Biochemical Pharmacology, Vol. 53, pp. 1883–1888, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(97)00058-0

β-Adrenergic Regulation of Renin Expression in Differentiated U-937 Monocytic Cells

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ABSTRACT. Previous studies from our laboratories demonstrated that human decidual macrophages and peripheral mononuclear cells express renin. In the present study, we found that U-937 monocytes, induced to differentiate into macrophage-like cells by treatment with phorbol dibutyrate (PDBU), express renin mRNA and release renin (95% of which is in the form of prorenin). Treatment of these PDBU-exposed cells with dibutyryl-cAMP (1 mM) caused a 20-fold increase in renin mRNA and a 10-fold increase in prorenin release. Forskolin (10 μM), an activator of adenylyl cyclase, and terbutaline (100 μM), a β_2-adrenergic agonist known to increase cAMP levels, also increased renin mRNA and prorenin release. The secretory response to terbutaline was potentiated by the type IV cyclic AMP-phosphodiesterase (PDE) inhibitor Ro 20-1724 (50 μM). Angiotensin II agonist inhibited the stimulatory effect of terbutaline on renin secretion as did the cytokines tumor necrosis factor-α and lipopolysaccharide plus interferon-γ. Since other studies have shown that U-937 cells possess β_2-adrenergic receptors and express mainly the type IV PDE, the present findings strongly suggest that β-adrenergic receptors in mononuclear cells are coupled to renin expression via the cAMP transduction pathway. The results support a possible role for the renin-angiotensin system in macrophage function and suggest potential autocrine regulatory mechanisms in prorenin expression. BIOCHEM PHARMACOL 53;12:1883–1888, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. renin; prorenin; U-937 cells; macrophage; β-adrenergic; cyclic AMP; cytokines (TNF-α, interferon-γ); phorbol ester; angiotensin

Renin is synthesized and released by many cell types, including JGA‖ cells, trophoblasts, and decidual stromal cells. JGA cells release renin in response to physiological stimuli primarily in the form of active renin, while extrarenal cells release renin primarily in the form of prorenin, the unprocessed precursor [1]. In JGA cells, renin is stored and released from large cytoplasmic granules, while renin in extrarenal cells appears to be released by a constitutive mechanism [2]. However, the mechanisms involved in the synthesis and release of extrarenal prorenin are poorly understood.

We have demonstrated that human decidual macrophages and peripheral mononuclear cells express and release renin, primarily in the form of prorenin [3]. To examine the regulation of prorenin in macrophage-like cells, we have used the U-937 cell line, which can be induced to terminal monocyte/macrophage differentiation by several agents, including phorbol ester. In addition, we have examined the effects of a number of agents on renin secretion from the differentiated cells. These include agents that modify intracellular cAMP, which is known to activate the renin gene [1]: dibutyryl-cAMP, forskolin, Ro 20-1724, and terbutaline. We have also examined the effects of agents that are known to decrease renin expression in extrarenal tissues: angiotensin II [4], TNF-α [5], IFN-γ [6], and LPS [7]. A preliminary report of our findings has appeared [8].

MATERIALS AND METHODS

Cell Culture

U-937 cells were obtained from the American Type Culture Collection. The cells were maintained with RPMI-1640 medium (Gibco BRL, Gaithersburg, MD) supplemented with 10% FBS (Hyclone, Logan, UT) at 37° in a humidified atmosphere of 95% air/5% CO_2. The cells (1×10^6 cells/well) were plated in 24-well culture plates and cultured with RPMI-1640 containing 5% FBS and 15 mM HEPES, and differentiated with 100 nM PDBU (Sigma Chemical Co., St. Louis, MO) for 3 days [9]. In our studies, we employed PDBU as a differentiation-inducing agent

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‖ *Abbreviations:* JGA, juxtaglomerular apparatus; cAMP, cyclic AMP; FBS, fetal bovine serum; RT–PCR, reverse transcription–polymerase chain reaction; GADPH, glyceraldehyde-3-phosphate dehydrogenase; ANGIO, Sar-1-angiotensin II; PDBU, phorbol dibutyrate; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; LPS, lipopolysaccharide; and PDE, cyclic adenosine 3′,5′-monophosphate phosphodiesterase.

Received 11 July 1996; accepted 2 January 1997.

because of its better aqueous solubility and its ability to be washed from cells [9]. After washing with PDBU-free medium, the cells were treated with control medium or with medium containing terbutaline (Sigma) in the presence or absence of Sar-1-angiotensin II [angiotensin II agonist (ANGIO), Sigma], dibutyryl-cAMP (Sigma), or cytokines for another 3 days with daily medium changes. All chemicals were sterilized before use, and there was no evidence of contamination.

RNA Extraction and Northern Analysis

RNA analysis was performed as previously described [3]. The extraction of total cellular RNA was based on the single-step method by Chomczynski and Sacchi [10]. Cells were lysed by the addition of 1.0 mL of TRI-Reagent solution (Molecular Research Center, Inc., Cincinnati, OH) for every 1×10^6 cells and solubilized by passing the lysate through a 1-mL pipette. RNA was extracted and precipitated according to the manufacturer's instructions. The final pellet was resuspended in 1 mM EDTA (pH 7.0).

Total cellular RNA from control and cytokine-exposed decidual cells was separated by electrophoresis on 1% agarose gel containing formaldehyde, transferred to a nylon membrane (Nytran; Schleicher & Schuell, Keene, NH), and cross-linked by UV irradiation. Hybridization was performed using a human renin 228 bp cDNA fragment (spanning exons 4–7) labeled with [^{32}P]dCTP by random priming according to the manufacturer's recommended protocol (Prime-It II; Stratagene, La Jolla, CA). Hybridization was performed at 57° overnight. The membranes were washed under stringent conditions (final wash: 0.5× SSC at 60°; 1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and autoradiographed at −70° for 12–72 hr with an intensifying screen. The renin cDNA probe was removed by washing in 0.1× SSC/0.5% SDS at 65° for 1 hr, and the membrane was rehybridized with a ^{32}P-labeled 195 bp human GAPDH cDNA fragment and autoradiographed as described above. Autoradiograms were analyzed by PhosphoImager (Molecular Dynamics, Inc., Sunnyvale, CA), using ImageQuant software (Molecular Dynamics).

Semi-quantitative RT–PCR

First strand DNA was synthesized at 42° for 60 min using 200 ng of total RNA, 100 U of M-MLV reverse transcriptase (Gibco BRL), 0.5 mg of oligo(dT) primer (Gibco BRL), dNTP (0.5 mM each of dGTP, dATP, dTTP, and dCTP; Gibco BRL) and 2 mCi of [^{32}P]dCTP in RT buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 10 mM dithiothreitol, 3 mM $MgCl_2$). The DNA was then amplified using 1.25 U of Taq DNA polymerase (Gibco BRL), each primer (renin: sense primer, 5′-TCAGCCAGGACATCAT CACC-3′; antisense primer, 5′-TCTCGGAATCTCTGT TGTAG-3′ for 228 bp products [exon 4–7]; or sense primer, 5′-AAATGAAGGGGGTGTCTGTGG-3′; antisense primer, 5′-CGAAGCCAATGCGGTTGTTAC-3′ for 402 bp products [exon 7–10]; GAPDH: sense primer, 5′-CCATGGAGAAGGCTGGGG-3′; antisense primer, 5′-CAAAGTTTGTCATGGATGACC-3′ [11, 12]) in PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl and 2 mM $MgCl_2$) for 25 cycles by Perkin–Elmer Cetus PCR Thermal Cycler (Norwalk, CT). The number of PCR cycles was based on kinetic studies performed to establish the optimum number of cycles, yet remain in the exponential part of the amplification curve, for both control and drug-treated samples (data not shown). The amplification profile involved denaturation at 94° for 1 min, primer annealing at 60° for 1 min, and extension at 72° for 2 min. Primers for GAPDH were added in the same tube, and the amount of GAPDH cDNA generated was used as an internal control. PCR products were separated by 5% polyacrylamide gel electrophoresis and autoradiographed. PCR products were confirmed by Southern hybridization and size.

Assay for Renin

Renin levels in the medium were measured by an immunoradiometric assay (IRMA) (Corning-Nichols Institute, San Juan Capistrano, CA) that measures total renin (prorenin plus active renin). This assay utilizes a renin inhibitor in combination with two specific antibodies [13, 14] and has a lower coefficient of variation for inter- and intra-assay than the conventional trypsin-activation method [13]. The sensitivity is about 2–4 μU/mL, but the amounts of renin released from the U-937 cells (200–10,000 μU/mL) usually required dilution to reach the linear portion of the standard curves. Although the 5% FBS did not interfere with the IRMA assay method, it was included in the standards so that standards and samples all had the same concentration. Earlier studies indicated that over 95% of the renin released from decidual cells is in the form of prorenin, and similar results were found with U-937 cells (95.1 ± 2.3%; N = 8). Therefore, renin results are expressed in terms of total renin (renin plus prorenin). In all cases, the total renin concentration in the medium in the final 24 hr of incubation is presented.

Statistical Analysis

All incubations were carried out in quadruplicate wells with results expressed as means ± SEM. All experiments were repeated at least twice. Statistical differences were determined by ANOVA followed by planned orthogonal contrasts or the Newman–Keuls test, depending on the design of the experiment, with $P < 0.05$ considered significant.

RESULTS

Effect of PDBU on Cell Morphology

As shown in Fig. 1, exposure of U-937 cells to phorbol ester (PDBU) resulted in marked morphologic changes. The exposed cells aggregated and showed more irregular shapes

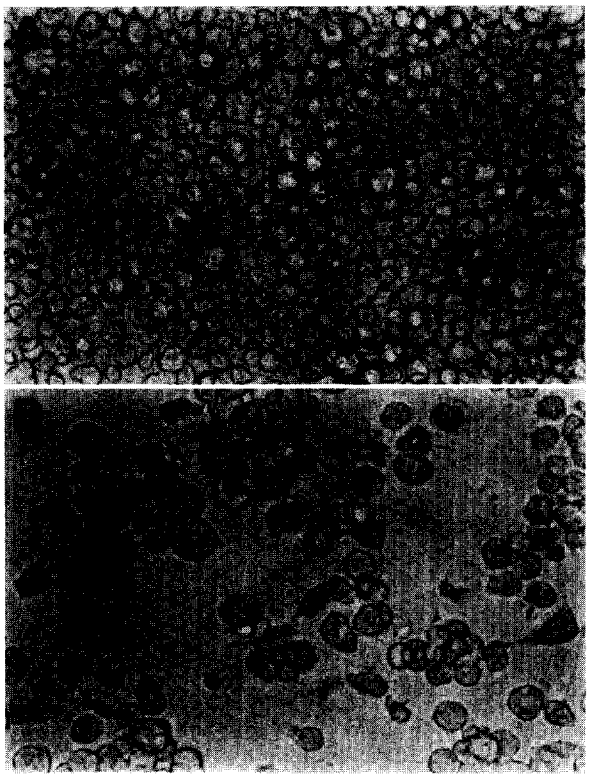

FIG. 1. U-937 cells after 3 days in the absence (A) or presence (B) of PDBU (100 nM). Magnification: 400×.

with a larger cell size and a decrease in number. These results are identical to previous studies on the effects of phorbol esters on the morphology of U-937 cells [9, 15].

Effect of Agents Altering cAMP on Renin Secretion and Expression

Treatment with dibutyryl-cAMP (1 mM) for 72 hr caused a 13-fold increase in renin secretion and a 22-fold increase in renin mRNA (Fig. 2). The size of the renin mRNA (1.5 kb) in U-937 cells was identical to that reported previously [16] (data not shown). Forskolin (10 μM), which activates adenylyl cyclase, induced an even larger increase in renin secretion (data not shown) and a 4.2-fold increase in renin mRNA (Fig. 3).

Effect of β_2-Adrenergic Activation on Renin Expression

To examine whether renin secretion induced by exogenous cAMP and forskolin could be mimicked by activation of membrane β_2-adrenergic receptors, we exposed the PDBU-treated cells to terbutaline, a selective β_2-adrenergic agonist. Previous studies have shown that β_2-adrenergic activation of U-937 cells increases cellular cAMP [17]. Terbutaline (100 μM), which is known to stimulate placental renin secretion, caused a 4-fold increase in renin release from U-937 cells ($P < 0.01$), and the terbutaline-induced renin release from U-937 cells was inhibited by ANGIO by 57% ($P < 0.05$) (Fig. 4). Since previous

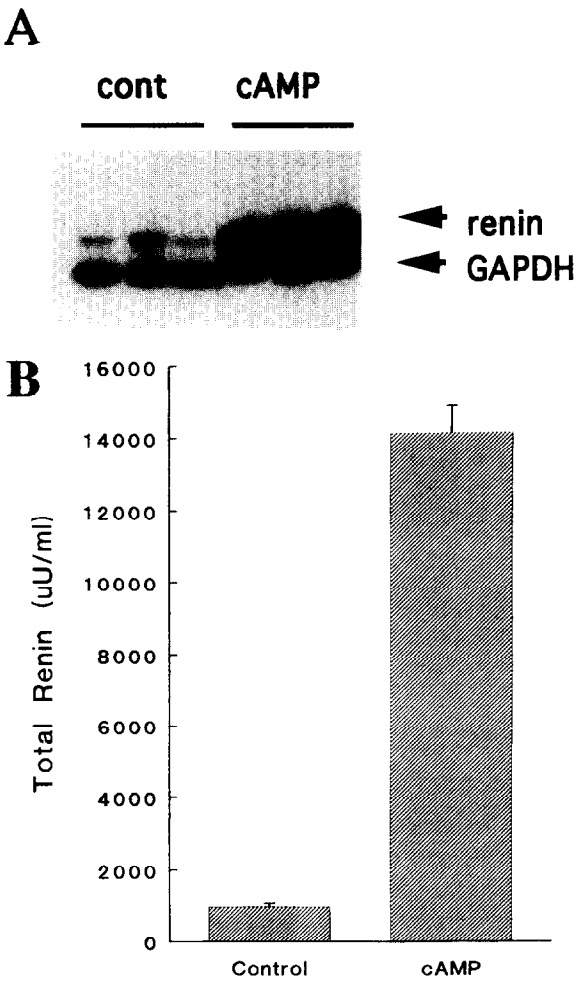

FIG. 2. Effect of dibutyryl-cAMP (cAMP) on renin mRNA (top panel) and renin release (bottom panel) from U-937 cells differentiated by PDBU. Cells were treated with or without dibutyryl-cAMP (1 mM) for 3 days. Values are means ± SEM, N = 4. The sizes of PCR products were 228 bp for renin and 195 bp for GAPDH (appropriate for the primers used).

evidence indicates that β-adrenergic stimulation of U-937 cells up-regulates phosphodiesterase [17], we tested whether the renin stimulation by terbutaline would be potentiated by phosphodiesterase inhibition. Ro 20-1724, a type IV PDE inhibitor, at a concentration of 50 μM increased basal renin release ($P < 0.005$) and potentiated the stimulant effect of terbutaline on renin secretion ($P < 0.05$) (Fig. 5). This finding with Ro 20-1724 is similar to that observed on placental renin secretion in response to β-adrenergic stimulation [18].

Effect of Cytokines and LPS on Terbutaline-Induced Renin Secretion

TNF-α completely blocked the stimulant effect of terbutaline on renin secretion ($P < 0.001$) (Fig. 6). While LPS and IFN-γ had no significant effect by themselves, the combination of LPS + IFN-γ reduced the stimulant effect of terbutaline by 93.7% ($P < 0.01$) (Fig. 6).

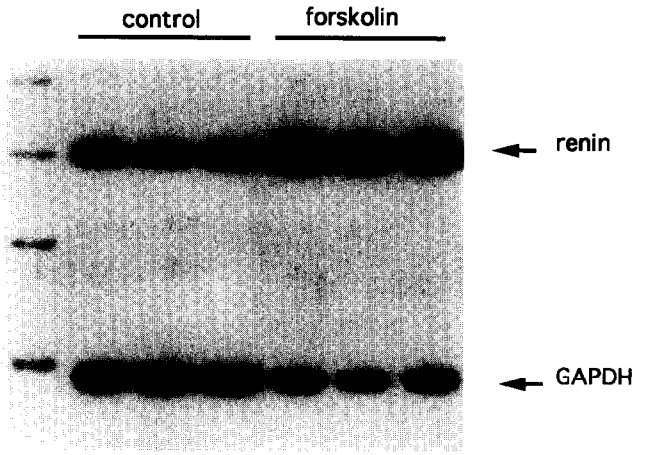

FIG. 3. Effect of forskolin on renin mRNA from U-937 cells differentiated by PDBU. Cells were treated with or without forskolin (10 μM) for 3 days. The sizes of PCR products were 402 bp for renin and 195 bp for GAPDH.

DISCUSSION

The present studies demonstrate that U-937 cells can be induced with PDBU to a macrophage-like phenotype that expresses renin. Renin release from the differentiated U-937 cells is similar to that of other extrarenal cells that express renin. Most of the renin released was in the form of prorenin. In addition, agents that directly or indirectly cause an increase in intracellular cAMP levels also stimulated an increase in prorenin release. ANGIO and cytokines, which are known to down-regulate renin release in other tissues, also inhibited renin release from U-937 cells.

Another feature of U-937 cells is that they possess

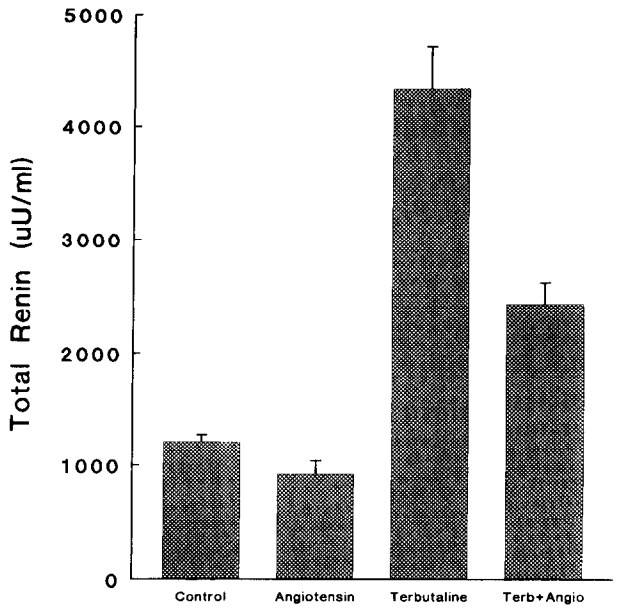

FIG. 4. Effects of terbutaline and angiotensin on renin release from U-937 cells. Cells differentiated by PDBU were incubated with terbutaline (100 μM) and/or ANGIO (10 μM) for 3 days. Release in the final 24 hr is shown. Values are means ± SEM, N = 8.

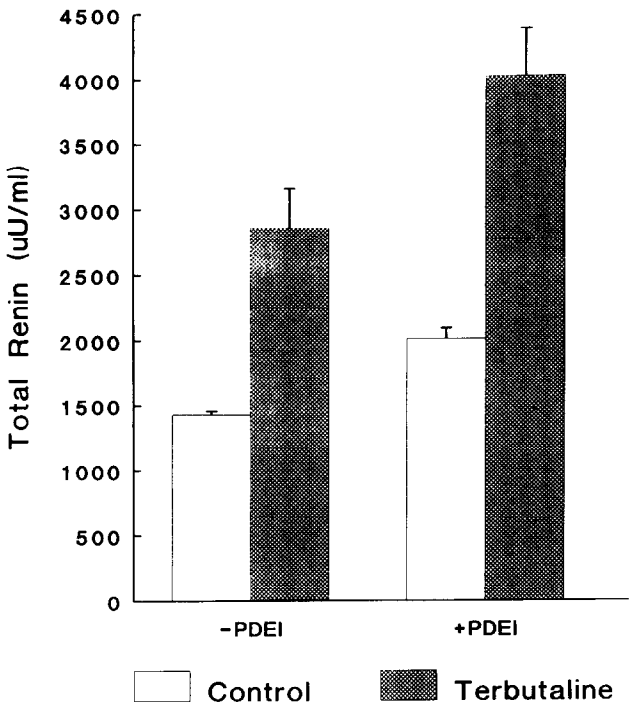

FIG. 5. Effect of type IV cAMP-phosphodiesterase inhibitor (PDEI, Ro 20-1724) on spontaneous and terbutaline-induced renin release from U-937 cells. Values are means ± SEM, N = 4.

β_2-adrenergic receptors coupled to cAMP generation [17]. Since we have found that renin secretion from human villous placenta is stimulated by β_2-adrenergic activation

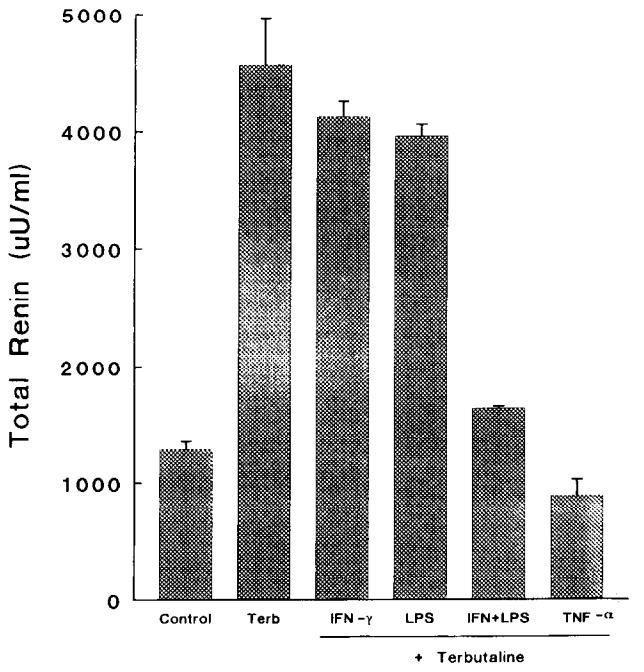

FIG. 6. Effects of cytokines on renin release induced by terbutaline. Cells differentiated by PDBU were exposed for 3 days to terbutaline (100 μM) alone or to terbutaline in the presence of IFN-γ (50 ng/mL), LPS (100 ng/mL), IFN-γ plus LPS, or TNF-α (10 ng/mL). Values are means ± SEM, N = 4.

[18], we examined the effect of terbutaline, a β_2-adrenergic agonist, on U-937 renin secretion. Terbutaline caused an increase in renin secretion from U-937 cells, and this was potentiated by Ro 20-1724, a type IV PDE inhibitor. Other studies have shown that inhibition of type IV PDE potentiates the cAMP response of U-937 cells to β_2-adrenergic stimulation [17]. These results are similar to our findings on human placental cells [18], and suggest mediation by cAMP.

Agents acting more directly on cAMP, such as forskolin and dibutyryl-cAMP, also increased the expression of renin in U-937 cells. There are reasons for expecting cellular levels of cAMP to increase renin gene expression. We have shown that β-adrenergic stimulation of placental renin secretion is mediated by protein kinase A [19]. Other studies have found that renin gene expression in kidney and fetal membranes is stimulated by forskolin and related agents [20–22]. Transfection studies on kidney [23] and chorio-decidual cells have shown that the renin gene contains cAMP-response elements (CRE) in the 5′ flanking region which regulate gene expression [24–26]. In addition, there is evidence that cAMP can also stabilize renin mRNA in mouse kidney [27]. Therefore, it is possible that cAMP may elevate renin mRNA in U-937 cells by more than one mechanism. The present results indicate that renin gene expression in U-937 cells can be regulated by β-adrenergic-coupled cAMP generation.

In addition to positive regulation of renin gene expression, there are a number of agents that are known to down-regulate expression. A potential autocrine regulator is angiotensin II, which acts through a negative feedback mechanism. Angiotensin II has been found to down-regulate renin expression in the kidney and placenta [28–31], and some of this regulation may be mediated by inhibition of adenylyl cyclase [29]. The presence of high affinity angiotensin II receptors has been reported on undifferentiated U-937 cells, although they were somewhat atypical [32].

We have shown previously that LPS and a number of cytokines decrease renin expression in decidual cells [5–7]. In the present study, TNF-α and a combination of LPS + IFN-γ inhibited the terbutaline-induced secretion of renin. LPS alone at the concentration that we used did not inhibit renin secretion, unlike our findings on decidual cells. Some factors that may be related to this decreased sensitivity include possible inhibition by down-regulation of protein kinase C associated with prolonged exposure to phorbol ester [33]. Some potential mechanisms for the effects of LPS and cytokines on renin expression include a decrease in cAMP [34], which is a known regulator of renal and extrarenal renin gene expression [1]; or an increase in nitric oxide [35], which has inhibitory and stimulatory effects on renin secretion [36, 37]. The synergism that was observed between LPS and IFN-γ on renin expression has been reported for other biological effects in a number of cell types, including U-937 cells [35, 38, 39]. Some of this synergism may be related to enhanced release of TNF-α [40, 41].

In conclusion, the present studies indicate that U-937 cells that have differentiated towards a macrophage/monocyte cell type express renin and secrete large amounts of prorenin under the influence of agents that activate cAMP. Thus, U-937 cells provide an excellent model system to study the regulation and expression of extrarenal renin since they behave similarly to other non-renal renin-producing cells. Furthermore, they are easy to maintain and can be induced to differentiate into different phenotypic types. The fact that these cells also respond to ANGIO suggests a possible role for the renin-angiotensin system in macrophage function.

This work was supported by NIH Grant HD15201.

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