Mechanism of Induction of Mouse Kidney Alcohol Dehydrogenase by Androgen

ANDROGEN-INDUCED STIMULATION OF TRANSCRIPTION OF THE Adh-1 GENE*

(Received for publication, February 22, 1988)

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The three alcohol dehydrogenase genes in the mouse are subject to developmental, hormonal, and genetic control as revealed by variation in expression among inbred strains. The primary purpose of this study was to determine the mechanism by which androgen regulates the expression of the Adh-1 gene in kidney. In addition, the fold-induction in several inbred strains was examined in a search for possible genetic variation in the induction process, and Adh-1 expression in several tissues was studied. Testosterone treatment of female mice results in a 10–12-fold increase in alcohol dehydrogenase activity and a corresponding increase in the rate of enzyme synthesis accounts for this induction. The induction of Adh-1 mRNA after androgen treatment is sufficient to account for the induction in enzyme synthesis. An increase in Adh-1 transcription accounts for a substantial part of the increase in Adh-1 mRNA level following androgen simulation. This conclusion was reached using nuclear “run-on” assays, in vivo labeling, and a kinetic analysis of Adh-1 mRNA accumulation and loss in response to hormone. This induction requires androgen receptor. The fold-induction by androgen of Adh-1 mRNA is similar in eight inbred mouse strains. There is almost a 100-fold variation in Adh-1 mRNA concentrations among various mouse tissues. Tissues with lowest level of expression are brain and heart, while liver and adrenals have the highest content of Adh-1 mRNA.

The alcohol dehydrogenase genes in the mouse are subject to interesting genetic, developmental, and hormonal control. The Adh-1, Adh-2, and Adh-3 genes encode alcohol dehydrogenase isozymes with different catalytic properties, and these genes are expressed in a tissue-specific manner (1, 2). Two regulatory loci are known which act in a tissue-specific manner to control expression of Adh-1 (3) or Adh-3 (4). The major liver and kidney enzyme is encoded by Adh-1, and both cDNA (5, 6) and genomic clones (7) of this gene have been isolated and characterized.

An important aspect of Adh-1 regulation is that testosterone increases kidney alcohol dehydrogenase enzyme activity (6, 8) and the concentration of Adh-1 mRNA (6). The mechanism by which androgen induces Adh-1 mRNA is not understood, and although a number of gene products are inducible in mouse kidney by testosterone, there is no generalized mechanism that accounts for this effect. However, most kidney enzymes which increase in activity after testosterone treatment are accompanied in large part by a corresponding induction of their respective mRNAs (9–15). For at least three androgen-responsive genes in mouse kidney, evidence indicated that regulation was post-transcriptional, and mRNA stabilization was suggested as the mechanism for induction (14). More recent evidence, however, indicated that these genes do respond to androgen by increasing rates of mRNA synthesis, and the possibilities remain that androgen mediates regulation of either gene transcription or processing of primary transcripts in the nucleus (16). In other tissues, such as mouse liver, androgen has been shown to induce gene products by increasing transcription (14, 17). In rat prostate the mRNAs for C1 and C3 proteins appear to be induced transcriptionally and also stabilized by androgen (18), although there is some question about the interpretation of these results (19).

Many steroid hormones exert a dramatic effect on responsive genes through interaction with a receptor protein which in turn binds to specific DNA sequences to promote transcription (20–22). It will be of interest to determine whether this mechanism is also used in the androgen response. The first goal is to determine conclusively which step or steps in the pathway from gene to protein are subject to androgen regulation.

Here we report a detailed analysis of the process of induction of kidney alcohol dehydrogenase by androgen. Induction of enzyme activity is shown to occur by an increase in enzyme synthesis, which correlates with an increased Adh-1 mRNA content. Using kinetic analysis, transcription “run-on” assays, and in vivo labeling of newly synthesized RNA, we demonstrate that the androgen-induced increase in Adh-1 mRNA content in mouse kidney occurs primarily by an increase in Adh-1 transcription.

EXPERIMENTAL PROCEDURES

Animals—Inbred strains of mice were obtained from the Jackson Laboratory. For most studies female mice of strain A/J were used except where indicated. Experimental animals were 2–4 months of age. The requirement for androgen receptor was examined using C3H mice carrying the Tfm mutation. Parabiotic mice were produced by joining pairs of mice at the scapula and allowing cross-circulation to develop. Red blood cells labeled with 51Cr and injected into one mouse were found to equilibrate between the two individuals of a pair in less than 30 min. Hypophysectomized C57BL/6J female mice were used to investigate pituitary requirements for androgen induction. Testosterone was administered subcutaneously as a 30-mg pellet in the nape of the neck which resulted in a continual chronic dose of the hormone. For deinduction, the pellet was surgically removed.

Enzyme Activity and Relative Rates of Synthesis—Alcohol deh-
Androgen Induction of Kidney Alcohol Dehydrogenase

drogenase activity was measured in the high speed supernatant of 20% kidney homogenates as previously described (3). Protein content was estimated (25) using bovine serum albumin as a standard. The relative rates of synthesis of alcohol dehydrogenase in kidney of control and testosterone-treated mice were determined in mice fasted overnight. Kidneys were homogenized in 160 ml of 1.5 M NaCl containing 1 mM NADH. Centrifugation and immunoprecipitation of alcohol dehydrogenase from heat-treated supernatants was done as previously described (3). Radioactivity in immunoprecipitated protein as well as in total protein was determined as previously reported (3).

Pasmids—The following plasmids were used in this study: pADHm9 contains a 1050-nucleotide Adh-1 cDNA insert (7); pIBADH-1 contains the same Sau3a/PstI fragment from pADHm9 (6) cloned into the BamHI/PstI site of pSP65 so that the SP6 promoter can be used to make a single-stranded RNA probe which hybridizes to Adh-1 mRNA; pIBADH-1 contains the same Sau3a/PstI fragment in pIB76, which has both T7 and SP6 promoters in opposing directions; and p91 contains a cDNA for mouse muscle a-actin mRNA (24).

Analysis of Tissue Levels of Adh-1 mRNA—Total cellular RNA was extracted using the guanidinium thiocyanate method (25) or the guanidinium HCl method (26) with comparable results. RNA preparations were routinely analyzed for quality by agarose gel electrophoresis as well as absorbance at 280 and 290 nm. RNA concentrations were determined from A260 values.

Determination of relative levels of Adh-1 mRNA by dot blot and Northern blot analysis was as previously described (6). Quantification of Adh-1 mRNA for the kinetic analysis (Fig. 4), in Tfm mice (Table 3), and RNA was isolated by adding 350 pl of 25 mM Tris-C1, pH 7.5, 5 mM MgCl2, and 0.1 mM EDTA and ethanol. The RNA was dissolved in sterile water, and aliquots were stored in aliquots at -80°C.

RESULTS

The administration of testosterone to A/J female mice results in a 12–15-fold increase in kidney alcohol dehydrogenase activity whether expressed per gram of tissue or per milligram of soluble protein (Fig. 1). The activity reaches a near maximum by 9–10 days with only a slight rise thereafter. Removal of androgen results in a 30% reduction in activity within 2 days (data not shown).

The induction of enzyme activity in the kidney is accomplished by an increase in the relative rate of specific enzyme synthesis in this tissue (Fig. 2). Within 3 days after androgen administration, the relative synthesis of kidney alcohol dehydrogenase reaches a maximum rate of about 0.7 which is more than a 10-fold increase over the synthesis rate in kidney of control mice. The synthetic rate remains elevated until androgen is removed. Two days after androgen withdrawal the relative rate of synthesis has dropped to slightly above basal levels.

The relative content of Adh-1 mRNA was determined in kidney tissue from other animals of this set (Fig. 2). There is an initial increase in the relative rate of specific enzyme synthesis. The induction of specific enzyme synthesis is followed by a reduced rate of synthesis.
Androgen Induction of Kidney Alcohol Dehydrogenase

14533

FIG. 2. Induction of enzyme synthesis and Adh-I mRNA by testosterone. Relative rates of synthesis were determined by in vivo labeling and immunoprecipitation techniques, and Adh-I mRNA relative concentrations were determined by hybridization to a specific probe as detailed under "Experimental Procedures." The arrow indicates the time of androgen removal. c, control animals.

A clear relationship between the synthesis of alcohol dehydrogenase and the content of Adh-I mRNA during the time course of induction and deinduction. The concentration of Adh-I mRNA clearly reached a plateau by the 3-day time point as did the synthesis of the protein. Enzyme activity does not reach a new steady-state level until 9-10 days of continuous androgen administration (Fig. 1). This is due to the effect of enzyme turnover on the time required to reach the new steady-state level of enzyme protein in the tissue following the hormone-induced increase in synthetic rate. The kinetics of enzyme activity accumulation suggest that alcohol dehydrogenase has a half-life of 3-4 days in mouse kidney.

Northern blot analysis of kidney Adh-I mRNA content was conducted on RNA samples isolated at various times after androgen treatment and withdrawal. These experiments show that noninduced and induced Adh-I mRNAs are of the same approximate size and that this species of mRNA induces sufficiently to account for the fold induction of alcohol dehydrogenase protein synthesis (Fig. 3).

A thorough kinetic analysis of Adh-I mRNA accumulation in kidney tissue after androgen treatment and its decline after subsequent withdrawal was examined to more fully understand the mechanism of induction. Assuming classical turnover kinetics, this approach can be used to estimate the half-life of the Adh-I mRNA in the presence or absence of hormone since the time course required to reach a new steady-state level after a perturbation is a function of the degradation constant of the macromolecule. This is mathematically described by the classical rate equation used to describe the kinetics of protein induction (30). The RNA turnover rate constants during induction ($k_d$) and deinduction ($k_d'$) can be evaluated by integrating and rearranging the basic rate equation (14, 31). The slopes of linear plots of these equations provide estimates of the degradation constants in the presence and absence of hormone.

The rate of accumulation of Adh-I mRNA after initial hormone administration is slower than the rate of decline from the fully induced level following removal of hormone (Fig. 4A). When these data are examined using the integrated form of the rate equation (Fig. 4B), the linear relationships obtained confirm that the induction and deinduction can be approximated by the model equation for first order turnover. The slopes of the lines obtained from the data for the induction and deinduction (Fig. 4B) provide values of 0.034 h⁻¹ and 0.075 h⁻¹ of hormone.

FIG. 3. Northern analysis of Adh-I mRNA content in response to testosterone. At various times after testosterone treatment or withdrawal, kidney RNA was isolated and separated by electrophoresis (10 µg/slot), and blotted onto nitrocellulose. Adh-I mRNA sequences were identified by hybridization to a specific probe.

FIG. 4. Kinetic analysis of the hormonal modulation of Adh-I mRNA levels in kidney. A, Adh-I mRNA concentrations at various times after androgen administration (○) or removal (○) were determined using RNA probes generated from pSP65ADH-1 as described under "Experimental Procedures." B, integration of the data in A resulting in linear plots where the slope is the degradation constant for the RNA in the presence (○, $k_d = 0.034$ h⁻¹) or absence (○, $k_d' = 0.075$ h⁻¹) of hormone.
0.075 h⁻¹ as values of the degradation rate constant in the presence (k_d) or absence (k'ₐₙ) of hormone, respectively. The biphasic nature of deinduction in Fig. 4B might indicate a small, more stable subpopulation of Adh-1 mRNA molecules; however, it should be noted that the mathematical treatment of the data tends to amplify experimental error as the new steady state is approached. Therefore, the calculated value of k'ₐₙ is based only on the slope of the first phase, which accounts for 80% of the Adh-1 mRNA. The values of kₐ and k'ₐₙ correspond to half-lives of 20 and 9.2 h, respectively. This suggests that testosterone has some effect on the stability of Adh-1 mRNA during induction although not a dramatic one. The appropriate degradation constant can be used to calculate the synthetic rate constant for Adh-1 mRNA synthesis in the presence (kₐ) or absence (k'ₐₙ) of hormone using the steady-state equation R = kₐ/kₐₙ where R is the concentration of Adh-1 mRNA. These values are 5.01 μg of Adh-1 mRNA·g total RNA⁻¹·h⁻¹ in the presence of hormone and 0.74 μg of Adh-1 mRNA·g total RNA⁻¹·h⁻¹ in the absence of hormone.

Transcription run-on reactions were performed on liver nuclei and kidney nuclei isolated from androgen-treated or control mice to determine if androgen stimulates transcription of the Adh-1 gene. Incorporation of labeled nucleotide into acid precipitable product was linear for 1 h in liver nuclei. Kidney nuclei from control and androgen-treated animals both exhibited a continuous increase in total incorporation from 1 h with a similar slight deviation from linearity. The Adh-1 transcripts were quantified by hybridization to immobilized pADHm16. Actin transcripts, which represent a non-inducible gene, were quantified by hybridization to the actin cDNA recombinant plasmid p91. Transcription rate of the Adh-1 gene is much higher in liver than control kidney (Fig. 5A) correlating with the difference in Adh-1 mRNA in these tissues. Androgen treatment results in an increase in Adh-1 transcription which is seen as a 3-4-fold induction as early as 12 h after administration. After 5 days of continuous androgen administration, the transcriptional rate of the Adh-1 gene in kidney has increased 6-fold over that of control male mice (Fig. 5B).

In vivo radiolabeling of newly synthesized RNA gave an independent assessment of the effect of androgen on the synthetic rate of Adh-1 mRNA (Table I). These data clearly indicate that androgen stimulates the fractional rate of Adh-1 synthesis and that the amount of the induction in synthesis correlates well with the fold induction in relative transcription rate.

To explore additional aspects of Adh-1 regulation, studies were undertaken to determine the requirement for androgen receptor during induction, to examine several inbred strains for possible genetic variation in the induction process, and to quantitate Adh-1 mRNA levels in various mouse tissues. The induction of Adh-1 mRNA in kidney tissue requires androgen receptor (Table II). Tfm mice lacking functional androgen receptor did not induce even when parabiosed with normal mice. This suggests that the receptor is needed in the target cells and cannot function to produce a secondary hormone which circulates to induce in the absence of functional receptor. Pituitary function is also not required for the induction of Adh-1 mRNA since hypophysectomized animals induce equally to controls.

The Adh-1 mRNA is induced in eight inbred mouse strains examined (Table III). Most strains induce 9-10-fold although BALB/cJ mice show a slightly greater induction primarily

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**TABLE I**

| Treatment | No. | Adh-1 mRNA | FRS* | Fold induction |
|-----------|-----|------------|------|---------------|
|           | ppm |            |      |               |
| Control   | 6   | 67-198     | 55.1 ± 9.4 |
| Induced   | 7   | 117-447    | 337 ± 55 |

*Fractional rates of synthesis (in parts/million) were determined by dividing the amount of label incorporated into Adh-1 mRNA by the amount of label incorporated into total RNA. Data were corrected for the efficiency of Adh-1 mRNA recovery by using a synthetic, internal, hybridization standard. Averages ± S.E. are shown for the indicated number of independent experiments.

**TABLE II**

| Conditions* | Strain | Testosterone* | Adh-1 mRNA* |
|-------------|-------|---------------|--------------|
|             |       | relative      |              |
| Parabiotic  | C3H (+/+)| + | 10.5 |
|             | C3H (Tfm/N)| | 0.7 |
| Control     | C57BL/6J| 0 | 1.0 |
| Control     | C57BL/6J| + | 6.3 |
| Hypophysectomized | C57BL/6J| + | 6.9 |

*All determinations were done in triplicate using at least two mice under each condition. RNA was prepared from the kidneys of individual mice and not pooled. Normal C3H females were parabiotically connected to Tfm mutants, which were genetically male but phenotypically female.

* Values are expressed relative to control C57BL/6J levels.
Androgen induction of Adh-1 mRNA in kidney tissue of recombinant inbred progenitor strains

| Strain   | Testosterone* | Adh-1 mRNA | Fold induction |
|----------|---------------|-------------|---------------|
| A/J      | 0             | 13          | 11            |
|          | +             | 148         |               |
| AKR/J    | 0             | 18          | 10            |
|          | +             | 177         |               |
| BALB/cJ  | 0             | 11          | 16            |
|          | +             | 179         |               |
| C3H/J    | 0             | 19          | 9             |
|          | +             | 169         |               |
| C57BL/6J | 0             | 17          | 10            |
|          | +             | 173         |               |
| C57L/J   | 0             | 15          | 10            |
|          | +             | 145         |               |
| DBA/2J   | 0             | 19          | 10            |
|          | +             | 195         |               |
| SWR/J    | 0             | 14          | 10            |
|          | +             | 132         |               |

*0 = no testosterone; + = testosterone.

All values are the average of two experiments.

**Table IV**

Adh-1 mRNA concentrations in tissues of C57BL/6J mice

| Tissue     | No.* | Adh-1 mRNA |
|------------|------|------------|
|            | µg/gr total RNA |
| Brain      | 4    | 3.2 ± 0.3  |
| Heart      | 3    | 3.1 ± 0.6  |
| Lung       | 1    | 16.7       |
| Liver      | 4    | 172 ± 46   |
| Kidney     | 4    | 15 ± 0.8   |
| Adrenals   | 1    | 284        |

*Number of animals on independent RNA preparations.

Average ± S.E. are given.

due to somewhat lower basal level. The variation in induction among the inbred strains examined does not appear to be enough to exploit genetically.

Adh-1 mRNA is also found in tissues other than liver and kidney (Table IV). Liver tissue has a 10-fold greater Adh-1 mRNA concentration than does kidney, and the level in lung is comparable to the kidney level. Brain and heart have much lower concentrations of Adh-1 mRNA. Adrenal tissue has the highest level of Adh-1 mRNA of the tissues examined. This was confirmed by Northern blot analysis on two additional RNA preparations (data not shown). It remains to be determined what processes from Adh-1 to mRNA are being regulated to lead to these diverse concentrations.

**DISCUSSION**

Androgens are known to increase the level of several gene products in mouse kidney (32). Some of these are identified only as increases in enzyme activity following androgen administration. However, with the increased availability of specific cloned DNA sequences, it has been possible to demonstrate that many of these enzyme inductions are accomplished by increasing the concentration of the respective mRNA.

The data presented here show that testosterone administration results in a 10–12-fold induction in alcohol dehydrogenase activity in mouse kidney. This induction is mediated by stimulating the synthesis of the protein, and the increased synthetic rate is sufficient to account for the total induction observed. This eliminates the need to suggest that androgen treatment decreases the degradation rate of this protein as occurs for ornithine decarboxylase protein in mouse kidney (33). Furthermore, using a specific Adh-1 cDNA probe to measure relative levels of Adh-1 mRNA, it was observed that during induction and deinduction, the level of Adh-1 mRNA correlates closely with the synthesis rate of this specific protein. Therefore, the level of Adh-1 mRNA increases with testosterone treatment in concert with the increase in specific alcohol dehydrogenase synthesis suggesting that androgen treatment exerts its effect on kidney alcohol dehydrogenase levels specifically by inducing Adh-1 mRNA concentration without any change in translational efficiency of this mRNA.

The major mechanism accounting for the androgen-induced increase in Adh-1 mRNA concentration is a stimulation of the transcription rate of Adh-1. This conclusion comes from three independent observations. First, direct support for this hypothesis was obtained in nuclear run-on experiments. Quantification of in vitro transcripts in nuclei obtained from kidney of control or androgen-treated animals showed that androgen induces Adh-1 transcription 6-fold. Second, measurements of the fractional rate of Adh-1 mRNA synthesis, determined by in vivo labeling, confirmed that androgen stimulates this rate by more than 6-fold. Although fractional rates of mRNA synthesis determined in intact animals are actually the products of both transcriptional and nuclear processing events (16), it appears that nuclear processing of Adh-1 transcripts is not regulated by androgen. The similarity between measurements of Adh-1 induction using both in vitro transcription and in vivo mRNA synthesis assays suggests that only transcription is androgen responsive. Third, the kinetic analysis of the time course of Adh-1 mRNA accumulation and loss as modulated by hormone suggests that the degradation constant for the Adh-1 mRNA is reduced in the presence of testosterone. However, the reduction in degradation rate is only 2-fold, thus the decrease is insufficient to account for the total increase in Adh-1 mRNA concentration which occurs with hormonal treatment. Using the calculated degradation constants and the steady-state levels of Adh-1 mRNA in the presence and absence of hormone, kinetic constants for Adh-1 mRNA synthesis were estimated and suggested that testosterone stimulates Adh-1 mRNA synthesis nearly 7-fold. There are several assumptions made in using the kinetic approach to determine synthesis and degradation constants of macromolecules (34). One is that the stimulus, in this case androgen, abruptly changes the kinetic constant(s) being affected. Clearly, the transcription rate of the Adh-1 gene is increased by 12 h, but it is not at the full level of induction ultimately achieved. Also, one assumes that the kinetic constants of synthesis and degradation do not continue to change during the entire time when the macromolecule is increasing in amount. Nevertheless, all three approaches used in this experimental system support the conclusion that androgen induces Adh-1 mRNA primarily by increasing its rate of synthesis by stimulating transcription of the Adh-1 gene. From these combined results, it appears clear that androgen-induced transcriptional activation of the Adh-1 gene plays a major role in the hormonal regulation of the level of this mRNA in mouse kidney.

These studies also confirm that androgen receptor is required for the testosterone-induced increase in Adh-1 mRNA concentration as demonstrated by the absence of induction in Tfm mice, which are deficient in the receptor protein (35). When a Tfm mouse is made parabiont with a normal mouse...
and both are supplied with hormone, the Tfm mouse still does not induce. This suggests that the receptor protein acts directly in androgen-responsive cells and is not involved in a secondary effect such as the production of another circulating hormone which functions as the true inducer. It was previously shown that pituitary function is not required for the induction of kidney alcohol dehydrogenase activity by testosterone (36), and, as expected, testosterone can induce Adh-1 mRNA in the absence of pituitary function. Interestingly, pituitary function is required for the testosterone-induced increase in kidney β-glucuronidase activity (36) suggesting that the mechanisms by which these two proteins are induced is different.

Induction properties of several androgen-regulated genes in mouse kidney are now known. The 10-fold induction of Adh-1 mRNA in mouse kidney is similar to that seen for KAP mRNA (37) and RP-2 mRNA, which is also known as MAKmRNA (38) and MK908 mRNA (14, 39). Ornithine decarboxylase mRNA concentration also increases 10–20-fold with androgen treatment (12) while androgen induces β-glucuronidase mRNA content as much as 100-fold (10, 13).

The androgen-induced increase in Adh-1 mRNA is fairly rapid and is evident as early as 12 h and peaks by around 75 h. In contrast, ornithine decarboxylase mRNA has slower induction kinetics and is maximal after about 120 h (14). β-Glucuronidase mRNA requires nearly 20 days of androgen treatment for full induction (19). Full induction of RP-2 mRNA is reported to occur by 36 h (38) and 90 h (14) in two independent studies. The reported differences in time required for full induction may be due to the use of different inbred strains in the two reports. Other steroid-responsive genes also exhibit variation in response time (40), possibly reflecting the time necessary to saturate multiple receptor-binding sites (31, 40). The variation in response time of the other androgen-responsive genes in kidney may simply reflect different mechanisms of induction. Although mRNA stabilization has been suggested as a major factor in the induction of ornithine decarboxylase, RP-2, and KAP mRNAs (14), it has recently been shown that androgen regulates either transcription or the nuclear processing of each of these mRNAs in mouse kidney (16). In contrast to the uncertainties about the mechanism of induction for other androgen-regulated mRNAs in mouse kidney, it is clear that transcriptional activation of the Adh-1 gene by androgen plays a dominant role in the induction of kidney alcohol dehydrogenase.

Androgen has been shown in other responsive tissues to act transcriptionally. Mouse liver major urinary protein mRNA is induced by transcriptional activation (14, 17) as is ovomucoid mRNA in chick oviduct (41). Using nuclear run-on assays, genes encoding rat prostatic binding proteins were shown to be transcriptionally activated by androgen, but the effect was too small to account for the large increase in mRNA concentrations and an effect on mRNA stabilization was also suggested (18). However, measurements of nuclear RNA using intron-specific probes indicated that testosterone acts primarily within the nucleus, implicating either transcription or processing of nuclear mRNA as the major androgen-regulated mechanism (19). Transcriptional activation of a gene by hormone treatment would presumably involve interaction between a hormone-receptor complex and DNA sequences in the target gene. Comparative sequence analysis of 5’-flanking regions of six androgen-regulated genes failed to clearly demonstrate any potential androgen-responsive sequences, although all had regions of dyad symmetry upstream of the promoter (42). Two rat genes regulated by androgen in prostate and seminal vesicle have been reported to share sequence homology in a 30-nucleotide sequence located in the 5’-end of these genes (43). More recently, systems for transfection into androgen-responsive cell lines have been developed in which the transfected sequences are androgen-regulated (44). Such systems are clearly needed to study sequences in the Adh-1 gene necessary for testosterone-induced transcriptional activation.

In summary, the Adh-1 gene appears to be the first androgen-responsive gene in the mouse kidney for which transcriptional activation by hormone has been demonstrated. Among several strains of laboratory mice, little variation in the extent of Adh-1 mRNA expression or induction was observed; however, in an examination of several closely related wild Mus species variation in the phenotype for androgen-induction of the Adh-1 mRNA was observed. Some species have a Adh-1 unresponsive to androgen, and the induction phenotype does not follow evolutionary relatedness between the species. It should be informative to exploit these other Adh-1 genes in determining the DNA sequences involved in androgenic steroid induction, and to study the evolution of this aspect of Adh-1 regulation.

Acknowledgments—We thank Susan Elliger and Mary Ann Hardwick for excellent technical assistance. We thank Dorothy Tabron for providing the parathyroid and hypophysectomized mice used in these experiments. Drs. K. Paigen, M. Dewey, F. Berger and L. Bracey participated in many helpful discussions. One of us (M. R. F.) is especially grateful to Dr. K. Paigen for the hospitality shown during a stay in his laboratory when part of this work was completed. We thank D. Williams and C. Cook for their help in the preparation of this manuscript.

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Androgen Induction of Kidney Alcohol Dehydrogenase

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