Effect of Chenodeoxycholic Acid on 11β-Hydroxysteroid Dehydrogenase in Various Target Tissues

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Glucocorticoids are metabolized by isoforms of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). There is some controversy concerning the bile acid, chenodeoxycholic acid (CDCA), as a potential endogenously produced inhibitor of 11β-HSD. The present experiments were designed to determine the relative specificity of CDCA for both isoforms of 11β-HSD and to assess the biological relevance of inhibition in vascular tissue. IC_{50} values (concentrations which inhibit 50% of the enzyme reaction) were calculated using rat liver microsomes as a source of 11β-HSD1 dehydrogenase, Leydig cells for 11β-HSD1 dehydrogenase and reductase, aorta for 11β-HSD1 dehydrogenase and reductase, and sheep kidney for 11β-HSD2 dehydrogenase. In each case, CDCA functioned as a potent inhibitor of 11β-HSD1 dehydrogenase with IC_{50} values of ranging from 0.2 to 7 μmol/L in contrast to 37 to 200 μmol/L for 11β-HSD1 reductase. CDCA exhibited relatively weak inhibitory activity against 11β-HSD2 from sheep kidney with an IC_{50} of 70 μmol/L. The effect of CDCA on vascular contraction was studied in aortic rings isolated from Sprague-Dawley rats incubated in medium containing corticosterone 10 nmol/L ± CDCA (1 μmol/L) for 24 hours. Rings were stimulated with graded concentrations of phenylephrine (PE) (10 nmol/L, 100 nmol/L, and 1 μmol/L). Rings exposed to corticosterone and CDCA consistently demonstrated a greater contractile response at lower doses of PE (63% at PE 10 nmol/L, $P < .001$; 20% at PE 100 nmol/L, $P < .025$; and 10% at PE 1 μmol/L, not significant [NS]) compared to control preparations incubated with cortiosterone alone. These studies demonstrate (1) that CDCA preferentially affects 11β-HSD1 dehydrogenase; (2) CDCA does inhibit 11β-HSD2 dehydrogenase and 11β-HSD1 reductase but only at high(er) concentrations exceeding 70 μmol/L and 37 μmol/L, respectively; and (3) inhibition of 11β-HSD1 dehydrogenase in aortic rings by CDCA (1 μmol/L) enhances the contractile response of corticosterone plus PE.

THE ISOFORMS OF 11β-hydroxysteroid dehydrogenase (11β-HSD) convert active endogenous glucocorticoids to their respective 11-dehydro derivatives.[1-3] These 11-dehydro metabolites do not appear able to directly activate glucocorticoid receptors and induce a primary biologic effect. 11β-HSD1 prefers NADP(H) and is capable of bi-directional activity, deactivating glucocorticoids in the dehydrogenase mode and regenerating glucocorticoids from 11-dehydro metabolites in the reductase mode.[3] The principal direction of 11β-HSD1 is tissue-specific; that is, in liver, lung, and several other tissues, it functions largely in the reductase mode,[4,5] but in testicular Leydig cells, brain, adipose cells and vascular tissue, the enzyme functions both as a dehydrogenase and as a reductase.[6-9] The NAD-dependent 11β-HSD2 is generally considered to function physiologically only in the dehydrogenase mode.[10]

There is considerable interest in the respective roles of 11β-HSD isoforms in both health and disease especially in the regulation of blood pressure. Vascular endothelial cells isolated from rats express both 11β-HSD1 and 11β-HSD2 while vascular smooth muscle (VSM) cells express 11β-HSD1 but not 11β-HSD2 mRNA.[11] Expression of 11β-HSD1 is far greater than 11β-HSD2 in rat endothelial cells.[10] Adult human vascular tissue also expresses significant levels of 11β-HSD1 and smaller amounts of 11β-HSD2.[12-14] Glucocorticoids are known to potentiate the vasoconstrictive effects of both catecholamines and angiotensin II.[15,16] Inhibitors of both 11β-HSD1 and 11β-HSD2 dehydrogenase alter vascular resistance; dehydrogenase inhibition enhances,[17-20] while reductase inhibition attenuates[17,18,20] phenylephrine (PE)-induced vascular contraction. More recent experiments conducted using 11β-HSD1 and 11β-HSD2 antisense oligonucleotide probes have suggested[20] that 11β-HSD1 dehydrogenase specifically plays a pivotal role in regulation of vascular tone in addition to 11β-HSD2 dehydrogenase.

Chenodeoxycholic acid (CDCA) possesses a 3α,5β-ring A steroidal configuration and has a structural resemblance to the 11β-HSD inhibitor glycyrrhetinic acid. CDCA is produced endogenously in micromolar concentrations in serum and CDCA levels can increase several-fold with liver disease.[21] There is some controversy in the literature concerning CDCA and its ability to inhibit the 2 known isoforms of 11β-HSD. Perschel et al and we have shown that CDCA preferentially inhibits 11β-HSD1 dehydrogenase derived from rat tissues.[22,23] Similar studies using human liver and kidney also demonstrated that CDCA only blocked 11β-HSD1; it did not appear to inhibit 11β-HSD2.[25] The controversy in isoform specificity arose from experiments performed by Stauffer et al.[24] In those contrasting studies, CDCA impeded 11β-HSD2 activity present in HEK-293 cells, which had been transfected with the enzyme. CDCA only affected 11β-HSD2 dehydrogenase at very high nonphysiological concentrations.[24] The present series of experiments were designed to clarify the specificity of CDCA as an inhibitor of 11β-HSD derived from several different tissues. We also wished to examine its potential biologic relevance in influencing vascular contractility given our prior observation that a chronic infusion of CDCA into adrenally intact normotensive rats leads to elevated blood pressure.[26]

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MATERIALS AND METHODS

Reagents

[1,2,6,7-^{3}H]-corticosterone with the specific activity of 70 Ci/mmol was obtained from NEN Life Science Products (Shelton, CT). Radioactive [1,2,6,7-^{3}H]-11-dehydrocorticosterone (specific activity, 80 Ci/mmol) was synthesized from [^{3}H]-corticosterone according to previously reported methods.[17,23] Methanol (high-performance liquid chromatography [HPLC]-grade) was obtained from Fisher Scientific (Houston, TX). Corticosterone and 11-dehydrocorticosterone were from Steraloids (Newport, RI). Corticosterone, glycyrrhetinic acid, carbenoxolone, CDCA, HEPES, Tris-HCl, NAD, NADP, and NADPH were all obtained from Sigma Chemical Co (St Louis, MO). Both corticosterone and CDCA were dissolved in a mixture of 0.154 mol/L NaCl and ethanol (90:10 vol/vol) prior to each experimental series.

HPLC

Methanol 750 μL was used to stop the enzyme reactions described below. After centrifugation, an aliquot of supernatant was analyzed by HPLC using a DuPont Zorbax C8 column (Wilmington, DE). The separated radioactive products (corticosterone and 11-dehydrocorticosterone) were detected and quantitated by flow-cell scintillation analysis.[23]

11β-HSD1 Assay for Dehydrogenase and Reductase Activity in Homogenates of Rat VSM Cells and Aortic Tissue Minces

Cultured VSM cells were homogenized in 25 mmol/L HEPES buffer, pH 7.4. 11β-HSD1 dehydrogenase was assayed by incubating homogenates (37.5 μg protein) with 10 nmol/L [^{3}H]-corticosterone in presence of 3 mmol/L NADP, 50 mmol/L Tris-HCl, pH 8.4, at 37°C for 90 minutes in a total volume of 250 μL; under these conditions, 65% [^{3}H]-11-dehydrocorticosterone was formed. 11β-HSD1 reductase was assayed by incubating homogenates (50 μg protein) with 10 nmol/L [^{3}H]-11-dehydrocorticosterone in presence of 3 mmol/L NADPH, 50 mmol/L Tris-HCl, pH 7.4, at 37° C for 90 minutes in a total volume of 250 μL; under these conditions, 60% [^{3}H]-corticosterone was formed.

11β-HSD1 dehydrogenase and 11β-HSD1 reductase were also assayed in freshly isolated male rat aorta. Minced tissue (30 mg) was incubated with either 10 nmol/L [^{3}H]-corticosterone or 10 nmol/L [^{3}H]-11-dehydrocorticosterone in the presence of 500 μL of Dulbecco's modified Eagles medium/F-12 HAM for 2 hours at 37°C in an atmosphere of 5% CO_2 and 95% O_2, pH 7.4. Under these conditions, 22% [^{3}H]-11-dehydrocorticosterone and 45% [^{3}H]-corticosterone, respectively, were made.

Assay of 11β-HSD1 Enzyme Activity in Intact Leydig Cells

The dehydrogenase and reductase activities were assessed in freshly prepared intact Leydig cells (50,000 to 100,000 cells) incubated in cell culture medium, pH 7.4 containing 25 nmol/L [^{3}H]-corticosterone or 31 nmol/L [^{3}H]-11-dehydrocorticosterone as substrate, respectively, at 37°C for 10 minutes. Under these conditions, 55% [^{3}H]-11-dehydrocorticosterone and 31% [^{3}H]-corticosterone were made, respectively.

Assay of 11β-HSD1 Enzyme Activity in Hepatic Microsomes

11β-HSD1 dehydrogenase and reductase activities were also assessed using rat hepatic microsomal fractions (7.5 μg protein). The microsomes were incubated with 600 nmol/L [^{3}H]-corticosterone in the presence of 3 mmol/L NADP, 50 mmol/L Tris-HCl, pH 8.4, at 37°C for 15 minutes or with 600 nmol/L [^{3}H]-11-dehydrocorticosterone in the presence of 3 mmol/L NADPH, 50 mmol/L Tris-HCl, pH 7.4, at 37°C for 20 minutes. Under these conditions, 61% [^{3}H]-11-dehydrocorticosterone and 57% [^{3}H]-corticosterone were made.

Assay of 11β-HSD2 Enzyme Activity in Sheep Kidney Microsomes

11β-HSD2 assay was performed as previously described[27] incubating sheep kidney microsomal fraction (6.5 μg protein) with 50 nmol/L corticosterone containing 1 μCi [^{3}H]-corticosterone, 50 mmol/L Tris-HCl buffer, pH 8.4, and 200 μmol/L NAD^+ for 10 minutes at 37°C, in a total volume of 0.25 mL. Under these conditions, 62% [^{3}H]-11-dehydrocorticosterone was made. The enzymatic reaction was terminated by addition of methanol and synthesis of 11-dehydrocorticosterone was measured by HPLC as described above.

To determine the IC_{50} for CDCA, the percentage inhibition of the reaction was calculated by measuring the decrease in product formation in the presence of varying concentrations of CDCA (0.1 to 250 μmol/L) as compared with product formed in the controls, the presence of vehicle without CDCA. Each concentration was tested in triplicate and the dose-response curve, showing percentage inhibition versus log concentration of CDCA was plotted. The data fitted a log-linear straight line. From these curves, the micromolar concentration (IC_{50}) of CDCA that caused a 50% inhibition of the reaction rate was determined.[23]

Vascular Ring Preparations

Male Sprague-Dawley rats weighing 150 to 200 g were euthanized using pentobarbital (50 mg/kg intraperitoneally). The thoracic aorta was then rapidly removed through a median sternotomy. The adventitia was resected but the endothelial cell layer was left intact. The aorta was sectioned into 2-to 3-mm rings and individual rings were placed into a 24-well culture plate, one ring per well. Each well contained 1 mL of MEM/F12 with 1% fetal bovine serum, streptomycin 100 μg/mL, penicillin 100 U/mL, and amphotericin 0.25 μg/mL. Half of the rings were incubated with corticosterone (10 nmol/L) alone, while the other half of the rings were treated with corticosterone (10 nmol/L) plus CDCA (1μmol/L). The concentration of corticosterone or 11-dehydrocorticosterone chosen was within the physiologic range and the concentration of CDCA used has been shown to be effective in the inhibition of 11β-HSD1. Rings were then incubated at 37°C under 95% O_2/5% CO_2 for 24 hours prior to study. Similar experiments were also conducted with 11-dehydrocorticosterone (10 nmol/L).

To assess contraction, aortic rings were suspended by tungsten wires with 1 g of tension. Rings were placed into a vessel bath maintained at 37°C containing MEM/F12 either corticosterone ± CDCA or 11-dehydrocorticosterone ± CDCA, but without serum, and aerated with 95% O_2/5% CO_2 at pH 7.4 as previously described.[20] Vascular rings were then allowed to equilibrate for 20 minutes before being stimulated with increasing doses of PE (10 nmol/L to 1 μmol/L). PE was chosen because it activates both α and β adreno-receptors and induces a contractile response. Changes in the force of vascular contraction were monitored using a Narishige micromanipulator and a model FT03 force transducer (Grass Instrument Co, West Warwick, RI). Measurements were recorded on a computer using Labview 4.1 Virtual Instrument System (National Instruments, Austin, TX). Vessel viability was tested by demonstrating the ability of the vessel to vigorously contract when exposed to known vasoconstrictors and relax back to baseline after treatment with acetylcholine. Where appropriate, data are expressed as mean ± SE and were analyzed using analysis of variance (ANOVA) and Student's *t* test with Bonferroni modification. *P* values less than .05 are considered significant.

RESULTS

Effects of CDCA on 11β-HSD1 Activity

VSM cells from rat aorta. CDCA was tested for its effects on both 11β-HSD1 dehydrogenase and reductase activities using homogenates of rat smooth muscle cell preparations. The

Table 1. IC_{50} (μmol/L) Values for CDCA Against 11β-HSD1 From Rat Tissues

Source of Enzyme	Dehydrogenase	Reductase
VSM cell homogenate	2.1	150
Aorta minced tissue	0.2	>100
Leydig cells	7.0	>200
Liver microsomes	4.0	37

assays were conducted in the presence of varying concentrations (0.1 to 250 μM) of CDCA. In cell homogenates, CDCA inhibited the dehydrogenase reaction with an IC_{50} value of 2.1 μmol/L. In contrast, the effect of CDCA on 11β-HSD1 reductase activities was much less with an IC_{50} value of 150 μmol/L.

Rat aorta minces. Similar results were obtained when CDCA was tested with tissue minces from rat aorta. CDCA inhibited the dehydrogenase reaction with an IC_{50} of 0.2 μmol/L compared to an IC_{50} of greater than 100 μmol/L in the reductase mode.

Rat testicular Leydig cells. In intact Leydig cell preparations, CDCA also inhibited both dehydrogenase and reductase activities with IC_{50}s of 7 μmol/L for dehydrogenase and greater than 200 μmol/L for reductase. In comparison, in intact Leydig cell preparations, glycyrrhetinic acid (GA) strongly inhibited 11β-HSD1 dehydrogenase and reductase activities with IC_{50}s of 0.45 μmol/L and 5.0 μmol/L, respectively. Carbenoxolone(CBX), the succinate ester of this licorice derivative, also inhibited both dehydrogenase and reductase activities with IC_{50}s of 4.75 μmol/L for dehydrogenase and 70 μmol/L for reductase.

Rat liver microsomes. Similarly, CDCA inhibited both dehydrogenase and reductase of hepatic microsomal preparations. Dehydrogenase, again, was more sensitive with an IC_{50} of 4 μmol/L compared to an IC_{50} of 37 μmol/L for the reductase (Table 1).

Effects of CDCA on Sheep Kidney Mirosomes (11β-HSD2)

When tested against sheep kidney microsomal 11β-HSD2, CDCA was a relatively weak inhibitor of dehydrogenase activity with an IC_{50} of 70 μmol/L. The other bile acids tested also possessed IC_{50}s greater than 10 μmol/L (Table 2).

Effect of CDCA on Vascular Contractile Response

Endothelium intact rat aortic rings were incubated for 24 hours with corticosterone (10 nmol/L) and either CDCA (1 μmol/L) or vehicle. Aortic rings exposed to corticosterone and CDCA demonstrated contractile responses to graded concentrations of PE which were significantly higher than those seen in rings preincubated with corticosterone alone (Fig 1). The

Table 2. IC_{50} (μmol/L) Values for Bile Acids Against Sheep Kidney Microsomal 11β-HSD2

Compounds	Dehydrogenase
Cholic acid (CA)	100
Lithocholic acid (LCA)	25
Chenodeoxycholic acid (CDCA)	70
Deoxycholic acid (DCA)	90

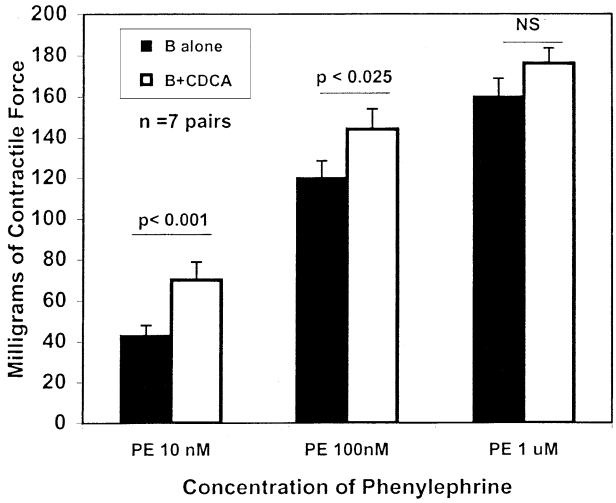

Fig 1. Effect of CDCA on the contractile response of rat aortic rings to PE. Rings were incubated for 24 hours with corticosterone (B) (10 nmol/L) and either CDCA (1 μmol/L) or vehicle, and then their contractile responses to graded concentrations of PE were measured. Values shown are mean ± SE; n = 7 rings per group.

enhanced response with corticosterone and CDCA was most notable when rings were stimulated with the lowest concentrations of phenylephrine reaching 63% with 10 nmol/L PE ($P < .001$) and 20% with 100 nmol/L PE ($P < .025$). The magnitude of the enhanced effect of corticosterone and CDCA on the aortic ring contractile response was similar to previously published experiments in which 11β-HSD1 dehydrogenase was inhibited in the presence of corticosterone and rings stimulated with PE.[18]

11-Dehydrocorticosterone, like its parent steroid corticosterone, also enhances the contractile effects of phenylephrine and angiotensin II in rat aortic rings.[18,19] These findings are most likely explained by the 11-dehydrocorticosterone being enzymatically transformed back to corticosterone by 11β-HSD1 reductase. If the conversion of 11-dehydrocorticosterone to corticosterone (11β-HSD1 reductase) is blocked as with carbenoxolone, 11-keto-progesterone, or 11β-HSD1 antisense, aortic rings demonstrate an attenuated contractile response to PE.[17,18,20] In a second series of experiments, rat aortic rings were coincubated with 11-dehydrocorticosterone and CDCA (1 μmol/L). No change in the contractile responses to graded concentrations of PE was observed when compared to rings preincubated with 11-dehydrocorticosterone alone (Fig 2).

DISCUSSION

Various investigators have demonstrated that CDCA exerts an inhibitory effect upon 11β-HSD. The controversy lies in isoform specificity and in the case of 11β-HSD1, directional effects of this bile acid. Diederich et al[25] observed that CDCA blocked 11β-HSD1 in human liver but had no effect on human kidney 11β-HSD2 dehydrogenase. In those studies, CDCA did inhibit 11β-HSD1 reductase when 11-dehydro-dexamethasone was used as a substrate. In our current experiments, performed with 11β-HSD1 derived from several different tissues, CDCA

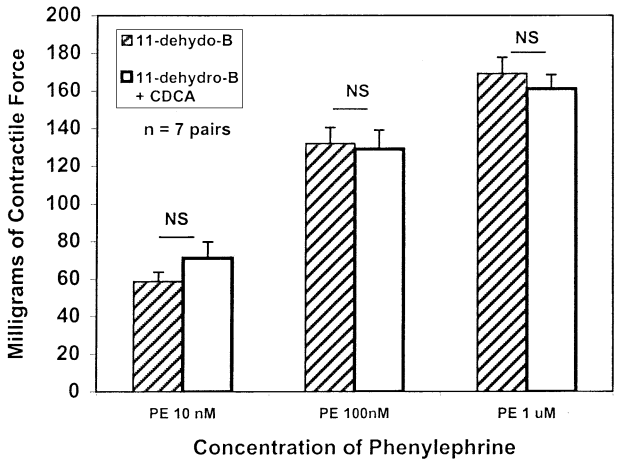

Fig 2. Effect of CDCA on the contractile response of rat aortic rings to PE. Rings were incubated for 24 hours with 11-dehydrocorticosterone (11-dehydro-B) (10 nmol/L) and either CDCA (1 μmol/L) or vehicle, and then their contractile responses to graded concentrations of PE were measured.. Values shown are mean ± SE; n = 7 rings per group.

preferentially inhibits 11β-HSD1 dehydrogenase at IC_{50} concentrations less than 4 μmol/L. CDCA suppresses 11β-HSD1 reductase activity in each of these tissues but only at higher concentrations in the range of 37 to 150 μmol/L. Similar results were observed in whole cell preparations.

Glycyrrhetinic acid, like CDCA, is a potent inhibitor which preferentially inhibits 11β-HSD1 dehydrogenase with an IC_{50} of 0.45 μmol/L for dehydrogenase relative to 5.0 μmol/L for reductase. Carbenoxolone, less potent than glycyrrhetinic acid, inhibits both dehydrogenase and reductase activities with IC_{50}s of 4.75 μmol/L for dehydrogenase and 70 μmol/L for reductase. We also re-examined the effects of CDCA on 11β-HSD2 derived from sheep kidney using 50 nmol/L corticosterone as substrate and confirmed that CDCA was only effective as an inhibitor at IC_{50} concentrations greater than 70 μmol/L. While the pattern established from our experiments superficially appears to conflict with several of the above 11β-HSD2 studies,[21,24] differences largely rest on the interpretation of which inhibitory concentrations of CDCA are relevant. In our earlier studies[27] using 50 nmol/L corticosterone as substrate, we designated 10 μmol/L as an arbitrary cut-off of for inactivity of 11β-HSD2 inhibitors. For purposes of comparison, we have shown that the inhibitor 11β-OH-progesterone has an IC_{50} of 0.05 μmol/L against 11β-HSD2.[27] Other technical issues may also contribute to the differences in the results, including pH of medium, substrates used, and whether 11β-HSD2 was naturally present or transfected into the cells. Nonetheless, two separate sets of investigations, one using human tissues,[25] the other rat tissues,[22,23] demonstrated preferential inhibitory effects of CDCA on 11β-HSD1. The earlier work of Perschel et al[22] also showed that other bile acids, including lithocholic acid, differentially affected the dehydrogenase activity of 11β-HSD1 relative to its reductase activity.

There has been considerable interest in the enzyme 11β-HSD2 as a modulator of vascular activity, but a clear additional role for the bi-directional isoform 11β-HSD1 in this tissue has yet to fully emerge. The 11β-HSD1 isoform is the predominant one found in rat vascular endothelial cells together with smaller amounts of 11β-HSD2, and is the only isoform expressed in rat VSM.[11] 11β-HSD1 is also the major isoform along with smaller amounts of 11β-HSD2 in human VSM cells and vascular tissue.[12-14] In vascular tissue preparations under physiological conditions, the reductase mode of 11β-HSD1, which converts 11-dehydro metabolites (11-dehydrocorticosterone and cortisone) back to their active counterparts (corticosterone and cortisol), is greater than the forward dehydrogenase mode demonstrating a ratio of approximately 3:1.[11,12,19]

Glucocorticoids are known to play an important role in the regulation of vascular tone and blood pressure.[15,16] Glucocorticoids can bind to and activate glucocorticoid receptors (and possibly mineralocorticoid receptors) to potentiate the vasoconstrictive effects of both catecholamines and angiotensin II.[10,19,28,29] Mineralocorticoid receptors and 11β-HSD have also been suggested to play a role in the rapid nongenomic effects of glucocorticoids and aldosterone in vascular tissue.[14] Glucocorticids have been shown to further amplify the contractile effects of PE and angiotensin II when 11β-HSD enzyme activity is inhibited.[17-19] We, and others, have demonstrated that 11-dehydrocorticosterone also potentiates (in a similar manner to corticosterone) the contractile effects of PE and angiotensin II in rat aortic rings,[18,19] an effect that is abolished by the selective inhibition of 11β-HSD1 reductase by 11-ketoprogesterone.[18] Given that the receptor binding of 11-dehydro-glucocorticoids is negligible, we have proposed that 11-dehydrocorticosterone is metabolized back to corticosterone by 11β-HSD1 reductase. Thus, since CDCA inhibits 11β-HSD1 dehydrogenase, 11β-HSD2 at high concentrations, and only suppresses 11β-HSD1 reductase similarly at high concentrations, we believe CDCA can be used as a tool to dissect the role 11β-HSD1 dehydrogenase plays in vascular tone.

Earlier experiments had shown that non-specific inhibition of the dehydrogenase reaction with agents that affected both 11β-HSD1 and 11β-HSD2 clearly enhanced the contractile response of vascular tissue.[17-19] The present studies are the first to show that specific inhibition of 11β-HSD1 dehydrogenase reaction by a bile acid, CDCA (1 μmol/L), can be associated with an enhanced contractile response. Thus under selected circumstances, the contribution of 11β-HSD1 dehydrogenase to overall local glucocorticoid metabolism, and hence local glucocorticoid concentrations in vascular tissue may be significant. Our results not only suggest a role for 11β-HSD1 in blood pressure regulation, but possibly also for interactions in these processes by the newly discovered bile acid receptors (BARs), which are present in many tissues.[30,31] The present study also supports our previous observations, which demonstrate that 11β-HSD1 antisense oligomers (as well as 11β-HSD2 antisense oligomers) enhance the ability of corticosterone to amplify the contractile responses of PE in rat aortic ring preparations.[20] This observation suggests that 11β-HSD1 dehydrogenase, in addition to 11β-HSD2, also operates to protect steroid receptors in vascular tissue from overactivation by glucocorticoids. Indeed, in these studies, 11β-HSD1 antisense significantly reduced the metabolism of corticosterone to 11-dehydrocortico-

sterone in aortic ring preparations.[20] Furthermore, when rat aortic rings were co-incubated with 11-dehydrocorticosterone and CDCA (1 μmol/L), there was no change in the contractile responses to graded concentrations of PE compared to controls pre-incubated with 11-dehydrocorticosterone alone. These results again support the claim that CDCA at this low concentration preferentially affects the dehydrogenase but does not appreciably influence the reductase function of 11β-HSD1. While vascular tissue has been shown to contain both steroid receptors,[11,28,29] we do not know whether glucocorticoid or mineralocorticoid receptors are involved in these effects.

The present studies confirm earlier work[22,32-34] that in several target tissues of corticosterone, including rat liver, kidney, testicular Leydig cells, and vascular tissue, CDCA preferentially inhibits 11β-HSD1 dehydrogenase. Leydig cells were chosen since they are unusual as targets of glucocorticoid action in that they express 11β-HSD1 as the predominant isoform, and appear to rely on its dehydrogenase activity to attenuate the suppressive effects of glucocorticoid on testosterone production. 11β-HSD1 is the most likely source for the observed dehydrogenase activity in vascular tissue and the target tissues studied here. In those tissues possessing both significant 11β-HSD1 dehydrogenase and reductase activity the relative contribution of each direction to the overall equilibrium concentrations of local corticosterone/11-dehydrocorticosterone attained will play an important role in the hormonal function(s) and response(s) of glucocorticoids in each target tissue.[35]

Whether CDCA itself is a biologically important mediator of glucocorticoid metabolism will need to be explored. Unlike many other proposed endogenous 11β-HSD inhibitors, CDCA is metabolically stable and can exist in the circulation in concentrations that could affect 11β-HSD1 dehydrogenase. Certainly, its action raises the possibility that it and other endogenously produced substances may be in the circulation or made locally altering the direction and functional equilibrium of 11β-HSD1 in vascular and other tissues. Such endogenously produced glycyrrhetinic acid–like substances (GALFs)[36] may have far reaching effects on vascular tone, as well as sodium homeostasis with a net effect on blood pressure.

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