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Inhibition of *CYP17* expression by adrenal androgens and transforming growth factor β in adrenocortical cells★

Justyna M. Biernacka-Łukanty, Tomasz P. Lehmann☼ and Wiesław H. Trzeciak✉

Department of Biochemistry and Molecular Biology, University of Medical Sciences, Poznań, Poland

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Cytochrome P450c17, encoded by the CYP17 gene, is a component of the 17α-hydroxylase/17,20-lyase enzyme complex essential for production of adrenal glucocorticoids and androgens as well as gonadal androgens. The expression of *CYP17* in adrenocortical cells is stimulated by corticotropin (ACTH) *via* the signal transduction pathway involving cAMP and protein kinase A (PKA). Thus, in addition to glucocorticoids, ACTH stimulates formation of adrenal androgens, which are known to induce transforming growth factor β (TGF-β) secretion. TGF-β in turn inhibits steroid hormone output by attenuating both basal and ACTH-dependent expression of *CYP17*. The present study revealed that treatment of bovine and human H295R adrenocortical cells with androgens resulted in a decrease in the basal level of *CYP17* transcript and cortisol secretion, without affecting forskolin-stimulated levels. We also demonstrated that in H295R cells TGF-β inhibited both basal and forskolin-stimulated accumulation of *CYP17* mRNA. Determination of promoter activity, directing luciferase reporter gene expression in H295R cells transfected with deletion fragments of bovine *CYP17* promoter, indicated that the –483 to –433 bp fragment of the promoter was necessary for the inhibitory action of TGF-β on *CYP17* expression.

It is concluded that in bovine and human adrenocortical cells, androgens inhibit basal *CYP17* expression probably at the transcriptional level and independently of the effect of TGF-β.

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✉Correspondence to: Wiesław H. Trzeciak, Department of Biochemistry and Molecular Biology, University of Medical Sciences, 6 Swiecickiego St., 60-781 Poznań, Poland; tel./fax (48 61) 865 9586; e-mail: trzeciak@amp.edu.pl

Abbreviations: CRS1, CRS2, cAMP-responsive sequences; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulphate; TGF-β, transforming growth factor β; SF-1, steroidogenic factor 1; StAR, steroidogenic acute regulatory protein.

Human cytochrome P450c17α is encoded by the *CYP17* gene (Picado-Leonard & Miller, 1987; Bhasker *et al.*, 1989) located on chromosome 10q24.3 (Fan *et al.*, 1992) and expressed in adrenals and gonads (Chung *et al.*, 1987). In the mammalian adrenal, except in rodents, zona fasciculata and reticularis produce P450c17 which is required for 17α-hydroxylation of pregnenolone and progesterone in cortisol and androgen biosynthesis (Sewer & Waterman, 2003). Recent studies of *CYP17* expression in human adrenocortical H295R cells have revealed that the distal sequence responsible for the basal expression is located between –184 and –206, while the proximal and cAMP-responsive element are located within the –63 and +1 fragment of *CYP17* promoter (Rodriguez *et al.*, 1997). These sequences are capable of binding steroidogenic factor 1 (SF-1). SF-1 is an activator of transcription of a number of genes involved in steroidogenesis, including those encoding cytochromes P450 (Parker & Schimmer, 1997; Lin *et al.*, 2001). A complex comprising SF-1, p54nrb/NonO and polypyrimidine tract-binding protein associated splicing factor (PSF) binds within the proximal fragment, between –63 and +1 bp and confers cAMP-dependent activation on human *CYP17* expression (Sewer *et al.*, 2002), whereas the Sp1 and Sp3 factors bind within the distal fragment, between –206 and –184 bp (Lin *et al.*, 2001). Studies in bovine adrenocortical cells have indicated that transcription of *CYP17* is regulated by ACTH. The effect of ACTH is mediated by cAMP (Zuber *et al.*, 1985) and cAMP-responsive sequences, CRS1 and CRS2, located in the *CYP17* promoter (Lund *et al.*, 1990; Bakke & Lund, 1992). CRS1 provides a binding site for the homeodomain proteins Pbx1 and Meis1 (Bischof *et al.*, 1998) and CRS2, binds orphan nuclear receptors, SF-1 or chicken ovalbumin promoter transcription factor 1 (COUP-TF1). SF-1, acting through CRS2, stimulates *CYP17* expression (Bakke & Lund, 1995), whereas COUP-TF1 binding to the same sequence acts to suppress transcription of this gene.

The protein product of *CYP17* plays a key role as a branch point of adrenocortical steroidogenesis between glucocorticoids and androgens. Dehydroepiandrosterone (DHEA) and its sulphate (DHEAS), in addition to androstendione, are secreted predominantly by zona reticularis. ACTH increases the conversion of cholesterol to pregnenolone and of pregnenolone to DHEA by activating *CYP11A1* and *CYP17* transcription (Orth & Kovacs, 1998; Rainey *et al.*, 2002). Studies in rodent adrenals have shown a direct correlation between testosterone treatment and a decrease in glucocorticoid production and inhibition of ACTH-dependent expression of *HSD3B* (Nowak *et al.*, 1995; Stalvey, 2002). It has been found that in bovine adrenocortical cells, glucocorticoids decrease CYP17 mRNA level in a receptor-mediated mechanism (Trzeciak *et al.*, 1993), nevertheless the effect of androgens on the adrenal *CYP17* expression has not been investigated yet.

It has been suggested that transforming growth factor β (TGF-β) and its receptor may be involved in androgen-induced inhibition of human adrenocortical H295R cell growth (Zatelli *et al.*, 1998) and TGF-β expression is stimulated by androgens in these cells (Zatelli *et al.*, 2000). TGF-α is a multifunctional regulator of an array of biological processes including cell differentiation, cell migration, extracellular matrix formation, immunosuppression, angiogenesis, and steroidogenesis (Shi & Massague, 2003). It has been observed in bovine and human cells that cortisol, dehydroepiandrosterone sulfate and androstendione production is inhibited by TGF-β (Lebrethon *et al.*, 1994; Le Roy *et al.*, 2000). A consequence of TGF-β action is the down-regulation of ACTH receptors and inhibition of steroidogenic acute regulatory protein (StAR), as well as *CYP17* and *CYP11B* genes expression (Perrin *et al.*, 1991; Brand *et al.*, 2000; Liakos *et al.*, 2003). Although the

regions responsible for the inhibition by TGF-β have been determined in the promoters of *StAR* and *CYP11B*, the localisation of the TGF-β-responsive sequence in the *CYP17* promoter remains unknown (Brand *et al.*, 2000; Liakos *et al.*, 2003).

The present study was designed to evaluate the effect of androgens on *CYP17* expression and to investigate a possible link between adrenal androgens, TGF-β and *CYP17* expression in adrenocortical cells.

MATERIALS AND METHODS

Cell culture. Cells were isolated from bovine adrenals by digestion with trypsin/EDTA. The cells were grown at 37°C in 5% CO_2/95% air in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DME/F-12, 1:1 mixture) (Invitrogen, Carlsbad, CA, U.S.A.) supplemented with 10% foetal calf serum, 1% Antibiotic Antimycotic (Sigma, St. Louis, MO, U.S.A.). After attaining confluence, the medium was changed and a defined medium supplemented with 1% ITS (insulin 10 μg/ml, transferrin 5.5 μg/ml and sodium selenite 5 ng/ml, Sigma) was applied. The cells were incubated for 24 h with 25 μM forskolin, 250 nM dehydroepinadrosterone (DHEA), 25 nM testosterone or 250 nM androstendione (all from Sigma).

Human adrenocortical cells H295R were cultured in DME/F-12 supplemented with 4% Ultroser G (BioSepra SA, Villeneuve la Garenne, France), 1% ITS and 1% Antibiotic Antimycotic at 37°C in 5% CO_2/95% air. After attaining confluence, cells were incubated in serum free DME/F-12 containing 0.1% Ultroser G for 24 h before treatment for 24 h with 25 μM forskolin, 250 nM DHEA, 25 nM testosterone or 250 nM androstendione. The cells were also incubated for 24 h with 25 μM forskolin and/or 2.5 ng/ml TGF-β (Promega, Madison, WI, U.S.A.).

Cortisol content in the incubation media was measured by direct radioimmunoassay using a commercially available kit (Orion Diagnostica, Finland).

RNA analysis. Total RNA was isolated from cultured cells by the use of TRI Reagent (Sigma) and poly(A)$^+$ RNA was reverse transcribed using AMV Reverse Transcriptase (Promega). The isolated RNA (5 μg) was incubated for 10 min at 70°C. Then a reaction mixture containing: 2 μl 10× Reverse Transcription Buffer, 1 μl (0.5 μg/μl) oligo(dT)$_{15}$, 2 μl (10 mM) dNTPs, 4 μl (25 mM) $MgCl_2$, 0.5 μl (40 U/μl) ribonuclease inhibitor and 0.75 μl AMV-RT was added. The reaction mixture was incubated for 15 min at 42°C and chilled on ice. The cDNA was amplified by PCR using 0.25 μl (1 U/μl) REDTaq™ DNA Polymerase (Sigma), 1 μl (10 mM) dNTPs, 0.6 μl (10 nmol) of each primer: bovine *CYP17* sense 3′-GAATGCCTTTGCCCTGTTCA-5′, antisense 3′-CGCGTTTGAACACAACCC - TT-5′, human *CYP17* sense 3′-TGGCCCC-ATCTATTCTGTTC-5′, antisense 3′-CTTCTC-CAGCTTCTGATCGC-5′, β-actin sense 3′-GT-GGGGCGCCCCAGGCACCA-5′, antisense 3′-CTCCTTAATGTCACGCACGATTTC-5′ and 1.25 μl 10× PCR buffer, in a total volume of 12.5 μl. The *CYP17* primers were designed based on bovine (NM_174304) and human (M31146 and M31147) sequences and synthesised by Sigma-Genosys Ltd (London, U.K.). The amplification was conducted under the following conditions: initial denaturation, 2 min at 94°C; 5 cycles (30 s at 94°C, 30 s at 65°C, 30 s at 72°C); 20 cycles (30 s at 94°C, 30 s at 62°C, 30 s at 72°C); final extension, 7 min at 72°C. The lengths of amplified cDNA fragments were: bovine *CYP17*, 330 bp; human *CYP17*, 254 bp, and *β-actin*, 539 bp.

Plasmid vectors. The bovine *CYP17* reporter constructs were generated using bovine genomic DNA. Genomic DNA was purified using the DNeasy Tissue Kit (Qiagen, Hilden, Germany). Bovine *CYP17* promoter fragments with truncated 5′ ends and a common 3′ end (+24) were amplified by PCR using forward primers: 5′-GGGGTACCTAGG-GCCCCTGATCTCAA-3′, 5′-GGGGTACCGC-

AGCAAACATGGAATGAAG-3′ and 5′-GGGGTACCGGCTGTGCTGCAGGATAA-3′, positioned at −483, −433 and −98 bp, respectively, and a common reverse primer 5′-CCCAAGCTTTGTGGAGTGGCTTCAGCCA-3′. The *CYP17* primers were designed basing on bovine gene sequence (X59613). Primers contained *Kpn*I or *Hin*dIII (reverse) restriction sites (underlined). The PCR products containing respective fragments of *CYP17* promoter were subsequently digested with *Kpn*I and *Hin*dIII (Roche Molecular Biochemicals, Indianapolis, IN, U.S.A.). The digested PCR products were purified on agarose gel, eluted with the GelElute™ Agarose Spin Column Kit (Sigma) and cloned into the *Kpn*I/*Hin*dIII restriction sites of the pGL2 plasmid (Promega). Plasmid pGL2 without the *CYP17* promoter was used as a control. The plasmids were purified using the Midi Prep kit (Qiagen) and sequenced (GenomeExpress, Meylan, France).

Cell transfection and reporter assay. The H295R cells (100 000 cells per well) were plated a day before onto 24-well dishes (Becton Dickinson Labware, Franklin Lakes, NJ, U.S.A.) and were transfected using DOTAP (Roche Molecular Biochemicals) according to the manufacturer's protocol, 2 μg of DNA was used per well. Co-transfection of the *Renilla* luciferase gene under the control of tyrosine kinase promoter (pRL-TK) was used as a control of transfection efficiencies. Transfected cells were treated with 25 μM forskolin or 2.5 ng/ml TGF-β for 24 h, harvested, lysed and both firefly and *Renilla* luciferase activities were sequentially measured with the Dual Luciferase Reporter Assay System (Promega) on a LUMAT LB 9507 luminometer (EGG Berthold, Bad Wildbad, Germany). Results were expressed as relative firefly luciferase light units (RLU) normalised to *Renilla* luciferase activity of the same sample.

Statistical analysis. Each experiment was performed in triplicate. The data presented are the mean ±S.E.M. The results were analysed by Student's *t*-test and $P < 0.05$ was taken as the level of significance.

RESULTS

The effect of androgens on the level of *CYP17* transcript and cortisol secretion in bovine adrenocortical cells

We examined whether the principal adrenal androgen, DHEA, as well as testosterone and androstendione, decrease basal and forskolin-stimulated CYP17 mRNA level and cortisol secretion in bovine adrenocortical cells in culture. Previous studies in these cells showed that ACTH- or forskolin-induced CYP17 mRNA level was maximal after 24 h of treatment (Le Roy *et al.*, 2000). In our experiment, the cells were treated with forskolin alone or with each androgen with or without forskolin for 24 h, and harvested for analysis of CYP17 mRNA levels, while the medium was collected for cortisol estimation. The concentration of DHEA and androstendione (250 nM) used was approximately 10-times above the physiological plasma level in humans, and testosterone concentration was 25 nM, close to physiological (Orth & Kovacs, 1998).

As expected, *CYP17* expression was significantly (2.5-fold ± 0.6) increased by forskolin whereas DHEA, testosterone and androstendione decreased the basal level of expression by 80% (±18.4), 30% (±6.9) and 50% (±11.5), respectively (Fig. 1, A and B). The androgens, however, did not affect the level of mRNA in forskolin-stimulated cells (Fig. 1, A and B). Under forskolin treatment for 24 h, cortisol level in the media was elevated 4-fold (±0.14) above the control (18 and 75 ng/ml respectively), whereas DHEA inhibited basal cortisol production by 35% (±1.2), testosterone by 18% (±0.6) and androstendione by 34% (±1.2). None of these steroids, however, caused any changes in cortisol output in forskolin-treated cells (Fig. 1C).

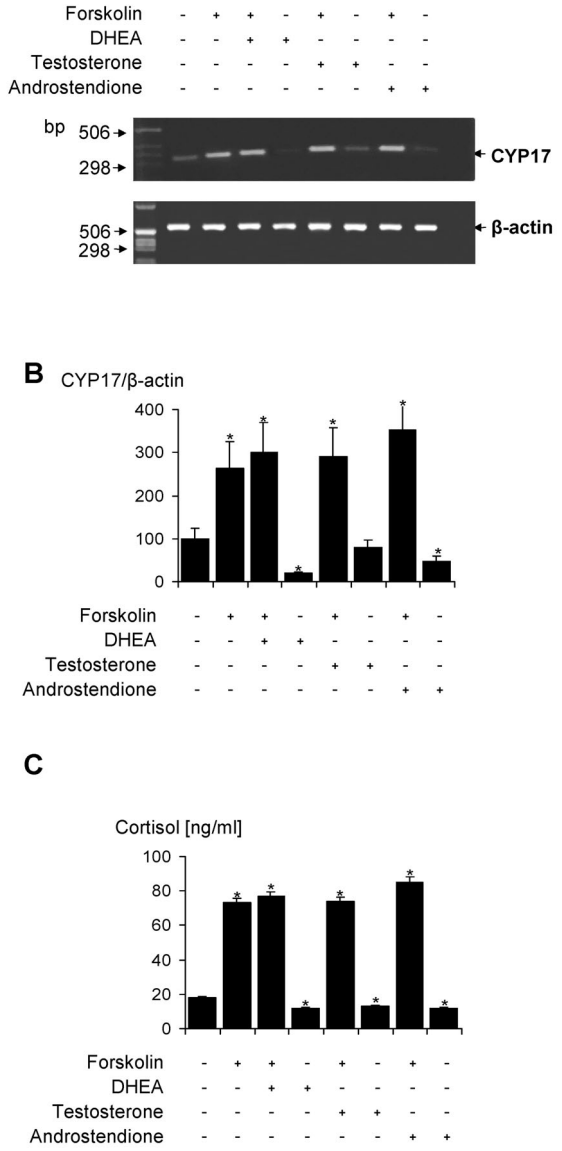

Figure 1. Effects of androgens and forskolin on *CYP17* transcript level and cortisol secretion in bovine adrenocortical cells.

Bovine adrenocortical cells were incubated for 24 h with 25 μM forskolin and/or 250 nM DHEA, 25 nM testosterone or 250 nM androstendione and concentration of cortisol in the culture media was determined. Total RNA was reverse transcribed, and specific fragments of CYP17 as well as β-actin cDNAs were amplified (RT-PCR) as described in Materials and Methods. **A**. Ethidium bromide staining of CYP17 and β-actin cDNAs from a typical experiment, after 25 PCR cycles. **B**. Densitometric analysis of the bands is expressed as percentage of untreated control after normalisation to β-actin mRNA. Values represent the mean ± S.E.M. of three independent experiments. **C**, Mean cortisol level ± S.E.M. in triplicate samples from three independent experiments. *Significantly different from control, $P < 0.05$.

The effect of androgens on the level of *CYP17* transcript in human adrenocortical cells

Human adrenal cortex tumour cells, line H295R, were used to investigate whether the effect of androgens on *CYP17* expression would be similar to that in bovine adrenocortical cells. It has been demonstrated that the H295R cells secrete mineralocorticoids, glucocorticoids and androgens, and express the appropriate steroidogenic genes (Rainey *et al.*, 1994). We observed that, like in bovine

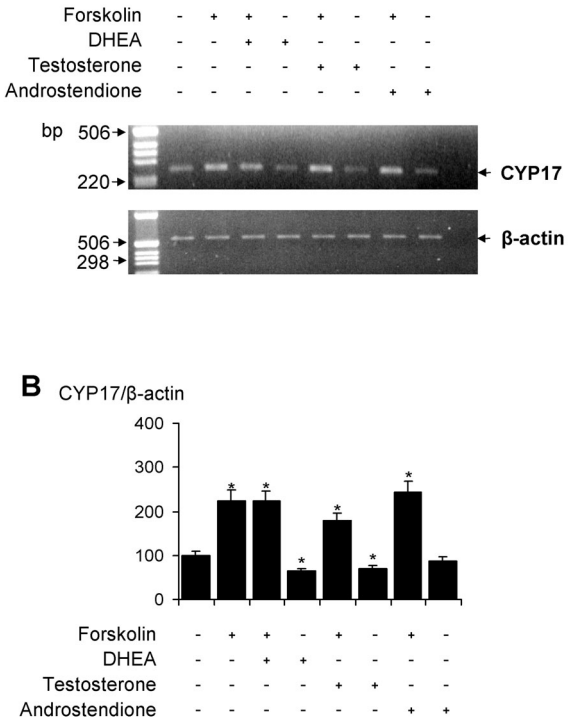

Figure 2. Effects of androgens and forskolin on *CYP17* transcript level and cortisol secretion in human adrenocortical cells.

H295R cells were incubated for 24 h with 25 μM forskolin alone or in combination with 250 nM DHEA, 25 nM testosterone or 250 nM androstendione and concentration of cortisol in culture media was determined. **A**. Ethidium bromide staining of CYP17 and β-actin cDNAs from a typical experiment, after 25 PCR cycles. **B**. Densitometric analysis of the bands is expressed as percentage of untreated control after normalisation to β-actin mRNA. Values represent the mean ± S.E.M. of three independent experiments. *Significantly different from control, $P < 0.05$.

adrenocortical cells, *CYP17* expression was significantly (2.2-fold±0.2) increased by forskolin, whereas DHEA, testosterone and androstendione, decreased the basal level of expression by 36% (±3.7), 31% (±3.2) and 12% (±1.2), respectively. Adrenal androgens, however, did not affect the level of CYP17 mRNA in forskolin-stimulated cells (Fig. 2).

The effect of forskolin and TGF-β on the level of *CYP17* transcript

Thus far, it has been demonstrated that H295R cells respond to TGF-β and androgens, and that androgens could increase TGF-β production in these cells (Zatelli *et al.*, 1998; 2000). To investigate the effect of TGF-β on the expression of *CYP17* we treated the H295R cells with TGF-β for 24 h and found that the basal level of *CYP17* transcript was decreased by about 50% (Fig. 3). Since in H295R cells the ACTH receptor (MC2R) is hardly detectable (Rainey *et al.*, 1994), forskolin was used to stimulate *CYP17* expression. As expected, forskolin induced *CYP17* expression over 1.5-fold (±0.2) after 24 h and the effect of forskolin was significantly reduced (40% ±5.6 inhibition) by co-treatment with TGF-β (Fig. 3).

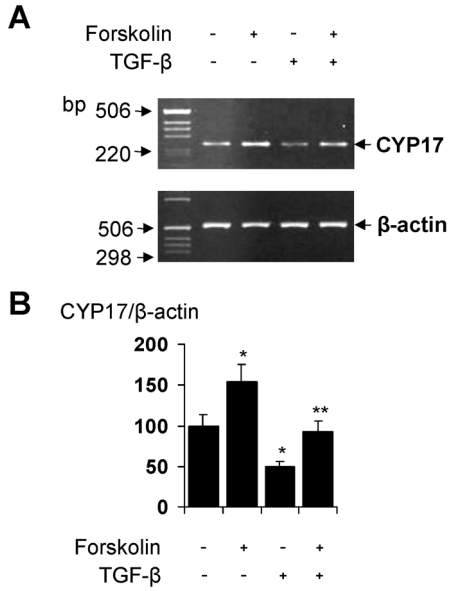

Figure 3. Regulation of human *CYP17* transcript level by forskolin and TGF-β.

H295R cells were incubated for 24 h with 25 μM forskolin and/or 2.5 ng/ml TGF-β. **A**. Ethidium bromide staining of CYP17 and β-actin cDNAs from a typical experiment, after 25 PCR cycles. **B**. Densitometric analysis of the bands is expressed as percentage of untreated control after normalisation to β-actin mRNA. Values represent the mean ± S.E.M. of three independent experiments. *Significantly different from control, **significantly different from forskolin, $P < 0.05$.

The influence of forskolin and TGFβ on transcriptional activity of bovine *CYP17* promoter

It was previously demonstrated that sequences designated CRS1 and CRS2, responsible for the basal expression and cAMP responsiveness of bovine *CYP17* promoter, are located –243/–225 bp and –80/–40 bp upstream from the transcription start site (Zanger *et al.*, 1991). However, localisation of the sequences responsible for the effect of TGF-β has not been determined in bovine *CYP17* promoter. To investigate the influence of forskolin and TGF-β on transcriptional activity of bovine *CYP17* promoter, H295R cells were transiently transfected with constructs containing deletion fragments spanning the region –483 to –98 bp upstream of the transcription start site. Transfection of these cells with the –483 fragment of the promoter resulted in about 9-fold (±0.9) higher basal expression, whereas upon transfection of these cells with the –433 fragment, a 1.6-fold (±0.2) higher basal expression of the reporter gene, comparing with the –98 construct, was observed. Incubation of the transfected cells with forskolin for 24 h resulted in stimulation of the transcriptional activity of the promoter 1.4, 2.0 and 2.3-fold for the –483, –433 and –98 fragments, respectively.

Transfection of the cells with the –483 construct followed by treatment with TGF-β resulted in a decrease of the basal expression of the reporter gene by about 18% (±0.2). Simultaneous treatment of the cells with forskolin and TGF-β also resulted in about 18% (±0.2)

inhibition of the reporter gene expression, compared with forskolin alone (Fig. 4).

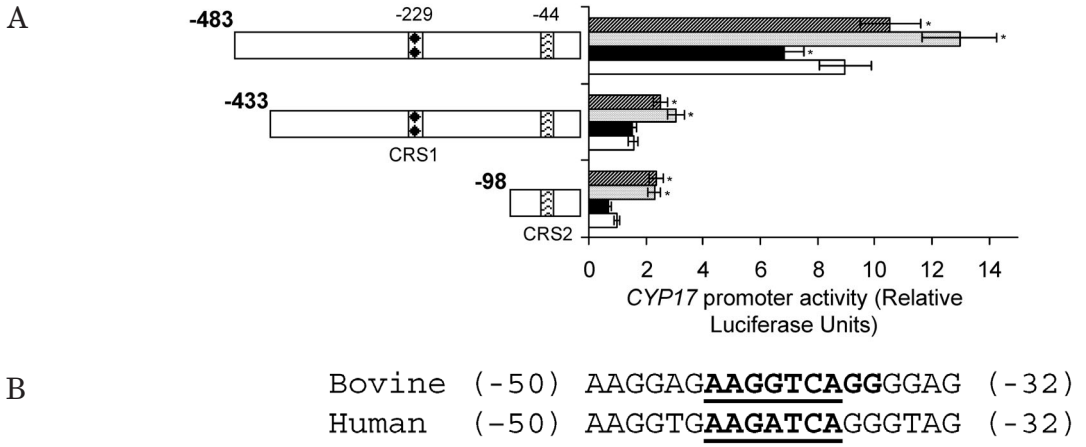

Figure 4. Effects of forskolin and TGF-β on *CYP17* promoter activity in human adrenocortical cells.

A. H295R cells were transiently transfected with vectors containing fragments of bovine *CYP17* promoter (depicted schematically on the left), directing luciferase reporter gene expression. Eighteen hours after transfection, the cells were placed in a serum-free medium for 24 h, then samples were either treated for 24 h with: 25 μM forskolin alone (dotted bars), 2.5 ng/ml TGF-β alone (black bars), forskolin plus TGF-β (dashed bars), or remained untreated (open bars). Luciferase activity was measured as described in Materials and Methods, then the fold induction relative to the basal activity of the –98 bp construct was determined. Data represent mean luciferase activity ±S.E.M. in triplicate samples from three independent experiments. *Significantly different from untreated control, $P < 0.05$. **B**. Sequence alignment of bovine CRS2 and human SF-1 response element in *CYP17* promoter.

DISCUSSION

It was previously reported that androgens inhibit both basal and cAMP-induced expression of *CYP17* in mouse Leydig cells *via* an androgen receptor-mediated mechanism (Burgos-Trinidad *et al.*, 1997), but a similar effect of androgens has never been reported in adrenocortical cells. It has also been demonstrated that in the primary bovine adrenocortical cells in culture, a synthetic glucocorticoid (dexamethasone) lowered the level of *CYP11A1* and *CYP17* transcripts, and that the effect of dexamethasone was mediated by glucocorticoid receptor (Trzeciak *et al.*, 1993). This suggested an intracrine mechanism of regulation of these genes, by the end products of the pathway. Since the core of the consensus glucocorticoid response element (GRE) sequence is identical with ARE (5'-GGTACANNNTGTTCT-3') (Mangelsdorf *et al.*, 1995) it can not be excluded that androgens, bound to their receptors, act to inhibit *CYP17* expression in bovine adrenocortical cells. Androgen receptors were detected in rat adrenocortical cells (Bentvelsen *et al.*, 1996), and human adrenal tumour cells, H295R line (Rossi *et al.*, 1998). In our study, the inhibitory action of androgens on the basal expression of *CYP17* in the primary culture of bovine adrenocortical cells and human H295R was shown. However, in contrast to the Leydig cells of mouse, there was no effect of androgens on the forskolin-induced level of *CYP17* transcript in bovine adrenocortical cells and H295R cells. The reason of this discrepancy is unknown. We noticed, however, that androgens decreased only basal cortisol secretion, without affecting forskolin-stimulated output, which is consistent with our results on *CYP17* expression in these cells.

Since it was demonstrated that H295R cells contain androgen receptors (Rossi *et al.*, 1998) and androgens induce expression of the gene encoding TGF-β (Zatelli *et al.*, 2000), it is possible that the inhibitory effect of andro-

gens on *CYP17* expression is mediated by TGF-β. TGF-β is a potent inhibitor of steroid hormone biosynthesis and strongly inhibits transcription of steroidogenic genes (Feige *et al.*, 1986). *CYP17* is the major target of TGF-β action in bovine (Le Roy *et al.*, 2000), ovine (Rainey *et al.*, 1990), and human adrenocortical cells (Lebrethon *et al.*, 1994). Although it was reported that TGF-β decreased 17α-hydroxylase/17,20-lyase activity in H295R cells (Liakos *et al.*, 2003), its effect on CYP17 mRNA level in these cells has not been demonstrated. We showed that TGF-β decreased the basal and forskolin-induced level of CYP17 mRNA in H295R cells. If the effect of androgens on *CYP17* was mediated by TGF-β, the androgens should affect both the basal and the forskolin-stimulated levels of *CYP17* transcript. However, we noticed that in both bovine and human adrenocortical cells, the forskolin-stimulated level of *CYP17* transcript was unaffected by androgens, and consequently, androgens did not affect forskolin-stimulated cortisol secretion. Our results indicate that the inhibitory effect of androgens on the expression of *CYP17* is not mediated by TGF-β, but androgens might inhibit transcription of *CYP17* by interaction with SF-1. This view is supported by recent findings that androgens, bound to their receptor (AR), directly interact with SF-1 to inhibit expression of *DAX-1* (Mukai *et al.*, 2002). The mechanism of TGF-β action on adrenal steroidogenesis is not fully understood. This prompted us to investigate expression of a reporter gene in H295R cells transfected with fragments of bovine *CYP17* promoter directing the expression of luciferase gene. The bovine *CYP17* promoter contains cAMP responsive sequences (CRS1 and CRS2) (Lund *et al.*, 1990) located –243/–225 bp and –80/–40 bp upstream from the transcription start site (Zanger *et al.*, 1991). The proximal sequence constitutes a binding the site for SF-1, a principal activator of *CYP17* transcription, whereas the distal sequence binds the transcription factors Pbx and Mais. Both sequences mediate cAMP-stimulated expression of *CYP17* (Bakke & Lund, 1995; Bischof *et al.*, 1998). Bovine SF-1 response element CRS2 is very similar to human SF-1 binding sequence (Fig. 4B) and these elements are located about 50 base pairs upstream from the transcription start site. Transfection of H295R cells with the –483 fragment of bovine *CYP17* promoter harbouring both CRS1 and CRS2 resulted in a 9-fold higher basal expression comparing with the –98 construct. In contrast, upon transfection of these cells with the –433 fragment, still containing both CRS, the basal expression of the reporter gene was only 1.6-fold higher than that driven by the –98 fragment, suggesting that between –483 and –433 there is a sequence responsible for maintaining the appropriate basal level of *CYP17* expression. We found that the cells responded to the stimulator of adenylyl cyclase (forskolin) known to increase intracellular cAMP concentration by a significant rise in the activity of the luciferase reporter. However, the fold-stimulation of the reporter gene by forskolin was smaller when longer constructs of the *CYP17* promoter were used. This suggested that sequences responsible for inhibition of the stimulated expression could be located in the longer constructs. These sequences might be a target for the inhibitory action of TGF-β on the expression of *CYP17*. Therefore we decided to investigate the effect of TGF-β on the expression of the reporter gene in transfected cells and found that TGF-β significantly reduced luciferase activity. The inhibitory effect of TGF-β on forskolin-stimulated expression of the reporter gene was evident only when the –483 construct was used. This indicated that the TGF-β responsive sequence was located within the fragment of *CYP17* promoter between the nucleotides –483 and –433. Although no typical Smad binding element (5′-GTCTGT-3′) was found within the fragment –483/–433, it contained a putative Sp1 site CCCAGGCAAAA (MYYMGCCYM) at –446, which might be responsible for the in-

hibitory effect of TGF-β on *CYP17* expression (Feng *et al.*, 2000).

It is concluded that in adrenocortical cells *CYP17* expression is negatively regulated by androgens and TGF-β, probably at the transcriptional level. The –483/–433 fragment of *CYP17* promoter, harbouring a putative Sp1 site, is required for the effect of TGF-β. Although androgens are known to promote TGF-β production, we conclude that the effect of androgens on *CYP17* expression is possibly TGF-β-independent. Nevertheless, further investigations are needed to identify the *CYP17* promoter sequences and proteins involved in androgen and TGF-β inhibition of *CYP17* expression in adrenocortical cells.

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