11β-Hydroxysteroid Dehydrogenase Type 2 in Mouse Aorta Localization and Influence on Response to Glucocorticoids

Clare Christy, Patrick W.F. Hadoke, Janice M. Paterson, John J. Mullins, Jonathan R. Seckl, Brian R. Walker

Abstract—Both isozymes of 11β-hydroxysteroid dehydrogenase, which interconvert active and inactive glucocorticoids, are expressed in the mouse aortic wall. Mice deficient in 11HSD type 2 (which converts active corticosterone into inert 11-dehydrocorticosterone) have hypertension and impaired endothelial nitric oxide activity. It has been suggested that 11HSD2 influences vascular function directly by limiting glucocorticoid-mediated inhibition of endothelium-derived nitric oxide. This study sought to determine (1) the cellular distribution of the 11HSD isozymes within the mouse aortic wall and (2) the influence of 11HSD2 on direct glucocorticoid-mediated changes in aortic function. Mouse aortas were separated into their component layers and RNA extracted for RT-PCR. Both types of corticosteroid (mineralocorticoid and glucocorticoid) receptors and both 11HSD isozymes were expressed in the aortic wall. 11HSD1 expression colocalized with α-smooth muscle actin (a marker for smooth muscle cells), whereas 11HSD2 colocalized with TIE-2 (a marker for endothelial cells). Functional relaxation responses of mouse aortic rings were unaltered after exposure to glucocorticoids for 24 hours. In the presence of l-arginine, glucocorticoids produced an endothelium-independent reduction of contraction; similar results were obtained with aortas from mice with genetic inactivation of 11HSD2. Incubation in medium containing l-arginine reversed the endothelial cell dysfunction associated with 11HSD2 inactivation. Thus, 11HSD2 is appropriately sited to modulate endothelial cell function, but endothelial dysfunction in 11HSD2 knockout mice cannot be explained simply by increased access of corticosterone to endothelial cell corticosteroid receptors. Therefore, additional mechanisms, possibly involving indirect effects of enhanced corticosterone action in the kidney and the resultant hypertension, must be involved. (Hypertension. 2003;42:580-587.)

Key Words: endothelium ■ glucocorticoids ■ mice ■ muscle, smooth, vascular ■ vasoconstriction ■ vasorelaxation

The access of glucocorticoids to their receptors is regulated by the 11β-hydroxysteroid dehydrogenase (11HSD) isozymes, which catalyze the interconversion of active glucocorticoids (corticosterone in rodents, cortisol in man) with their inactive metabolites (11-dehydrocorticosterone and cortisone). In most if not all tissues, 11HSD type 1 is a predominant reductase in vivo,1 regenerating active corticosterone from inert 11-dehydrocorticosterone. 11HSD1 is widely expressed and plays an important role in enhancing glucocorticoid receptor (GR) activation in metabolically important sites (eg, liver, adipose tissue). 11HSD type 2 is an exclusive dehydrogenase2 and catalyzes the reverse reaction, inactivating corticosterone. In the adult, 11HSD2 expression is largely restricted to mineralocorticoid target tissues, such as the colon and the distal convoluted tubule of the nephron, where it protects intrinsically nonselective mineralocorticoid receptors (MR) from inappropriate activation by glucocorticoids.3

Both 11HSD isozymes are present in blood vessels, suggesting that concentrations of active glucocorticoid are regulated within the vessel wall. This is important because both GR and MR are present in vascular tissue,4 and glucocorticoids may enhance smooth muscle cell contractility, inhibit endothelium-derived nitric oxide activity, and stimulate vascular hypertrophy.5–6 Indeed, congenital 11HSD2 deficiency, 11HSD inhibition after excessive consumption of liquorice,7,8 and antisense inactivation of 11HSD29 are associated with increased vascular contractility. In aortas from mice homozygous for a disrupted 11HSD2 gene,10 contractility is enhanced as a result of impaired endothelium-derived nitric oxide activity.11 In contrast, genetic inactivation of 11HSD1 has no effect on blood pressure or vascular function in mice.11 Consequently, it was proposed that 11HSD2 expression in endothelial cells influences vascular function by limiting glucocorticoid-mediated inhibition of endothelium-derived nitric oxide activity.

However, there remain uncertainties in this hypothesis. First, the precise cellular distribution of 11HSD isozymes in the vessel wall is uncertain. In cells in primary culture, 11HSD1 and 11HSD2 have been detected in vascular smooth muscle, suggesting that concentrations of active glucocorticoid are regulated within the vessel wall. This is important because both GR and MR are present in vascular tissue,4 and glucocorticoids may enhance smooth muscle cell contractility, inhibit endothelium-derived nitric oxide activity, and stimulate vascular hypertrophy.5–6 Indeed, congenital 11HSD2 deficiency, 11HSD inhibition after excessive consumption of liquorice,7,8 and antisense inactivation of 11HSD29 are associated with increased vascular contractility. In aortas from mice homozygous for a disrupted 11HSD2 gene,10 contractility is enhanced as a result of impaired endothelium-derived nitric oxide activity.11 In contrast, genetic inactivation of 11HSD1 has no effect on blood pressure or vascular function in mice.11 Consequently, it was proposed that 11HSD2 expression in endothelial cells influences vascular function by limiting glucocorticoid-mediated inhibition of endothelium-derived nitric oxide activity.

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muscle cells, adventitial fibroblasts, and endothelial cells. However, histological studies in rat vessels suggest that immunoreactivity for 11HSD1 is restricted to vascular smooth muscle; data for 11HSD2 are inconsistent, probably on account of the varied characteristics of different antisera. The inconsistencies in 11HSD isozyme localization in the literature may also be due to differences in expression in arteries from distinct anatomical sites, differences between species, and the use of cultured cells. Studies of liver and adipose cells have indicated that 11HSD isozyme expression is sensitive to culture conditions. Second, it is not established whether effects of glucocorticoids on vascular function are direct (mediated by either GR or MR) or indirect; it is possible that 11HSD2 manipulations alter other determinants of vascular function or alter vascular structure without increasing activation of corticosteroid receptors in endothelial cells.

In the experiments here, we aimed to clarify the role of 11HSD isozymes in the mouse vessel wall by establishing the cellular distribution of 11HSDs in vivo in cell types of the aortic wall and to determine whether increased exposure (of GR or MR) to glucocorticoid is responsible for endothelial cell dysfunction in the aorta when 11HSD2 is impaired.

**Methods**

Studies used male MF1 (Harlan Olac) and 11HSD2−/− mice. These were maintained under controlled conditions with free access to standard chow (Special Diet Services) and water. Animals 12 to 16 weeks of age were killed by asphyxiation with CO2; all experiments conformed to standards defined in The Principles of Animal Care (NIH publication No. 85-23, revised 1985).

**Drugs**

Salts were obtained from BDH; Noradrenaline hydrochloride, 5-hydroxytryptamine creatinine sulfate, and acetylcholine chloride were from Sigma; 3’-morpholinosydnonimine (SIN-1) was from Alexis. Stock solutions (10−3 mol/L) were prepared in distilled water, frozen as 1 mL aliquots at −20°C and thawed as required.

**Localization of 11HSD Isozymes in Aortic Wall**

Thoracic aorta from MF1 mice were cleaned of fat and connective tissue. Some vessels were rinsed, perfused with collagenase IV (1 to
ethidium bromide and visualized under UV light. Product sizes for
were analyzed by electrophoresis on a 1.8% agarose gel containing
72 °C), with a final extension period of 10 minutes. RT-PCR products
costeroid receptors; 62 °C for 11HSD2), and extension (90 seconds at
Reverse Transcription Kit (Promega) for 45 minutes at 42
resultant cDNA templates underwent PCR amplification (35 cycles)
/H9251
specific for
/H9251
stored at
were then separated mechanically. Tissues were snap-frozen and
minutes) to digest endothelial cells. The medial and adventitial layers
° C with carbogen (5 to 10
2 mg/mL, Sigma), and incubated at 37 °C with carbogen (5 to 10
minutes) to digest endothelial cells. The medial and adventitial layers
were then separated mechanically. Tissues were snap-frozen and
stored at −80°C. Frozen tissues were crushed in liquid nitrogen, total
RNA was extracted with the use of TRIzol Reagent (Gibco) and
quantified by UV spectroscopy.

Total RNA (1 μg) was reverse-transcribed with the use of a
Reverse Transcription Kit (Promega) for 45 minutes at 42°C. The
resultant cDNA templates underwent PCR amplification (35 cycles)
with Taq polymerase (Promega) in the presence of oligonucleotides
specific for α-smooth muscle actin (α-SMA; 5’-TTGGAAAA-
GATCTGGCACCACT’–3’, and 5’-GCACTATGTCACGAGG-
AATAG’–3’), TIE-2 (5’-TTACTCTAATCCACGTCAAGCC-3’
and 5’-CAGCTGGTTCCTTCTCAGTT-3’), 11HSD1 (5’-AAAC-
GCTGTTCACWGGGGCCAGCAA-3’ and 5’-AGGATC-
CARAGCACAATTTGCTTG-3’), 11HSD2 (5’-ACCCCTGCTTG-
GCAAGCCTACG GCA-3’), 11HSD2 (5’-SMA, TIE-2, GR, MR, 11HSD1, and 11HSD2 were 370 bp, 296
bp, 700 bp, 440 bp, and 144 bp, respectively.

Thoracic aortas from MF1 or 11HSD2−/− mice were divided into
on Aortic Endothelial Cell Function
Influence of In Vitro Glucocorticoid Manipulation
on Aortic Endothelial Cell Function
Thoracic aortas from MF1 or 11HSD2−/− mice were divided into
3 sections. One section was used immediately for functional investi-
gation; the others were incubated in Dulbecco’s minimum essential
medium (DMEM) containing either vehicle, corticosterone (100
nmol/L), or dexamethasone (100 nmol/L). Some experiments used
modified DMEM without L-arginine. Incubations were performed at
37 °C in an atmosphere of 95% O₂/5% CO₂ for 24 hours. Each section
was then divided into 2 rings and assessed by means of small-vessel
myography. Cumulative concentration-response curves were ob-
tained for KCl (2.5 to 320 mmol/L) and NE (1 mmol/L to 3 μmol/L).
VASodilator responses were obtained for acetylcholine (ACh; 1
nmol/L to 30 μmol/L) and β-morpholinosydnonimine (SIN-1; 1
nmol/L to 30 μmol/L) after contraction with 0.1 to 1.0 μmol/L 5-HT.
For vessels incubated in the presence of glucocorticoids, functional
experiments were performed in the continued presence of the
appropriate steroid.

Statistics
Data expressed as mean±SEM and analyzed by means of paired or
unpaired Student t test or 2-way ANOVA as appropriate. Differences
were considered significant at a level of P<0.05.

Results
Localization of 11HSD Isozymes in Aortic Wall
Vascular Smooth Muscle and Endothelial Cell Marker
Expression in Aortic Layers
Expression of α-SMA (the specific marker for vascular
smooth muscle cells) was detected in intact aorta and also in
isolated medial and adventitial layers (Figure 1a). In contrast,
the endothelial cell–specific marker, TIE-2, was detected in
endothelium-intact aorta but not in medial or adventitial
fractions (Figure 1b). Thus, removal of the endothelium was
complete, but RNA could not be successfully extracted from
preparations of isolated endothelial cells.
mRNAs for Corticosteroid Receptors and 11HSD Isozymes in Aortic Layers

GR and MR mRNAs were both detected in mouse aortas (Figures 1c and 1d). Expression of both receptors was also detected in the medial layer after removal of the endothelial and adventitial layers. 11HSD1 mRNA was detected in all samples shown to express α-SMA, for example, intact aorta, the isolated medial layer, and the adventitia (Figure 1e). In contrast, expression of 11HSD2 mRNA was only detected in the intact aorta, which was also the only sample to show TIE-2 expression (Figure 1f).

Influence of In Vitro Glucocorticoid Manipulation on Endothelial Cell Function in Mouse Aorta

Preliminary investigations confirmed that contractile and relaxant responses of mouse aortic rings were maintained after incubation in DMEM for 24 hours. After 24-hour incubation, exposure of denuded aortic rings to L-NOARG (10^-4 mol/L) had no effect on contractile responses to NE (data not shown), suggesting no significant activation of inducible nitric oxide synthase (iNOS).

MF1 Aortas

In the absence of added L-arginine, incubation with corticosterone had no effect on contractile responses to NE or potassium chloride (Figure 2 and Table 1). In contrast, in the presence of L-arginine (0.85 mmol/L), incubation with corticosterone (100 nmol/L) reduced the amplitude of NE-mediated contraction in denuded aortic rings and in intact rings in the presence of L-NOARG but did not affect NE-mediated contraction of intact aortic rings (Figure 3 and Table 1). The amplitude of potassium chloride–mediated contraction was also attenuated in intact aortic rings incubated with corticosterone; a trend toward reduced contraction did not achieve significance in denuded vessels (Figure 3 and Table 1). The sensitivity of contractile responses was unaffected by exposure to corticosterone.

Incubation with corticosterone had no effect on endothelium-dependent relaxation, either in the presence or absence of L-arginine (Figure 4 and Table 1). Corticosterone had no effect on endothelium-independent relaxation in the presence of L-arginine (Figure 4 and Table 1), but in the absence of L-arginine there was evidence that SIN-1–mediated relaxation was enhanced (Table 1).

Incubation with dexamethasone (100 nmol/L) in the presence of L-arginine produced effects broadly consistent with those of corticosterone (Table 2). Dexamethasone attenuated NE-mediated contraction in intact as well as in denuded aortic rings and attenuated KCl-mediated contraction in intact but not denuded vessels. Sensitivity to vasoconstrictors and responses to vasodilators were unaffected by dexamethasone.

### Table 1. Effect of Incubation With (100 nmol/L) Corticosterone on Mouse Aortic Function

<table>
<thead>
<tr>
<th>Drug</th>
<th>Endothelium</th>
<th>Control (4–5)</th>
<th>Cort (5–6)</th>
<th>Sensitivity (−logEC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NOARG</td>
<td>Intact</td>
<td>82.8±6.1</td>
<td>83.6±7.9</td>
<td>7.23±0.25</td>
</tr>
<tr>
<td></td>
<td>Denuded</td>
<td>10.4±5.8</td>
<td>18.6±10.4</td>
<td>...</td>
</tr>
<tr>
<td>SIN-1</td>
<td>Intact</td>
<td>84.1±3.2</td>
<td>87.2±3.3</td>
<td>6.03±0.10</td>
</tr>
<tr>
<td></td>
<td>Denuded</td>
<td>77.7±5.2</td>
<td>90.1±3.6*</td>
<td>5.67±0.14</td>
</tr>
<tr>
<td>NA</td>
<td>Intact</td>
<td>1.16±0.19</td>
<td>0.95±0.18</td>
<td>7.08±0.06</td>
</tr>
<tr>
<td></td>
<td>Denuded</td>
<td>3.10±0.25</td>
<td>2.62±0.29</td>
<td>7.17±0.22</td>
</tr>
<tr>
<td>Intact+L-NOARG</td>
<td></td>
<td>3.63±0.59</td>
<td>3.24±0.38</td>
<td>7.07±0.12</td>
</tr>
<tr>
<td>KCl</td>
<td>Intact</td>
<td>2.43±0.49</td>
<td>2.28±0.17</td>
<td>71.06±3.49</td>
</tr>
<tr>
<td></td>
<td>Denuded</td>
<td>2.16±0.15</td>
<td>2.01±0.24</td>
<td>41.56±5.17</td>
</tr>
</tbody>
</table>

(a) Incubation in DMEM without L-arginine

Values are mean±SEM; n=4–8. Cort indicates corticosterone; Emax, maximum response (mN/mm or % relaxation); intact/denuded, with/without endothelium; L-NOARG, N²-nitro-L-Arginine (10^-4 mol/L); sensitivity, −logEC50 or pD2, except KCl (mM).

*P<0.05 using Student unpaired t test.
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0.85 mmol/L -arginine produced no significant alterations in tile or relaxant function (Table 3). Furthermore, incubating mice in the presence of corticosterone had no effect on contrac-

As with wild-type mice, incubation of aortas from 11HSD2 mice showed impaired endothelium-dependent relaxation (Emax, 47.4 ± 10.6%; -logEC50, 6.78 ± 0.11; n = 4) and enhanced NE-mediated relaxation (Emax, 76.7 ± 3.2%; Emax, 7.60 ± 0.04, P = 0.005; n = 4). Incubation of intact aortas from 11HSD2 mice in DMEM containing L-arginine for 24 hours resulted in a reduction in the contractile response to NE (Emax, 0.63 ± 0.23 mN/mm; pD2, 7.08 ± 0.01; P = 0.10; n = 4) (Figure 5b) and an enhancement of acetylcholine-mediated relaxation (Emax, 76.7 ± 3.2%, P = 0.023; -logIC50, 7.54 ± 0.27, P = 0.04; n = 4). In denuded vessels, functional responses of aortas from 11HSD2 mice were normal at baseline and unaffected by incubation in DMEM, regardless of the presence of L-arginine (data not shown).

Discussion

The detection of both GR and MR mRNA in mouse aorta and medial smooth muscle, consistent with previous investigations, indicates that colocalized 11HSDs may modulate

### Table 2. Effect of Incubation With (100 nmol/L) Dexamethasone on Mouse Aortic Function

<table>
<thead>
<tr>
<th>Drug</th>
<th>Endothelium</th>
<th>Control</th>
<th>Dexamethasone</th>
<th>Control</th>
<th>Dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>Intact</td>
<td>82.0±6.0</td>
<td>81.7±8.4</td>
<td>7.35±0.21</td>
<td>7.37±0.25</td>
</tr>
<tr>
<td></td>
<td>Denuded</td>
<td>27.9±6.4</td>
<td>19.9±8.9</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>SIN-1</td>
<td>Intact</td>
<td>76.2±3.8</td>
<td>89.7±2.5</td>
<td>5.70±0.18</td>
<td>6.06±0.09</td>
</tr>
<tr>
<td></td>
<td>Denuded</td>
<td>89.8±3.2</td>
<td>92.3±1.3</td>
<td>6.08±0.09</td>
<td>6.12±0.13</td>
</tr>
<tr>
<td>NA</td>
<td>Intact</td>
<td>1.43±0.39</td>
<td>0.52±0.13</td>
<td>7.44±0.05</td>
<td>7.10±0.13</td>
</tr>
<tr>
<td></td>
<td>Denuded</td>
<td>2.69±0.21</td>
<td>2.49±0.28</td>
<td>7.34±0.07</td>
<td>6.98±0.06**</td>
</tr>
<tr>
<td>KCl</td>
<td>Intact</td>
<td>3.25±0.16</td>
<td>2.79±0.22</td>
<td>34.40±1.40</td>
<td>42.17±2.86</td>
</tr>
<tr>
<td></td>
<td>Denuded</td>
<td>2.51±0.30</td>
<td>2.83±0.22</td>
<td>27.00±1.44</td>
<td>29.60±2.09</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=5–8.

*P<0.05; **P<0.005 using Student unpaired t test.

Our results have not determined whether 11HSD1 is expressed in mouse aortic endothelial cells. However, immunohistological studies in other species suggest that 11HSD1 is not present in the endothelium of intact arteries. In contrast with 11HSD1, 11HSD2 mRNA was only detected in aortic fractions that expressed the marker for endothelial cells (TIE-2). This demonstration of 11HSD2 expression, for the first time in freshly isolated vessels, suggests its localization to be restricted to the endothelium, which supports findings in cultured rat aortic cells. These localization studies indicate that 11HSD1 and 11HSD2 are available for intracrine and perhaps paracrine modulation of corticosteroid receptor activation in the vessel wall. Previous studies using knockout mice or 11HSD inhibitors have suggested that the major influence of 11HSDs on vascular function is determined by dehydrogenase inactivation of glucocorticoids, which is predominantly (if not exclusively) attributable to 11HSD2. Therefore, the influence of endothelial 11HSD2 on vascular responses to corticosterone was explored in greater detail.

The attenuated contraction observed in wild-type mouse aortic rings, after exposure to a physiological concentration of glucocorticoid for 24 hours in vitro, occurred only in the presence of L-arginine but was the result of changes in the vascular smooth muscle. Impaired contractility was evident in denuded aortas and was also unmasked in vessels with an intact endothelium after inhibition of nitric oxide synthase (NOS). Activation of iNOS in the vascular smooth muscle did not contribute to the reduction in contraction as nonselective NOS inhibition did not enhance contractile responses in denuded aortic rings; moreover, glucocorticoids have previously been shown to prevent induction of iNOS. The fact that both receptor-dependent and receptor-independent vasoconstriction were attenuated suggests that alterations in signaling within the smooth muscle; glucocorticoids can influence G protein–receptor interactions and ion channel activity. Glucocorticoid-mediated alterations in ion transport in the vasculature have been reported, but most studies suggest that these changes would result in enhanced rather than attenuated contractility. The current findings in mice are consistent with our previous work with rat aorta exposed to glucocorticoids in vitro. However, some reports on rat vessels show enhanced contraction attributed to glucocorticoid-mediated inhibition of endothelium-derived nitric oxide, an effect that may also be important in human vessels. The present results indicate that neither endothelium-dependent nor endothelium-independent relaxation of mouse aortas were altered by exposure to glucocorticoids in

### TABLE 3. Influence of Corticosterone on the Functional Responses of Aortic Rings From 11HSD2−/− Mice in the Absence or Presence of L-Arginine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Without L-Arginine</th>
<th>With L-Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Corticosterone</td>
</tr>
<tr>
<td></td>
<td>(a) Without Endothelium</td>
<td>(b) With Endothelium</td>
</tr>
<tr>
<td></td>
<td>(a) Without L-Arginine</td>
<td>(b) With L-Arginine</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
<td>Control</td>
</tr>
<tr>
<td>ACh</td>
<td>63.5 ± 14.2</td>
<td>66.9 ± 11.3</td>
</tr>
<tr>
<td>SIN-1</td>
<td>97.0 ± 1.8</td>
<td>98.3 ± 1.1</td>
</tr>
<tr>
<td>NA</td>
<td>0.84 ± 0.19</td>
<td>0.71 ± 0.18</td>
</tr>
<tr>
<td>KCl</td>
<td>2.12 ± 0.46</td>
<td>2.31 ± 0.26</td>
</tr>
</tbody>
</table>
Discrepancies in this literature may relate to differences between species or variable experimental protocols. Investigators have used widely varying steroid concentrations and length of incubation. Moreover, availability of nitric oxide in different preparations may vary; dexamethasone has been shown to inhibit iNOS activity, which is induced in many organ bath experiments but was shown not to be relevant in our preparations. Finally, these results suggest that enhanced arterial contraction in response to \( \alpha \)-adrenoceptor agonists after in vivo exposure to glucocorticoids is not mediated by a direct interaction between the steroid and the cells of the vascular wall.

One interpretation of the current findings is that mouse aortic endothelial cells, which we show here express 11HSD2, are protected from the actions of corticosterone. In the absence of glucocorticoid-induced endothelial dysfunction, attenuated contraction may become apparent. However, similar effects were obtained with the use of dexamethasone, a synthetic steroid that is less susceptible to inactivation by 11HSD2 but is a GR rather than mixed MR/GR agonist. This might suggest that the attenuating effects of these steroids on contractile responses are mediated by GR in vascular smooth muscle and that the endothelial effects are MR-mediated; corticosterone might not gain access to MR within endothelial cells because they are protected by 11HSD2. However, to support this attractive hypothesis requires evidence that in the absence of 11HSD2, corticosterone does then induce endothelial dysfunction and hence enhanced contractile responses. In fact, in aortas from 11HSD2 knockout mice, we found that in vitro incubation with corticosterone, even in the absence of L-arginine supplementation, had no effect on endothelial function or contractile responses. Furthermore, with L-arginine incubation in vitro, the endothelial dysfunction in 11HSD2 knockout mouse vessels could be washed out over 24 hours. There are discrepancies between the lack of effect of corticosterone in vitro in vessels from 11HSD2 knockout mice and the potentiation of corticosterone action in vitro previously reported with pharmacological inhibition of 11HSDs in rat vessels. However, pharmacological inhibitors have nonspecific actions, including toxicity to endothelial cells, which suggest that the present results may more reliably indicate the influence of 11HSD2. We conclude that the endothelial dysfunction in 11HSD2 knockout mice cannot be explained simply by increased access of corticosterone to endothelial

![Figure 4](image-url)

**Figure 4.** Influence of corticosterone on relaxation of mouse aortic rings in response to \( a \), acetylcholine (n=4 to 7), and \( b \), SIN-1 (n=5 to 8). Cumulative concentration-response curves were obtained after constriction with 5-HT (0.1 to 1 \( \mu \)mol/L) in endothelium-intact (○/●) and endothelium-denuded (■/▲) mouse aortic rings after incubation (24 hours) in DMEM containing either corticosterone (100 nmol/L; circles) or vehicle (squares). All points represent mean±SEM; curves were compared by 2-way ANOVA.

![Figure 5](image-url)

**Figure 5.** Influence of corticosterone and nitric oxide supplementation on contraction of aortic rings from 11HSD2\(^{-/-} \) mice. \( a \), Cumulative concentration-response curves to norepinephrine were obtained in endothelium-intact (filled symbols) and endothelium-denuded (open symbols) 11HSD2\(^{-/-} \) aortic rings after incubation with either corticosterone (100 nmol/L; ●/●) or vehicle (○/○). \( b \), Cumulative concentration-response curves to norepinephrine were obtained in endothelium-intact 11HSD2\(^{-/-} \) aortic rings either immediately after isolation (●) or after incubation (24 hours) in DMEM with (solid line) or without (dashed line) L-arginine. All points represent mean±SEM (n=4 to 8); curves were compared by 2-way ANOVA; **P<0.0001.
Perspectives

Our current results suggest that 11HSDs in the vessel wall do not directly modulate corticosterone effects on vascular function. What, then, is the role of these enzymes in vessels? Both the level of mRNA expression and the activity expressed per milligram of protein are low in vessels relative to many other tissues. Nonetheless, there are many other important effects of glucocorticoids within the vessel wall, including modulation of vascular development, structure, and inflammatory responses, which may be regulated by 11HSDs. Indeed, these effects may only be apparent in stressed conditions, such as during an inflammatory response, when there is dramatic upregulation of 11HSD1 in particular. It appears that more diverse experimental approaches will be required to understand fully the role of 11HSDs in the vessel wall.

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