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Regulation of human renin expression in chorion cell primary cultures

(gene expression/placenta/hypertension/cyclic AMP)

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ABSTRACT **The human renin gene is expressed in the kidney, placenta, and several other sites. The release of renin or its precursor, prorenin, can be affected by several regulatory agents. In this study, primary cultures of human placental cells were used to examine the regulation of prorenin release and renin mRNA levels and of the transfected human renin promoter linked to chloramphenicol acetyltransferase reporter sequences. Treatment of the cultures with a calcium ionophore alone, calcium ionophore plus forskolin (that activates adenylate cyclase), or forskolin plus a phorbol ester increased prorenin release and renin mRNA levels 1.3- to 6-fold, but several classes of steroids did not affect prorenin secretion or renin RNA levels. The transfected renin promoter (584 or 100 base pairs of 5′-flanking DNA) initiated at the correct start site in these cells and forskolin increased its expression 2.5- to 4-fold. Constructs containing renin 5′-flanking DNA linked to a heterologous promoter cotransfected into HeLa cells with either glucocorticoid or estrogen receptor expression vectors were not regulated by dexamethasone or 17β-estradiol. These results suggest that (*i*) the first 584 base pairs of the renin gene 5′-flanking DNA do not contain functional glucocorticoid or estrogen response elements, (*ii*) placental prorenin release and renin mRNA are regulated by calcium ion and by the combinations of cAMP with either C kinase or calcium ion, and (*iii*) the first 100 base pairs of the human renin 5′-flanking DNA direct accurate initiation of transcription and can be regulated by cAMP. Thus, some control of renin release in the placenta (and by inference in other tissues) occurs via transcriptional influences on its promoter.**

The renin–angiotensin system is important for regulating blood pressure, fluid volume, and other responses (1, 2). Circulating renin of renal origin is rate limiting for generation of angiotensin II, the potent vasoconstrictor and stimulator of aldosterone release (1, 2). However, renin, its precursor prorenin, and other components of the renin–angiotensin system are also produced in extrarenal sites (2–9). Renin levels are affected by several factors, but the mechanisms for such influences are not well defined.

The placenta is a major extrarenal site of renin gene expression. It releases prorenin into the amniotic fluid, where prorenin levels can exceed those in plasma 100-fold (4). Prorenin is also synthesized by cultured placental cells (5–8). Whether chorionic cytotrophoblasts (5–8) or uterine decidual cells present in the preparations are responsible for this prorenin synthesis (9) is a controversial subject. Nevertheless, those cells that produce prorenin contain factors that activate the human renin promoter and are useful for studying its control.

Candidate signals that may affect renin gene transcription include the second messengers for polypeptide and catecholamine hormones, such as cAMP (activated by forskolin; refs. 10 and 11), calcium ion, C kinase (activated by phorbol esters; ref. 12), and steroid hormones (13). Several stimuli that activate adenylate cyclase affect renin or prorenin release: β-adrenergic stimuli enhance renal renin release (2, 14); chorionic gonadotropin and luteinizing hormone increase plasma prorenin levels (15, 16); and forskolin increases prorenin release from placental preparations (5). Angiotensin II inhibits renal renin release and its actions may be mediated by calcium ion and protein kinase C (2, 17–19). Estrogens can increase plasma renin levels (20) and may stimulate prorenin release from the human ovary (15, 21). Mineralocorticoid hormones block renin release through their salt-retaining actions (2). The renin gene 5′-flanking DNA contains sequences that have some homology to estrogen and glucocorticoid response elements (22), but it is unclear whether any of these steroids affect renin gene transcription.

In the current studies, human chorion cell primary cultures have been used to study the control of both the endogenous renin gene and of transfected genes containing human renin gene 5′-flanking DNA. The results show that endogenous renin gene expression can be regulated by calcium ion alone or with forskolin, and forskolin plus a phorbol ester, but it is not affected by steroid hormones. They further show that the activity of the transfected renin promoter containing as few as 100 base pairs (bp) of 5′-flanking DNA can be regulated by forskolin, but the first 584 bp of renin 5′-flanking DNA do not appear to contain estrogen or glucocorticoid response elements.

MATERIALS AND METHODS

Primary Cell Culture, Renin Assays, and RNA Analysis. Cell cultures were prepared from 30–50 g of human chorion laeve essentially as described by Poisner *et al.* (6). Minced membranes were incubated overnight at 8°C in Dulbecco's modified Eagle's medium H21 (DMEM H21) containing 10% fetal calf serum (FCS) and then treated with 0.05% trypsin for 1 hr at 22°C. The tissue was washed three times, resuspended in DMEM H21 containing 100 μg of bacterial collagenase per ml (Worthington CLSII), and incubated for 3 hr at 37°C. The cells were washed three times with phosphate-buffered saline (PBS), sequentially filtered through 1.0- and 0.5-mm sterile nylon mesh filters, pelleted, resuspended in 10 ml of PBS, and separated by Percoll (Pharmacia) step gradients. The cells at the 15–37% interface were collected and plated at 40–50% confluency on 150-mm plates in DMEM H21 containing 10% FCS. Cells were treated with drugs after 7–10 days in culture. For renin assays, cells were incubated in fresh DMEM H21 containing 10% FCS with or without drugs for 24 hr. The media were removed and fresh medium with drugs was

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Abbreviations: CAT, chloramphenicol acetyltransferase; TK, thymidine kinase; PMA, phorbol 12-myristate 13-acetate.

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added. After an additional 24 hr, aliquots of media were assayed for prorenin levels, after activation of prorenin by digestion with trypsin (50 μg/ml) for 1 hr at 22°C, with a Gammacoat ^{125}I-labeled renin radioimmunoassay kit (Baxter Travenol Diagnostics, Cambridge, MA). Total cellular RNA was prepared (23), denatured with glyoxal and dimethyl sulfoxide, and 30 μg was loaded per well on a 1.2% agarose gel (24). After electrophoresis, the RNA was transferred to nitrocellulose by blotting and was baked in a vacuum oven at 70°C for 2 hr. Membranes were prehybridized in 1 M NaCl/1% SDS/10% dextran sulfate/tRNA (50 μg/ml) at 60°C for 2 hr. Hybridization was in fresh buffer at 60°C for 24 hr with ^{32}P (3×10^6 cpm/ml) end-labeled 27-base oligonucleotide probe (specific activity, 10^8 cpm per μg of DNA) complementary to human preprorenin mRNA encoding amino acids 10–18 (22). Membranes were washed in 2× standard saline citrate (SSC) (24)/0.1% SDS for 20 min at 22°C and then in 1× SSC/0.1% SDS for 30 min at 60°C, and exposed to x-ray film.

Plasmid Constructions. Plasmid hR-584CAT contained a 595-bp *Sau*3A fragment of the human renin gene (−584 to +11; numbering with respect to the transcriptional start site) (22) inserted into the *Bam*HI site of the pUC9 polylinker. DNA containing the chloramphenicol acetyltransferase (CAT) gene coding sequence and the simian virus 40 splice and polyadenylylation signals was introduced into the plasmid at the *Bam*HI site on the 3′ end of the renin promoter. The 5′ deletion of the renin 5′-flanking sequences was produced by BAL-31 digestion followed by *Eco*RI linker addition and cloning of the deleted fragment (−100 to +11) into the *Eco*RI/*Bam*HI sites of the pUC19 polylinker (24). Renin gene 5′-flanking DNA fragments were also inserted adjacent to the herpes simplex virus thymidine kinase (TK) promoter (−109 to +52) linked to the CAT gene in pUC19 by blunt-end ligation into the *Bam*HI site at its 5′ end. Individual plasmids contained renin gene DNA: from the *Sau*3A1 site at −584 to the *Kpn* I site at −146 in its native orientation (phR17TKCAT), and in the opposite orientation (phR3TKCAT); from −146 to +11 (*Kpn* I to *Sau*3A1 sites) in its native orientation (phR2TKCAT), and in the opposite orientation (phR8TKCAT). Plasmid hRCATGM4 was constructed by subcloning the *Kpn* 1/*Eco*RI fragment of phR584CAT (−146 in the renin 5′-flanking DNA to the *Eco*RI site 250 bp into the CAT gene) into pGEM4 (Promega). Other plasmids used were as follows: pATG2 and pAER, containing human glucocorticoid (25) and estrogen (26) receptor expression vectors; pTATCAT and pMMTVCAT, which contain glucocorticoid response elements from the rat tyrosine aminotransferase gene (27) and mouse mammary tumor virus long terminal repeat (28), respectively, upstream of TKCAT; pA$_2$CAT, the *Xenopus laevis* vitellogenin A$_2$ gene estrogen response element (−331/−89) upstream of a TKCAT gene (29); phMThGH, human growth hormone coding sequences downstream of the human metallothionein II$_A$ promoter (30); pTESΔNH152CAT, human chorionic gonadotropin α-subunit cAMP response element (−152/+4) upstream of a TKCAT gene (31).

Transient Expression Assays. Transfections were performed by calcium phosphate precipitation (32) on 150-mm plates of chorion cells cultured for 10 days in 30 ml of DMEM H21 with 10% FCS. Each plate received 30–40 μg of test plasmid in 1 ml of buffer. After 20 hr, the cells were "shocked" with 15% (vol/vol) glycerol in DMEM H21 for 3 min, and fresh medium either with or without drugs was added. After 8 hr, the cells were harvested by freeze–thawing in 0.2% Triton X-100/0.25 M Tris·HCl, pH 7.5, for CAT assays or extraction with 5 M guanidine isothiocyanate for RNA purification. HeLa cell transfections (10 or 20 μg of plasmid) were done in 100- or 150-mm plates in 15 or 20 ml of DMEM H21 with 2% FCS, which had been stripped of steroids (33). For receptor cotransfection, a 3:1 ratio of test to receptor expression plasmid was used. After 6 hr, the cells were glycerol shocked and fresh DMEM H21 with 2% stripped FCS with or without steroids was added. Cells were harvested 20 hr later. CAT assays were performed with 100 μg of protein in 150–200 μl of 150 mM Tris·HCl, pH 7.5/1 mM acetyl coenzyme A/0.2 μCi of [^{14}C]chloramphenicol (50 mCi/mmol; 1 Ci = 37 GBq; Amersham) for 4 hr at 37°C (in the liner range). Protein concentrations were determined by using the Pierce protein assay reagent. The extent of acetylation was determined by thin-layer chromatography and liquid scintillation counting of radioactive spots. In some experiments, phMThGH was cotransfected at 1/10th the concentration of the test plasmid and human growth hormone levels in the media were measured with a Tandem-RHGH immunoradiometric assay kit (Hybritech). Statistical analyses were performed by using the Wilcoxon paired-sample test (34).

RNase Protection Assay. CAT mRNA levels were measured and transcriptional start sites were mapped by RNase protection assays. Plasmid hRCATGM4 was transcribed *in vitro* by SP6 polymerase (Promega) according to the manufacturer's directions. Full-length probe was isolated on a 5% polyacrylamide sequencing gel and eluted in 1 ml of 10 mM Tris·HCl, pH 8.0/1 mM EDTA/300 mM NaCl/1% SDS/proteinase K (40 μg/ml) at 37°C. After 1 hr, the probe was extracted and hybridized with 50 μg of chorion RNA for 20 hr in 30 μl of 80% formamide/400 mM NaCl/40 mM Pipes, pH 6.7/1 mM EDTA at 60°C. Samples were digested by addition of 300 μl of 10 mM Tris·HCl, pH 8.0/5 mM EDTA/300 mM NaCl/RNase A (10 μg/ml). After 1 hr at 37°C, 20 μl of 10 mM Tris·HCl, pH 8.0/5 mM EDTA/1% SDS/proteinase K (2 μg/ml)/tRNA (1 μg/ml) was added. After 30 min, the samples were extracted, precipitated, and electrophoresed on 6% polyacrylamide gels.

RESULTS

Endogenous Renin Gene. Chorion cell primary cultures produced negligible renin and 50–350 ng of angiotensin I per ml per hr of trypsin-activatable renin activity (prorenin) per 24 hr per confluent plate of cells (≈0.2 g of wet cell pellet), confirming previous results (5–7). Fig. 1 shows the responses to forskolin, phorbol ester (phorbol 12-myristate 13-acetate; PMA), calcium ionophore (A23187), and various combina-

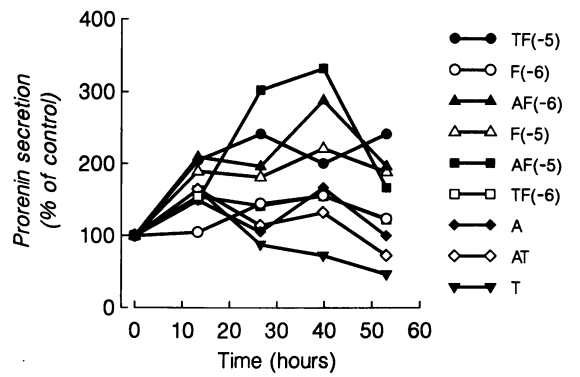

FIG. 1. Time courses of various agents on prorenin release. Results are expressed as prorenin secreted per the preceding 13.25-hr period, relative to untreated levels. Fresh drugs and media were added at each time point. A (◆), 1 μM calcium ionophore; F(−6) (○), 1 μM forskolin; F(−5) (△), 10 μM forskolin; T (▼), 10 nM PMA; AF(−6) (▲), 1 μM calcium ionophore + 1 μM forskolin; AF(−5) (■), 1 μM calcium ionophore + 10 μM forskolin; AT (◇), 1 μM calcium ionophore + 10 nM PMA; TF(−6) (□), 10 nM PMA + 1 μM forskolin; TF(−5) (●), 10 nM PMA + 10 μM forskolin. Results are expressed as percent of control (untreated) cells at each time point. Control prorenin ranged from 200 to 300 ng of angiotensin I·ml^{-1}·hr^{-1}. The control is 100% at each time point.

Table 1. Effect of drug treatments of chorion cell cultures on prorenin release

Treatment	Prorenin release, ng of AI·ml^{-1}·hr^{-1} (range)	Relative prorenin release ± SE	*n*	*P**
Untreated	67–330	1.0	6	—
A23187 (1 μM)	86–375	1.34 ± 0.11	5	<0.05
Forskolin (10 μM)	68–410	1.16 ± 0.09	6	>0.10
PMA (0.1 μM, 10 nM)	41–165	0.75 ± 0.08	6	<0.05
A23187 + forskolin (10 μM)	236–650	2.48 ± 0.34	4	<0.05
A23187 + PMA (0.1 μM)	47–300	0.80 ± 0.17	4	>0.20
PMA (0.1 μM) + forskolin (1 μM)	110–405	1.55 ± 0.14	4	<0.05

Cells were incubated with drugs for 40 hr (one experiment), for 48 hr (four experiments), or for 53 hr (one experiment). Results are given as means ± standard errors of two to six experiments relative to untreated levels. AI, angiotensin I.
*Two-tailed *t* test.

tions. Most responses were observed by 13 hr, were maximal between 27 and 40 hr, and tended to decline after 40 hr. Forskolin at 10 μM stimulated prorenin secretion by ≈2-fold; 1 μM forskolin gave a lower response. The calcium ionophore had a modest effect and PMA tended to be inhibitory after a modest early stimulation. The effect of forskolin plus the ionophore exceeded that of forskolin alone, whereas the effect of PMA plus forskolin differed only modestly from that of forskolin alone.

Table 1 shows the results from a number of experiments of the effects of these drugs after 40–53 hr. The calcium ionophore increased prorenin secretion slightly but significantly (1.3-fold; $P < 0.05$). Forskolin at 1 μM did not significantly affect prorenin secretion. PMA had a small inhibitory effect. Forskolin plus either calcium ionophore or PMA showed synergistic stimulations of prorenin secretion of 2.5- and 1.6-fold, respectively ($P < 0.05$). By contrast, dexamethasone, progesterone, or estradiol did not affect prorenin secretion (data not shown).

Fig. 2 shows the effects of these drugs for 8 or 48 hr on prorenin mRNA levels. The calcium ionophore had no effect at 8 hr and had a small stimulatory effect at 48 hr. The lanes had equal quantities of RNA based on the 18S and 28S RNA content (see legend to Fig. 2), in spite of the appearance of more control lamin mRNA in the A and AF lanes, which probably results from smearing of the renin signal due to the large amounts of RNA (30 μg) applied. Forskolin at 1 μM had no effect and PMA had no or a slightly stimulatory effect (1.2-fold) at 8 hr and decreased renin mRNA levels at 48 hr. All three combination treatments increased prorenin mRNA by 8 hr and the effects were greater at 48 hr, at which time the relative renin mRNA levels for each drug or combination correlated fairly well with the relative amounts of prorenin secreted into the medium (Table 1 and Fig. 1). Forskolin plus calcium ionophore or PMA increased renin mRNA levels by 6.3- and 3.1-fold, respectively. Dexamethasone, progesterone, estradiol, aldosterone, or testosterone at 0.1 μM had no effect on renin mRNA levels (data not shown).

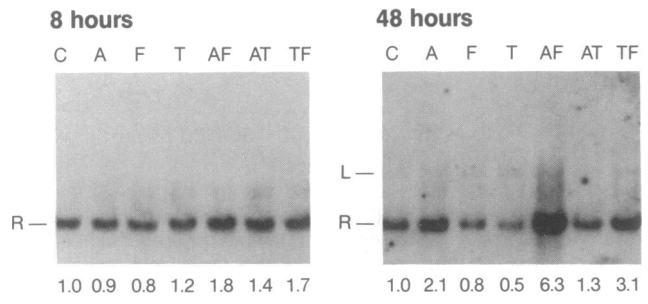

FIG. 2. Effect of various agents on renin mRNA levels. The following drugs were used: A, 1 μM calcium ionophore: F, 1 μM forskolin: T, 0.1 μM PMA. Levels of renin mRNA (R) relative to untreated cells (C) (determined by densitometric scanning) are given beneath each lane. As internal controls, the gels showed equal amounts of 28S and 18S RNA by UV shadowing and the 48-hr samples were probed for human lamin mRNA (L).

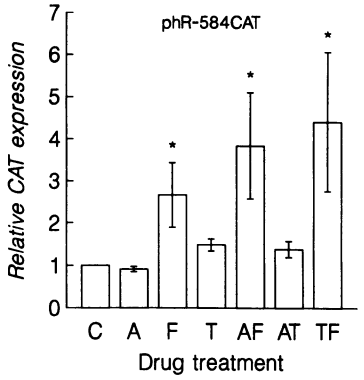

FIG. 3. Effect of various drugs on the transfected human renin promoter. The following drugs were used: A, 1 μM calcium ionophore; F, 1 μM forskolin; T, 10 nM PMA. Each experiment was performed on a chorion cell preparation by splitting one calcium phosphate precipitate onto seven plates of cells. Results are the means ± standard errors of eight experiments relative to untreated cells (C). *, $P < 0.05$ (Wilcoxon test).

Transfected Renin Gene Promoter. Effects on the transfected renin promoter were assessed after an 8-hr incubation, at which time endogenous renin mRNA levels were increased by forskolin plus either a calcium ionophore or PMA and were not decreased by PMA (Figs. 3 and 4). The transfections involved calcium phosphate uptake, which can stimulate the transcription of other genes (35), and which may act in synergy with forskolin or a phorbol ester. Activity of phR-584CAT or phR-100CAT was stimulated 2.7- to 3.8-fold by forskolin alone (F; $P \leq 0.05$) and ≈1.4-fold by PMA (T; $P < 0.05$ for phR-584CAT but insignificant for phR-100CAT). The combination of the calcium ionophore or PMA with forskolin also increased promoter activity by 3- to 4-fold ($P \leq 0.05$), although these increases were not significantly greater than those with forskolin or PMA alone. PMA with the ionophore resulted in statistically insignificant increases in phR-

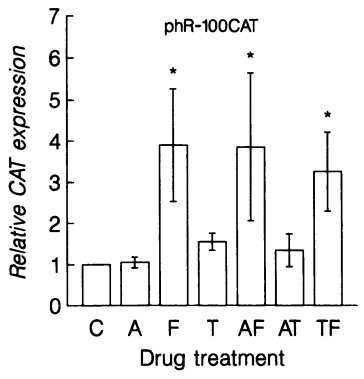

FIG. 4. Effects of drugs on the transfected renin promoter deleted to −100. The following drugs were used: A, 1 μM calcium ionophore; F, 10 μM forskolin; T, 10 nM PMA. Experiments were performed and the results are expressed as described in Fig. 3. *, $P < 0.05$ (Wilcoxon test).

584CAT or phR-100CAT activity. Since forskolin (at 10 μM) plus calcium ionophore did not affect the activity of ph-MThGH (see *Materials and Methods*; data not shown), the effect of forskolin and calcium ionophore on the renin promoter is specific. As shown in Fig. 5, transcripts from phR-100CAT are initiated at the start site mapped for the kidney (22). Furthermore, transcripts initiated from this start site were stimulated by forskolin alone (4.4-fold) or in combination with calcium ionophore (4.5-fold).

Heterologous Promoter Analysis. Hybrid genes containing the renin gene 5′-flanking DNA linked to the TK promoter and CAT gene coding sequences transfected into chorion cells did not show either basal or forskolin plus calcium ionophore-inducible expression to a level greater than pTKCAT, whereas pTESΔNH152CAT, containing a cAMP response element (see *Materials and Methods*), was induced >8-fold (data not shown). Thus, the cAMP response element of the human renin gene does not appear to function as a classical enhancer element. HeLa cells were cotransfected with the renin–TK–CAT constructs and vectors that express either the human glucocorticoid or estrogen receptors. None of the constructs containing portions of the renin gene 5′-flanking DNA was regulated by either 0.1 μM 17β-estradiol or by 0.1 μM dexamethasone after 20 hr of drug treatment, whereas the control plasmids pTATCAT and pMMTVCAT were induced 15- and 4-fold, respectively, by dexamethasone, and pA2CAT was induced ≈17-fold by 17β-estradiol (data not shown). These results suggest that the renin 5′-flanking DNA (−584 to +11) does not contain sequences that respond to these steroids.

DISCUSSION

In these studies, primary cultures of human chorion laeve cells were used to study human renin gene expression. Confirming previous results (5), these cells released prorenin but not renin. Prorenin secretion was increased by the calcium ionophore A23186 and by forskolin in the presence of the ionophore or PMA, whereas PMA or forskolin (1 μM) alone had little effect. However, Poisner *et al.* (5) found that forskolin at 50 μM stimulated prorenin secretion by ≈1.7-fold, and we noted a 2-fold effect of 10 μM forskolin on prorenin release (Fig. 1). Thus, calcium ion stimulation may be required for effects on prorenin production by lower but not higher concentrations of cAMP. The stimulation of prorenin release by 1 μM forskolin and PMA under conditions in which neither agent alone gave a response may indicate that forskolin either blocks a down-regulation of C kinases (since PMA alone may have a transient effect; Fig. 1) or acts synergistically with it. The activities of PMA and calcium ion could be interrelated in that calcium might increase C kinase activity (36) since both agents enhance the response to 1 μM forskolin. The latter (at 1 μM) given with either the ionophore or PMA also increased prorenin mRNA levels by 6.3- and 3.1-fold, respectively, by 48 hr, suggesting that these three mediators affect prorenin release by increasing prorenin mRNA levels.

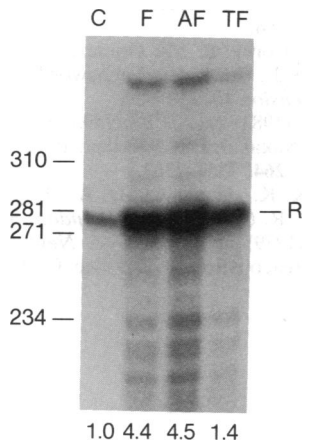

FIG. 5. Effect of drugs on correctly initiated RNA transcripts of phR-100CAT. The following drugs were used: F, 10 μM forskolin; AF, 10 μM calcium ionophore plus 10 μM forskolin; TF, 10 μM forskolin plus 10 nM PMA. RNA levels were measured by the RNase protection assay. Correctly initiated transcripts are indicated (R). Relative RNA levels are given at the bottom of each lane.

These results imply that renin mRNA levels could also be regulated by the same mediators in other cells. The control of renal renin release by β-adrenergic stimulation (presumably via cAMP) and the increase in rat adrenal renin content in response to calcium ion (37, 38) could be due in part to increases in renin mRNA. By contrast, calcium ion may act by a different mechanism in the kidney, where it inhibits renal renin release (39). Systems that respond to cAMP and Ca^2 have been classified as unidirectional or bidirectional depending on whether the two messengers act in the same or opposite directions (40). Chorion laeve may be a unidirectional system with respect to their calcium and cAMP regulatory pathways, since both messenger systems positively regulate prorenin synthesis.

The placenta cells were also used to study the transfected renin promoter. The promoter containing 100 bp of 5′-flanking DNA linked to CAT coding sequences was found to initiate transcription at the same start site as the kidney (22). Forskolin (10 μM) increased the expression of transfected renin–CAT genes containing either 100 or 584 bp of 5′-flanking DNA by 2.5- to 4-fold and this response was elicited at the correct start site on phR-100CAT (4.5-fold). It was difficult to assess the influence of Ca^2 uptake, since this was stimulated by the DNA transfections. Thus, the calcium ionophore or PMA alone or in combination with forskolin had no or a statistically insignificant effect. Nevertheless, these results indicate that the renin promoter can respond to cAMP, and this can explain cAMP effects on renin mRNA levels and prorenin release by the placenta and on renin and prorenin release in other tissues (see Introduction).

It is not clear how the cAMP responsiveness of the first 100 bp of the renin 5′-flanking DNA is mediated. There are no significant DNA sequence homologies with the cAMP response element core consensus (10, 31) in this region of the renin promoter, and the renin DNA containing the cAMP-responsive sequences did not behave as a cAMP response element when linked to a heterologous promoter. Sequences in the −80 to −90 region of the renin promoter resemble the AP2 binding site, which mediates cAMP and phorbol ester induction of the simian virus 40 enhancer, but we failed to detect an effect of cAMP in these cells on a transfected metallothionein promoter that contains an AP2 site (12). Burt *et al.* (41) reported that the expression of constructs containing 584 bp of the human renin 5′-flanking DNA linked to the TK promoter and transfected into JEG-3 cells was increased by cAMP, but the stimulation was only 60%. The authors suggested that cAMP responsiveness was due to sequences located ≈140 bp upstream of the CAP site, based on a stimulation of <30% with constructs containing renin gene 5′-flanking DNA linked to a heterologous promoter. Our observation that constructs bearing only 100 bp of human renin 5′-flanking DNA are induced by cAMP make the −140 region an unlikely candidate as the major cAMP-responsive region in the renin promoter. Furthermore, unlike Burt *et al.* (41), we did not obtain cAMP effects on transfected plasmids carrying this region of the renin 5′-flanking DNA linked to a heterologous promoter. Taken together, these data imply that cAMP induction of renin gene expression occurs through the action of trans-acting factors distinct from cAMP response element binding protein (42) and that the spatial relationship

of factor binding sites in the first 100 bp of the renin promoter is important for this stimulation.

Our studies also addressed whether renin gene expression can be affected by steroid hormones. Placental prorenin release and renin mRNA levels were not affected by dexamethasone, progesterone, or estradiol. Whereas this could mean that the prorenin-expressing placental cells do not respond to steroids, the placenta is known to contain steroid hormone receptors (43). To address this issue further, vectors containing renin 5′-flanking sequences linked to TKCAT were cotransfected with estrogen or glucocorticoid receptor expression plasmids into HeLa cells and were found not to respond to estradiol or dexamethasone, whereas plasmids containing known estrogen or glucocorticoid response elements did respond to the respective steroid. Thus, the first −584 bp of the renin gene 5′-flanking DNA, including the sequences centered around −351 and −275, which were previously proposed as steroid response elements (22), do not appear to contain estrogen or glucocorticoid response elements. Although functional steroid response elements could exist elsewhere in the renin gene, the combined data raise the possibility that most of the effects of steroid hormones on renin release are indirect.

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