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ORIGINAL ARTICLE

Blood pressure decrease in spontaneously hypertensive rats folowing renal denervation or dopamine β-hydroxylase inhibition with etamicastat

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Overactivity of the sympathetic nervous system has an important role in the development and progression of arterial hypertension. Catheter-based renal nerve ablation for the treatment of drug-resistant hypertension has recently been developed. An alternative strategy for the modulation of sympathetic nerve function is to reduce the biosynthesis of noradrenaline (NA) by inhibiting dopamine β-hydroxylase (DβH), the enzyme that catalyzes the conversion of dopamine (DA) to NA in the sympathetic nerves. Renal denervation (RDN) surgery was performed in spontaneously hypertensive rats (SHR) to evaluate the effect of RDN on the DA and NA levels and on blood pressure over a 28-day period. The selective peripheral DβH inhibitor etamicastat ($30 \, mg \, kg^{-1} \, day^{-1}$) was administered to another cohort of SHR. RDN and etamicastat treatment had no effect on the renal function, as assessed by measuring the water balance response, renal function and urinary electrolyte levels. RDN significantly decreased the systolic blood pressure (SBP) and the diastolic blood pressure (DBP). A gradual return of the SBP and the DBP to the high baseline levels was observed over time. Conversely, treatment with etamicastat resulted in a significant decrease in the SBP and the DBP at all time points. On the last day of the assessment, NA levels in renal tissue were significantly decreased in both RDN and etamicastat-treated groups. In contrast, the NA levels in the left ventricle were decreased only in the etamicastat-treated group. Thus, RDN produces transitory decreases in blood pressure, whereas prolonged downregulation of sympathetic drive with the DβH inhibitor etamicastat results in a sustained decrease in the SBP and the DBP.

INTRODUCTION

Hypertension is one of the most prevalent cardiovascular risk factors.[1] Overall, 26.4% of the adult population in 2000 had hypertension, and the number of adults with hypertension is predicted to increase by ~60% to a total of 1.6 billion in 2025.[2] Despite the use of generally effective antihypertensive therapy, blood pressure (BP) often remains above target levels because of poor adherence, an inadequate treatment regimen and/or treatment resistance.[3] Resistant hypertension is currently defined as the failure to achieve a goal of BP < 140/90 mm Hg in patients who are compliant with the maximal tolerated doses of a minimum of three antihypertensive drugs, one of which is a diuretic. The prevalence of resistant hypertension is unknown, but clinical trials suggest that it is not rare and may involve perhaps 20–30% of the study participants.[4]

The activation of the sympathetic nervous system, which involves an increased spillover of noradrenaline (NA) in specific organs such as the heart and kidney, has a major role in the pathophysiology of hypertension[5–7] and congestive heart failure[8,9] and is associated with an increased mortality.[10–12] Recently, catheter-based renal nerve ablation has been developed for the treatment of drug-resistant hypertension.[13–17] Most of these systems direct a radiofrequency energy along the lengths of the main renal arteries to ablate the renal sympathetic nerves that lie within and just beyond the adventitia.[18] Catheter-based renal nerve ablation results in a sustained reduction of BP that can still be observed 3 years post denervation.[19] Nonetheless, the first prospective, single-blind, randomized, sham-controlled trial showed no difference in office or 24-hour ambulatory BP at 6 months between the renal denervation (RDN) and sham procedure groups.[20] Moreover, limitations to the procedure exist, including patient eligibility criteria. The patients who are eligible based on criteria used in published studies represent an exceedingly small proportion of the total hypertensive population; such criteria thus prevent the use of RDN in a broader range of hypertensive patients.[21]

Therefore, alternative strategies to modulate sympathetic nerve function are attractive. Among such alternative strategies, the inhibition of dopamine β-hydroxylase (DβH), which is the enzyme

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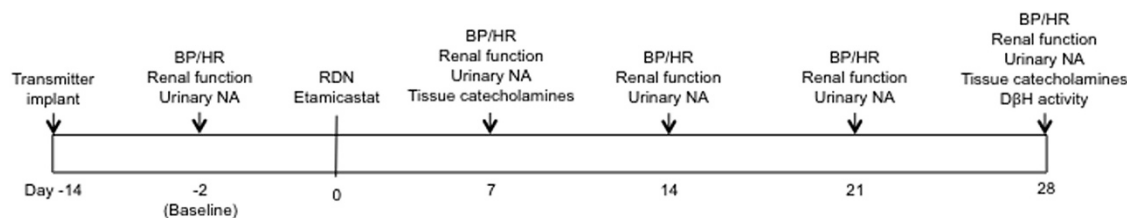
Figure 1 Timeline of the experimental protocol. DβH, dopamine β-hydroxylase; HR, heart rate; BP, blood pressure; NA, noradrenaline; RDN, renal denervation.

responsible for the conversion of dopamine (DA) to NA in sympathetic nerves, has emerged as the most promising.[22] DβH inhibition has the potential to cause a gradual sympathetic slowdown instead of an acute inhibition, as is caused by β-blockers, thereby reducing the negative hemodynamic impacts.[23] DβH inhibition also increases the availability of DA,[24,25] which improves renal function by causing renal vasodilatation and inducing diuresis and natriuresis.[26–28] Several DβH inhibitors have been reported,[29–31] but none have yet achieved the marketing approval because of their low potency, poor DβH selectivity[32] and/or significant adverse effects.[33] Etamicastat (also known as BIA 5-453; (R)-5-(2-aminoethyl)-1-(6,8-difluorochroman-3-yl)-1,3-dihydroimidazole-2-thione hydrochloride) is a new-generation DβH inhibitor in development by BIAL-Portela & Cª, S.A. (S. Mamede do Coronado, Portugal) as a potential new drug therapy for cardiovascular disorders. Etamicastat is a reversible DβH inhibitor that has limited access to the brain and acts mainly on the periphery by decreasing NA levels in the sympathetically innervated tissues.[34,35] Etamicastat has been shown to reduce both systolic BP (SBP) and diastolic BP (DBP), when administered alone or in combination with other antihypertensive drugs, and to reduce urinary NA excretion in spontaneously hypertensive rats (SHR); no changes in BP have been reported in normotensive Wistar–Kyoto rats.[36,37] Recently, etamicastat has been shown to have BP-lowering effects in hypertensive patients.[38]

The present study was designed to compare the efficacy of RDN and DβH inhibition by etamicastat in the reduction of peripheral sympathetic activity and the amelioration of high BP in SHR.

METHODS

Animals and general procedures

Male 12-week-old SHR (Charles River Laboratories, Domaine des Oncins, France) were housed in Macrolon cages with free access to food and tap water under controlled environmental conditions in a colony room (12 hours light–dark cycle, room temperature $22 \pm 1\,°C$ and humidity $55 \pm 15\%$). All animal procedures conformed to the guidelines in Directive 2010/63/EU of the European Parliament concerning the protection of animals used for scientific purposes and to the Portuguese law on animal welfare (Decreto-Lei 113/2013). Etamicastat was synthesized in the Laboratory of Chemistry of BIAL-Portela & Cª, S.A. All other chemicals and materials were obtained from Sigma-Aldrich (St Louis, MO, USA), unless noted otherwise. For all surgeries, rats were anesthetized with pentobarbital sodium ($50\,mg\,kg^{-1}$ IP). Gentamicin sulfate ($10\,mg\,kg^{-1}$ IM; Pfizer, New York, NY, USA) was administered before the surgery. Carprofen ($5\,mg\,kg^{-1}$ SC; Pfizer) was given twice daily for 2 days post surgery.

Experimental protocol

The timeline for the experimental protocol is shown in Figure 1. SHR were instrumented with radio telemeters (TA11PA-C40, DSI, St Paul, MN, USA) as previously described.[36] Rats were allowed to recover individually for 2 weeks before sympathetic nervous system modulation. Animals were randomly assigned to the control ($n=7$), RDN ($n=6$) or etamicastat ($n=6$) treatment groups. An additional, satellite group of animals was used to evaluate tissue

catecholamines 7 days post intervention (control group, $n=6$; RDN group, $n=5$; etamicastat treatment group, $n=6$).

Renal denervation

RDN surgery was performed as previously described.[39] Briefly, a lateral incision was made on each side of the abdominal wall to expose the renal arteries and veins of each kidney. Under a dissecting microscope, visible renal nerves, fat and connective tissue were stripped from the renal vessels. Renal vessels were then painted with 10% phenol in 96% ethanol to ensure the destruction of any remaining nerves.

Chronic treatment with etamicastat

Rats were administered $30\,mg\,kg^{-1}\,day^{-1}$ body weight of etamicastat in drinking water. The daily dose of etamicastat attained for the 7-days period (for the satellite tissue catecholamine-measurement experiments) was $31.9 \pm 2.4\,mg\,kg^{-1}$ body weight and for the 28-days period (all other experiments) was $30.2 \pm 1.7\,mg\,kg^{-1}$ body weight.

Evaluation of renal function

Rats were placed in individual metabolic cages (Tecniplast, Varese, Italy), and 24-hour urine samples were collected before (baseline) and 7, 14, 21 and 28 days after the start of the protocol. All biochemical assays were performed by using a Cobas Mira Plus analyzer (ABX Diagnostics for Cobas Mira, Geneva, Switzerland).

Cardiovascular evaluation

SBP, DBP, mean arterial pressure (MAP) and heart rate (HR) telemetry recordings were recorded before (baseline) and 7, 14, 21 and 28 days post sympatholytic treatment. The data were collected for 40 s every 10 min and averaged over 48 hours by using the Dataquest A.R.T. acquisition and analysis system 4.0 (DSI).

Pharmacokinetic evaluation

Pharmacokinetic evaluation was performed after the completion of the experimental protocol. Blood was collected from the vena cava (S-Monovette, Sarstedt, Nümbrecht, Germany), and plasma etamicastat levels were quantified by a tandem mass spectrometer as previously described.[40]

Catecholamine assay

NA and DA levels were quantified in the frontal cortex, kidney cortex, left ventricle of the heart and vas deferens on days 7 and 28 of the protocol. Catecholamine (DA, NA and adrenaline) content was assessed in right adrenal gland samples. Twenty-four-hour urine samples from rats housed individually in metabolic cages (Tecniplast) were collected for NA quantification before (baseline) and 7, 14, 21 and 28 days after the start of the protocol. Samples were analyzed by using a high performance liquid chromatography system with electrochemical detection as previously described.[41]

Measurement of DβH activity

Determination of DβH activity was performed according to the method of Nagatsu and Udenfriend[42], with some modifications. In brief, on the final day of the protocol, left adrenal glands were removed, placed in $200\,\mu l$ of $50\,mM$ Tris, pH 7.4 and stored at $-80\,°C$ until analysis. Samples were thawed on ice

and homogenized (Precellys bead beater, Bertin Technologies, Montigny-le-Bretonneux, France) for two cycles per 5 s at 5000 r.p.m., after which the total protein content in the homogenates was quantified (Bradford reagent, Bio-Rad, Hercules, CA, USA). Samples were diluted to $1.5\,mg\,ml^{-1}$ and mixed with 700 μl of incubation buffer (18.0 ml of 1 M sodium acetate, pH 5; 4.5 ml of 0.2 M sodium fumarate; 4.5 ml of 0.2 M ascorbic acid; 4.5 ml of 0.02 M pargyline; 13.5 ml of 0.2 M N-ethylmaleimide; 9.0 ml of catalase, $14\,200\,U\,ml^{-1}$; 4.5 ml of Milli-Q water (Milli-Q Advantage A10 Ultrapure Water Purification System, Merck Millipore, Darmstadt, Germany); 4.5 ml of 20 μM copper (II) sulfate). The enzymatic reaction was initiated at 37 °C with 50 μl of 0.5 M tyramine. After 45 min, the reaction was stopped with 200 μl of 2 M perchloric acid. Samples were then centrifuged for 5 min at 9000 g to pellet the protein, and 800 μl of the supernatant was transferred into SPE cartridges (Isolute SCX-3, 100 mg fixed-well plate, Biotage, Uppsala, Sweden). Samples were eluted with 600 μl of 4 M ammonium hydroxide and then derivatized with 200 μl of 2% sodium periodate; after 6 min, the reaction was stopped with 200 μl of 10% sodium metabisulfite. An amount of 200 μl of the derivatized samples was then transferred to a 96-well plate, and absorption was measured by spectro-photometry at 330 nm (Spectramax Plus, Molecular Devices, Silicon Valley, CA, USA). DβH activity was assessed by measuring the quantity of octopamine formed (expressed in $ng\,mg^{-1}$ of protein per min).[43]

Statistical analysis

The data are presented as the mean ± s.e.m.. Data analyses were performed using Prism 6 (GraphPad Software, San Diego, CA, USA). The data for the experimental group were compared to those of the control group using one-way or repeated-measures analysis of variances followed by Dunnett's multiple comparison tests. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Fluid balance and renal function responses to peripheral sympathetic modulation

Water intake, urine output and renal function were assessed at baseline and 7, 14, 21 and 28 days post sympatholytic therapy. RDN had no significant effect on fluid balance. Prolonged etamicastat

treatment ($30\,mg\,kg^{-1}\,day^{-1}$) decreased the water intake and urinary output (Table 1). No global changes in fluid balance were observed. Observations from our laboratory showed that decreased water intake may be related to palatability (unpublished data). Etamicastat was found in the plasma of all animals. The concentrations of etamicastat in plasma were $145.8 \pm 21.2\,ng\,ml^{-1}$ (satellite) and $154.5 \pm 25.8\,ng\,ml^{-1}$ (28-day period). Renal function was unaffected by RDN or etamicastat treatment (Tables 2 and 3).

Cardiovascular responses to peripheral sympathetic modulation

As shown in Figures 2a–c, the control group of SHR had established hypertension, and no difference in SBP, DBP or MAP compared with baseline values occurred throughout the protocol (SBP = 178.6 ± 1.0, DBP = 129.0 ± 2.3, MAP = 153.3 ± 1.4 mm Hg). The baseline BP was similar between groups. In RDN-treated animals, SBP, DBP and MAP values were significantly ($P < 0.05$) decreased on days 7 and 14 post denervation compared with the baseline. By day 21, the BP values were no longer significantly different from baseline values or the corresponding values in control animals. SBP, DBP and MAP returned to baseline values on the final day of the assessment (day 28 after targeted sympathetic ablation; SBP = 178.1 ± 10.5, DBP = 125.1 ± 10.1, MAP = 150.7 ± 10.1 mm Hg). In contrast, periph-eral sympathetic modulation by etamicastat induced a sustained BP decrease throughout the entire 28-days period.

The effects of sympathetic modulation on HR are depicted in Figure 2d. The baseline pulse rate was similar between the groups. The HR of control SHR fell transiently from the baseline (314.2 ± 2.9 beats per min) on days 14 and 28 (−9 beats per min at both time points). After RDN surgery, rats' HRs were not different from baseline (310.0 ± 3.4 beats per min) 7 days post surgery but were significantly increased 14 days after RDN (+14 beats per min), after which they returned to baseline values by days 21 and 28 of the assessment. Prolonged etamicastat treatment in the drinking water caused a small,

Table 1 Body weight and water balance response after renal denervation or etamicastat treatment in the SHR

	Control	Renal denervation	Etamicastat
Body weight (g)			
Baseline	361.9 ± 8.7	369.0 ± 5.7	365.3 ± 7.3
7 days	371.7 ± 9.6	358.0 ± 10.3	376.0 ± 8.2
14 days	376.9 ± 9.8	365.5 ± 11.1	380.8 ± 8.6
21 days	380.1 ± 10.0	383.3 ± 10.8	384.0 ± 8.1
28 days	382.0 ± 9.8	389.2 ± 11.0	387.0 ± 8.6
Water intake (ml per 24 h)			
Baseline	19.3 ± 1.3	12.9 ± 1.2*	20.3 ± 1.5
7 days	20.5 ± 2.1	17.1 ± 1.0	13.4 ± 1.4*
14 days	17.4 ± 1.7	14.4 ± 0.9	11.7 ± 1.1*
21 days	17.4 ± 2.1	11.5 ± 1.2*	10.7 ± 0.7*
28 days	16.1 ± 2.0	16.4 ± 0.9	8.6 ± 0.9*
Urine output (ml per 24 h)			
Baseline	7.6 ± 1.4	6.6 ± 1.3	8.5 ± 1.0
7 days	8.0 ± 2.2	6.9 ± 1.0	3.3 ± 0.6
14 days	8.8 ± 1.4	6.3 ± 0.8	4.1 ± 0.5*
21 days	8.8 ± 1.8	6.9 ± 0.6	2.9 ± 0.2*
28 days	9.3 ± 1.2	7.2 ± 0.5	4.4 ± 0.4*

Abbreviation: SHR, spontaneously hypertensive rats.
Mean ± s.e.m., $n = 6–7$ per group.
*$P < 0.05$ vs control group. One-way analysis of variance followed by Dunnett's multiple comparison test.

Table 2 Renal function after renal denervation or etamicastat treatment in the SHR

	Control	Renal denervation	Etamicastat
Urinary protein (mg per 24 h)			
Baseline	10.6 ± 0.8	11.2 ± 1.1	16.1 ± 2.9
7 days	10.1 ± 0.7	10.3 ± 1.8	10.9 ± 3.3
14 days	11.3 ± 0.8	8.4 ± 1.3	10.0 ± 2.2
21 days	10.0 ± 1.2	9.9 ± 0.6	9.3 ± 2.0
28 days	12.5 ± 1.3	10.6 ± 0.3	8.4 ± 0.6*
Urinary creatinine (mg per 24 h)			
Baseline	9.3 ± 0.8	8.7 ± 0.5	9.2 ± 0.5
7 days	8.3 ± 0.6	9.0 ± 0.4	7.5 ± 0.8
14 days	10.1 ± 0.6	8.9 ± 0.5	8.3 ± 0.5*
21 days	8.8 ± 0.9	9.8 ± 0.3	7.4 ± 0.3
28 days	10.8 ± 0.8	10.1 ± 0.6	8.9 ± 0.4
Urinary urea (mg per 24 h)			
Baseline	325.5 ± 35.4	357.9 ± 14.8	363.2 ± 15.1
7 days	327.7 ± 19.7	376.0 ± 17.5	304.8 ± 35.4
14 days	379.4 ± 24.7	337.3 ± 17.5	317.6 ± 18.4
21 days	343.7 ± 42.9	345.9 ± 11.9	290.8 ± 11.0
28 days	415.9 ± 27.8	364.9 ± 7.5	356.4 ± 16.2

Abbreviation: SHR, spontaneously hypertensive rats.
Mean ± s.e.m., $n = 6–7$ per group.
*$P < 0.05$ vs control group. One-way analysis of variance followed by Dunnett's multiple comparison test.

Table 3 Urinary electrolytes after renal denervation or etamicastat treatment in the SHR

	Control	Renal denervation	Etamicastat
Urinary Na⁺ (µmol per 24 h)			
Baseline	255 ± 33	349 ± 23	530 ± 98*
7 days	381 ± 159	557 ± 88	404 ± 48
14 days	351 ± 84	403 ± 23	266 ± 18
21 days	578 ± 248	531 ± 120	290 ± 22
28 days	698 ± 209	567 ± 116	356 ± 41
Urinary K⁺ (µmol per 24 h)			
Baseline	892 ± 125	803 ± 26	1021 ± 95
7 days	1013 ± 133	1174 ± 130	716 ± 94
14 days	1072 ± 90	970 ± 108	747 ± 78*
21 days	1067 ± 187	950 ± 67	621 ± 72
28 days	979 ± 88	1027 ± 113	697 ± 56
Urinary Cl⁻ (µmol per 24 h)			
Baseline	1409 ± 200	1644 ± 137	2032 ± 341
7 days	1729 ± 222	2432 ± 302	1507 ± 249
14 days	1184 ± 162	1860 ± 189*	990 ± 122
21 days	1158 ± 176	1682 ± 149*	924 ± 41
28 days	1698 ± 97	1470 ± 100	2160 ± 326
Urinary Na⁺-to-K⁺ ratio			
Baseline	0.38 ± 0.07	0.41 ± 0.04	0.51 ± 0.07
7 days	0.54 ± 0.19	0.46 ± 0.05	0.59 ± 0.08
14 days	0.32 ± 0.05	0.48 ± 0.05*	0.37 ± 0.03
21 days	0.62 ± 0.17	0.55 ± 0.11	0.50 ± 0.07
28 days	0.53 ± 0.10	0.58 ± 0.14	0.51 ± 0.04

Abbreviation: SHR, spontaneously hypertensive rats.
Mean ± s.e.m., $n = 6$–7 per group.
*$P < 0.05$ vs control group. One-way analysis of variance followed by Dunnett's Multiple Comparison Test.

but significant, decrease in HR throughout the protocol (baseline: 311.8 ± 3.5, 7 days: 295.3 ± 2.0, 14 days: 284.4 ± 0.7, 21 days: 287.6 ± 1.9 and 28 days: 287.2 ± 2.8 beats per min).

Catecholamine content in tissues
NA and DA tissue levels were assayed on days 7 and 28 of the protocol. As depicted in Figures 3a and c, RDN resulted in a sustained and marked depletion ($\approx$95% decrease) of NA levels in the renal cortex of both the left and right kidneys compared with control SHR. No effects on NA levels in the frontal cortex or vas deferens were observed, but there was a transient 1.5-fold increase in NA content in the left ventricle of the heart on day 7 of the protocol. On the other hand, sympathetic modulation via treatment with etamicastat resulted in a prolonged, $\approx$50% decrease in NA content in all peripheral tissues 7 and 28 days post intervention. No differences in tissue DA levels were observed after RDN surgery. In contrast, prolonged etamicastat treatment resulted in a significant 3–15-fold increase in DA content in all peripheral tissues compared with controls throughout the protocol (Figures 3b and d).

As shown in Figure 4, DA levels in the adrenal glands were significantly increased after etamicastat treatment on days 7 ($\approx$17-fold increase) and 28 ($\approx$18-fold increase) of the protocol. No difference in the DA content of the adrenal glands was observed after RDN. Targeted RDN significantly increased NA (1.4-fold increase) and adrenaline (1.3-fold increase) levels in the adrenal glands 7 days post
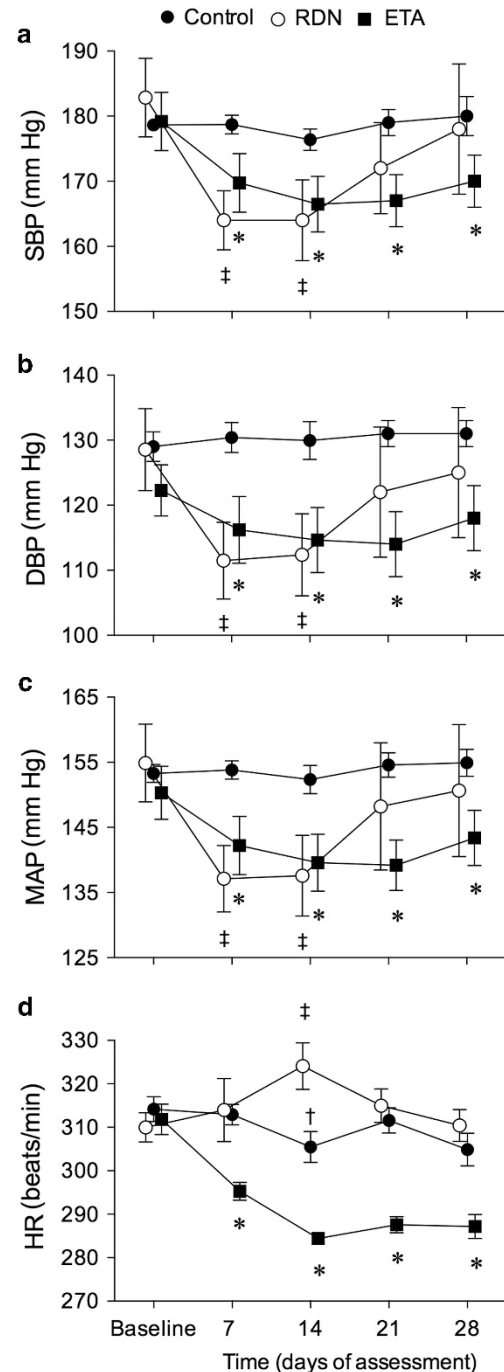
Figure 2 Effect of renal denervation and etamicastat treatment on (**a**) systolic blood pressure, (**b**) diastolic blood pressure, (**c**) mean arterial blood pressure and (**d**) heart rate. Values were determined to be significantly different from the corresponding baseline values by a repeated-measures analysis of variance followed by Dunnett's multiple comparison test (†$P < 0.05$ for Control, $n = 7$; ‡$P < 0.05$ for renal denervation, $n = 6$; *$P < 0.05$ for etamicastat treatment, $n = 6$). The data are shown as the mean ± s.e.m.

surgery. NA and adrenaline levels returned to control levels on the final day of the assessment after targeted sympathetic ablation. Etamicastat treatment resulted in a sustained, $\approx$30% decrease in NA and adrenaline levels in the adrenal glands that was observed 7 and 28 days post intervention compared with the control rats.

7 days post-intervention

28 days post-intervention
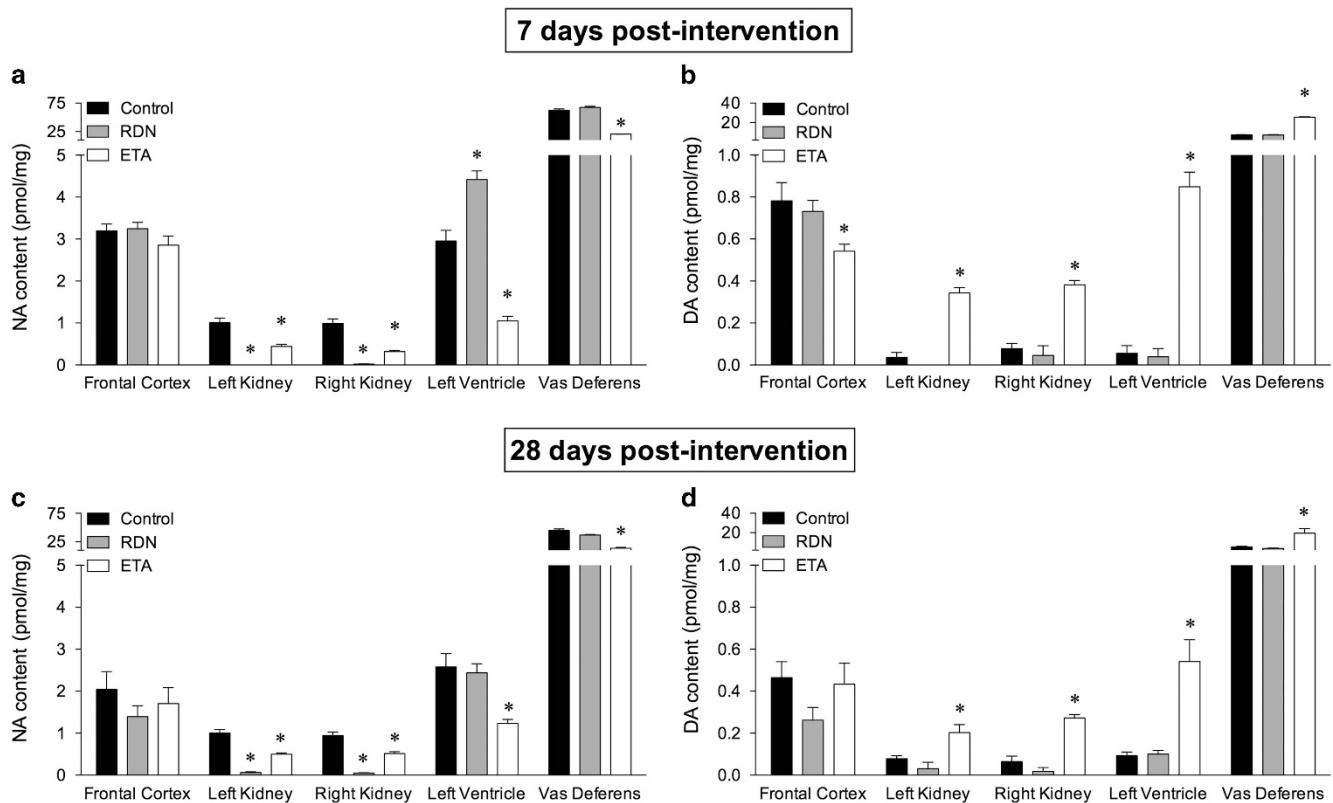
Figure 3 Effect of renal denervation (*n* = 5–6) and etamicastat treatment (*n* = 6) on (**a** and **c**) noradrenaline and (**b** and **d**) dopamine levels in the frontal cortex, kidney (renal cortex), heart (left ventricle) and vas deferens 7 and 28 days post intervention. Values were determined to be significantly different from corresponding control values (*n* = 6–7) by a one-way analysis of variance followed by Dunnett's multiple comparison test (*$P < 0.05$). The data are shown as the mean ± s.e.m.

Urinary NA levels are shown in Figure 5. RDN had no effect on the urinary NA excretion. Conversely, etamicastat treatment resulted in an ≈80% decrease in 24-h urinary NA levels at all time points of the protocol compared with the baseline levels.

DβH activity

Tyramine can be oxidized to octopamine in a reaction that is catalyzed by DβH. DβH activity can be defined based on the rate of formation of octopamine in rat adrenal gland homogenates. This assay was performed to demonstrate the target-specific effect of chronic etamicastat treatment on peripheral enzymatic DβH activity at the end of the study. Figure 6 displays the DβH activity at the end of the protocol. The RDN surgery protocol did not alter adrenal DβH activity (46.2 ± 6.4 ng mg^{-1} protein per min) compared with control animals (65.5 ± 11.4 ng mg^{-1} protein per min). However, prolonged oral etamicastat treatment led to a significant decrease (≈62%) in adrenal DβH activity (24.8 ± 8.4 ng mg^{-1} protein per min) compared with control animals.

DISCUSSION

Renal sympathetic denervation has recently been introduced in the clinical setting for treatment-resistant hypertension. Although a long-term follow-up (36 months) confirms that reductions in BP are sustained,[19] the placebo-control SYMPLICITY HTN-3 study found no significant effect of RDN on office or 24-hour ambulatory SBP.[20] Moreover, patient selection constraints limit the use of these systems in the general therapy-resistant hypertensive population.[21] Failure to achieve a complete circumferential denervation by using the

SYMPLICITY single-tip catheter has been proposed as a reason for the failure of this study to achieve its primary efficacy end point. The EnligHTN I was the first-in-human study, using a multielectrode ablation system, in patients with drug-resistant hypertension.[14] Twelve months of data, together with a complete analysis of efficacy and safety information, including office, 24-hour ambulatory, and home BP measurements, are now available and continue to demonstrate the safety and efficacy of the EnligHTN ablation system in patients with drug-resistant hypertension.[44] The EnligHTN I study was, however, an unblinded and single-arm study.

The present study aimed to determine the efficacy of RDN and DβH inhibition via a chronic etamicastat treatment in the reduction of peripheral sympathetic activity and high BP in SHR, which are a rat model of genetic essential hypertension. The data presented here clearly demonstrate that in SHR, whereas RDN surgery, which caused a decrease in renal NA levels by more than 95% throughout the assessment period (28 days), resulted in only a transitory decrease in high BP, the reduction in peripheral sympathetic activity produced by prolonged treatment with etamicastat resulted in a sustained amelioration of high BP over the entire assessment period. A decrease in NA release as a result of DβH inhibition gradually slows down the sympathetic drive and thus leads to an indirect decrease in the activation of α- and β-adrenoceptors.[24] Indeed, etamicastat treatment resulted in a ≈50% decrease in NA content and a marked increase in DA content in peripheral tissues with sympathetic innervation. Increased DA availability[24,25] may result in additional benefits, as DA improves renal function by causing renal vasodilatation and by inducing diuresis and natriuresis.[26–28] Nonetheless, the renal effect of
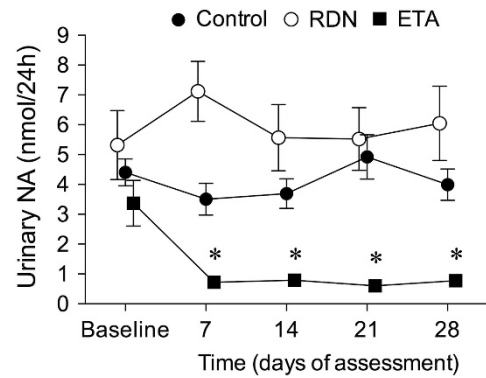
a 7 days post-intervention

b 28 days post-intervention
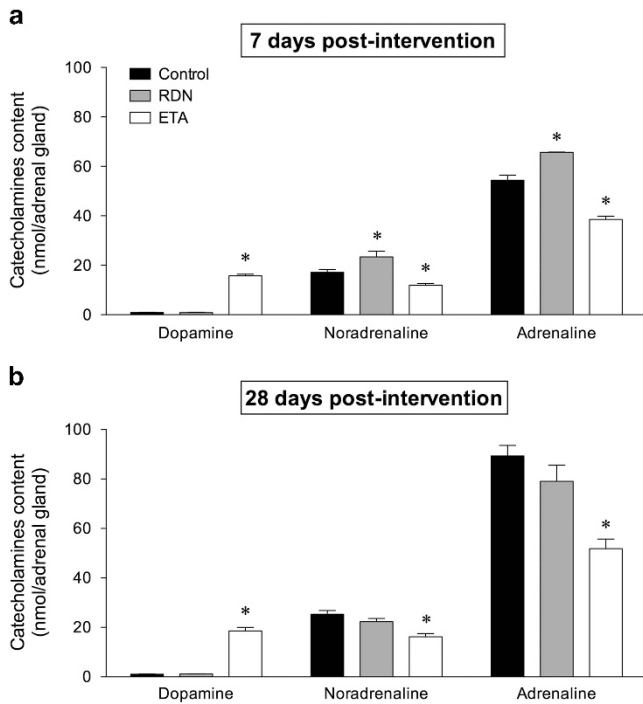
Figure 4 Effect of renal denervation (*n*=5) and etamicastat treatment (*n*=6) on catecholamine (dopamine, noradrenaline and adrenaline) content in the adrenal glands 7 and 28 days post intervention. Values were determined to be significantly different from corresponding control values (*n*=6) by a one-way analysis of variance followed by Dunnett's multiple comparison test (*$P<0.05$). The data are shown as the mean±s.e.m.

Figure 5 Effect of renal denervation (*n*=6) and etamicastat treatment (*n*=6) on urinary noradrenaline excretion. Values were determined to be significantly different from the corresponding baseline values by a repeated-measures analysis of variance followed by Dunnett's multiple comparison test (*$P<0.05$ for etamicastat treatment; control group, *n*=7). The data are shown as the mean±s.e.m.

Figure 6 Effect of renal denervation (*n*=6) and etamicastat treatment (*n*=6) on the inhibition of dopamine β-hydroxylase activity in rat adrenal glands on the final day of the protocol. Values were determined to be significantly different from the corresponding control (*n*=7) values by using a one-way analysis of variance followed by Dunnett's multiple comparison test (*$P<0.05$). The data are shown as the mean±s.e.m.
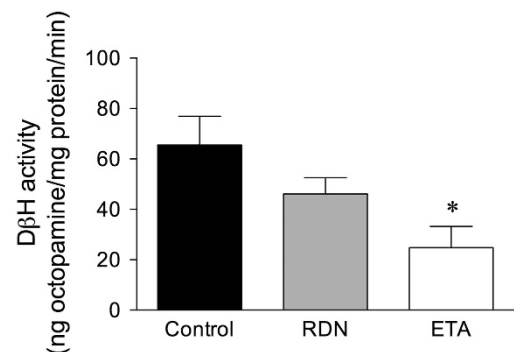
DA is impaired in SHR because of defects in renal transduction of the DA signal.[45]

SHR represent an experimental model of hypertension; many characteristics of SHR resemble those found in essential hypertension in humans. Hypertension in SHR is a heterogeneous, multifactorial disorder.[46,47] In agreement with previous reports,[48–50] in this study, we showed that specific renal sympathetic nervous system ablation causes a transitory decrease in BP in SHR. Transient increases in NA levels were observed 7 days post RDN in the left ventricle of the heart and in the adrenal glands, which resulted in an increase, albeit not significant, in urinary NA levels (7 days), before returning to values similar to those in control SHR by the end of the assessment period. Although RDN has a target-specific effect on renal sympathetic nerves,[51] ablation of renal sympathetic nervous system efferents and afferents has been shown to induce a transient increase in peripheral sympathetic drive, which can trigger compensatory systemic factors and mechanisms that cause a gradual return to high BP.[46,52] Experimental evidence suggests that after denervation, a return of functional activity precedes the complete regrowth of nerve fibers. For example, the functional activity of the rat heart has been shown to approach control levels two weeks after sympathectomy, when myocardial catecholamine stores were still 80% depleted.[53] Moreover, in the chronically denervated rat kidney, supersensitivity increases renal responsiveness to NA.[54,55]

Etamicastat treatment produced a significant reduction in urinary NA levels (≈80%), whereas RDN failed to significantly alter urinary NA levels. However, both treatments reduced kidney tissue levels of NA; this effect was more marked in the RDN group. Total chronic denervation of one kidney has been shown to have no effect on the rate of excretion of endogenous NA, and the NA that is subsequently excreted in the urine appears to be derived solely from the circulating catecholamines.[56,57] Indeed, etamicastat treatment resulted in a general reduction in peripheral noradrenergic drive, as evidenced by the reductions in NA levels observed in the heart, vas deferens and adrenal gland. Given that NA outputs correlate positively with creatinine clearance,[58,59] the reduction in urinary NA levels in etamicastat-treated animals may be attributed to a reduction in peripheral sympathetic drive.

Chronic etamicastat treatment resulted in a sustained reduction in BP and in a small, but significant, decrease in pulse pressure in SHR. The effects of etamicastat on BP may be attributed to a reduced activation of both peripheral α- and β-adrenoceptors.[26] The observed decrease in HR values may be accounted for by the diminished activation of β-adrenoceptors; β-blockers are known to decrease pulse pressure through negative inotropic and chronotropic actions.[60] The effect of etamicastat treatment on HR that was observed in this study was not observed in prior studies.[36,37] Nonetheless, It should be noted that previous studies evaluated the effects of etamicastat on BP and HR in SHR after a single dose, with measurements made by monitoring telemetry signals[36] or by using the tail-cuff plethysmography method.[37] Although similar BP outcomes were obtained in all of

these studies, differences in the methodology and in the study protocol may explain the differences observed in the pulse pressure.

In conclusion, these results support the use of peripheral DβH inhibitors as a promising approach for the treatment of cardiovascular disorders such as hypertension and chronic heart failure, where a reduction in NA levels may be of therapeutic benefit.

CONFLICT OF INTEREST

NMP, BI, EM, LCW and PSS are/were employees of BIAL-Portela & Cª, S.A. (the sponsor of the study) at the time of the study.

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