0013-7227/02/$03.00/0
Printed in U.S.A.

Endocrinology 143(1):198–204
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11β-Hydroxysteroid Dehydrogenase 1 Transforms 11-Dehydrocorticosterone into Transcriptionally Active Glucocorticoid in Neonatal Rat Heart

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The ability of cells to directly respond to glucocorticoids and aldosterone is a function of GR and MR expression, and coexpression of 11β-hydroxysteroid dehydrogenases (11βHSDs), which convert glucocorticoids and their 11-ketometabolites into either receptor inactive or active derivatives. The aim of the present study was to determine the cellular expression of GR, MR, 11βHSD1, and 11βHSD2 in neonatal rat heart and determine the role these enzymes play in modulating glucocorticoid and aldosterone action. Ribonuclease protection analysis and steroid binding assays showed that GR is expressed in both cardiac myocytes and fibroblasts, whereas MR is expressed only in myocytes. 11βHSD2 was not detected in cardiac cells, but 11βHSD1 was expressed at high levels in both cardiac myocytes and fibroblasts. Enzyme activity studies demonstrated that 11βHSD1 acted as a reductase only, converting biologically inactive 11-dehydrocorticosterone to corticosterone, which then stimulated serum and glucocorticoid-induced kinase gene transcription via GR. In both cardiac myocytes and fibroblasts, aldosterone stimulated serum and glucocorticoid-induced kinase gene expression exclusively via GR, but not MR, indicating that aldosterone can have glucocorticoid-like actions in heart. The ability of cardiac cells to use both circulating corticosterone and 11-dehydrocorticosterone as a source of glucocorticoid suggests that the heart is under tonic glucocorticoid control, implying that glucocorticoids play important homeostatic roles in the heart. (*Endocrinology* 143: 198–204, 2002)

CARDIAC HYPERTROPHY IS an important compensatory mechanism of the heart in response to chronic increases in hemodynamic load; sustained hemodynamic load, however, eventually causes a transition from compensatory hypertrophy to heart failure. Elevated levels of both endogenous glucocorticoids and mineralocorticoids can induce increases in hemodynamic load by stimulating sodium retention, extravascular fluid expansion, and increasing total peripheral resistance (1). In addition to these effects, there is evidence for direct actions of these steroids on the heart that may contribute to the altered phenotypes associated with cardiac hypertrophy and failure (2, 3). Furthermore, elevated aldosterone has been implicated in the development of cardiac fibrosis (4); although, whether this is a direct aldosterone effect on cardiac fibroblast collagen synthesis is controversial (5–7).

Direct effects of mineralocorticoids and glucocorticoids on cardiac cells require the presence of MRs and GRs. GRs are ubiquitously expressed and have been described in heart (8). Specific binding of aldosterone (9) and MR mRNA (10) have been demonstrated in rat heart, whereas both MR mRNA and MR protein have been detected in human cardiomyocytes (11). MR has equally high affinity for both endogenous glucocorticoids and aldosterone; and, given that circulating concentrations of glucocorticoids are usually three orders of magnitude higher than those of aldosterone, a cellular mechanism is required to allow aldosterone to bind to this nonselective receptor. The enzyme 11β-hydroxysteroid dehydrogenase (11βHSD)2 converts endogenous glucocorticoids corticosterone (B) and cortisol to MR inactive 11-ketometabolites, 11-dehydrocorticosterone (11-DHB), and cortisone, thus enabling aldosterone to access MR *in vivo* (12, 13). In the absence of 11βHSD2, MRs bind and can be activated by endogenous glucocorticoids (14, 15). The 11-ketometabolites of B and cortisol are also GR inactive, so that 11βHSD2 also regulates steroid access to GR (16).

In addition to 11βHSD2, other 11βHSD isoforms have been reported, of which one (11βHSD1) has been cloned. In contrast to 11βHSD2, which is NAD-dependent and operates as an exclusive dehydrogenase for B and cortisol, 11βHSD1 in tissue homogenates is NADP/NADPH-dependent and catalyzes the reversible conversion of B and cortisol to 11-DHB and cortisone, respectively (17). In intact cells, and *in vivo*, 11βHSD1 is thought to act only as a reductase and thus can potentiate glucocorticoid action by increasing the local tissue concentration of endogenous glucocorticoids (18, 19). 11βHSD isoforms thus play a critical role in modulating corticosteroid hormone action by interconverting endogenous glucocorticoids, B, and cortisol to GR- and MR-inert 11-ketometabolites. In human and rat heart homogenates, 11βHSD activity is present (20, 21). Both cofactor dependence analysis (20, 22) and expression of specific mRNA suggest that 11βHSD1 is the isoform predominantly expressed in heart, though 11βHSD2 may be present at low levels (20, 23).

The ability of the heart to respond to endogenous glucocorticoids and aldosterone is not only a function of the presence of GR and/or MR but also expression of the enzymes 11βHSD1 and 11βHSD2 in the same cells. Previous studies have suggested that both 11βHSD1 and 11βHSD2 are

Abbreviations: B, Corticosterone; BdU, bromodeoxyuridine; 11βHSD, 11β-hydroxysteroid dehydrogenase; 11-DHB, 11-dehydrocorticosterone; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; RNase, ribonuclease; SGK, serum and glucocorticoid-induced kinase.

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expressed in heart, although the cell(s) in which these enzymes is expressed, and the potential coexpression with MR and/or GR has not been reported. In addition, the role these enzymes play in modulating glucocorticoid and aldosterone action has not been addressed. Therefore, the present study has determined the expression of MR, GR, and 11βHSD isoforms in cardiac myocytes and fibroblasts. In addition, 11βHSD activity was assessed to determine whether access of steroids to these receptors is modulated and whether this affects receptor function. We demonstrate that cardiac myocytes and fibroblasts express functional GR, whereas MR expression is limited to myocytes. The absence of 11βHSD2 and the presence of high levels of 11βHSD1 reductase activity in both cardiac myocytes and fibroblasts allow these cells to use both 11-DHB and B as a source of transcriptionally active glucocorticoid and suggest that myocyte MRs mediate glucocorticoid effects *in vivo*.

Materials and Methods

Cells and tissues

Primary cultures of cardiac myocytes and fibroblasts were prepared from 1- to 2-d-old Sprague Dawley rats as described (24). After preplating, to remove fibroblasts, nonattached myocytes were plated at an initial density of 750 cells/mm^2 and incubated for 18 h in DMEM (ICN, Aurora, OH) containing 10% FCS + 5% horse serum and 0.1 mM bromodeoxyuridine (BdU). Cardiomyocytes were maintained in DMEM containing 0.1 mM BdU, 10 μg/ml bovine transferrin, and 2.5 U/ml human insulin, for a further 48 h. Experiments were performed by incubating cells in the same medium in the absence of BdU. Adherent cells, obtained from preplating, were used to prepare nonmyocyte cultures (predominantly cardiac fibroblasts), which were grown to confluence in DMEM containing 10% FCS before passaging into 6-well culture plates. Fibroblasts were transferred to DMEM, without serum, 24 h before each experiment. Tissues were from male Sprague Dawley rats weighing 200–300 g.

Ribonuclease (RNase) protection analysis

Total RNA was prepared from cells and tissues by the guanidinium isothiocyanate method (25). ^{32}P-Labeled riboprobes were generated from a 177-bp rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA; rat 11βHSD1 cDNA corresponding to nucleotides 590-1265; rat serum and glucocorticoid-induced kinase (SGK) cDNA corresponding to nucleotides 314–985; and cDNAs as previously described for GR, MR, and 11βHSD2 (26). RNase protection analysis (26) on 3–4 μg total RNA was used to quantify mRNA levels, and [^{32}P]-labeled hybrids were visualized and quantified on a Fujix Bio-Imaging Analyzer. Because GR and MR and 11βHSD2 riboprobes were uniformly labeled with [^{32}P]uridine 5′-triphosphate, but differed in the number of nucleotides protected (294 for GR, 188 for MR, and 628 for 11βHSD2), specific activity [in molar terms (cpm/mol riboprobe)] differed between the riboprobes. To allow for assessment of relative expression of MR, GR, and 11βHSD2 mRNA, protected hybrids were corrected for specific activity.

Binding assay

Cardiac cells were incubated at 37 C with [^{3}H]dexamethasone (25–30 nM) or [^{3}H]aldosterone (25–30 nM) for 75 min. Specific binding to GR was [^{3}H]dexamethasone binding displaced by the GR antagonist RU38486 (6 μM). Specific MR binding was [^{3}H]aldosterone binding in the presence of 6 μM RU38486 displaced by 6 μM aldosterone. After incubation with steroids, nuclei were separated from cytoplasm as previously described (26).

11βHSD activity

[^{3}H]11-DHB was produced by incubating 11βHSD2, containing Ishikawa cells (27), with [^{3}H]B for 24 h at 37 C. After the incubation, medium was ethylacetate-extracted and reconstituted in ethanol, and the percent [^{3}H]B and [^{3}H]11-DHB values were determined by TLC followed by phosphorimage analysis as previously described (26). For 11βHSD activity studies, cells were incubated with DMEM/HEPES (Sigma, St. Louis, MO) containing a combination of both 17 nM [^{3}H]B and 24 nM [^{3}H]11-DHB, with media sampled at 1 and 6 h. Steroids were then ethylacetate-extracted from medium and separated by TLC, and [^{3}H]labeled steroids were visualized and quantified by phosphorimage analysis (26). Cells were collected at the end of the 6-h incubation, and DNA content was measured (28).

Statistics

Data were compared by one-way ANOVA, followed by Fisher's protected least significant differences test. Differences of $P < 0.05$ were considered significant. All data are expressed as mean ± SEM.

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Results

MR, GR, and 11βHSD expression

MR, GR, and 11βHSD2 mRNA were measured simultaneously in liver, heart, cardiac cells, and epithelial cells isolated from ileum. Ileum RNA was used as a positive control for GR, MR, and 11βHSD2 mRNA. Liver, which expresses very little MR or 11βHSD2, was used as an indicator of assay sensitivity. Fig. 1A shows a typical phosphorimage, after simultaneous RNase protection analysis of GR, MR, and 11βHSD2 mRNA. In Fig. 1B, relative expression of GR, MR, and 11βHSD2 was assessed by correcting values for riboprobe specific activity and micrograms of total RNA assayed. As illustrated, GR mRNA expression was ubiquitous, with significantly lower levels of expression in cardiac fibroblasts, compared with both whole heart and cardiac myocytes (Fig. 1B). MR mRNA was expressed in whole heart and cardiac

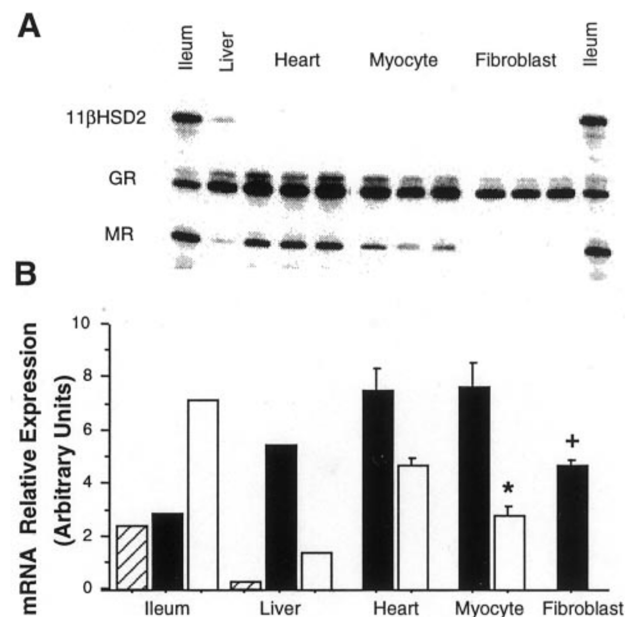

FIG. 1. A, Phosphorimage of GR, MR, and 11βHSD2-protected [^{32}P]hybrids after RNase protection analysis of 3 μg total RNA for cardiac myocytes and 4 μg for heart, cardiac fibroblasts, ileum cells, and liver. B, Relative expression of 11βHSD2 mRNA (*hatched bar*), GR mRNA (*solid bar*), and MR mRNA (*open bar*) after correcting data for riboprobe specific activity and micrograms of total RNA. n = 4. *, $P < 0.05$, compared with MR mRNA in heart; +, $P < 0.05$, compared with GR mRNA in heart and myocytes.

myocytes but not fibroblasts. MR mRNA concentration was significantly ($P < 0.05$) greater in whole heart than in myocytes (Fig. 1B), suggesting that MR is expressed in other cardiac cells that are lost during the cell isolation procedure. 11βHSD2 mRNA was not detected in whole heart or cultured cardiac cells. As illustrated in Fig. 2, two 11βHSD1 mRNA species were detected in liver, heart, and cardiac cells. The 11βHSD1 riboprobe used in the present study is complementary to the 3′ untranslated region of 11βHSD1 mRNA, thus the two protected bands cannot be explained by heterogeneity in the 5′ region as described previously (29). Further studies are required to determine whether the protected species reflect 3′ heterogeneity in 11βHSD1 mRNA. The variation in the larger 11βHSD1 mRNA species, observed between cardiac cells and heart, reflected the same changes in the shorter 11βHSD mRNA. Both 11βHSD1 mRNA species were significantly higher in myocytes than in fibroblasts and whole heart.

Consistent with the expression of the specific mRNAs, GR binding was present in both cardiac myocytes and fibroblasts, and MR binding was only detectable in cardiac myocytes. In cardiac myocytes, specific GR binding was 7-fold higher than MR binding, with GR binding being slightly higher ($P < 0.05$) in myocytes than in fibroblasts (Fig. 3).

11βHSD activity

RNase protection analysis indicated that 11βHSD1, but not 11βHSD2, is present in cardiac myocytes and fibroblasts. To assess whether 11βHSD1 was acting as a reductase or dehydrogenase, cells were incubated with a combination of [^{3}H]B (17 nM) and [^{3}H]11-DHB (24 nM), and conversion to either B or 11-DHB was assessed. As illustrated in Fig. 4A, 11βHSD1 acted as a reductase, converting 11-DHB to B. After a 1-h incubation, 39 ± 2% and 42 ± 3% of 11-DHB was converted to B in myocyte and fibroblast cultures, respectively; after 6 h, 73 ± 3% and 75 ± 1% of 11-DHB had been converted (Fig. 4B). DNA and protein levels were measured

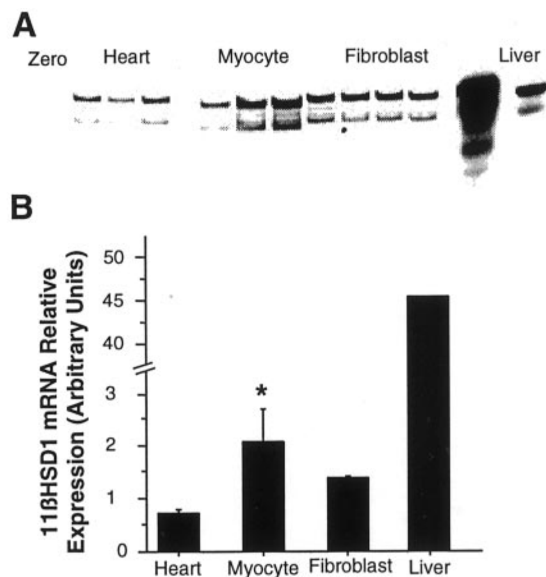

FIG. 2. A, Phosphorimage of 11βHSD1-protected [^{32}P]hybrids after RNase protection analysis of 3 μg total RNA for cardiac myocytes and 4 μg for heart, cardiac fibroblasts, and liver. Two specific bands are detected in samples, except in the zero (no RNA) control. Two different phosphorimage exposures for liver are shown, the first being the same exposure time as the other samples and the second being a shorter exposure time showing the two protected [^{32}P]hybrids. B, Relative expression of 11βHSD1 mRNA in the different tissues and cells after being corrected for micrograms of RNA loaded. n = 4. *, $P < 0.05$, compared with heart.

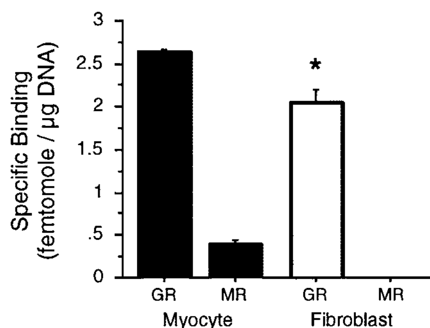

FIG. 3. Specific binding of [^{3}H]dexamethasone to GR and [^{3}H]aldosterone to MR in cardiac myocytes and fibroblasts. Cardiac cells were incubated with [^{3}H]dexamethasone (25–30 nM) or [^{3}H]aldosterone (25–30 nM) for 75 min. [^{3}H]dexamethasone binding, displaced by 6 μM RU38486, was taken as specific GR binding, and MR binding was [^{3}H]aldosterone binding displaced by aldosterone (6 μM) in the presence of RU38486. Results are mean ± SEM, n = 3. *, $P < 0.05$, compared with myocyte GR binding, n = 3; *, $P < 0.05$, compared with myocyte GR binding.

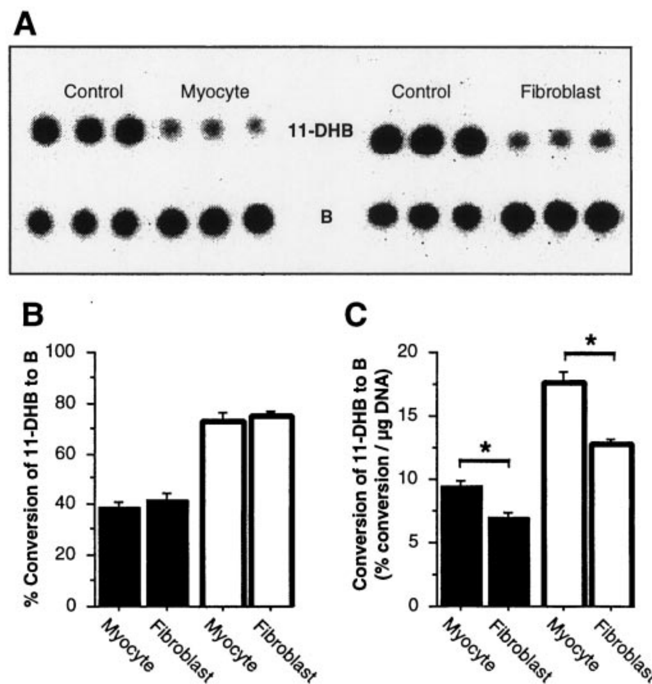

FIG. 4. 11βHSD activity in cardiac myocytes and fibroblasts. Cells were incubated with a cocktail containing both [^{3}H]corticosterone (B, 17 nM) and [^{3}H]11-DHB (24 nM). Media was sampled at 1 h (*solid bars*) and 6 h (*open bars*). Control is media incubated in the absence of cells and reflects the starting concentration of both [^{3}H]B and [^{3}H]11-DHB in the cocktail. A, Phosphorimage of the TLC profile of extracted [^{3}H]steroids after 6 h of incubation in the absence (Control) and presence of cardiac cells. B, Percentage of [^{3}H]11-DHB converted to [^{3}H]B by cells. C, Percentage of [^{3}H]11-DHB converted to [^{3}H]B, normalized to DNA recovered. n = 3. *, $P < 0.05$.

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in cell cultures and were 4.1 ± 0.5 μg and 82 ± 3 μg, respectively, for myocytes and 5.9 ± 0.1 μg and 153 ± 2 μg, respectively, for fibroblasts. When conversion was expressed per microgram of DNA, and therefore corrected for cell number, 11βHSD activity was significantly higher, at both 1 h and 6 h, in myocytes than in fibroblasts. Given the 2-fold higher protein levels in fibroblast, the difference between myocyte and fibroblast 11βHSD activity was even more pronounced when the results are expressed per microgram protein (data not shown).

MR and GR induction of SGK mRNA

SGK, an immediate early gene, contains a GRE in its promoter region (30), and is transcriptionally induced by serum, glucocorticoids (31), and aldosterone (32, 33). To determine the ability of glucocorticoids and aldosterone to activate GR and/or MR and induce gene transcription, cardiac fibroblasts and myocytes were incubated with various combinations of steroids, and SGK mRNA was determined by RNase protection assay. In cardiac myocytes, maximum induction of SGK mRNA by the synthetic glucocorticoid dexamethasone (500 nM) occurred at 1 h and was still maximally elevated at 3 h (data not shown); all further incubations with steroids were therefore performed for 2 h. Illustrated in Figs. 5 and 6 are typical phosphorimages generated by the SGK and GAPDH RNase protection assay, with the *arrow* indicating the specific SGK mRNA-protected [^{32}P]hybrid. GAPDH mRNA levels were used as a comparative control and did not change with any of the steroid treatments. The *upper band*, at relatively high abundance in Figs. 5C and 6 and at low levels in Fig. 5, A and B, is nonspecific and can be seen in the zero (no RNA) lanes. This nonspecific RNase-resistant band is generated during SGK riboprobe synthesis and usually reflects both the amount and specific activity of the SGK antisense probe added to the RNase protection assay. As illustrated in Fig. 5A, when cardiac fibroblasts were incubated for 2 h with the mineralocorticoid aldosterone or glucocorticoids (dexamethasone, B) at a concentration of 500 nM, SGK mRNA increased 3- to 4-fold. Because MR were undetectable in cardiac fibroblasts by both RNase protection analysis and binding assays, we determined whether both the B and aldosterone effects were mediated via GR, by using specific GR and MR antagonists. As illustrated in Fig. 5B, the GR antagonist RU38486, but not the MR antagonist RU28318, completely inhibited B induction of SGK mRNA in fibroblasts. Spironolactone, an aldosterone antagonist, also inhibited B-induced SGK mRNA, supporting previous data (34) that this drug can also act as a GR antagonist. Aldosterone-induced SGK mRNA was inhibited by both RU38486 and spironolactone, demonstrating that the aldosterone-induced response in cardiac fibroblasts is via GR (Fig. 5C).

When cardiac myocytes were treated with B (100 nM), SGK mRNA was stimulated 4-fold, whereas aldosterone (100 nM) led to a 1.7-fold stimulation. Both the B and aldosterone responses were blocked by RU38486, suggesting that both steroids mediated their effects via GR, not MR, in cardiac myocytes (Fig. 6).

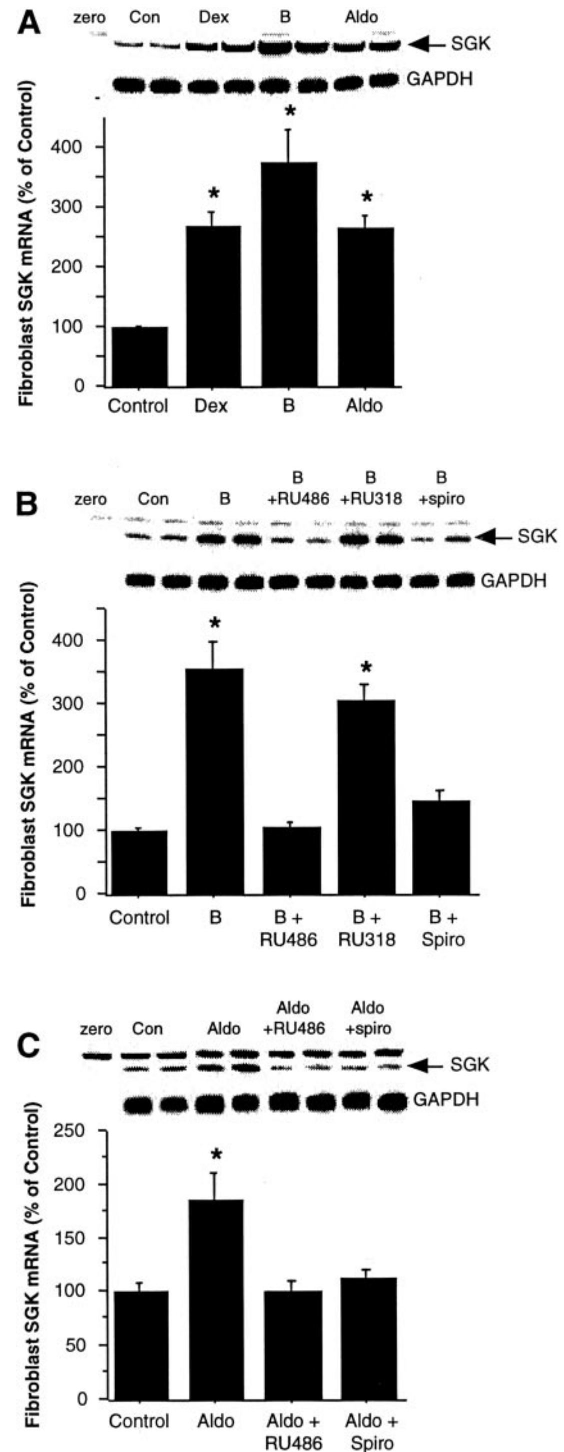

FIG. 5. SGK and GAPDH mRNA expression in cardiac fibroblasts after treatment of cells with steroids for 2 h at 37 C. Typical phosphorimages generated by the SGK and GAPDH RNase protection assay are shown in A, B, and C; the *arrows* indicate the specific SGK mRNA-protected [^{32}P]hybrid. Graphs represent the relative expression of SGK mRNA when expressed as percent of control (Con; no steroid treatment). A, Cells were treated with 500 nM dexamethasone (Dex), corticosterone (B), or aldosterone (Aldo). B, Cells were treated with 100 nM B in the presence or absence of 10 μM RU38486 (RU486), 5 μM RU28318 (RU318), or 10 μM spironolactone (spiro). C, Cells were treated with 100 nM aldosterone in the presence or absence of 5 μM RU486, or 10 μM spiro. n = 3–4. *, $P < 0.05$, compared with control.

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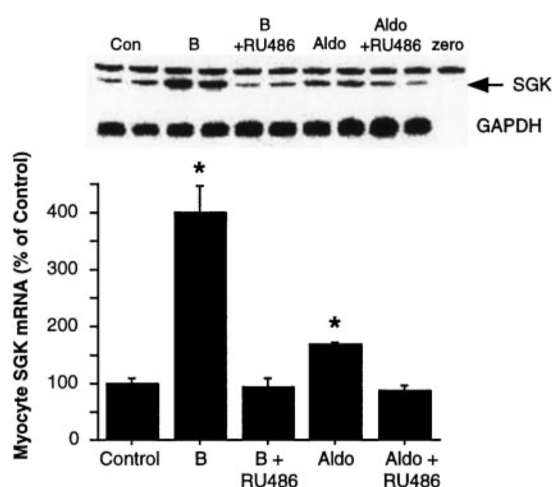

FIG. 6. SGK and GAPDH mRNA expression in cardiac myocytes after treatment with 100 nM B or Aldo in the presence or absence of 5 μM RU486. Phosphorimages generated by the SGK and GAPDH RNase protection assays are shown; the *arrow* indicates the specific SGK mRNA-protected [^{32}P]hybrid. The graph represents the relative expression of SGK mRNA when expressed as percent of control (no steroid treatment). n = 3. *, $P < 0.05$, compared with control.

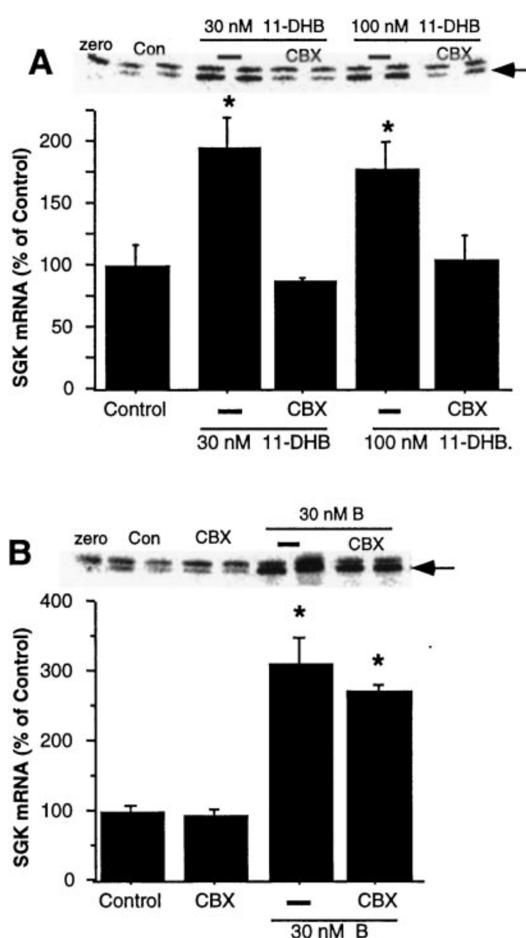

FIG. 7. 11-DHB regulation of cardiac fibroblast SGK mRNA expression. Panel A, Cells were treated with either 30 nM or 100 nM11-DHB in the presence or absence of 3 μM carbenoxolone (CBX) for 5 h at 37 C. Panel B, Cells were treated with 3 μM carbenoxolone in the presence or absence of 30 nM corticosterone (B) for 2 h at 37 C. Phosphorimages generated by the SGK RNase protection assay are shown; the *arrow* indicates the specific SGK mRNA-protected [^{32}P]hybrid. The graph represents the relative expression of SGK mRNA when expressed as percent of control (no treatment). n = 3. *, $P < 0.05$, compared with control.

11-DHB and SGK mRNA

The presence of 11βHSD1 and 11-reductase activity in cardiac cells suggests that the heart may use circulating 11-ketometabolites of B and cortisol as a source of glucocorticoid. To test whether SGK mRNA induction is responsive to 11-DHB and whether conversion to B is required, cardiac fibroblasts were incubated for 5 h with 30 nM or 100 nM 11-DHB in the presence or absence of the 11βHSD inhibitor, carbenoxolone (3 μM). As illustrated in Fig. 7A, 30 nM and 100 nM 11-DHB induced a 2-fold increase in SGK mRNA. Inhibition of 11βHSD1 activity, and thus conversion of 11-DHB to B with carbenoxolone, inhibited SGK mRNA induction by 11-DHB, suggesting that active conversion of 11-DHB to B is mandatory for induction of SGK gene expression. To ensure that carbenoxolone itself does not alter SGK gene expression, fibroblasts were incubated for 2 h with 3 μM carbenoxolone in the presence or absence of 30 nM B. As illustrated in Fig. 7B, carbenoxolone did not alter basal or B-induced SGK gene expression.

Discussion

The main findings of the present study are that: 1) GR and 11βHSD1 are coexpressed in both cardiac myocytes and fibroblasts, with only myocytes expressing low levels of MR; 2) glucocorticoids and aldosterone induce SGK gene transcription exclusively via GR; and 3) cardiac 11βHSD1 converts inactive 11-DHB to B, to generate a ligand that can activate gene transcription via GR. These data are of potential interest at a number of levels, including cardiac aldosterone effects being mediated through GR and the ability of the heart to use circulating 11-DHB as a source of glucocorticoid.

There is evidence, from *in vivo* studies, that both glucocorticoids and mineralocorticoids may play a pathophysiological role in cardiac hypertrophy and fibrosis (4, 35). Direct effects of glucocorticoids and aldosterone on cardiac cells have been demonstrated; although whether these effects are mediated via MR and/or GR is unclear. In cardiac myocytes, there is relatively high expression of GR and low expression of MR; whereas in cardiac fibroblasts, only GR is present. 11βHSD2 enables circulating aldosterone access to MR by metabolizing endogenous glucocorticoids to their inactive 11-ketometabolites. The absence of this enzyme in cardiac myocytes suggests that *in vivo*, B (rather than aldosterone) binds MR in these cells. This is in agreement with previous *in vivo* studies in which cardiac MR were shown to bind B (9). In addition, 11βHSD1 (which acts as a reductase, converting 11-ketoglucocorticoids to active glucocorticoids in both cardiac myocytes and fibroblasts) increases local intracardiac B concentration, therefore making it less likely that endogenous circulating aldosterone gains substantial access to MR. Thus, under normal physiological conditions, direct effects of circulating aldosterone, via MR, on cardiac myocytes is unlikely, and not possible in cardiac fibroblasts that lack MR expression.

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There is some evidence that the heart itself may synthesize B and aldosterone (36), raising the possibility that local production of aldosterone may enable this steroid to be at high enough concentration to occupy cardiac myocyte MR. The lower levels of aldosterone synthesis in heart, relative to local production of B, however, still argue against physiological aldosterone occupancy of cardiac MR. After myocardial infarction, cardiac expression of aldosterone increases, whereas local B production is decreased (37), suggesting that aldosterone may play a role in the heart, primarily under pathological conditions.

SGK is an immediate early gene containing a functional GRE in its promoter region (30) and which is transcriptionally induced by glucocorticoid-activated GR and aldosterone-activated MR (31–33). In addition, SGK is regulated posttranscriptionally by phosphorylation mediated via the phosphoinositide 3-kinase signaling pathway (38), a kinase that was recently shown to be involved in myocyte hypertrophy (39). Thus, in heart, SGK is potentially a functional convergence point between steroid signaling and other ligands that activate phosphoinositide 3-kinase. To further address the potential role GR and MR play in cardiac cells, we used SGK gene expression as an indicator of GR- or MR-mediated effects on gene transcription. In the present study, we show that both B and aldosterone increased SGK mRNA in cardiac myocytes and fibroblasts, albeit to differing degrees. The responses to both B and aldosterone were totally blocked by the GR antagonist RU38486, but not the MR antagonist RU28318, demonstrating that the effect was mediated via GR, not MR. Previous studies have demonstrated that cellular steroid receptor concentration can directly impact on the response (40), so that the inability of activated MR to stimulate SGK mRNA expression in cardiac myocytes may be attributable to the low level of MR expression in these cells. Several studies have demonstrated that aldosterone can directly effect cardiac cells, although most studies have not clearly demonstrated that the aldosterone effects are mediated via MR. Previous studies have required high concentrations of aldosterone for a maximum response (3, 41, 42), suggesting that the effect is via GR, not MR. Spironolactone has been used in some studies as a specific MR antagonist (42), although it can antagonize both MR and GR responses (34). Furthermore, other studies have shown aldosterone effects within a relatively short latency (2, 43), suggesting that these responses are via a nongenomic action of MR or alternatively via a membrane receptor. In addition, the aldosterone induced effects that have been reported (3, 42) can also be induced by glucocorticoids (44, 45), further suggesting that the aldosterone effects may be mediated via GR. In one study, however, high glucose concentrations potentiated aldosterone effects via MR in cardiomyocytes (46), suggesting that, under pathological conditions, these cells may become more sensitive to aldosterone-activated MR.

In vivo, aldosterone excess, in combination with a high-salt diet, increases collagen deposition in the heart only after several weeks of treatment (4), arguing against an initial direct effect of aldosterone on heart. Previous studies addressing direct effects of aldosterone on collagen synthesis in rat cardiac fibroblasts have been inconsistent (5–7). In the present study, we demonstrate that MR mRNA and binding are not present in cardiac fibroblasts, which would support the conclusions from *in vivo* studies that a direct action of aldosterone on cardiac fibroblast collagen production is unlikely. Paracrine effects of aldosterone via other cell types, however, cannot be discounted, and induction of MR under pathological conditions is also possible.

An important finding of the present study is the ability of relatively low concentrations of 11-DHB to induce gene transcription via GR. 11-DHB itself is unable to activate GR or MR and is therefore considered to be biologically inert. The presence of 11βHSD1, acting as a reductase in cardiac cells, enables these cells to use circulating 11-DHB. 11-DHB in rat, and the human equivalent cortisone, circulate at a concentration that is approximately 4-fold less than the endogenous glucocorticoids B and cortisol (47, 48). Considering that 11-DHB and cortisone have much lower affinity than B and cortisol for the two major plasma-binding proteins CBG and albumin, they therefore provide a major pool of available glucocorticoid for tissues such as the heart. This suggests that the heart is constantly exposed to glucocorticoids at higher-than-circulating levels, providing essentially complete occupancy of cardiac MR and at least double the GR occupancy, compared with other tissues.

In conclusion, we have demonstrated that rat heart does not express 11βHSD2 and that there is no 11-dehydrogenase activity in intact cells. These data suggest that myocyte MR would physiologically mediate glucocorticoid (rather than aldosterone) effects, although the present study indicates that the low level of MR expression may render them transcriptionally inactive, at least in terms of SGK induction. In contrast, both cardiac myocytes and fibroblasts express 11βHSD1, which enables these cells to use 11-DHB as a source of glucocorticoid. The ability of the heart to mediate glucocorticoid effects via MR and GR and to use both endogenous glucocorticoids and their 11-ketometabolites suggests that the heart may be under tonic glucocorticoid control, which implies that glucocorticoids play an important homeostatic role in heart.

Acknowledgments

We thank Rebecca Ridings for technical assistance, and Professor John Funder for constructive criticism.

Received February 26, 2001. Accepted September 12, 2001.

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This work was supported by a block grant to the Baker Medical Research Institute from the National Health and Medical Research Council of Australia.

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