05 versus VEH/VEH), and this increase was completely inhibited by pretreatment with α-MPT (Fig. 6B).

Discussion

Our study describes the development of an animal model to study hypertension induced by the chronic administration of DEX. Mainly, by choosing radiotelemetry for recording blood pressure and an oral route for chronic administration of DEX in rats, our model circumvents the confounding factors seen in earlier studies (Iijima and Malik, 1988; Wallwork et al., 2003; Mondo et al., 2006; Ong et al., 2007). These include stress from restraint that was used for blood pressure recording and administration of DEX, which may have had an effect on the arousal state of the animal, possibly altering blood pressure.

Fig. 3. Effects of pair feeding (n = 3) with respect to VEH (n = 3)- and DEX-treated rats (0.16 mg/kg/d, n = 3) on food intake (A), Δbody weight (B), and ΔMAP (C). Data for body weight and MAP are presented as the difference between the average of 3 days baseline and each day of recording for body weight and MAP (24-hour mean ± S.E.M.). The administration of drugs is indicated with arrows below the y-axis. Two-way analysis of variance with repeated measurements and Holm-Sidak post hoc analysis revealed significant differences between the groups. (A) shows differences within DEX treatment and day 2 of baseline (**P < 0.001) as well as VEH group (**P < 0.05). Also, it shows differences within pair feeding and day 3 of baseline recording (*P < 0.01) as well as VEH group (**P < 0.05). In (B) and (C), differences are indicated against day 3 of baseline (**P < 0.001) and VEH group (**P < 0.01 and *P < 0.001).

Fig. 4. TH mRNA in the adrenal medulla (A) and brainstem (B) of animals treated with VEH or DEX medium dose of 0.3 mg/kg/d after 10 days of DEX treatment (**P < 0.01). Results are shown as the relative expression calculated from the average difference in cycle threshold between the VEH and DEX groups in the adrenal medulla (n = 5 and 6/group, respectively) and brainstem (n = 3 and 4/group, respectively). Data were analyzed using Mann–Whitney U test.
results. We found that MAP increases in a cumulative and dose-dependent manner over the period of continuous oral DEX administration (Fig. 2A), with significant increases (+11 mmHg) seen after 6 days of treatment with a medium dose of DEX (0.3 mg/kg/d). In an attempt to relate our experimental protocol to a clinically relevant dose/time frame, we can say our treatment paradigm would be approximately equivalent to 6 months of medium-dose GC therapy in humans (Quinn, 2005; Andreollo et al., 2012). Interestingly, patients affected by rheumatoid arthritis receiving long-term GC treatment (>6 months) are more prone to develop hypertension when using a medium dose of prednisone (0.1–4 mg/kg/d) than those with limited GC exposure (<0.1 mg/kg/d for <6 months) (Panoulas et al., 2008).

Concomitant with an increase in MAP, a 10-day continuous oral administration of DEX reduces food intake, but not water intake (data not shown), and is accompanied by a decrease in body weight in rats. However, the pair-fed group, which has similar reduction in body weight as in the DEX-treated group, does not show any change in MAP. This suggests food intake and body weight reductions due to DEX may not be directly related to its pressor effect through the sympathoadrenal system. Studies in the literature point to a complex interplay between GCs and other hormonal factors that regulate feeding behavior and subsequent body weight changes (Jahng et al., 2008; Cummings et al., 2013). For instance, DEX was shown to increase insulin secretion via improvement of β cell function (Cummings et al., 2013), in addition to affecting leptin release and its hypothalamic actions (Jahng et al., 2008), enhancing energy expenditure and anorexia. In contrast, GC therapy in humans is often associated with weight gain, in addition to increase in blood pressure (Whitworth et al., 2005).

Many factors, including diet, exercise, stress, and aging itself, affect adrenal expression of TH (Fluharty et al., 1983; Tumer et al., 1992; Kvetnansky et al., 2003; Patel et al., 2005). Several studies have explored to understand the relationship
between peripheral TH expression and hypertension (Guo et al., 2005; Burgi et al., 2011; Congo Carbajosa et al., 2015). Recent studies have uncovered natural polymorphic variations in the human TH gene that influence transcription, autonomic function, and hypertension (Rao et al., 2007, 2010). A causal relationship was revealed when acute DEX treatment in rats (1 mg/kg for 2 days) was shown to upregulate TH mRNA in the adrenal medulla and cause hypertension in rats (Kumai et al., 2000), and antisense TH gene therapy in rats was effective in causing hypotension in the SHR model (Kumai et al., 2001).

Because our model allows monitoring long-term effects of DEX treatment on blood pressure and assessing the underlying mechanism, we find that, similar to acute DEX treatment, chronic DEX exposure also causes an increase in TH mRNA and protein levels, most likely via GR in chromaffin and ganglionic cells of the adrenal medulla (Phillips et al., 2001). This increase in TH transcription, perhaps, leads to synthesis of new enzyme molecules that in turn enhance TH activity and catecholamine synthesis. The exact medullary cell type involved in TH transcription in this model system is not known and will need to be determined in future studies. Of note, we did not find an increase in TH mRNA in the brainstem. This indicates that the long-term genomic effect of DEX on TH occurs mainly in the periphery, including adrenal medulla and other sympathetic nerve terminals.

Fig. 6. TH protein and pSer<sup>40</sup>-TH in the adrenal medulla after DEX and α-MPT. (A) shows TH protein Western blot and densitometry percentage from control. (B) presents pSer<sup>40</sup>-TH Western blot and pSer<sup>40</sup>-TH/β-actin ratio. There were significant differences between VEH/VEH and VEH/DEX, but not with α-MPT–treated groups. Results were analyzed using one-way analysis of variance with Dunnett’s multiple comparison test (*P < 0.05 and **P < 0.01 versus VEH/VEH, n = 4/group).

DEX crossed into the brain, its effects on blood pressure may have been opposite, based on the report that intracerebroventricular injection of DEX reduces blood pressure (Tonolo et al., 1993). We never observed a decrease in blood pressure during the entire period of DEX administration, consistent with peripheral, not central effects.

We did not explore the role of TH in superior cervical ganglion (SCG) and carotid body, although they could also be potential peripheral sites for DEX effects; indeed, TH mRNA at these sites increases in response to stressful conditions (Chen et al., 1995). However, the duration and robustness of these increases are different, and, more importantly, they entail different signaling pathways (Nankova et al., 1999). For example, activator protein-1 has a lower binding to the TH promoter motif upon immobilization in the SCG than in the adrenal medulla (Sabban et al., 2004). The genomic effect of DEX on TH via the atypical glucocorticoid response element might not occur at the SCG, and was less likely to be of stimulatory nature that we saw in vitro (Rani et al., 2009; Sheela Rani et al., 2013). Consistent with this, systemic α-MPT administration generates a greater decrease in blood pressure than guanethidine, an inhibitor of the norepinephrine uptake transporter (Chiou-Tan et al., 1994). This suggests that noradrenergic chromaffin cells that contain TH, but lack this transporter (Phillips et al., 2001), contribute to blood pressure regulation to a greater extent than adrenergic
chromaffin cells, ganglion cells, or nerve fibers that have the transporter.

We found that TH protein in the adrenal medulla did not change in the α-MPT/VEH group, indicating that basal levels of catecholamine synthesis in the adrenal medulla may not be affected by α-MPT. This is in keeping with the stability of baseline MAP during treatment with this drug. However, total TH protein and pSer40-TH decreased in the α-MPT/DEX group compared with the VEH/DEX group, suggesting that α-MPT might reduce only DEX-induced TH expression and phosphorylation. Indeed, this is the first study to show such an effect of this drug, α-MPT. It is thus possible that DEX affects TH enzyme not only at a transcriptional level, but also posttranslationally, perhaps involving the N terminus of the catalytic domain (Nakashima et al., 2009).

However, the interpretation of these results is complicated due to the ability of α-MPT to cross into the blood brain barrier and inhibit endogenous TH, both peripherally and centrally. In that case, our results could resemble studies in which the reduction of central catecholamine concentrations alters sympathetic outflow and elevates blood pressure (Doba and Reis, 1974; Fujino, 1984). In our study, such a consequence of α-MPT administration, although we had expected it to be reduced due to a decrease in cardiac sympathetic activity. As mentioned above, this could also be associated with α-MPT central effects. More importantly, HR maintenance could be due to opposing responses of the parasympathetic nervous system to reduced sympathetic activity (Dickson et al., 1981; Levy, 1984; Laeroix et al., 1990), in addition to potential cardiovascular supersensitivity to catecholamines that may occur immediately after α-MPT administration (Brus et al., 1970; Tripovic et al., 2010). In contrast, decreased HR upon DEX administration is probably a reflex central response to the elevated blood pressure.

In summary, in this study we developed an animal model to study chronic DEX-induced hypertension in which the catabolic effects of DEX are independent of its effects on blood pressure. The chronic administration of DEX modified the peripheral catecholamine biosynthetic pathway at the level of TH in the adrenal medulla. Furthermore, the levels of TH in sympathetic nerve terminals and ganglia, although challenging to measure due to small sample, may be necessary to tease out their relative contributions in DEX-induced hypertension. In particular, the adrenal medulla and sympathetic nerves are important sources of excessive catecholamine levels that lead to cardiovascular alterations and hence hypertension. Because of their repercussion on blood pressure, they are potential targets of intervention to prevent cardiovascular side effects of glucocorticoid therapy.

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Authorship Contributions

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References


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A Novel Model of Dexamethasone-Induced Hypertension 535

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