ACTH depletion represses vascular endothelial-cadherin transcription in mouse adrenal endothelium in vivo

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Abstract

Vascular endothelial-cadherin (VE-cadherin) is an endothelial cell-specific adhesion protein that is localised at cell–cell contacts. This molecule is an important determinant of vascular architecture and endothelial cell survival. In the adrenal cortex, steroidogenic and endothelial cells form a complex architecture. The adrenocorticotropic hormone (ACTH) regulates gland homeostasis whose secretion is subjected to a negative feedback by adrenocorticosteroids. The aim of the present study was to determine whether VE-cadherin expression in the adrenal gland was regulated by hormonal challenge. We demonstrated that VE-cadherin protein levels were dramatically decreased (23·5±3·7%) by dexamethasone injections in the mouse and were restored by ACTH within 7 days (94·9±18·6%). Flow cytometry analysis of adrenal cells showed that the ratios of endothelial versus total adrenal cells were identical (35%) in dexamethasone- or ACTH-treated or untreated mice, suggesting that VE-cadherin expression could be regulated by ACTH. We demonstrate the existence of a transcriptional regulation of the VE-cadherin gene using transgenic mice carrying the chloramphenicol acetyl transferase gene under the control of the VE-cadherin promoter. Indeed, the promoter activity in the adrenals, but not in the lung or liver, was decreased in response to dexamethasone treatment (40±1·3%) and was partially restored after gland regeneration by ACTH injection (82±3%). In conclusion, our results show that transcription of a specific endothelial gene is controlled by the hypothalamo–pituitary axis and the data expand the knowledge regarding the role of ACTH in the regulation of the adrenal vascular network.

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Introduction

Vascular endothelial (VE)-cadherin (CD144) has been shown to play important roles in the establishment and maintenance of endothelium integrity (Lampugnani et al. 1993). VE-cadherin is a member of the cadherin superfamily, which is exclusively localised at interendothelial junctions (Lampugnani et al. 1995, Dejana et al. 1999). The extracellular domain of VE-cadherin is required for calcium-dependent homophilic adhesion and its cytoplasmic domain allows association with the catenins and the cytoskeleton. This interaction is needed for endothelium integrity, which is exclusively localised at interendothelial junctions (Lampugnani et al. 1995, Dejana et al. 1999). The extracellular domain of VE-cadherin is required for calcium-dependent homophilic adhesion and its cytoplasmic domain allows association with the catenins and the cytoskeleton. This interaction is needed for endothelium integrity, which is exclusively localised at interendothelial junctions (Lampugnani et al. 1995, Dejana et al. 1999). In embryos lacking VE-cadherin, the extension of primitive vascular structures was dramatically altered, thereby indicating a crucial role for this protein in vascular morphogenesis as well (Gory-Faure et al. 1999). Accordingly, the VE-cadherin cytoplasmic domain was recently shown to regulate endothelial protrusive activity in vitro, suggesting that VE-cadherin may be essential for the invasive process (Kouklis et al. 2003). In addition, ablation experiments strongly suggested that VE-cadherin might be involved in the vascular endothelial growth factor (VEGF)-induced survival pathway (Carmeliet et al. 1999).

The steroidogenic adrenal gland is an endocrine tissue characterised by an intense capillary network of highly permeable, often fenestrated vessels that allows the transportation of the endocrine hormones to the blood circulation (Kikuta & Murakami 1982). Adrenal alteration may lead to various disorders such as Addison’s disease, involving an intrinsic alteration of the adrenal gland cortex, or adrenal failure attributable to hypophyseal or hypothalamic pathology (Oelkers et al. 1992, Mayenknecht et al. 1998). The pituitary adrenocorticotropic hormone (ACTH) is the major trophic factor regulating and maintaining adrenocortical function, affecting such diverse processes as steroidogenesis, cell proliferation, migration, and survival (Gallo-Payet & Payet 2003). Given the variety of the biological events triggered by ACTH, it has been proposed that these effects are induced by multiple relay proteins synthesised and secreted by the steroidogenic cells (Fan & Iseki 1998, Feige et al. 1998). Recent studies have shown that several angiogenic growth factors are produced and
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secreted by normal endocrine cells and are increased in pathological states of endocrine glands, including inflammation, hyperplasia, and neoplasia (Katoh 2003). Of interest, carcinomas and adenomas in human adrenal cortex differ in their angiogenic patterns as visualised by CD34 labelling (Bernini et al. 2002).

As VE-cadherin is an important determinant of vascular architecture and endothelial survival, we reasoned that this molecule could be regulated during hormonal control of adrenal trophic changes. To investigate this hypothesis, we induced hypothalmo–pituitary axis deficiency in mice by dexamethasone injections (Keller-Wood & Dallman 1984, Dallman et al. 1987). Subsequently, adrenocortical functions were regenerated by ACTH administration. We showed that alteration of adrenal morphogenesis induced by hypothalmo–pituitary axis deficiency was correlated with a decrease in VE-cadherin content. However, endothelial/total cell ratios were maintained during one or both hormone treatments, suggesting that VE-cadherin expression was downregulated in endothelial cells of hypotrophic adrenals. Using VE-cadherin-chloramphenicol acetyl transferase (CAT) transgenic mice, we demonstrated that regulation occurred at the level of transcription. The dexamethasone-induced transcriptional reduction was specific to the adrenal gland, thereby demonstrating that a direct effect of dexamethasone on VE-cadherin promoter could not account for this regulation. After treatment with ACTH, the adrenal regained normal VE-cadherin content. Our results demonstrate that VE-cadherin and thus adrenal endothelial junctions are under the control of pituitary ACTH and our data expand the knowledge regarding the role of ACTH in the regulation of the vascular network in the adrenal gland.

Materials and methods

Reagents

ACTH(1–39), acetyl-coenzyme A (CoA), benzamidine, dexamethasone, leupeptin, pepstatin A, fluorescein (FITC)-labelled dextran (2 × 10^6 average molecular weight), Triton X-100 and Tween 20 were purchased from Sigma Chemical Co. (St Louis, MO, USA). Collagenase B, and DNase I were from Roche (Meylan, France). OCT compound was from Miles Scientific (Elkhart, IN, USA). The rat monoclonal antibody to mouse VE-cadherin (clone 11D4-1) and the isotypic non immune rat IgG2b were from Becton-Dickinson-Pharmingen (San Diego, CA, USA). The CD31 antibody (clone MEC 13-3) was from (Vecchi et al. 1994). Secondary antibodies were from Jackson Immunoresearch Laboratories (West Grove, PA, USA), except the horseradish peroxidase-conjugated goat anti-rat IgG which was from Sigma Chemical.

Animals

All protocols in this study were conducted in strict accordance with the Ministère de l’Education Nationale, de la Recherche et de la Technologie Guidelines for the Care and Use of Laboratory Animals. NMRI mice were purchased from Charles River (Les Oncins, France). The animals were allowed to acclimatise for a period of 1 week before experimental manipulation. VE-cadherin-CAT transgenic mice (Gory et al. 1999) were bred and maintained homozygous in the animal facility.

Dexamethasone and ACTH treatment

Animals were divided into three groups (n=5 per group) with equal average body weight between the three groups. The first group (control, CTL) was treated daily by intraperitoneal (i.p.) injection (200 µl) of sterile 0-9% saline solution for 6 consecutive days. Animals of the second group were treated daily by i.p. injection of dexamethasone (DEX; 5 mg/kg body weight) in 0-9% saline for 6 consecutive days. Animals of the third group received a daily i.p. injection of DEX like the second group and were then treated daily by i.p. injection of ACTH(1–39) (30 IU/kg) for 7 consecutive days. After the treatment, animals were weighed and then killed by i.p. application of an overdose of pentobarbital.

Adrenal histochemistry and morphometry

For immunohistochemistry, adrenal glands were fixed in 4% paraformaldehyde in phosphate buffer saline (PBS) and treated as described in Gaillard et al. (2000). The slides were subsequently incubated with a primary rat monoclonal anti-VE-cadherin antibody (0-5 µg/ml), followed with a biotinylated donkey anti-rat immunoglobulin and horseradish peroxidase-labelled steptavidin. Peroxidase was revealed using the metal enhanced diaminobenzidine substrate kit.

For FITC-dextran labelling, mice were anaesthetised with 100 mg/kg ketamin and 10 mg/kg xylazin and then perfused through the left ventricle with PBS containing 50 µg/ml FITC-dextran. Animals died during perfusion. Immediately after perfusion, adrenals...
were removed and fixed for 1 h in 4% formaldehyde-
PBS at 4°C. Adrenal glands were then embedded in
OCT compound after overnight cryoprotection in 20% (wt/vol) sucrose at 4°C. Adrenal morphology was
analysed as the width of the fasciculata-reticularis zone
from FITC-dextran injected mice on adrenal section.
Frozen sections (10 µm thick) were mounted in FluorSave and photographed using a fluorescence
microscope (Axioplan, Zeiss) equipped with a digital
camera (Spot 2, Diagnostic Instruments).

**CAT assay**

CAT assays were carried out as previously described (Gory et al. 1999). Briefly, mice were killed by CO₂
inhalation and the adrenals were carefully dissected from
connective tissues. Extracts were prepared by homogenisation using a Polytron in cold PBS and centrifuged for
20 min at 15 000 g. Proteins were added to 100 µl reaction mixture containing 0.25 M Tris–HCl (pH 7.8),
1 mM EDTA, 4·5 µl 100 mM acetyl-CoA and 2·5 µl [14C]chloramphenicol (2·5 Ci/ml). To quantify CAT
activity, radioactive spots representing acetylated and
nonacetylated forms of chloramphenicol were integrated
using a PhosphorImager (Molecular Dynamics, Sunnyvale,
CA, USA) and data were expressed as the percentage of
acetylated forms over total chloramphenicol.

**Western blotting**

Adrenals were lysed in lysis buffer, and protein
concentration was estimated by the bichinchoninic acid
method (Chabre et al. 1995). Proteins (20 µg) from the
various adrenocortical extracts were analysed by
SDS/PAGE (12% acrylamide). Proteins were then
transferred from the gel to nitrocellulose for 1 h and the
residual binding sites were blocked by incubating the
filters for 1 h in PBS containing 0·05% (v/v) Tween 20
and 5% (w/v) non-fat milk. The blots were subsequently
incubated overnight at 4°C with primary rat monoclonal
anti-VE-cadherin antibody (2 µg/ml in PBS/5% milk/0·05% Tween 20) or with mouse monoclonal
anti-β-tubulin antibody (dilution 1:250 000). After being
washed, the blots were incubated for 1 h with horse-
radish peroxidase-conjugated rabbit anti-mouse IgG
diluted in PBS containing 0·05% (v/v) Tween 20.
Immunoreactive proteins were visualised by chemilumi-
nescence (ECL). Signal visualisation was performed by
film exposure. The densitometric analysis was performed
under the low exposure time of the film.

**Flow cytometry analysis**

Adrenal glands were dissociated for 1 h at 37°C with a
PBS solution containing 0·2% collagenase B, 200 U/ml
DNAse I and 10% fetal calf serum and by repeated
flushing through a 21-gauge needle. After two washes
in PBS, aliquots containing 1×10⁶ cells were incubated for
1 h at 4°C with 100 µl PBS containing 2% bovine
serum albumin, followed by incubation with a rat
monoclonal anti-mouse CD31 antibody. After two
washes in PBS, the cells were incubated at 4°C for 1 h
with 100 µl PBS containing 2% bovine serum albumin
and 13 µg/ml FITC-conjugated (Fab’2) fragments of
goat anti-rat IgG. Isotypic non immune rat IgG2b
(10 µg/ml) was used as a negative control. The stained
cells were washed twice, resuspended in PBS and
analysed in a FACScaliber flow cytometer (Becton
Dickinson).

**Data presentation and statistical analyses**

The data are presented as the mean ± s.e.m., and the
numbers of subjects in each experimental group are
indicated in the figure legends. Significant changes in
hormone concentration, adrenal weight and CAT
activity were determined by a one-way ANOVA
followed by Tukey–Kramer multiple comparisons test.

**Results**

**Detection of VE-cadherin expression in adrenal
doendothelial structures of mouse adrenal**

The vasculature of the adrenal gland was examined by
systemic injection of fluorescein isothiocyanate (FITC)-
dextran in mouse heart. As shown in Fig. 1A, the
cortical capillaries arise directly from the capsule,
initially forming an anastomotic network lining the cells
of the zona glomerulosa, then continuing as longitudinal
sinusoids running radially between columns of cells
within the zona fasciculata.

To determine VE-cadherin localisation in the adrenal
gland, we performed indirect immunohistochemistry
staining using a rat monoclonal antibody raised against
the extracellular domain of VE-cadherin. VE-cadherin
was detectable in all adrenal capillaries as shown by
the positive brown staining (Fig. 1B). There is a direct
continuity between cortical and medullary capillaries.
Thus, VE-cadherin expression pattern in the adrenal
paralleled the vascular network revealed by FITC-
dextran labelling, indicating that VE-cadherin repres-
ents a uniform marker of adrenal vasculature.

The presence of VE-cadherin was confirmed by
immunoblotting analysis of adrenal extract. Total
adrenal proteins (40 µg) were analysed by SDS-PAGE.
As shown in Fig. 1C, the antibody recognised a band of
appropriate molecular mass of 125 kDa corresponding
to VE-cadherin. Equivalent amounts of VE-cadherin
were detected in right and left adrenals.

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Figure 1 Detection of VE-cadherin expression in mouse adrenal endothelium. (A) Vascular staining with FITC-dextran. Mice were injected in the left ventricle with FITC-labelled dextran at 50 mg/ml in PBS. Immediately after perfusion, the adrenals were removed and fixed. Frozen-embedded sections (10 µm thick) of whole adrenal were prepared and examined by fluorescence microscopy. (B) Immunolocalisation of VE-cadherin protein. Paraffin sections of mouse adrenal glands were immunostained with the rat monoclonal antibody to mouse VE-cadherin as described in Materials and methods. Immunoreactivity is shown in brown. Caps, capsule; Glo, zona glomerulosa; Fasc, zona fasciculata; Med, medulla. (C) Adrenal proteins were extracted and 40 µg were analysed by SDS-PAGE (12% acrylamide). Immunoblot analysis was performed with an anti-VE-cadherin antibody. The arrow shows the mature form of VE-cadherin protein (125 kDa). A cleavage product is also visible below. The migration of molecular mass markers (kDa) is shown on the left. L, left; R, right.
Variation of VE-cadherin expression in response to hormonal treatment

To examine further the role of ACTH on VE-cadherin expression, hypothalamo–pituitary deficiency was induced in mice by dexamethasone administration. Daily i.p. injection of DEX (5 mg/kg body weight) for 6 days induced a significant decrease (P<0.001) in adrenal weights from 6.3 ± 0.5 mg on day 1 to 3.4 ± 0.2 mg on day 6. The concentration of circulating corticosterone was decreased by 50% (data not shown). On day 6 of DEX administration, mice were injected with FITC-dextran, and adrenals from perfused mice were analysed by fluorescence microscopy. Figure 2A shows a composite fluorescent micrograph of median sections of the adrenals from treated (DEX) or untreated (CTL) mice. A specific decrease in the widths of zonae fasciculata and reticularis was observed associated with a disorganisation of the longitudinal vascular network (Fig. 2B).

Figure 2 Adrenal morphometry and VE-cadherin content after dexamethasone treatment. (A) Composite light micrograph of median sections of the adrenal cortex of FITC-dextran injected mice. The zona fasciculata–reticularis region of the adrenal glands from dexamethasone-treated mice (DEX) is significantly smaller than that of glands from untreated mice (CTL). (B) Higher magnification showing disorganisation of longitudinal vasculature. Abbreviations as in Fig. 1. (C) Total adrenal proteins (40 µg) were prepared from the adrenals of untreated (CTL), or dexamethasone (DEX)-, or DEX/ACTH-treated adrenals, analysed by SDS-PAGE and immunoblotted with anti-VE-cadherin (a) and anti-β-tubulin (50 kDa) (b) antibodies. (D) Densitometric analysis of immunoblots from seven adrenals. Data are the means±S.E.M. of the densitometric level of VE-cadherin expressed as a percentage of controls.
To analyse whether DEX- and DEX/ACTH treatments modified VE-cadherin protein levels in adrenals, immunoblot analyses were performed on adrenal extracts using a rat monoclonal anti-VE-cadherin antibody. As illustrated in Fig. 2C, the intensity of the VE-cadherin band was significantly decreased (23.5 ± 3.7%) after steroid treatment of mice (Fig. 2D) and restored after secondary treatment with ACTH (94.9 ± 18.6%). We thus conclude that the morphological changes observed in adrenals after dexamethasone treatment are correlated with the dramatic reduction in VE-cadherin.

Endothelial cell analysis by flow cytometry

To determine whether the observed variations of VE-cadherin were caused by a modification of the endothelial versus total cell ratio, we performed flow cytometry analyses of adrenal cells from treated and untreated mice after collagenase dissociation and CD31 labelling. CD31 is a uniform marker of endothelial cells, resistant to collagenase treatment (DeLisser et al. 1994). The percentage of endothelial cells was obtained using light scatter and fluorescence characteristics. In untreated (CTL) adrenals, antibodies to CD31 recognised a distinct subpopulation (Fig. 3A, CTL, right panel), whereas cells incubated with non-immune rat immunoglobulins harboured background staining (Neg). The percentages of CD31-positive cells in adrenal tissues were highly reproducible; variations were found to be less than 5% of the mean when three independent measurements were performed on one adrenal. Representative examples of flow cytometry analyses of cells from untreated, DEX- or DEX/ACTH-treated adrenals are shown in Fig. 3A. Mean percentages of CD31-positive cells (Fig. 3B) were remarkably stable after one or both hormone treatments: CTL, 35.11 ± 2.77%; DEX, 34.8 ± 3.7%; DEX/ACTH, 35.77 ± 4.8% (n=10 independent glands in each group). These data show that ACTH coordinately regulates the extent of the steroidogenic and vascular systems in the adrenal cortex. Furthermore, this experiment allowed us to conclude that changes in VE-cadherin levels observed after dexamethasone treatment did not result from variable proportions of endothelial cells in adrenals during regression-regeneration experiments. Rather, these data suggest that VE-cadherin expression was decreased in endothelial cells after dexamethasone administration.

VE-cadherin promoter activity in adrenal tissue extracts

To analyse whether the decrease in VE-cadherin expression was caused by a transcriptional inhibition of the VE-cadherin gene, we used transgenic mice carrying the CAT gene under the control of the VE-cadherin promoter (Gory et al. 1999). Measurement of CAT enzymatic activity in organ extracts from these mice is a precise and very sensitive way to quantify VE-cadherin promoter activity and to evaluate its regulation.

Although the transgene was previously shown to be expressed in a number of organs, expression in adrenals had never been examined. Thus, protein extracts from adrenal glands were prepared and assayed for CAT enzymatic activity. Figure 4A shows that CAT activity was detectable in adrenal extract from 1 to 20 µg. As illustrated in Fig. 4B, the reaction was linear from 2 to 10 µg after 120 min of incubation. The time course of CAT activity using 10 µg protein revealed a linear reaction that was detectable after 10 min of incubation and was linear up to 120 min (Fig. 4C). Enzyme activity was assayed in the right and left adrenals of the same animals and, at each tested time of incubation, the same levels were obtained for 2, 5, or 10 µg protein (data not shown). Lung and liver were used as control organs and tested in the same enzymatic kinetic analyses. As shown in Fig. 4D, we found that CAT activities of individual organs from different mice (n=12) were repeatedly similar: lung 65.0 ± 5.0%, adrenal 29.8 ± 4.2%, liver 3.1 ± 0.4% of conversion/10 µg/30 min, indicating that comparison of CAT activity is possible between mice receiving different treatments.

Glucocorticoid negative feedback decreased VE-cadherin promoter activity in adrenals

Transgenic mice received one or both hormone treatments and CAT activity was analysed in the adrenal, lung and liver in each group. In the following experiments, CAT activities were determined in linear conditions (120-min incubation), using 2 µg extracts from eight adrenals (four mice) in each group. As illustrated in Fig. 5, CAT activity in the adrenal gland of the DEX group was significantly decreased (40%; P<0.05) as compared with the CTL group (100%). Treatment of transgenic mice with ACTH for 7 days restored the CAT activity of the DEX group, almost to the control level (82%). As the observed decrease in CAT activity in the adrenals may be caused by a direct effect of dexamethasone on VE-cadherin promoter, we determined transgene expression in lung and liver, two organs that are not regulated by ACTH. As shown in Fig. 5, no significant variation in CAT activities could be measured in lungs and livers from each group. These data indicate that a direct effect of dexamethasone on
Figure 3 Endothelial cell population in adrenals from dexamethasone-treated and untreated mice. Mouse adrenals were examined for their endothelial cell content by flow cytometry, after immunostaining with an endothelial cell-specific marker, CD31. Adrenal cells from the three groups of mice were isolated as single cell suspensions with collagenase, as described in Materials and methods. Representative profiles of CD31 immunoreactivity are illustrated in (A). Treatments are abbreviated as in Fig. 2. Neg, control with non-immune immunoglobulins. (B) Quantification of CD31-positive cell percentage in each group (n=8 adrenals). Data were not statistically significantly different between groups (by one-way ANOVA, followed by Tukey–Kramer multiple comparisons test).
VE-cadherin promoter activity could not account for the observed decrease in the adrenals. Altogether, our results show that VE-cadherin expression in adrenal vessels is controlled at the transcriptional level by hypothalamus–pituitary inputs.

Discussion

Although our understanding of the mechanisms of junction assembly has advanced significantly (Dejana 2004), much less is known about the regulation of cadherin cellular levels. The capillary network in the adrenal gland is a key component in steroid delivery into the systemic circulation (Sasano 1998). However, the regulation of expression of VE-cadherin in the adrenal gland has not been investigated, although it is considered a very significant molecule in the organization and maturation of neovessels and in the protection of endothelium integrity (Hynes 1992, Dejana et al. 2000). The present study is the first to demonstrate that VE-cadherin expression is reversibly regulated in the adrenal gland upon hormonal challenge. The experimental model used in this study reproduced some of the
morphological changes observed in long-term glucocorticoid treatment in humans, namely a decrease in adrenal weight associated with the retraction of the fasciculata/reticularis zonae. Several other studies have reported that a downregulation of VE-cadherin expression could be associated with severe pathological situations. For example, VE-cadherin expression is decreased in malignant angiosarcomas whereas it is maintained in benign hemangiomas (Martin-Padura et al. 1995). In diabetic retinal vessels, VE-cadherin downregulation was correlated with a rise in transvascular leakage (Davidson et al. 2000). In intimal vessels of atherosclerotic plaques, a decrease in VE-cadherin was accompanied by increased entry of immunocompetent cells and atherogenesis (Bobryshev et al. 1999). In each case, VE-cadherin downregulation was associated with a bad prognosis. Variations of VE-cadherin expression have also been documented in different in vitro systems. In human umbilical vascular endothelial cells, gamma linoleic acid, a potent inhibitor of tumour-induced angiogenesis, has been shown to decrease VE-cadherin expression and reduce tube formation in matrix (Cai et al. 1999). Hepatocyte growth factor/scatter factor, a cytokine increasing migration, motility, and dissociation of human umbilical vascular endothelial cells, was also shown to decrease VE-cadherin expression (Martin et al. 2001). Altogether, these findings suggest that VE-cadherin is not a constitutive gene, but may be subjected to regulation in response to various physiopathological stimuli.

In the adrenal gland, our results demonstrate that VE-cadherin expression is regulated by ACTH. The hormone action depends on the expression of its specific receptors. To date, five melanocortin (MC) receptors have been identified and shown to have a wide distribution throughout the body and probably many diverse functions (Magenis et al. 1994). ACTH preferentially binds to the MC2 receptor (Hofmann et al. 1988, Mizuno et al. 1989, Penhoat et al. 1993), hence it is considered to be the main ACTH receptor. MC2 receptor is mainly expressed in the zona fasciculata (the site of glucocorticoid production) and in zona glomerulosa (the site for mineralocorticoid production) in the adrenal cortex (Mountjoy et al. 1992, Xia & Wikberg 1996, Reineke et al. 1998). To our knowledge, no MC2 receptor has been identified in adrenal endothelial cells or in other types of endothelium. Furthermore, treatment of human umbilical vein endothelial cells with ACTH or dexamethasone did not alter VE-cadherin mRNA content in these cells (data not shown). It is thus likely that VE-cadherin regulation in the adrenal is mediated by steroidogenic cells, through an indirect effect of the hormone. Indeed, ACTH was described to induce the expression of several growth factors and proteins of the extracellular matrix in cultured adrenocortical cells (Feige et al. 1998). However, future studies are necessary to get further insights into the molecular mechanisms regulating adrenal VE-cadherin expression.

Our flow cytometry analysis of adrenal cells indicates that the hormonal treatment did not modify the ratio of endothelial over total adrenal cells. This feature suggests that the observed modifications in VE-cadherin content were not caused by a variation of the endothelial/adrenal cell ratio. To examine whether this regulation occurred at the transcriptional level, we used VE-cadherin-CAT transgenic mice to monitor VE-cadherin gene

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**Figure 5** VE-cadherin promoter activity in adrenal, lung and liver from hormonally treated mice. After the hormone treatment, the different organs were homogenised. Transgene expression was determined in the organs (adrenal, lung, liver) of the three groups using a protein sample of 2 µg. The reaction was performed for 2 h at 37 °C. Each data point represents the mean±S.E.M. of triplicates from five independent mice. **P<0·01 (one-way ANOVA).**
regulation (Gory et al. 1999). Measurement of CAT activity in several tissues proved to be a very sensitive and reproducible test to monitor VE-cadherin promoter activity. This assay is specific to transcriptional activity, independent of potential VE-cadherin post-transcriptional regulation such as mRNA stabilization. CAT activity was highly detectable in the adrenal of adult transgenic mice. We demonstrated the existence of regulation of VE-cadherin promoter in treated adrenals. In contrast, in lung and liver, two organs that are not regulated by the hypothalamo–pituitary axis, no modification in VE-cadherin promoter activity was detected, which is in agreement with the tissue specificity of ACTH action. These data further demonstrate that the VE-cadherin promoter is not under direct glucocorticoid regulation. Our data are consistent with a model where glucocorticoids and ACTH are not independent regulators of VE-cadherin promoter. We speculate that the level of mouse ACTH is the only important parameter in this process. Previous studies showed that tumour necrosis factor-alpha could also downregulate VE-cadherin transcription in cultured endothelial cells (Hofmann et al. 2002). Altogether, these findings suggest that VE-cadherin is subjected to regulation in response to various physiological stimuli.

Although mechanisms underlying adrenal capillary regulation have not been clearly elucidated, data from several investigations demonstrated that steroidogenic adrenal cells release angiogenic factors (Feige et al. 2000, Feraud et al. 2003). More recently, a human endocrine gland-derived vascular endothelial growth factor (EG-VEGF) was described as an endothelial cell mitogen with a selective activity on steroidogenic gland endothelial cells (LeCouter & Ferrara 2003). However, the study of the coordinated interactions and the identity of the soluble factors involved in vivo has been very difficult. A cross-talk between VEGF R2 and VE-cadherin was previously shown to stimulate endothelial cell survival by the phosphorylation of Akt (phospho-kinase B) and the inactivation of Beclin (Carmeliet et al. 1999). VE-cadherin deficiency increased endothelial apoptosis and abolished transmission of the survival signal induced by VEGF-A through reduced complex formation between VEGF-R2, beta-catenin, and phosphatidylinositol 3-kinase (Carmeliet et al. 1999). Thus, it is likely that a coordinated regulation of both proteins might be at the origin of the vascular regulation accompanying the structural modifications of the adrenal gland.

In conclusion, these findings indicate that VE-cadherin expression is regulated during vascular reorganisation triggered by physiological agonists. Given that VE-cadherin is involved in vascular morphogenesis and endothelial survival, the fact that its expression is regulated by hormonal treatment strongly suggests that VE-cadherin may be a key player in the coordination of vascular and steroidogenic systems.

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