11-hydroxysteroid dehydrogenase type 2 deficiency accelerates atherogenesis and causes proinflammatory changes in the endothelium in apoe-/- mice

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Mineralocorticoid receptor (MR) activation is proinflammatory and proatherogenic. Antagonism of MR improves survival in humans with congestive heart failure caused by atherosclerotic disease. In animal models, activation of MR exacerbates atherosclerosis. The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) prevents inappropriate activation of the MR by inactivating glucocorticoids in mineralocorticoid-target tissues. To determine whether glucocorticoid-mediated activation of MR increases atheromatous plaque formation, we generated ApoE−/−/11β-HSD2−/− double-knockout (E/b2) mice. On chow diet, E/b2 mice developed atherosclerotic lesions by 3 months of age, whereas ApoE−/− mice remained lesion free. Brachiocephalic plaques in 3-month-old E/b2 mice showed increased macrophage and lipid content and reduced collagen content compared with similar sized brachiocephalic plaques in 6-month-old ApoE−/− mice. Crucially, treatment of E/b2 mice with eplerenone, an MR antagonist, reduced plaque development and macrophage infiltration while increasing collagen and smooth muscle cell content without any effect on systolic blood pressure. In contrast, reduction of systolic blood pressure in E/b2 mice using the epithelial sodium channel blocker amiloride produced a less-profound atheroprotective effect. Vascular cell adhesion molecule 1 expression was increased in the endothelium of E/b2 mice compared with ApoE−/− mice. Similarly, aldosterone increased vascular cell adhesion molecule 1 expression in mouse aortic endothelial cells, an effect mimicked by corticosterone only in the presence of an 11β-HSD2 inhibitor. Thus, loss of 11β-HSD2 leads to striking atherogenesis associated with activation of MR, stimulating proinflammatory processes in the endothelium of E/b2 mice. (Endocrinology 152: 236–246, 2011)
a consequence of prerenreceptor metabolism of glucocorticoids to intrinsically inert 11-keto-glucocorticoids by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) (6). The pathophyslogic importance of 11β-HSD2 is demonstrated in patients with the syndrome of apparent mineralocorticoid excess caused by mutations in Hsd11b2, the human gene encoding 11β-HSD2 (7). Loss of 11β-HSD2 results in inappropriate activation of MR by glucocorticoids in the distal nephron causing hypokalemia and hypertension (8). Similarly, 11β-HSD2−/− mice are also hypertensive, with activation of MR in the distal nephron causing increased sodium reabsorption and potassium excretion (9).

Evidence supports a proatherogenic action of cortisol within the vessel wall (10). Glucocorticoid pharmacotherapy in humans is associated with increased cardiovascular events (11).

We and others have demonstrated the existence of the MR/11β-HSD2 system in nonepithelial tissues, including the vasculature (12). Thus, proatherogenic effects of MR activation could be mediated directly by increased mineralocorticoid hormones or through by-pass or reduced activity of vascular 11β-HSD2, permitting glucocorticoid activation of vascular MR. All known inhibitors of 11β-HSD2 can also inhibit 11β-HSD1 activity (13) and compromise the endothelial barrier by interacting with tight junction proteins (14). Therefore, we have investigated the underlying mechanism in Apoe−/−/Hsd11b2−/− double-knockout (E/b2) mice, lacking both apolipoprotein E (ApOE) and 11β-HSD2.

Materials and Methods

Generation of ApoE/Hsd11b2 double-knockout animals

All animal studies were conducted in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals under the auspices of the Animals (Scientific Procedures) Act UK 1986 after prior approval by the local ethical committee. The previously targeted Hsd11b2 allele (9) was transferred to C57BL/6J by nine generations of consecutive backcrosses. Two C57BL/6J females homozygous for deletion of the Hsd11b2 allele were crossed with an ApoE-knockout (ApoE−/−) male on the C57BL/6J background (Charles River, L’Arbresle Cedex, France) derived from the original knockout line (15). Double heterozygous male offspring (ApoE+/−/Hsd11b2+/−) were backcrossed to ApoE−/− females; progeny was subsequently intercrossed to produce double homozygous knockout animals (ApoE−/−/Hsd11b2−/−). The colony was maintained by crossing heterozygous ApoE−/−/Hsd11b2−/− knockout males (E/b2) to ApoE−/−/Hsd11b2−/− females; double-knockout E/b2 progeny was selected for experimental protocols. Genotyping was performed by PCR using genomic DNA extracted from ear clips. Apoe primers were located outside the neocassette inserted into exons 3 and 4 (ApoeEx3f, AACTTA TTC TAC ACA GGA TGC C; Apoe ex4r, CGT CAT AGT GTC CAT CTC CAT CAG TGC). These primers amplify both the wild-type allele (584 bp) and the knockout allele (1500 bp). PCR conditions were as follows: denaturation at 94°C for 5 min, then 3 min of elongation at 72°C, followed by 32 cycles of 94°C for 30 sec, 58°C for 1 min, and 72°C for 2 min. The Hsd11b2 allele (981 bp) was amplified with primers 11b2_679f, AGG CTG ATG ATA GAT TCA CGA GAC; and 11b2_1660r, CGA ATG TGT CTA TAA GCA GTG. The knockout allele was amplified using the genomic primer 11b2_679f (above) and primer Neof1441, GCG ATT GGG CTA ACC GCT TCC TCG, complementary to the Neo gene sequence in the targeting cassette inserted in the reverse orientation.

Animal treatments

Male Apoe−/− and E/b2 mice were maintained on normal chow diet with water ad libitum and a 12-h light, 12-h dark cycle. Systolic blood pressure (SBP) was measured in conscious, restrained mice by noninvasive tail cuff plethysmography (16). This procedure was repeated on a weekly basis during the first month followed by monthly measurements until termination. Representative Apoe−/− and E/b2 mice were killed by asphyxiation with CO2 at age 3 and 6 months for assessment of atherosclerotic lesion formation. To evaluate the effects of drugs, 2-month-old male E/b2 and Apoe−/− mice were randomized to receive normal chow diet containing the MR antagonist eplerenone (200 mg/kg·d), the epithelial sodium channel (ENaC) blocker amiloride (1 mg/kg·d; n = 9) that acts downstream of renal MR to lower blood pressure (17), or vehicle for 3 months.

Tissue preparation for assessment of atherosclerosis

After euthanasia, the vasculature was perfusion fixed in situ with 10% neutral-buffered paraformaldehyde via the left ventricle. Arteries removed included the aorta and the following major branches: brachiocephalic (innominate) artery and its branches, the right subclavian and right common carotid arteries, the left common carotid and left subclavian arteries, and the major branches of the abdominal aorta, including the celiac, superior mesenteric, and renal arteries.

Semiquantitative gross assessment of atherosclerosis in the arterial tree

Adventitia were dissected from fixed arteries under a dissecting microscope. Atherosclerotic lesions were visualized through the translucent arterial wall as yellowish-white opaque deposits. A semiquantitative scoring system was applied for atherosclerotic deposits at the following sites: 1) lesser curvature of the aortic arch, 2) origins of principal branches of thoracic aorta (brachiocephalic, left common carotid, and left subclavian arteries), 3) origin of right and distal right and left common carotid arteries, 4) distal right and left subclavian arteries, and 5) principal branches of abdominal aorta (celiac, superior mesenteric, and renal arteries). Scoring was based on the following criteria: 0, absent; 1, trace; 2, mild; 3, moderate; and 4, severe.

Arterial trees from all mice of both genotypes were coded and read blind by two independent observers. For each site, the arterial tree with the most severe deposit was assigned a score of 4. The remaining coded arterial trees were then assigned a score based on that sample.
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![Figure 1](image.png)

**FIG. 1.** Accelerated atherosclerosis in Apoe<sup>−/−</sup> mice on a standard low-fat diet. A, Atherosclerotic lesions and outward remodeling were evident in the brachiocephalic arteries of 3-month-old E/b2 mice but not in Apoe<sup>−/−</sup> mice (UST stain; magnification, ×10). B, At 6 months of age, lesion development and outward remodeling were significantly greater in E/b2 than in Apoe<sup>−/−</sup> mice. The example used for Apoe<sup>−/−</sup> shows the largest plaque seen at this age (UST stain; magnification, ×10). Lesions in E/b2 were more complex with evidence of buried fibrous caps (black arrows). Scale bar, 250 μm. C, Quantitative analysis confirmed that lesion sizes were increased, and lumens were reduced in E/b2 mice compared with Apoe<sup>−/−</sup> mice. Data are means ± SEM; †, P < 0.05; ‡, P < 0.01 compared with Apoe<sup>−/−</sup> using Student's unpaired t test (n = 4–8).

**TABLE 1.** Increased atherogenesis in E/b2 mice

<table>
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<tr>
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<th>3 months</th>
<th>6 months</th>
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<tr>
<td></td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (4)</td>
<td>E/b2 (8)</td>
</tr>
<tr>
<td>Area inside EEL (×10&lt;sup&gt;3&lt;/sup&gt; μm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>100 ± 18</td>
<td>243 ± 30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medial area (×10&lt;sup&gt;3&lt;/sup&gt; μm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>39.6 ± 9.1</td>
<td>100.2 ± 10.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lesion area (×10&lt;sup&gt;3&lt;/sup&gt; μm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1.4 ± 1.4</td>
<td>48.1 ± 13.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lumen area (×10&lt;sup&gt;3&lt;/sup&gt; μm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>59.0 ± 10.0</td>
<td>95.0 ± 12.0</td>
</tr>
<tr>
<td>Buried caps</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Macrophages % plaque area</td>
<td>0 (no plaques)</td>
<td>13.9 ± 5.2 (4)</td>
</tr>
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Histological analysis of brachiocephalic arteries identified extensive atherosclerotic lesions in 3-month-old E/b2 mice but not in age-matched Apoe<sup>−/−</sup> mice. At 6 months of age, lesion size remained significantly increased in E/b2 compared with Apoe<sup>−/−</sup> mice. The differences in lesion size were significant whether expressed as mm<sup>2</sup> or as a percentage of the lesion area (lesion/area inside the external elastic lamina × 100) (Fig. 1). Increased lesion development in E/b2 mice reduced the percentage luminal area (lumen/area inside the external elastic lamina × 100), compared with Apoe<sup>−/−</sup> mice at both 3 and 6 months of age (Fig. 1). Absolute lumen area (mm<sup>2</sup>) was not reduced in E/b2 mice due to extensive outward remodeling of the vessel (indicated by increased total area within the external elastic lamina). Buried fibrous caps were evident only in 6-month-old E/b2 mice. These may be an indication of lesion ‘vulnerability.’ Data are mean ± SEM, with group sizes shown in parentheses (n). EEL, External elastic lamina.

<sup>a</sup> P < 0.05.

<sup>b</sup> P < 0.01 compared to age-matched Apoe<sup>−/−</sup> mice (Student’s unpaired t test).

**Immunohistochemistry**

Plaque collagen content was assessed by Picrosirius red staining and extracellular lipid content by quantification of the holes left behind during histiologic processing of UST-stained sections (18). Plaque macrophage and smooth muscle cell (SMC) content was assessed using Mac-2 (VH BIO, Gateshead, UK) and smooth muscle α-actin (Sigma, Poole, UK) antibodies, respectively. Vascular cell adhesion molecule 1 (VCAM-1) (Cambridge Biosciences, Cambridge, UK) antibody was used to investigate adhesion molecule expression (for details, please see Supplemental data, published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org). Staining was imaged using a light microscope (Zeiss Axioskop) coupled to a Photometrics CoolSnap camera (Tucson, AZ). Photoshop CS3 Extended software was used to quantify staining, and data are expressed as stained areas relative to total plaque size (percentage).

A semiquantitative scoring method (carried out blind to genotype) was employed to assess VCAM-1 expression, where an arbitrary score of 0–4 was given to each section based on the percentage of the circumference of the specific endothelial staining.

**Cell culture**

Mouse aortic endothelial cells (MAECs cell line) (19) were maintained in endothelial basal medium 2 (Lonza, Slough, UK) supplemented with 10% fetal calf serum and Pen/Strep. Cells were treated overnight in serum-free medium with one of the following: 10 ng/ml TNF-α (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), 1 nM aldosterone in the presence/absence of 1 μM spironolactone, 1 nM corticosterone in the presence/absence of 1 μM glycyrretinic acid, and 1 μM spironalactone (all from Sigma). VCAM-1 expression was quantified by counting the number of positively stained cells per field (at magnification, ×40) in four separate fields for each treatment.
Plasma lipid and lipoprotein analysis

Terminal blood samples were taken for lipid analysis from the left ventricle of vehicle- and drug-treated E/b2 and Apoe−/− mice. Total plasma cholesterol was measured by a colorimetric reaction using the Cholesterol/Cholesteryl Ester Quantification kit (Merck, Whitehouse Station, NJ).

Statistical analyses

Data were analyzed by the GraphPad Prism analysis package (GraphPad, San Diego, CA). Data are expressed as means ± SEM. For analysis of unpaired datasets, Student’s unpaired t test was used for comparison of two groups, and one-way ANOVA with Tukey’s post hoc test was used for more than two groups. Repeated measures ANOVA was used for comparison of matched datasets. Two-way ANOVA followed by Bonferroni post hoc tests was used for analyzing the effects of drug treatments between groups. Scored data were analyzed by a nonparametric Kruskal-Wallis one-way ANOVA followed by Dunn’s post hoc test. A P value of less than 0.05 was considered to be statistically significant.

Results

E/b2 mice show accelerated atherosclerosis

E/b2 mice were born in the expected Mendelian numbers to Apoe−/− Hsd11b2+/− females mated with E/b2 males and showed no difference in weight gain to Apoe−/− Hsd11b2+/− littermates or age-matched Apoe−/− mice.

At 3 months of age, Apoe−/− mice raised on a standard chow diet displayed few, if any, signs of atherosclerosis in the brachiocephalic artery (Fig. 1A). In contrast, by 3 months of age on the same diet, E/b2 mice lacking both 11β-HSD2 and ApoE displayed atheroma (lipid core and proliferating SMCs) with occasional thin fibrous caps, affecting the aortic arch and its major branches, including the brachiocephalic trunk (Fig. 1A), a site particularly susceptible to plaque development in these animals when fed an atherogenic “Western” diet (20). At 3 months, some severe lesions were already present in E/b2 mice, with extended necrotic areas, cholesterol clefts, neointimal expansion, and elastic lamina remodeling.

By 6 months of age, E/b2 mice displayed complex severe atherosclerotic...
lesions with multiple fibrous caps (Fig. 1B). Importantly, these included buried caps, which have been suggested as being indicative of earlier plaque instability (Fig. 1B and Table 1) (21). However, in 6-month-old Apoe<sup>−/−</sup> mice raised on the same chow diet, atherosclerosis remained sporadic (only one of the six animals in this group had a notable plaque; example shown in Fig. 1B) in the brachiocephalic artery (Fig. 1B). This remarkable increase in atherosclerosis with 11β-HSD2 deficiency was despite similar plasma cholesterol levels between E/b2 (306 ± 54 mg/dl) and Apoe<sup>−/−</sup> (288 ± 46 mg/dl) mice.

Quantitative analysis of the areas inside the external elastic lamina, lesion areas, and lumen areas of the brachiocephalic artery revealed significantly larger atherosclerotic lesions in E/b2 mice at 3 and 6 months of age compared with age-matched Apoe<sup>−/−</sup> mice (Fig. 1C and Table 1). There was also a significant reduction of lumen size relative to the size of the vessel (Fig. 1C and Table 1), although the absolute lumen size was maintained (vessel diameter was also increased), suggestive of expansive remodeling in E/b2 mice.

**Plaque composition is altered in E/b2 mice**

Immunohistochemical staining revealed that atherosclerotic plaques in brachiocephalic arteries of 6-month-old E/b2 mice contained dense accumulations of macrophages and foam cells (Fig. 2A), most notably at the “shoulder” of lesions and near buried fibrous caps. Macrophage infiltration (Fig. 2B), and lipid content of plaques (Fig. 2C) were both significantly greater in E/b2 than in Apoe<sup>−/−</sup> mice. Furthermore, plaques in 3-month-old E/b2 mice contained significantly more macrophages (Fig. 2B) and lipid (Fig. 2C) than those in plaque size-matched Apoe<sup>−/−</sup> counterparts (examples of rare plaques from 6-month-old Apoe<sup>−/−</sup> mice selected to show composition). SMC content of plaques, assessed by α-smooth muscle actin (SMA) immunoreactivity, did not differ between E/b2 and Apoe<sup>−/−</sup> mice (Fig. 2D). Picrosirius red staining of collagen was significantly lower in plaques from 3- and 6-month-old E/b2 mice compared with plaques from 6-month-old Apoe<sup>−/−</sup> mice (Fig. 2E). Thus, 11β-HSD2 deficiency promotes the formation of collagen-poor, lipid-, and macrophage-rich plaques, suggestive of a “vulnerable” plaque phenotype.

**Accelerated atherosclerosis in E/b2 mice is associated with increased expression of VCAM-1**

VCAM-1 immunoreactivity was significantly higher in unaffected areas of brachiocephalic artery endothelium in both 3- and 6-month-old E/b2 mice compared with age-matched Apoe<sup>−/−</sup> controls (Fig. 3) both in the endothelium covering the plaque and in plaque-free regions of the vessel wall. Importantly, comparison of similar-sized vessels from 3-month-old E/b2 mice and 6-month-old Apoe<sup>−/−</sup> mice showed significantly higher VCAM-1 expression in E/b2 mice, despite their younger age (Fig. 3, A and B).

**Atheroprotective effects of eplerenone and amiloride do not correlate with their ability to lower blood pressure**

As anticipated from the previously reported phenotype of Hsd11b2<sup>−/−</sup> mice (9), E/b2 animals were moderately hypertensive compared with Apoe<sup>−/−</sup> mice, most likely due to overactivation of MR in the kidney (SBP 141.8 ± 2.5 mm Hg in E/b2 mice vs. 112.6 ± 1.7 mm Hg in Apoe<sup>−/−</sup> mice; P < 0.001). Thus, the exacerbated athero-
sclerosis in E/b2 mice could be attributable to hypertension as a result of renal MR activation by glucocorticoids, or to a direct effect of 11β-HSD2-deficiency within the vascular wall or a combination of these factors. To address MR involvement and to determine the dependency of the phenotype on hypertension, 2-month-old mice were fed chow diet containing vehicle or one of two pharmacologic inhibitors for 3 months: amiloride, an ENaC blocker that acts downstream of renal MR to lower blood pressure, and eplerenone, a highly selective MR antagonist. The dose chosen for the latter (200 mg/kg · d in chow) was previously shown to reduce atheroma formation in Apoe−/− mice administered aldosterone (22). We found no effect of any drug treatment on blood pressure of Apoe−/− mice (data not shown). Eplerenone had no significant effect on SBP in E/b2 mice, whereas amiloride reduced it by approximately 11 mm Hg, almost halving the blood pressure difference between E/b2 and Apoe−/− mice (Fig. 4A). However, despite being less effective as a hypotensive drug, eplerenone was more effective than amiloride in reducing overall plaque score as estimated by blinded, semi-quantitative scoring assessed across five sites (Fig. 4, B and C). Quantitative histological analysis showed that eplerenone also dramatically reduced plaque size and the expansive remodeling in subclavian arteries, which are particularly prone to large occlusive plaques in E/b2 mice (Fig. 4, D and E). Both eplerenone and amiloride reduced lesion size in brachiocephalic arteries (Fig. 4, F and G, and Table 2). The pattern of expansive remodeling evident in untreated E/b2 mice was also reduced after treatment with both eplerenone and amiloride (Table 2). Thus, the stronger atheroprotective effect of eplerenone in comparison with amiloride, with the latter producing a larger reduction in blood pressure, indicates that the predominant mechanism leading to accelerated atherosclerosis in E/b2 mice is unlikely to be attributable simply to hypertension after renal MR activation.

MR activation contributes to the altered plaque composition in E/b2 mice

To investigate whether MR antagonism altered plaque composition in addition to reducing lesion size in E/b2 mice, macrophage infiltration, α-SMA, collagen, and lipid content were measured in brachiocephalic plaques of E/b2
mice after 3 months of treatment with eplerenone or vehicle. Macrophage content was significantly reduced by eplerenone treatment (Fig. 5, A and B). Despite the increase in lipid content of plaques in E/b2 mice compared with Apoe<sup>-/-</sup> mice, MR blockade with eplerenone had no effect on lipid content in E/b2 mice (Fig. 5, A and C). Surprisingly, although there was no difference in plaque α-SMA content between E/b2 and Apoe<sup>-/-</sup> mice, SMC content was significantly increased by MR blockade in E/b2 mice (Fig. 5, A and D). Eplerenone treatment also significantly increased plaque collagen content compared with vehicle-treated E/2b mice (Fig. 5, A and E). There was a trend toward a reduction in the incidence of buried fibrous caps in the brachiocephalic artery of eplerenone-treated E/b2 mice (1.10 ± 0.31 buried caps for vehicle-treated mice vs. 0.56 ± 0.18 for eplerenone-treated E/b2 mice).

**Glucocorticoids in the presence of 11β-HSD2 inhibitor cause MR-mediated up-regulation of VCAM-1 expression in vitro**

MAECs were used to test whether mineralocorticoids or glucocorticoids acting through MR can affect VCAM-1 expression and whether 11β-HSD2 inhibition may be important in regulation of MR specificity. Similar to TNF-α, which potently increases VCAM-1 expression in endothelial cells (23), aldosterone markedly increased the number of MAEC expressing VCAM-1 (>7-fold) (Fig. 6), an effect blocked by pretreatment with the MR antagonist, spironolactone, suggesting an MR-mediated mechanism. Corticosterone alone had no effect on VCAM-1 expression. However, inhibition of 11β-HSD2 by pretreatment with glycyrrhetinic acid [a widely used 11β-HSD inhibitor (24), which had no effect on VCAM-1 expression on its own] allowed corticosterone to induce a more than 9-fold increase in the number of VCAM-1-stained cells (Fig. 6). Using RT-PCR, we have confirmed that 11β-HSD1 is not expressed in MAEC (data not shown). Thus, in this cell line, as in kidney, 11β-HSD2 protects MR from activation by glucocorticoids. Consistent with MR involvement, VCAM-1 up-regulation by corticosterone in the presence of glycyrrhetinic acid was reversed by blockade of MR with spironolactone (Fig. 6B).

**Discussion**

Early development of severe occlusive atheromatous plaques in E/b2 mice provides a new experimental model of atherosclerosis, mechanistically underpinned by glucocorticoid-mediated activation of MR. In contrast to most existing models, high fat/Western diet is not required for the development of atherosclerosis in Eb/2 mice. Indeed, when fed a chow diet, Apoe<sup>-/-</sup> mice exhibit moderate hyperlipidemia and only develop mature atheromatous plaques at 8–10 months of age (25). Feeding Apoe<sup>-/-</sup> mice a Western diet (containing cholesterol) increases plasma cholesterol, induces systemic inflammation, and accelerates atherosclerosis so that lesions appear within 5 wk (20). It therefore appears that progressive atherosclerosis in Apoe<sup>-/-</sup> mice is strictly dependent on systemic inflammation and elevated plasma cholesterol associated with the Western diet.

Atherogenesis in E/b2 mice was associated with early and increased macrophage infiltration of brachiocephalic lesions, even when matched with similar-sized lesions in older Apoe<sup>-/-</sup> mice. An increase in the number of buried caps in plaques of older E/b2 animals could reflect multiple events of plaque rupture and repair in E/b2 mice, typical of vulnerable plaques. Thus, for investigation of the processes leading to a vulnerable plaque phenotype, E/b2 mice may be a better experimental platform than Apoe<sup>-/-</sup> mice.

The ability of eplerenone administration to reduce plaque size and alter plaque composition implicates MR
activation as mechanistically important in accelerating atherosclerosis in E/b2 mice. This is consistent with previous data showing that aldosterone is proatherogenic in animal models (23, 26), although this has not been replicated in all studies (27). Moreover, eplerenone has beneficial effects on experimental atherosclerosis in non-human primates (28), again implicating MR activation in pathogenesis of atherosclerosis. This raises the possibility that the proatherogenic effects of MR activation are through cortisol (a glucocorticoid) rather than the mineralocorticoid, aldosterone.

Crucially, the effect of eplerenone on atherosclerosis in E/b2 mice was independent of MR effects on blood pressure (which was unaltered in E/b2 mice by this modest dose of eplerenone). In the absence of 11β-HSD2, MR are not protected from activation by glucocorticoids, and their activation in the distal nephron increases activity of ENaCs and Na/K ATPase, leading to hypertension (17). The extent of hypertension in E/b2 mice was comparable with that previously reported in single-knockout Hsd11b2−/− mice (9).

Bypassing MR activation with the ENaC blocker amiloride reduced blood pressure in E/b2 mice but was less effective in diminishing atherosclerosis than eplerenone. Hypertension in Hsd11b2−/− mice is initiated by MR activation and volume expansion, but already after 2.5 months of age, activity of ENaC was returned to the basal level, and high blood pressure was maintained by activation of α 1-adrenergic receptors (26). Hence, eplerenone cannot effectively normalize blood pressure at these later stages, whereas amiloride still can. Another possible contributor to hypertension in Hsd11b2 deficiency is the vasoconstrictive effect mediated by glucocorticoid receptors (29, 30), which will not be affected by the MR antagonist, eplerenone.

Thus, although hypertension may play a part in the accelerated atherogenesis in E/b2 mice, there is clearly a component of atherogenesis that is not merely due to the hypertension of “apparent mineralocorticoid excess” produced by MR overactivation in the distal nephron. This concurs with two previous studies in which blood pressure played little or no role in atherogenesis: in mice deficient for both ApoE and endothelial nitric oxide synthase (31) and Apoe−/− mice with renovascular hypertension (one kidney/one clip and two kidneys/two clips models) (32). In contrast, blood pressure per se played only a minor role in atheroma progression in Apoe−/− mice with renovascular hypertension compared with the large effect of angiotensin II (33).

Our data implicate activation of nonrenal MR in the pathogenesis of accelerated atherogenesis in E/b2 mice with the vascular wall being the most likely candidate site. In humans, 11β-HSD2 immunoreactivity has been re-
11β-HSD2 inhibitor. VCAM-1 increases monocyte/macrophage adhesion to endothelial cells (39) and is mechanistically linked to inflammatory processes (40) and atheroma development in Apoe−/− (41) and Ldlr−/− mice (42). Increased expression of VCAM-1 in E/b2 mice may be responsible for the increased macrophage infiltration leading to accelerated atherogenesis and may also stimulate expansive vessel remodeling (43).

Overall, these findings indicate that loss of function of 11β-HSD2 leads to striking atherogenesis in E/b2 mice mediated by activation of nonrenal MR. Endothelial expression of VCAM-1 and massive infiltration of the atherosclerotic plaques by macrophages at moderately elevated levels of plasma cholesterol are the characteristic features of this new mouse model of atherosclerosis.

We have shown for the first time that 11β-HSD2 is atheroprotective. In its absence, activation of MR mainly by glucocorticoids enhances inflammatory processes in the atherosclerotic plaque. This effect of MR is not solely attributable to changes in blood pressure. Whether it is associated with the altered activation mechanisms of MR in the endothelium remains to be investigated in the future experiments with tissue specific knockout of MR.

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