Regulation of human renin secretion and gene transcription in Calu-6 cells

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Abstract Calu-6 cells were characterized for studying the transcriptional regulation of the human renin gene. Analysis of *cis*-acting elements of the renin promoter showed the highest activity within the first 582 bp in serum-free conditions and of the 892 bp in the presence of serum. cAMP activates renin mRNA synthesis parallel to renin production (20-fold increase) as well renin promoter activity (2-fold). cAMP response element and the (−77 to −67) element are both necessary for activation of the renin promoter but do not act independently. Functional analysis of Intron A revealed the presence of a silencer specific to renin-producing cells.

Key words: Renin; cAMP; Promoter; Intron; Silencer; Serum

1. Introduction

Renin (EC 3.4.23.15) is an aspartyl protease which plays a major role in blood pressure control and electrolytic regulation by catalyzing the first and rate-limiting step of the renin–angiotensin system, i.e. the conversion of angiotensinogen to angiotensin I. Juxtaglomerular (JG) cells of the kidney are the primary source of circulating renin [1,2]. Recently, Petrovic et al. [3] showed that As4.1 cells, an immortalized cell line isolated from transgenic mice kidneys obtained by targeted oncogenesis, express mouse renin and regulate the mouse promoter in transient transfection experiments. Nevertheless, the lack of an established human JG cell line has greatly hampered the study of the transcriptional regulation of the human renin gene.

The renin gene, from human or rodent origin, is also expressed in several extra-renal sites [4] as for the other essential components of the renin–angiotensin system, thereby raising the hypothesis of differential transcriptional regulations in these different tissues. Primary cultures of human renin-expressing chorionic cells [5,6] have been the model of choice to study the transcriptional regulation of the human renin gene. Our studies have enabled the identification of *cis*-regulatory elements of the proximal promoter and *trans*-acting factors involved in basal and cyclic AMP (cAMP)-induced transcription in these cells [7,8].

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Abbreviations: bp, base pair; CAT, chloramphenicol acetyl transferase; CRE, cAMP-responsive element; CREB, CRE-binding protein; FCS, fetal calf serum; JG, juxtaglomerular

Renin has been shown to be expressed in the lung in transgenic mice [9] and in rare cases of hypertension due to renin-secreting pulmonary tumors [10–12]. Finally, recent studies by Lang et al. [13] showed that Calu-6 cells, a pulmonary carcinoma cell line express human renin mRNA and are a potentially interesting model for studying the regulation of the transcription of the gene.

The aim of this study was to elucidate the mechanisms controlling renin gene expression in Calu-6 cells. We determined the rate of endogenous human renin secretion and gene transcription as well as the *cis*-acting elements of the proximal promoter involved in basal and cAMP-induced transcription. Since sequences downstream from the transcription start site, particularly within the first intron, have been described which regulate the expression of various other genes in transient transfection experiments [14,15] and in transgenic studies [16], the role of intron A on human renin gene transcriptional regulation was studied ex vivo. In addition, the regulation by cAMP of human renin secretion and gene expression was studied in this model and compared to results previously published using primary cultures of chorionic cells [17] and juxtaglomerular cells [18]. The regulation of proximal promoter activity by cAMP was also investigated as contradictory results were published by the same group [19,20].

2. Materials and methods

2.1. Cell culture

The Calu-6 cell line was obtained from the ATCC (HTB 56) and grown in Minimal Essential Medium (MEM) with Earle's salts supplemented with sodium pyruvate, non-essential amino acids, 1 mM glutamine and 10% fetal calf serum (FCS).

2.2. Renin immunostaining

Indirect immunofluorescence studies were performed as described previously [21]. Calu-6 cells, incubated or not in the presence of 10^{-6} M forskolin, were grown in labtek coverslip flasks, rinsed in Tris-buffered saline (TBS: 0.1 M Tris and 0.25 M NaCl) solution, fixed in 4% paraformaldehyde with 3% sucrose for 45 min at room temperature and rinsed in TBS. The cells were then incubated for 1 h with the primary antibody R15 (polyclonal renin antiserum). R15 was used at a dilution of 1:50. As a negative control, cells were incubated with preimmune serum. Cells were then rinsed in TBS and incubated for 1 h with a fluorescinated anti-rabbit immunoglobulin antibody at a dilution of 1:10.

2.3. Determination of renin content and renin secretion

Calu-6 cells were grown in 25 cm^2 flasks. At confluency, culture medium was saved for renin secretion measurement and cells were rinsed 3 times with PBS. Cells were collected by scraping, resuspended in 500 μl of PBS and sonicated twice for 30 s at 4°C. An aliquot was removed for total protein quantification before renin measurement. Total renin (prorenin and active renin) was measured by ELISA as described by Ménard et al. [22].

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2.4. Measurement of human renin mRNA by quantitative RT/PCR

Calu-6 cells were plated on to 12-well plates. At confluency, cells were washed twice with PBS and then incubated in 750 μl of serum-free medium for 24 h. Forskolin (10^{-5} M) was added after renewal of serum-free medium and cells were incubated for 24 h. Then, culture medium was saved for renin measurement and cells were washed once with ice-cold PBS, lysed by the addition of 100 μl of an ice-cold buffer containing 2% Nonidet (NP40), 10 mM Tris-HCl (pH 8), 10 mM NaCl, 3 mM $MgCl_2$, and 0.7% (v/v) β-mercaptoethanol to each culture well and shaked for 10 min. Cell nuclei were removed by centrifugation at 12000×*g* for 3 min at 4°C. Each cytoplasmic fraction (3 μl), or 3 μl of 1:10 diluted fraction of 10^{-5} M forskolin-treated cells was used for reverse transcription followed by PCR amplification as described previously [17]. All results are given as mean of three independent experiments performed in triplicate wells ± SEM. Levels of significance were calculated by the Student's *t* test ($P < 0.05$ was considered significant; NS, non-significant).

2.5. Transfection analysis

The transfected plasmids were constructed as follows: the human renin gene promoter fragments were excised from pGL_2 reporter plasmids and fused with the luciferase reporter gene of pGL_3-basic (Promega) [8]. Intron A of the human renin gene was amplified by PCR from a genomic clone for 30 cycles using the Expand high fidelity kit (Boehringer Mannheim) using the primers 5′-TCCTGTACCTTTGGTCTCCCGACA-3′ and 5′-ATCACCTCTGTCACTGAGGGCCTC-3′. The PCR product was subcloned into the pCRII.1 vector (Invitrogen) and Intron A was then subcloned in the forward orientation upstream of the luciferase gene in pGL_3basic at the *Kpn*1/*Xba*1 sites creating pGL_3-Intron A. The promoter fragments controlling luciferase expression of pGL_3-582, pGL_3-892 and pGL_3-2824 plasmids were subcloned downstream of Intron A by *Bgl*II/*Kas*1 digestion creating pGL_3-582-Intron A, pGL_3-892-Intron A, and by *Xho*1/*Kas*1 digestion creating pGL_3-2824-Intron A. Intron A was excised from the pGL_3-Intron A vector by *Kpn*1/*Xho*1 digestion and subcloned in the forward orientation upstream the SV40 promoter of the pGL_3-promoter vector (Promega), allowing the comparison of pGL_3-SV40 and pGL_3-SV40-Intron A activities. All constructions were verified by the dideoxy sequencing method (Sequenase Version 2.0, DNA sequencing kit, USB).

Transient DNA transfections were performed using Dosper liposomal transfection reagent (Boehringer Mannheim). Calu-6, Hela and CHO cells were plated in 12-well plates and the culture medium was replaced by serum-free medium shortly before transfection. Dosper/DNA mixture, consisting of 6 μg of Dosper plus 1.5 μg renin reporter construct and 50 ng RSVCAT (as an internal control for plate-to-plate transfection efficiency), was then added. After overnight incubation, the medium was replaced with supplemented medium or serum-free medium when testing the effect of forskolin. In the latter case, the cells were then incubated for 24 h in the presence or absence of 10^{-5} M forskolin. Transfected cells were washed 3 times with PBS, lysed by adding 100 μl of passive lysis buffer (Promega). Luciferase activity was estimated by measuring luminescence for 10 s with a Bio.Orbit 1250 luminometer, 1 min after addition of 50 μl of luciferase reporter buffer (Promega).

Quantitative determination of chloramphenicol acetyl transferase (CAT) was performed by a sandwich immunoassay (Boehringer Mannheim).

All results are given as the mean of at least three independent transfection experiments ± SEM. Levels of significance were calculated by Student's *t* test ($P < 0.05$ was considered significant; NS, non-significant).

3. Results and discussion

Even though the main site of human renin synthesis is the juxtaglomerular cells of the kidney, renin gene expression has also been reported in a few malignant pulmonary tumors [10–12]. Until now, the extra-renal model, chorionic cells, have been used to determine human renin secretion and transcriptional regulation [17], to delineate the *cis*-regulatory elements of the proximal promoter [7] and to determine the sequences binding *trans*-acting factors responsible for cAMP induction of the transcription of the human renin gene promoter [8]. Recently, Lang et al. [13] described that Calu-6, a cell line isolated from a human anaplastic pulmonary carcinoma expressed the human renin gene. It was therefore interesting to compare renin gene expression and renin gene regulation in both cell types.

3.1. Characterization of Calu-6 cells

First, we characterised renin gene expression in Calu-6 cells. The average production of renin (prorenin and renin) in early passages (passage 36 to 47) was 5 ng/ml/24 h. By comparison, human chorionic cells in primary cultures produced 30–50 ng/ml/24 h, which is at least 3 times the highest rate produced by Calu-6 cells. In addition, our results demonstrated that renin synthesis falls to a very low level (0.9 ng/ml/24 h) around passage 48 in Calu-6 cells and accordingly, all our experiments were performed before this passage. Intracellular renin represents less than 5% of the total renin produced by Calu-6 cells. Immunofluorescence studies performed with the polyclonal antibody R15, which recognizes both renin and prorenin, also showed that there is no storage in these cells, as only a weak renin immunoreactivity was observed in basal culture conditions (Fig. 1A), whereas no staining was detected with the preimmune serum (data not shown). In conclusion, Calu-6 cells are an established cell line which represent a useful model for human renin gene expression studies, as long as experiments are restricted to early passages.

3.2. Human renin gene proximal promoter activity

To characterise *cis*-acting elements of the proximal promoter necessary for basal transcription, we performed transient transfection analysis of different renin promoter lengths fused to a luciferase reporter gene in Calu-6 cells. Transfection efficiency was verified by plate-to-plate co-transfection with RSVCAT. In addition, a higher transfection efficiency of Calu-6 was observed compared to chorionic cells. Our results demonstrate that pGL_3-582, which contains the fragment −582 to +16 has the highest promoter activity in Calu-6 cells (Fig. 2). Therefore, results were expressed as percentage of its activity. Transfection of the pGL_3-145 plasmid showed that its activity was 80% compared to pGL_3-582. In addition, no potent enhancers seem to be present in the proximal promoter up to -2824 since the pGL_3-2824 plasmid had 50% activity as compared to pGL_3-582. These results are in good accordance with those previously reported by Lang et al. [13] who showed that maximal activity was reached by transfecting the −896 construct, whereas the −148 and −2750 exhibit respectively 80 and 60% of the activity of the −896 plasmid. In chorionic cells, pGL_3-145 had 150% activity and pGL_3-2824 did not show any statistically different activity (both compared to pGL_3-582), suggesting a slightly differential regulation of the human renin gene proximal promoter in both cell types. Sequences of the human renin proximal promoter required for basal activity have been precisely defined in chorionic cells by transfecting a series of chimeric genes in which nucleotides corresponding to the cAMP responsive element (CRE) (−234 to −214) and to the site (−77 to −67), which does not bind to Pit-1 site despite bearing homology with a Pit-1-binding site, were mutated [8]. Transfection of these mutant plasmids demonstrated that the CRE and the (−77 to −67) region are essential for basal activity of the human renin gene proximal promoter in Calu-6 cells. pGL_3-145Δ1, where the

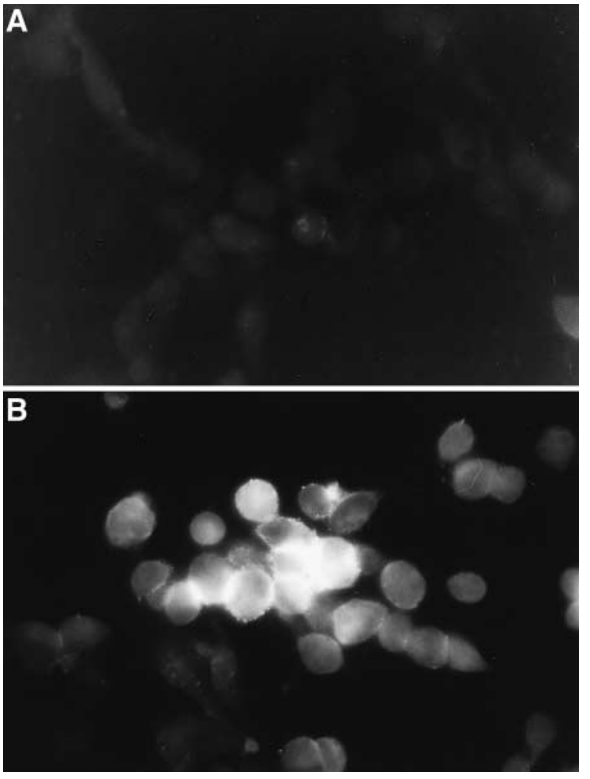

Fig. 1. Immunofluorescence photomicrographs of human Calu-6 cells in culture. Specific cytoplasmic staining with renin antiserum (R15) of non-treated cells (A) or 10^{-6} M forskolin-treated cells (B).

consensus binding site for Pit-1 (−77 to −67) was replaced by the Δ1 mutation [23] lost all activity and is comparable to pGL_3-basic. Mutation of this region in pGL_3-582, leading to pGL_3-582Δ1 resulted in a strong loss of activity as compared to the native pGL_3-582. In addition, disruption of the CRE (−226 to −219) in pGL_3-582MUT to a site that no longer binds CRE-binding protein (CREB), as described previously [8] allowed to create pGL_3-582MUT. This mutated construct had ~35% of the activity of the wild-type pGL_3-582 construct. Finally, all promoter activity was completely abolished by transfecting the pGL_3-582Δ1MUT where both the CRE and the (−77 to −67) region are mutated.

3.3. *Intron A contains cis-regulatory elements*

In mammalian genes, gene expression is regulated by multiple *cis*-acting elements which are present in the promoter, whether distal or proximal, but also within regions of the gene including introns and 3Õ untranslated regions. Among them, it has been shown for several genes that regulatory sequences are present in the first intron. To identify the role of Intron A [24] on the transcription rate of the human renin gene driven by the proximal promoter, its sequence was inserted in the forward orientation in pGL_3-basic and upstream the renin promoter fragment in the pGL_3-582, pGL_3-892, pGL_3-2824 constructs, creating respectively pGL_3-582-Intron A, pGL_3-892-Intron A, pGL_3-2824-Intron A. Calu-6 cells were transiently transfected with an equal mass (1.5 μg) or an equimolar amount (6.5 fmol) of each construct, the same results were obtained whatever the protocol we used and it was verified that Intron A showed no significant effect on pGL_3-basic. Our results showed that the presence of Intron A, which was inserted upstream and in the sense orientation, was associated with a repression of activity directed by the (−582 to +16) and the (−892 to +16) fragments and a weaker effect on the (−2824 to +16) (Fig. 3). Intron A had the highest inhibitor effect on the (−892 to +16) fragment. This result demonstrates that Intron A has a transcriptional silencing

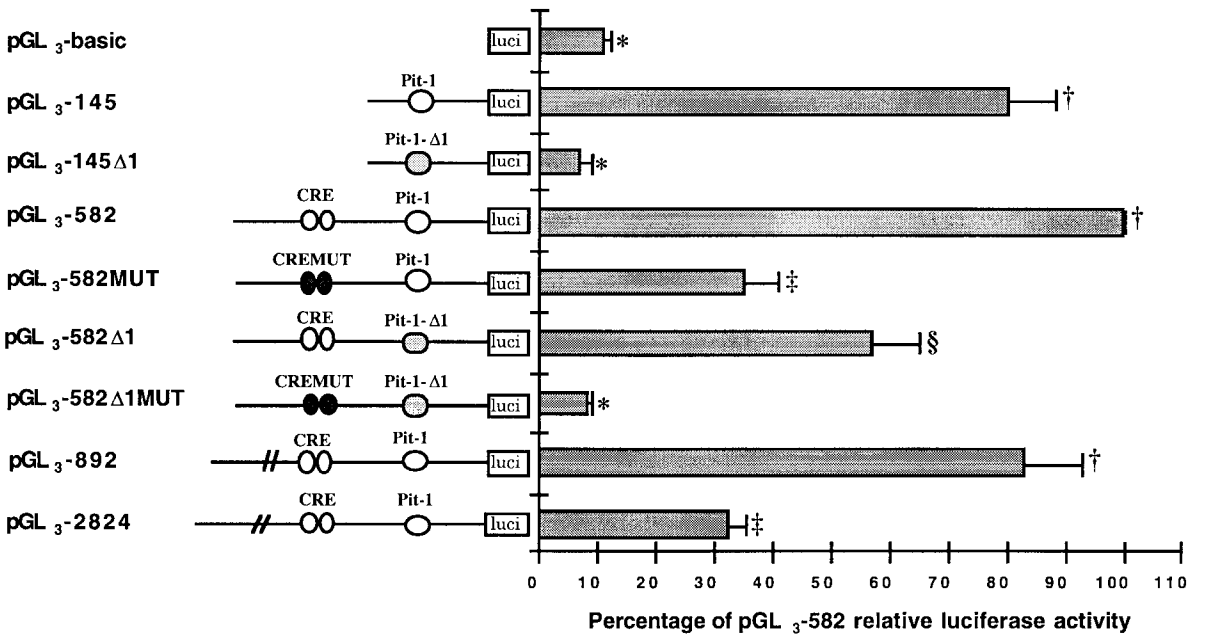

Fig. 2. Analysis of different renin promoter/luciferase constructs transfected into Calu-6 cells. Results (mean ± SEM) are given as percentage of pGL_3-582 luciferase activity. The CRE (CGTCA) was mutated to *tcgga* and the Pit-1 binding site core sequence TAATAAATCAG was replaced by TAAT*gggc*G*c*G as previously described. Each box represents the results of three independent transfection experiments performed in triplicate flasks. Levels of significance were calculated by the Student's *t* test. Groups, significantly different ($P < 0.05$) from each other are indicated by various location keys (*,†,‡,§).

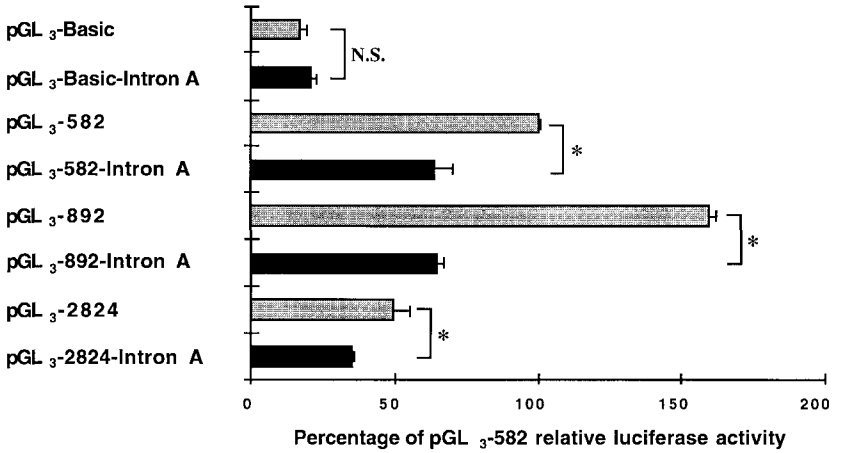

Fig. 3. Analysis of different renin promoter-Intron A/luciferase constructs transfected into Calu-6 cells. Results (mean ± SEM) are given as percentage of pGL_3-582 luciferase activity. Each box represents the results of three independent transfection experiments performed in triplicate wells. Levels of significance were calculated by the Student's t test. *Significantly different from control (without intron) ($P < 0.05$). NS, non-significant.

activity independent of its orientation and of its insertion site. Recently, during the course of this study, Lang et al. [19] showed a decrease in transcriptional activity which occurred in an orientation independent manner and that sequences between −149 and −896 may be required for the full silencing effect of Intron A when it was inserted downstream from the human renin promoter fragments. Intron A was also inserted upstream the SV40 promoter contained in pGL_3-promoter vector to check for potential promoter specificity of the silencing effect of intron A. Our results show that intron A also displays a comparable silencing effect on the activity of the SV40 promoter when transfected in renin-producing Calu-6 cells (Fig. 4A). In the context of this heterologous promoter, the silencing effect occurs in the same order of magnitude than the one observed on the (−892 to +16) fragment of the human renin promoter. Similarly, a cell-specific negative regulatory element has been identified in the first intron of the gene for bovine elastin [25]. To verify if any cell-specificity occurred in the silencing effect of intron A, the SV40-intron A construct was transiently transfected in non-renin-producing cells such as Hela and CHO cells. Interestingly, these results show that intron A has a positive regulatory effect in these cells (Fig. 4B,C) suggesting that the silencing effect is specific to renin-producing cells.

To determine more precisely the silencing properties of Intron A, we tested its effect in the presence (Fig. 3) or in the absence of FCS in the culture medium. We could observe that, in absence of FCS, renin promoter/luciferase constructs containing Intron A completely lost any promoter activity (data not shown). This suggests the presence of a putative regulatory element depending on the presence of FCS in the culture medium. This repression and serum dependence could be due to a down-regulation via chromatin constraints on the access of *trans*-acting factors, or at the level of interaction with the promoter, where the silencing elements compete for control of the transcription machinery.

3.4. Cyclic AMP regulation

The influence of cAMP on the regulation of human renin expression in Calu-6 cells was then studied. At the protein level, renin production was increased by forskolin, as shown by the strong renin immunostaining observed in the cytoplasm of treated cells (Fig. 1B) compared to cells grown in basal conditions. This was correlated with the dose-dependent increase in renin secretion. As shown in Fig. 5, forskolin induced a dose-dependent stimulation of renin production in culture medium. In the same experiments, renin mRNA was measured by quantitative RT/PCR and forskolin-induced stimulation of renin mRNA synthesis in the same dose-dependent manner. Maximum effect was observed at 10^{-5} M forskolin, whereas no effect could be detected between 10^{-8} M and 10^{-10} M. Our results clearly confirm that, as in other described human renin-producing cells, forskolin is a potent inducer of endogenous renin gene expression. Interestingly, this effect differs from that observed in chorionic cells by Caroff et al. [17] where forskolin alone had no effect on renin mRNA by quantitative RT/PCR, as it had also been observed by Duncan et al. [26] using Northern blot techniques. Nevertheless, forskolin acted synergistically with 12-myristate 13-acetate phorbol to increase renin mRNA levels and renin secretion in chorionic cells [17]. The regulation by forskolin in Calu-6 resembles to that observed by Della Bruna et al. [18] in primary cultures of mouse JG cells where forskolin stimulated renin mRNA level and renin secretion. These observations suggest that, in this respect, Calu-6 cells, when compared to JG cells, represent a good model to study the renin gene regulation by forskolin.

In addition, it has been shown, by us and others, on human [8] and mouse renin genes [27], that the renin proximal promoter can confer cAMP responsiveness. Recent results demonstrated that both the CRE and the (−77 to −67) region of the human renin gene are necessary to confer this inducibility in chorionic cells. In Calu-6 cells, a previous report by Lang et al. [19], was in contradiction with our results, showing that treatment of Calu-6 cells with 10^{-5} M forskolin caused a 100-fold increase in endogenous renin mRNA but did not increase the renin proximal promoter activity in transient transfections. They suggested that the increase in renin mRNA by cAMP may be mediated by post-transcriptional mechanisms. However, all transient transfections were performed in the presence of FCS containing medium, which could explain why they could not observe a statistical stimulation of pro-

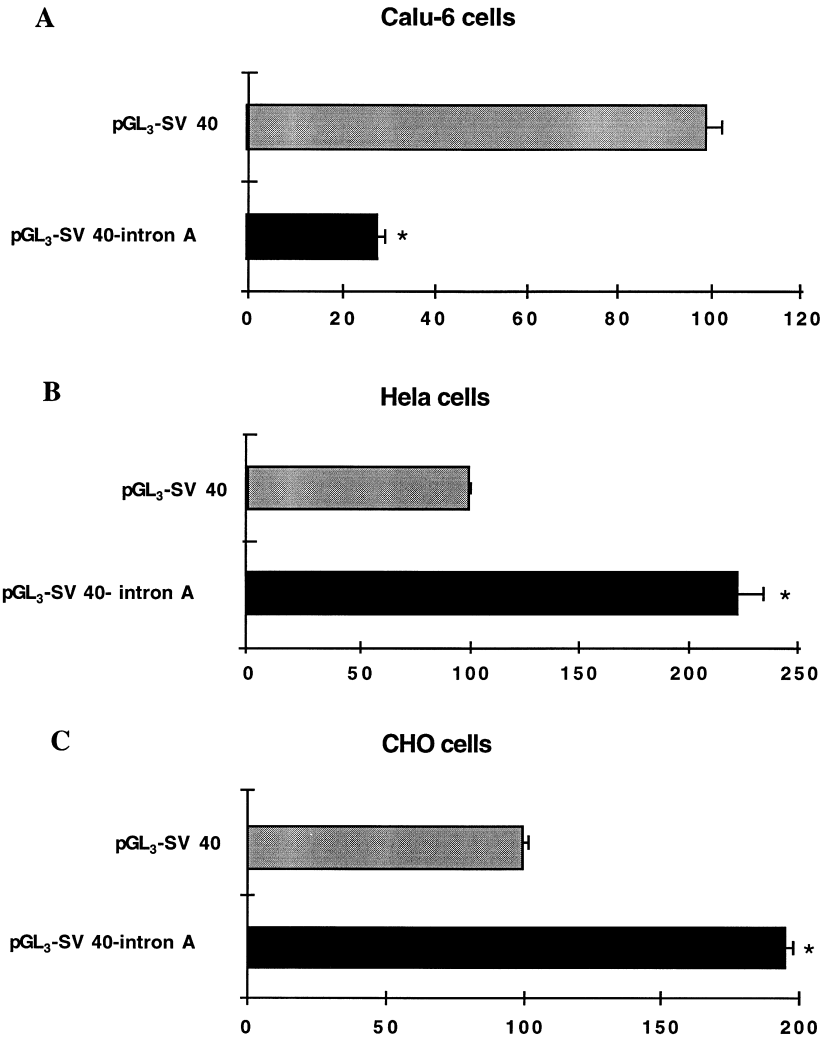

Fig. 4. Analysis of SV40 promoter-Intron A/luciferase constructs transfected into Calu-6 (A), Hela (B) and CHO (C) cells. Results (mean ± SEM) are given as percentage of pGL3SV40 promoter luciferase activity. Each box represents the results of four independent transfection experiments performed in triplicate wells. Levels of significance were calculated by the Student's t test. *Significantly different from control (without intron) ($P < 0.05$). NS, non-significant.

moter activity by forskolin, because FCS has been shown to be able to stimulate the transcription of several genes via different serum responsive elements [28]. In our case, we always examined the activity of these constructs after 10^{-5} M forskolin treatment in serum-free culture medium. Our results show that forskolin treatment induces a 2.5-fold increase in transcription of the pGL3-2824 construct (Table 1). This result was confirmed by showing an increase in transcription of the pGL3-892 and pGL3-582 constructs and further demonstrates that the proximal promoter contains *cis*-acting elements directing forskolin-induced transcriptional activation. These results show that as in chorionic cells, the proximal promoter could confer cAMP responsiveness with the same order of magnitude [7]. In order to delineate these functional *cis*-acting sequences, we transfected pGL3-582MUT where the renin CRE spanning the region (−226 to −219) was mutated. This mutation reduced the basal activity to 35% and completely abolished the forskolin stimulation (Table 1). This result differs from that observed in chorionic cells where the same mutation of the CRE only diminished the basal activity to a minor extent and did not suppress the forskolin stimulation. Therefore we examined the forskolin-induction activity of the (−77 to −67) region which has been shown to be partially responsible of the cAMP induction in chorionic cells [8]. By transfecting the pGL3-145 plasmid we showed that this sequence could confer a stimulation (1.6-fold) which was abolished by the mutation of the (−77 to −67) element to a Δ1 site (Table 1). In addition, in the context of the pGL3-582 plasmid the only mutation of this region abolished the induction whereas the basal activity was only 40% reduced. Recently, the same group [19,20], showed contradictory results concerning forskolin stimulation of the human renin gene promoter in Calu-6 cells. Surprisingly, in the latter paper, these authors observe that forskolin caused a 2–3-fold activation of the renin promoter involving both CRE-dependent and CRE-independent mechanisms. In summary, we conclude that whereas both elements can confer a part of the forskolin response in chorionic cells, disruption of either one element completely disrupt the forskolin response suggesting a differential regulation in Calu-6 cells than those reported by us and others [8,27] along the renin gene proximal promoter using different cell models. The potential effect of Intron A on

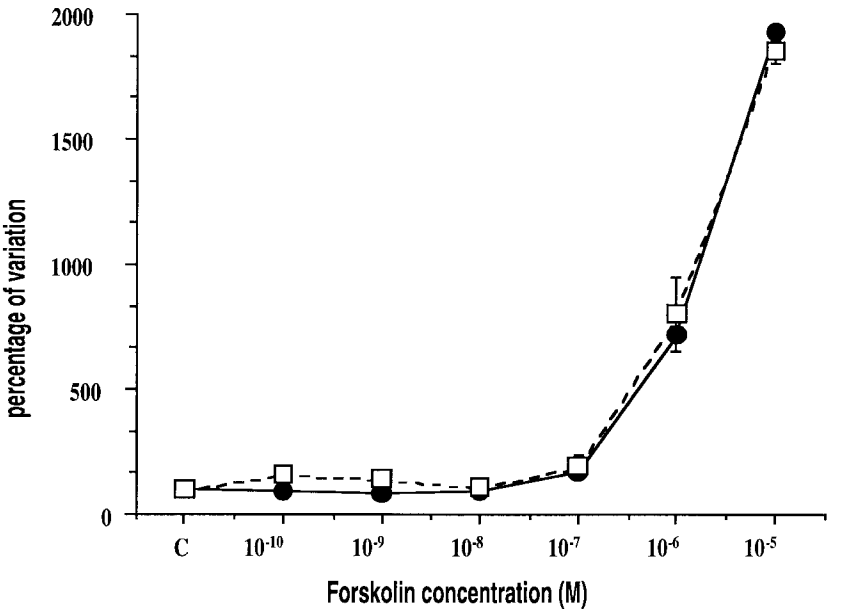

Fig. 5. Regulation of renin mRNA (□ - □) and renin production (● - ●) by forskolin. Calu-6 cells were incubated for 24 h without any agents (c) or with increasing concentrations of forskolin. Values are expressed as a percentage of the control and represents the results of three independent experiments performed in triplicate wells (means ± SEM). Renin determinations were performed in culture medium of the cells used for renin mRNA determinations.

cAMP-induced activation of the transcription rate of renin promoter/luciferase reporter constructs was also studied. Transient transfections of Calu-6 cells were performed in serum-free defined conditions with 10^{-5} M forskolin. Even though Intron A completely inhibits the promoter activity of the renin/luciferase constructs with which it is associated, in these serum-free culture conditions, despite a minimal basal activity, pGL_3-582-Intron A, pGL_3-892-Intron A and pGL_3-2824-Intron A can still be stimulated by forskolin in a range similar to the stimulation observed on parental constructs (not shown). These results suggest that Intron A does not interfere with the regulation by cAMP directed by the proximal promoter.

In conclusion, our results suggest that the pulmonary carcinoma cell line, Calu-6 cells, is an interesting model to study the transcriptional regulation of the human renin gene. These cells are the first described established cell line to synthesize relatively high amounts of endogenous renin and to regulate renin gene transcription. We show that cAMP is a potent activator of mRNA synthesis and renin production. Our results show a differential regulation in the proximal promoter as compared to chorionic cells. The CRE and the (−77 to −67) element are both necessary for cAMP activation but do not act independently since mutation of either one element abolishes it. A cell-specific negative regulatory element was determined in the first intron of the human renin gene. In addition, Intron A inhibits renin promoter activity of any tested constructs in the absence of serum and does not interfere with the forskolin stimulation. This suggests the presence of one silencer and one putative regulatory element depending on the presence of serum in Intron A. In addition, we suggest that the 20-fold stimulation by forskolin of mRNA synthesis parallel to renin secretion cannot be conferred entirely by the proximal promoter, which only confers a 2-fold activation, strongly suggesting that more distant regions from the transcription start site play a major role in the regulation of the

Table 1
Normalized luciferase activity of different renin promoter/luciferase constructs in serum-free conditions

Renin promoter/luciferase constructs	% Basal unstimulated luciferase activity of pGL_3-582 construct	Fold stimulation
pGL_3-basic	9 ± 1*	NS
pGL_3-145	130 ± 13†	×1.6
pGL_3-145Δ1	9 ± 2*	NS
pGL_3-582	172 ± 8‡	×1.7
pGL_3-582MUT	37 ± 6§	NS
pGL_3-582Δ1	54 ± 8¹¹	NS
pGL_3-582Δ1MUT	9 ± 2*	NS
pGL_3-892	153 ± 6‡	×1.8
pGL_3-2824	80 ± 8¶	×2.5

Luciferase activity is normalized to co-transfected RSVCAT activity. Each value represents the results of three independent transfection experiments performed in triplicate. Results (mean ± SEM) are expressed as percentage of basal unstimulated luciferase activity of pGL_3-582 plasmid. Fold stimulations were calculated as the ratio of normalized luciferase activity in forskolin-treated cells versus untreated cells.
Levels of significance were calculated by the Student's t test. Groups, significantly different ($P < 0.05$) from each other are indicated by various location keys (*,†,‡,§,¹¹,¶,). NS, non-stimulated.

human renin transcriptional regulation. Our study demonstrates that these *cis*-acting sequences mediating this activation are not located within the first intron. Molecular characterisation of each of these points are currently under study and should provide important new insights concerning the regulation of the human renin gene transcription.

References

[1] L. Barajas, Am J Physiol 236 (1979) F333–F343.
[2] E. Hackenthal, M. Paul, D. Ganten, R. Taugner, Physiol Rev 70 (1990) 1067–1116.
[3] N. Petrovic, et al. J Biol Chem 271 (1996) 22499–22505.
[4] M. Ekker, D. Tronik, F. Rougeon, Proc Natl Acad Sci USA 86 (1989) 5155–5158.
[5] A.M. Poisner, P. Agarwal, R. Poisner, Trophob Res 2 (1987) 45–60.
[6] F. Pinet, M.T. Corvol, J. Bourguignon, P. Corvol, J Clin Endocrinol Metab 67 (1988) 1211–1220.
[7] P. Borensztein, S. Germain, S. Fuchs, J. Philippe, P. Corvol, F. Pinet, Circ Res 74 (1994) 764–773.
[8] S. Germain, T. Konoshita, J. Philippe, P. Corvol, F. Pinet, Biochem J 316 (1996) 107–113.
[9] C.D. Sigmund, C.A. Jones, C.M. Kane, C. Wu, J.A. Lang, K.W. Gross, Circ Res 70 (1992) 1070–1079.
[10] J. Genest, et al. Trans Assoc Am Phys 88 (1975) 192–200.
[11] B.J. Morris, F. Pinet, J.B. Michel, F. Soubrier, P. Corvol, Clin Exp Pharmacol Phys 14 (1987) 227–231.
[12] G.M. Taylor, H.T. Cook, E.A. Sheffield, C. Hanson, W.S. Peart, Am J Pathol 130 (1988) 543–551.
[13] J.A. Lang, G. Yang, J.A. Kern, C.D. Sigmund, Hypertension 25 (1995) 704–710.
[14] F. Foufelle, N. Lepetit, D. Bosc, N. Delzenne, J. Morin, M. Raymondjean, P. Ferre, Biochem J 308 (1995) 521–527.
[15] S. Kurachi, Y. Hitomi, M. Furukawa, K. Kurachi, J Biol Chem 270 (1995) 5276–5281.
[16] S. Jallat, F. Perraud, W. Dalemans, A. Balland, A. Dieterle, T. Faure, P. Meulien, A. Pavirani, EMBO J 9 (1990) 3295–3301.
[17] N. Caroff, R. Della Bruna, J. Philippe, P. Corvol, F. Pinet, Biochem Biophys Res Comm 193 (1993) 1332–1338.
[18] R. Della Bruna, A. Kurtz, P. Corvol, F. Pinet, Circ Res 73 (1993) 639–648.
[19] J.A. Lang, L.H. Ying, B.J. Morris, C.D. Sigmund, Am J Physiol 271 (1996) F94–F100.
[20] L. Ying, B.J. Morris, C.D. Sigmund, J Biol Chem 272 (1997) 2412–2420.
[21] J.P. Camilleri, V. Phat, J. Bariety, P. Corvol, J. Ménard, J Histochem Cytochem 28 (1980) 1343–1346.
[22] J. Ménard, J. Bews, C. Heusser, J Hypertens 2 (Suppl 3) (1984) 275–278.
[23] J. Sun, C. Oddoux, A. Lazarus, M.T. Gilbert, D.F. Catanzaro, J Biol Chem 268 (1993) 1505–1508.
[24] P.M. Hobart, M. Fogliano, B.A. O'Connor, I.M. Schaefer, J.M. Chirwing, Proc Natl Acad Sci USA 81 (1984) 5026–5030.
[25] A. Manohar, R.A. Anwar, Biochem J 300 (1994) 147–152.
[26] K.G. Duncan, M.A. Haidar, J.B. Baxter, T.L. Reudelhuber, Proc Natl Acad Sci USA 87 (1990) 7588–7592.
[27] K. Tamura, S. Umemura, S. Yamaguchi, T. Iwamoto, S.-I. Kobayashi, A. Fukamizu, K. Murakami, M. Ishii, J Clin Invest 94 (1994) 1959–1967.
[28] S. Faisst, S. Meyer, Nucl Acids Res 20 (1992) 3–26.