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Zone-specific regulation of two messenger RNAs for P450c11 in the adrenals of pregnant and nonpregnant rats

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ABSTRACT **Adrenal mitochondria possess two steroidogenic cytochrome P450s. P450c11 converts deoxycorticosterone to corticosterone and aldosterone, and P450scc converts cholesterol to pregnenolone. These P450s receive electrons from NADPH via adrenodoxin reductase and adrenodoxin. A single bovine P450c11 protein has 11-hydroxylase, 18-hydroxylase, and 18-oxidase activities, but this series of enzymatic steps may be mediated by more than one enzyme in rats. Enzymatic assays of purified rat mitochondrial proteins have suggested that one enzyme found in all zones of the adrenal cortex has both 11- and 18-hydroxylase activities, whereas another enzyme, found exclusively in the zona glomerulosa, catalyzes 18-hydroxylation and 18-oxidation of corticosterone. We studied the number and zonal distribution of P450c11 mRNA species in the rat adrenal and how these mRNAs are regulated in the adrenals of normal and pregnant rats. Rats synthesize two similar, but distinct, P450c11 mRNAs. One, P450c11A, is found in both the zona glomerulosa and fasciculata/reticularis, whereas the second, P450c11B, is found only in the zona glomerulosa. The abundance of neither P450c11A mRNA nor P450c11B mRNA is affected by a high-salt diet. However, when rats receive a low-salt diet, P450c11A mRNA decreases and P450c11B mRNA increases. Dexamethasone decreases the amount of P450c11A mRNA without affecting P450c11B mRNA. The combination of a high-salt diet and dexamethasone decreases the amount of both mRNAs further to almost undetectable amounts. Rats given a low-salt diet and dexamethasone have a dramatic increase in the abundance of P450c11B mRNA. Thus both forms of P450c11 mRNA are regulated independently in the rat adrenal cortex. *In situ* hybridization studies show that only the P450c11 found in the zona glomerulosa is regulated by salt treatment *in vivo*, whereas glucocorticoid treatment *in vivo* regulates P450c11 in all zones. In the adrenals of pregnant rats, P450c11B is regulated in a similar fashion to its regulation in the nonpregnant rat adrenal, despite major differences in sodium retention and intravascular volume in pregnant and nonpregnant rats. In the pregnant rat, a low-salt diet increases the abundance of P450c11B to a greater degree than in the nonpregnant rat. By contrast, dexamethasone does not diminish the abundance of P450c11A mRNA in the pregnant rat but reduces it to an almost undetectable amount in the nonpregnant rat. Thus, the regulation of glucocorticoid and mineralocorticoid production in the pregnant and nonpregnant rat occurs by different mechanisms.**

The last three steps of aldosterone synthesis, 11-hydroxylation, 18-hydroxylation, and 18-oxidation, appear to be catalyzed by a single enzyme, cytochrome P450c11. Adrenal mitochondria from cattle and pigs appear to contain one species of P450c11 having 11β-hydroxylase, 18-hydroxylase, and 18-hydroxymethyloxidase activities (1, 2). However, rat adrenal mitochondria contain two distinct, but very similar, proteins of about 51 and 49 kDa (3–6). The 51-kDa protein, isolated from mitochondria of the zona fasciculata, catalyzed 11β- and 18-hydroxylations of 11β-deoxycorticosterone (DOC) to yield corticosterone and 18-OH-DOC but did not catalyze 18-hydroxylation or 18-hydroxydehydrogenation of corticosterone (3, 4). By contrast, the 49-kDa protein isolated from mitochondria of the zona glomerulosa from sodium-depleted rats converted DOC to 18-OH-DOC and corticosterone and to 18-hydroxycorticosterone and aldosterone (3, 4, 7). Immunoblots of mitochondrial proteins from preparations of zona glomerulosa show that sodium depletion or potassium loading increases the abundance of the 49-kDa protein and decreases the abundance of the 51-kDa protein (4). The complete amino acid sequence of the 49- and 51-kDa proteins is unknown, but partial amino acid sequencing of the amino-terminal 20 amino acids of a mixed preparation of these proteins showed that there are some differences between the two proteins, thereby excluding the possibility that these two proteins result from posttranslational modification of a single protein (4).

Both humans (8) and cattle (9) have two genes for P450c11, termed *CYP11A* and *CYP11B* (10). In humans, only one of these genes appears to be expressed in the adrenal, whereas in cattle, both genes are expressed, resulting in the formation of two different P450c11 mRNAs. Recently, three different rat P450c11 cDNAs were cloned and sequenced (11, 12). One was isolated from a cDNA library from whole adrenal tissue from corticotropin (ACTH)-stimulated rats (11), and two were isolated from a cDNA library derived from adrenal capsular tissue from sodium-depleted, potassium-replete rats (12). The latter two cDNAs are virtually identical and differ only in their 5′ termini and in amino acid 320; each of these two cDNAs has 81% nucleotide sequence identity to the P450c11 cDNA isolated from the intact adrenals of ACTH-stimulated rats.

The regulation of P450c11 mRNA has not been studied in detail. The 5′ flanking DNA of a mouse P450c11 gene contains tissue-specific regulatory elements and may contain cAMP regulatory elements (13). Comparison of the P450c11 gene sequence used in those studies (13) with the three P450c11 cDNAs recently published (11, 12) suggests that the gene transfer studies used the mouse P450c11A gene.

Adrenal steroidogenesis changes during pregnancy. Serum concentrations of aldosterone, but not corticosterone, are greater throughout rat gestation (14), whereas both glucocorticoid and mineralocorticoid concentrations increase late in the third trimester of human pregnancy (15). In the rat, corticosterone concentrations have been reported to be increased (16) or decreased (17) or remain unchanged (18) toward term, possibly reflecting circadian fluctuations (19). Pregnant rodents increase their plasma volume by about 60%, which is accompanied by an increase in sodium retention (20,

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21), but the role of mineralocorticoids and the potential regulation of P450c11 in these changes is unknown.

We determined how the pregnant rat regulates the production of glucocorticoids and mineralocorticoids by monitoring the synthesis of P450c11 mRNA in adrenals from late-gestation rats, and we determined the zonal distribution of expression of the two P450c11 mRNAs in the rat adrenal. We regulated mineralocorticoid production *in vivo* by altering dietary sodium and regulated glucocorticoid production by administering dexamethasone. Rat adrenals synthesize two distinct P450c11 mRNAs that are highly homologous. One is synthesized exclusively in the zona glomerulosa, while the other is synthesized throughout the adrenal cortex. Glucocorticoids and salt regulate both P450c11 mRNAs independently and in a zone-specific fashion. In contrast to the nonpregnant rat adrenal, P450c11 mRNA abundance in the pregnant rat adrenal is less sensitive to glucocorticoid inhibition and more sensitive to salt treatment.

MATERIALS AND METHODS

Animals. Sprague–Dawley rats, 200–250 g, received one of six regimens for 7 days. (*i*) Control (normal): animals received rat chow ad libitum and water. (*ii*) High salt: animals received rat chow ad libitum and 2% NaCl in their drinking water. (*iii*) Low salt: after receiving 2 mg of furosemide subcutaneously for 2 days, animals received sodium-deficient rat chow (Teklad, Madison, WI) and water. (*iv*) Dexamethasone: animals received daily subcutaneous injections of 20 μg of dexamethasone and rat chow and water ad libitum. (*v*) High salt + dexamethasone: animals received daily subcutaneous injections of 20 μg of dexamethasone, rat chow, and 2% NaCl in drinking water. (*vi*) Low salt + dexamethasone: animals were treated for 2 days with 2 mg of furosemide and were given daily subcutaneous injections of 20 μg of dexamethasone, sodium-deficient rat chow, and water. Similar experiments were performed with pregnant animals, starting approximately on day 13 or 14 of pregnancy and continuing for 7 days. Experiments were conducted in accordance with the principles and procedures as outlined by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Preparation of RNA. After decapitation, one adrenal from each animal was frozen immediately in liquid N_2 for RNA isolation; the other adrenal was placed in Tissue-Tek O.C.T. compound (Miles) and frozen on methanol/dry ice for *in situ* hybridization experiments. In one experiment, adrenals from normal animals were separated into zona glomerulosa and zona fasciculata/reticularis (and medulla) by decapsulation. The adrenal capsule (glomerulosa) and the remaining cells (decapsulated—fasciculata/reticularis and medulla) were frozen separately in liquid N_2. Tissues were sonicated in 5 M guanidinium isothiocyanate in 50 mM Tris Cl, pH 7.5/10 mM EDTA/5% 2-mercaptoethanol; CsCl was added at 0.45 g/ml, and 2 ml of this solution was layered on 750 μl of 5.7 M CsCl in 10 mM EDTA (pH 8) and centrifuged 3 hr at 166,000 × *g*. The pelleted RNA was resuspended in 10 mM Tris Cl, pH 7.5/1 mM EDTA (TE), extracted four times with phenol/chloroform, precipitated with ethanol, and resuspended in TE/0.1% $NaDodSO_4$.

Rat P450c11 cDNA. We cloned a segment of rat P450c11 cDNA by PCR amplification of rat adrenal cDNA. Double-stranded cDNA was prepared from 1 μg of rat adrenal RNA by reverse transcription as described (22). The 3′ PCR primer, 5′-CAGCTGCAGTCGGTTGAAG-3′, corresponds to nucleotides 414–432 of rat P450c11 cDNA (11) and contains a natural *Pst* I site used in cloning (underlined); the 5′ primer, 5′-GGCAAGCTTCCAAGAGCTGGGGC-3′, corresponds to nucleotides 213–229 (underlined) and contains a synthetic *Hin*dIII site for use in cloning. PCR amplification was performed in 50 mM Tris Cl, pH 9.0/2.5 mM $MgCl_2$/20 mM ammonium sulfate for 30 cycles as follows: denaturation at 94°C for 30 sec, primer annealing at 46°C for 30 sec, and extension at 72°C for 1 min. Amplified products were digested with *Hin*dIII and *Pst* I and separated on 6% polyacrylamide gels, and the 220-base-pair fragment was excised and cloned into the *Hin*dIII/*Pst* I sites of pBluescript KS (Stratagene). Positive clones were verified by double-stranded DNA sequencing on both strands (23). The sequence of the clone used in these studies was identical to a published sequence for rat P450c11 (11).

RNA Assays. RNase protection assays were done as described (24). pBluescript KS containing our 220-base-pair rat P450c11 *Hin*dIII/*Pst* I cDNA fragment was linearized with *Hin*dIII, and a 280-nucleotide ^{32}P-labeled RNA probe, corresponding to nucleotides 213–432 of rat P450c11 cDNA (11) (numbering starts at the initiator methionine codon) and containing 60 nucleotides of vector, was synthesized with T7 RNA polymerase. RNA samples from adrenal (2.5 μg) were combined with tRNA (50 μg) and precipitated with ≈1 × 10^6 cpm of probe, resuspended in 80% (vol/vol) formamide/400 mM NaCl/40 mM Pipes, pH 6.4/1 mM EDTA, boiled 5 min, and incubated overnight at 42°C. Samples were diluted 10-fold with 10 mM Tris Cl, pH 7.9/300 mM NaCl/5 mM EDTA/RNase A at 20 μg/ml for 30 min at 42°C, treated with proteinase K at 0.25 mg/ml and 0.5% $NaDodSO_4$ for 15 min at 37°C, extracted with phenol/chloroform, and precipitated with ethanol. Protected fragments were separated by electrophoresis on 6% polyacrylamide/7.5 M urea gels. *In situ* hybridization to tissue RNAs was done as described (25, 26).

RESULTS

P450c11 mRNA from Different Adrenal Zones. Normal rat adrenals were decapsulated to separate zona fasciculata/reticularis cells (decapsulated adrenal) from capsular (mainly zona glomerulosa) cells. P450c11 mRNA was assayed in each preparation by RNase protection (Fig. 1). By using a 280-base ^{32}P-labeled RNA probe, total rat adrenal RNA protected two fragments, termed A and B. Fragment A migrated at ≈220 nucleotides, the expected size for a full-length protected fragment. Fragment B, of ≈190 nucleotides, was also protected by total rat adrenal RNA. Other bands seen below fragments A and B varied in intensity and in number among different assays and RNA preparations, indicating that they represent nonspecific artifacts in the RNase protection assay. When RNA was analyzed from decapsulated adrenals, mainly containing cells from the zona fasciculata/reticularis, only the 220-base fragment A was detected. When RNA was analyzed from the adrenal capsule, mainly containing cells from the zona glomerulosa, both the 220-base and the 190-base fragments were protected. Thus, fragment B (190 bases) is a glomerulosa-specific form of P450c11 mRNA. We call this mRNA P450c11B to distinguish it from the P450c11 cDNA previously cloned and sequenced (11), which we call P450c11A. It is not clear if the P450c11 mRNA protecting the 220-base fragment is present in the adrenal in a zone-specific manner. The presence of the 220-base fragment A in RNA from adrenal capsules may be due to the presence of that form of P450c11 mRNA in the glomerulosa, or it may be due to contamination of the capsular preparation with fasciculata/reticularis cells. These data indicate that there are two distinct P450c11 mRNAs expressed in the normal rat adrenal, as is true for bovine adrenals. The relative intensities of the protected fragments indicate that P450c11A mRNA is expressed throughout the adrenal. P450c11B is expressed only in the zona glomerulosa, but even here it is less abundant that P450c11A. The zonal distribution of these P450c11 mRNAs suggests that P450c11B may be involved in aldosterone synthesis, whereas P450c11A mRNA appears to be involved in both aldosterone and corticosterone synthesis.

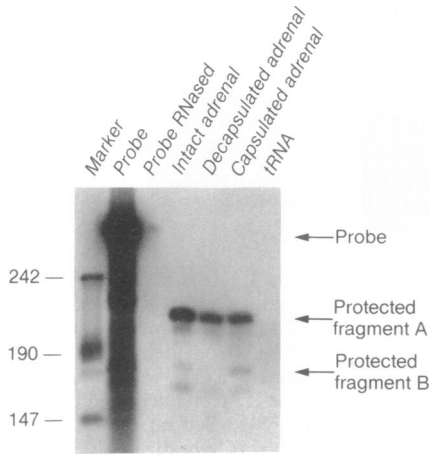

FIG. 1. RNase protection assay of RNA from normal rat adrenals. Adrenals were untreated ("Intact adrenal") or decapsulated and separated into decapsulated ("Decapsulated adrenal", zona glomerulosa) and capsular ("Capsulated adrenal", zona fasciculata/reticularis) samples, and 1 μg of RNA from each was combined with 50 μg of tRNA and hybridized with a 280-nucleotide ^{32}P-labeled rat P450c11 RNA probe. The probe is indicated by an arrow, and two protected fragments, A (220 nucleotides) and B (≈190 nucleotides), are also indicated. The lane marked "Probe RNased" contains 1 × 10^6 cpm of RNA probe treated with RNase A (20 mg/ml), and the lane marked "tRNA" contains 50 μg of tRNA treated exactly as all adrenal samples. Markers (in nucleotides) are ^{32}P-labeled *Msp* I-digested pUC19 DNA.

RNA from Normal, Salt-Depleted, and Salt-Loaded Rats. To determine if the two forms of P450c11 mRNA are regulated in a zone-specific manner, we used six groups of rats in which mineralocorticoid and/or glucocorticoid synthesis was altered by dietary and hormonal manipulation, and RNA from intact adrenals was analyzed by RNase protection assays (Fig. 2). Rats receiving a high-salt diet did not change the abundance of either form of P450c11 mRNA. The intensities of protected fragments A and B in the "High salt" lane in Fig. 2 were equivalent to those in the "Normal" lane in Fig. 2: the ratio of protected fragment A/B (P450c11A/P450c11B) was ≈10:1 in adrenals from both experimental groups. In rats receiving a low-salt diet, the amount of P450c11A mRNA decreased to approximately half of the amount in controls, while the amount of P450c11B mRNA increased ≈5-fold. Therefore, low-salt treatment affects accumulation of P450c11A and P450c11B mRNAs in opposite directions. When rats were given dexamethasone to suppress glucocorticoid synthesis, the abundance of P450c11A mRNA decreased drastically, to ≈1/50th the control. However, the abundance of P450c11B in rats treated with dexamethasone was about the same as (or slightly greater than) in control rats. Thus, dexamethasone inhibits the accumulation of P450c11A mRNA but does not affect the accumulation of P450c11B mRNA.

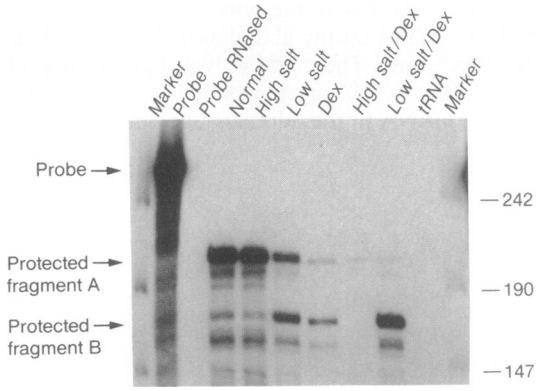

FIG. 2. RNase protection of adrenal RNA from rats placed on high- or low-salt diets and treated with dexamethasone (Dex). Two and five-tenths micrograms of RNA from the adrenal of each animal was combined with 50 μg of tRNA and 1 × 10^6 cpm of ^{32}P-labeled rat P450c11 RNA probe. The 280-base probe and protected fragments A and B are noted. The lane marked "Probe RNased" contains probe that was treated with RNase A (20 μg/ml), and "tRNA" contains 50 μg of tRNA that was treated identical to adrenal samples. Markers (in nucleotides) are ^{32}P-labeled *Msp* I-digested pUC19 DNA.

The combination of dexamethasone and salt-loading or salt-depleting aimed at regulating both mineralocorticoid and glucocorticoid synthesis simultaneously acts synergistically to regulate the accumulation of both P450c11A and P450c11B mRNAs. The combination of salt-loading and dexamethasone (Fig. 2, "High salt/Dex" lane) rendered P450c11B mRNA undetectable and decreased P450c11A mRNA to a concentration equivalent to that seen when rats were treated with dexamethasone alone. The most dramatic result was obtained when rats received a low-salt diet and dexamethasone, thereby reducing glucocorticoid synthesis and increasing mineralocorticoid synthesis (Fig. 2, "Low salt/Dex" lane). P450c11A mRNA remained barely detectable, while the abundance of P450c11B mRNA increased dramatically over that found in adrenals from normal rats or from rats receiving a low-salt diet alone. Therefore, salt and glucocorticoids synergistically modulate the abundance of P450c11B mRNA. The results (Fig. 2 and Table 1) were obtained in three separate experiments with six different animals per group.

***In Situ* Hybridization Histochemistry.** We determined directly which zones of the adrenal cortex responded to treatment with salt or glucocorticoids by performing *in situ* hybridization histochemistry on adrenal sections from the animals described above (Fig. 3). In the normal rat adrenal, P450c11 mRNA was found throughout the adrenal, with a slightly increased concentration in some regions of the outer zone (Fig. 3*A*). The distribution of P450c11 mRNA in the adrenals of rats receiving a high-salt diet decreased in the outer zone, so that the overall abundance in all zones of the adrenal was equivalent (Fig. 3*C*). When rats received a low-salt diet, P450c11 mRNA accumulated in the outer region of the adrenal (Fig. 3*E*). The cellular morphology seen in the light-field photomicrograph indicates that this region corresponds to the zona glomerulosa. Since our probe hybridizes

Table 1. Approximate relative abundances of the mRNAs for P450c11A and P450c11B as estimated from the RNase protection data

Regimen	P450c11A	P450c11B
Nonpregnant		
Control	10	1
High salt	10	1
Low salt	5	5
Dexamethasone	0.2	1–2
High salt + dexamethasone	0.2	0
Low salt + dexamethasone	0.2	10
Pregnant		
Control	10	1
High salt	10–15	1
Low salt	5	3–5
Dexamethasone	5	1
High salt + dexamethasone	5	0.5
Low salt + dexamethasone	3–5	10–15

The bands of protected fragments A and B from the gels used for Figs. 2 and 4 were scanned densitometrically, yielding, in arbitrary units, the relative intensities of the bands. For the nonpregnant rats, the data are from Fig. 2; for the pregnant rats, the data are from Fig. 4.

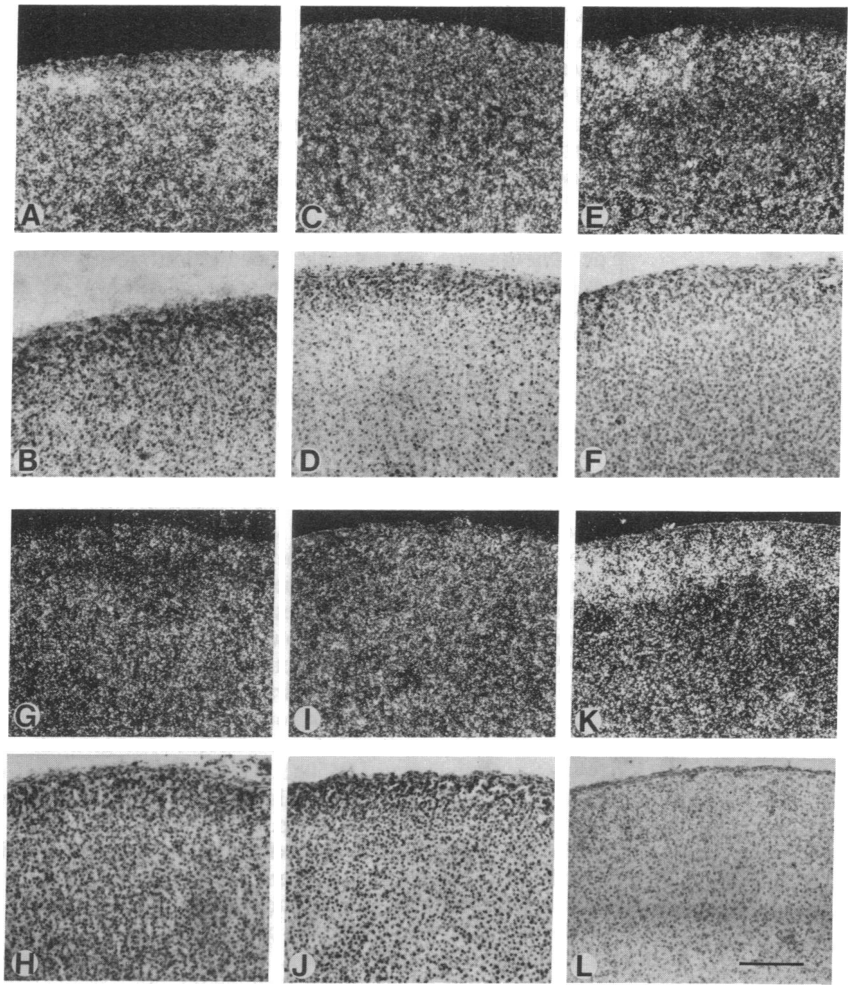

FIG. 3. *In situ* hybridization of rat adrenal sections with ^{35}S-labeled rat P450c11 RNA probe. Ten-micrometer sections, fixed, dehydrated, and stored as described (25), were treated with proteinase K, acetylated, and prehybridized (26). ^{35}S-labeled rat P450c11 cRNA (100 pg/μl) was hybridized at 37°C for 16 hr, washed (26), digested 30 min with RNase (20 μg/ml), coated with Ilford K% nuclear emulsion, and autoradiographed for 2 weeks before counterstaining with cresyl violet. Representative dark-field photomicrographs (*A*, *C*, *E*, *G*, *I*, and *K*) are of adrenals from control rats (*A*) or rats given a high-salt diet (*C*), a low-salt diet (*E*), dexamethasone treatment (*G*), a high-salt diet plus dexamethasone treatment (*I*), or a low-salt diet plus dexamethasone treatment (*K*). Positive signals are seen as white grains on a black background. All dark-field sections were taken at the same magnification and all were exposed to film for identical lengths of time. Light-field photomicrographs of each tissue section (*B*, *D*, *F*, *H*, *J*, and *L*) are shown below each dark-field photograph. Nuclei are seen as dark spots; silver grains cannot be seen on these light-field micrographs. (Bar = 100 μm.)

to both P450c11A and P450c11B, *in situ* hybridization experiments do not distinguish between these two mRNAs. However, the RNase protection experiments show that P450c11B, and not P450c11A, increased with low-salt treatment.

When rats were given dexamethasone, the abundance of P450c11 mRNA decreased in all zones (Fig. 3 *G* vs. *A*), consistent with a decrease in P450c11A mRNA shown by RNase protection (Fig. 2). The distribution and abundance of P450c11 mRNA in adrenals from rats given a high-salt diet and dexamethasone (Fig. 3*I*) was similar to the profile from rats given dexamethasone alone (Fig. 3*G*); thus, we could not detect a further decrease in P450c11 mRNA, as seen by RNase protection assays (Fig. 2). In rats given a low-salt diet and dexamethasone, P450c11 mRNA in the outer zone increased (Fig. 3*K*) relative to adrenals from rats given dexamethasone alone (Fig. 3*G*), with no further change in the abundance of P450c11 mRNA in the inner zones. Thus, low-salt treatment increased, and high-salt treatment decreased, the abundance of P450c11 mRNA only in the outer zone of the adrenal, whereas dexamethasone treatment decreased the abundance of P450c11 mRNA in all zones.

P450c11A and P450c11B mRNA in Pregnant Rat Adrenals. Midgestation (13 or 14 days) pregnant rats were subjected to the same 7-day salt and glucocorticoid protocols described above, and adrenal RNA from individual animals was analyzed for P450c11A and P450c11B by RNase protection assays. The adrenal of the normal, pregnant rat contained P450c11A and P450c11B mRNAs in essentially the same ratio as in the normal, nonpregnant rat adrenal: P450c11A mRNA was about 10 times more abundant than P450c11B mRNA (Fig. 4, "Normal" lane vs. Fig. 2, "Normal" lane). Treatment with high salt increased P450c11A mRNA slightly and did not affect P450c11B mRNA (Fig. 4, "High salt" lane). Adrenals from rats receiving a low-salt diet (Fig. 4, "Low salt" lane) had less P450c11A and more P450c11B mRNA than did adrenals from normal, pregnant rats. This was similar to the results with adrenals from normal, nonpregnant rats (Fig. 2, "Low salt" lane). Differences between the adrenals from pregnant and nonpregnant rats were greatest in response to dexamethasone. In the pregnant rat, unlike the nonpregnant rat, dexamethasone had little effect on the abundance of P450c11A mRNA (Fig. 4, "Dex" lane). There was only a slight decrease in the abundance of this mRNA, in comparison to an ≈98% decrease in P450c11A mRNA in the adrenals of normal, nonpregnant

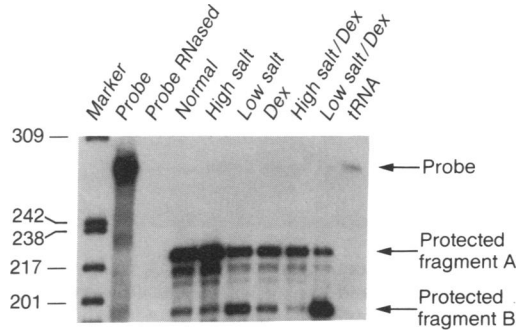

FIG. 4. RNase protection of RNA from adrenals from pregnant rats. Each lane contains 2.5 μg of adrenal RNA from individual animals and 50 μg of tRNA. "Probe RNased" and "tRNA" lanes are as described in the legends to Figs. 1 and 2. Protected fragments A and B are indicated. Markers (in nucleotides) are ^{32}P-labeled *Msp* I-digested pBR322 DNA. Dex, dexamethasone.

rats treated with dexamethasone. Thus, there appears to be no feedback inhibition on the synthesis of P450c11A mRNA in the adrenals of pregnant rats. This lack of feedback inhibition occurs irrespective of whether the rats are given a normal diet, high-salt diet, or low-salt diet. Conversely, P450c11B increased to a greater degree in the adrenals of pregnant rats than in those of normal rats receiving a low-salt diet and dexamethasone (Fig. 4, "Low salt/Dex" lane vs. Fig. 2, "Low salt/Dex" lane).

DISCUSSION

Rat adrenals synthesize two distinct P450c11 mRNAs: P450c11B is synthesized exclusively in the zona glomerulosa and P450c11A is synthesized in all zones. These two mRNAs are distinguished by RNase protection assays using a small fragment of P450c11 cDNA as probe. Since the RNA hybridizations in these protection assays were performed under highly stringent conditions, the degree of similarity between the two species of mRNA in the region of the probe is great. After this work was completed, the complete sequences of rat P450c11A and B became available (11, 12), showing that only 8 of the 220 bases in our probe differ in the A and B sequences. Inspection of these sequences shows that because RNase A does not detect G·U pairs and generally does not cut 3′ of mispaired purines, a 190-base fragment of B mRNA (bases 213–403) is protected by our 220-base A antisense probe. Thus the newly available sequence data confirm the predictions of our RNase protection data.

P450c11A and P450c11B mRNAs are found in the adrenal of normal rats in a ratio of ≈10:1. The abundance of these mRNAs is regulated independently (Table 1), consistent with their originating from separate genes. In the control rat, the 49-kDa protein found in the glomerulosa is undetectable, consistent with the low abundance of P450c11B mRNA in the control animal. Rats given a low-salt diet had an increased abundance of P450c11B mRNA and a decreased abundance of P450c11A. The relative abundances of these mRNAs most likely reflect the abundances of the 51-kDa and 49-kDa proteins found in mitochondria from enriched preparations of zona glomerulosa from rats given low-salt diets (3, 4, 7). Dexamethasone only affects the abundance of P450c11A mRNA in rats given a normal diet. Thus, P450c11A mRNA is probably involved in glucocorticoid production, and the decrease in that mRNA may reflect specific feedback inhibition in the synthesis of P450c11A mRNA.

Although the roles of renin, angiotensin, salt, and mineralocorticoids in the regulation of blood pressure have been studied extensively, previous studies have not directly addressed the physiologic regulation of P450c11. Complete deficiency of P450c11 activity due to severe lesions in the *CYP11* gene encoding human P450c11 (8, 27) causes a hypertensive form of congenital adrenal hyperplasia due to overproduction of deoxycorticosterone, one of the substrates for P450c11 (28). By contrast, disorders in later steps in aldosterone synthesis (e.g., in the corticosterone methyl oxidase step) result in mineralocorticoid deficiency and hypotension (29–31). The presence of these genetic disorders in the distal steps of aldosterone synthesis has long suggested that these steps require other enzymes in addition to 11-hydroxylase, even though clinical (32) and biochemical (2, 33) studies suggested that 11-hydroxylase, 18-hydroxylase, and possibly 18-methyloxidase activities were mediated by a single enzyme. The discovery that cattle and rats have two expressed genes for P450c11 initially seemed to confirm this (9, 12). However, it is not yet clear if the less abundant P450c11B is expressed in the human adrenal.

Our data suggest that in the rat, P450c11A mRNA is involved solely in glucocorticoid synthesis, whereas P450c11B mRNA, which encodes a protein with the potential to synthesize both glucocorticoids and mineralocorticoids, is involved solely in mineralocorticoid synthesis. The clear differences in the glucocorticoid suppressibility of P450c11A mRNA and salt enhancement of P450c11B mRNA abundances in pregnant vs. nonpregnant rats suggest that these may be important factors in increasing intravascular volume and salt retention in pregnancy. The potential role of the regulation of these enzymes in hypertension and human preeclampsia may merit further investigation.

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