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Sex Steroidal Regulation of Vessel Permeability Associated with Vessel Endothelial Cadherin (V-cadherin)

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In an attempt to understand the roles of cadherins in the placenta, mRNA expression and biological function of cadherins in 3A(tPA-30-1) cells (derived from human term placenta and transformed by SV40), and in HUV-EC-C cells (derived from the endothelial cells in human umbilical cord) were studied under the influence of sex steroids. Estradiol transiently decreased the endothelial cell barrier properties (ECBP) of HUV-EC-C cells, and progesterone reversed the changes induced by estradiol. However, neither estradiol nor progesterone demonstrated any effect on cell aggregation of either 3A(tPA-30-1) or HUV-EC-C cells. Estradiol transiently decreased the level of V-cadherin and its mRNA in HUV-EC-C cells, and progesterone reversed the level decreased by estradiol. However, neither estradiol nor progesterone demonstrated any effect on the level of E-cadherin mRNA in 3A(tPA-30-1) cells. Therefore, a sex steroidal role for placental development and function related to cadherins seems to focus on the endothelial cells, plausibly via vessel permeability for the utilization of placental products. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

It is known that sex steroids, especially estrogen and its receptors, regulate reproductive functions, even those of the placenta [1]. Homophilic cell-to-cell adhesion activated by a calcium-dependent adhesion molecule, namely cadherin, might contribute to morphogenesis [2]. In addition, sex steroids alter the expression of E-cadherin in reproductive tissues [3–6]. These phenomena prompted us to study the functional role of cadherins in the placenta, since the increased level of sex steroids during pregnancy [7, 8] might be associated with placental function.

The placenta consists of syncytiotrophoblasts and interstitial tissues including vessel endothelial cells [9]. Therefore, the expression and function of 3A(tPA-30-1) cells, derived from human term placenta and transformed by SV40, and HUV-EC-C cells, derived from the endothelial cells in human umbilical cord, were used in this study.

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MATERIALS AND METHODS

Chemicals

Estradiol and progesterone were purchased from Sigma (St. Louis, MO). All other chemicals and reagents were of the experimental grade. These hormones were solubilized in ethanol, and added to the culture media to obtain a final concentration of ethanol below 0.1% (v/v).

Cell culture

3A(tPA-30-1) cells (ATCC CRL 1583), derived from human term placenta and transformed by SV40, were cultured in 90% Alpha MEM and 10% fetal bovine serum (FBS). The cells express transformed phenotype at permissive temperature (33°C) and non-transformed phenotype at non-permissive temperature (40°C). Therefore, 3A(tPA-30-1) cells were cultured before experiments at 40°C. HUV-EC-C cells (ATCC CRL 1730), derived from the endothelial cells in human umbilical cord, were cultured in 90% (v/v) F12 K medium and 10% (v/v) FBS with 100 μg/ml heparin and 30 μg/ml endothelial cell growth supplement. Afterwards, the culture was pro-

ceeded in the conventional medium without FBS or phenol red. Forty-eight hours later, estradiol or progesterone alone, or estradiol plus progesterone were added to the culture dishes. The steroid concentrations are indicated in the corresponding figure legends.

Cell aggregation activity

This bioassay of activity is designed to determine the function of cell-to-cell adhesion activity, namely the activity of adhesion molecules [10–13]. Cells dispersed (1.5×10^5 cells) by treatment with 0.01% trypsin and 1 mM $CaCl_2$ (able to conserve the expression of cadherin on the cell membrane) were seeded to HCMF buffer [1 mM $CaCl_2$, 150 mM NaCl, 5.5 mM KCl, 5.5 mM glucose, 0.35 mM $Na_2HPO_4 \cdot 12H_2O$, 10 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.4] in a 24-well plate coated with 1% (wt/v) albumin. The plate was rocked at 80 rpm at 37°C for 60 min. The number of cell clusters and single cells was counted in five high-power fields using a phase-difference microscope. Aggregation index was defined as the ratio of the sum of the number of single cells and cell clusters to the initial number of dispersed cells [14, 15].

Measure of endothelial cell barrier properties (ECBP) [16, 17]

The upper face of filters in a chemotaxicell (Krabo, Osaka) was coated with 10 μg/ml human fibronectin (60 μl/filter) for 1 h at room temperature, and rinsed with serum-free F12K medium. Dispersed cells (10^5 cells) in 200 μl of the conventional medium (90% F12K medium and 10% FBS with 100 μg/ml heparin and 30 μg/ml endothelial cell growth supplement) were seeded in the upper compartment. The lower compartment was filled with 600 μl of the conventional medium. Culture was continued for 5 d with daily changing of the medium. Before the experiment, the culture medium in both the upper and lower compartments was replaced with serum-free and phenol red-free medium. Horseradish peroxidase (1.1 μl, HRP, 22.7 μM, final concentration: 0.126 μM) was added to the upper compartment. After 30 min, 30 μl of the culture medium in the lower compartment was transferred to a 2 ml-tube containing 860 μl of reaction buffer (50 mM NaH_2PO_4 and 5 mM guaiacol). The reaction to measure the concentration of HRP was started by adding 100 μl H_2O_2 and proceeded for 25 min at room temperature, then the absorbance was measured at 470 nm.

Reverse transcription–polymerase chain reaction (RT-PCR) to amplify E- and V-cadherin mRNAs

Total RNA was isolated from the cells by the acid guanidium thiocyanate–phenol–chloroform extraction method [18]. Total RNA (3 μg) was reverse transcribed with Moloney murine leukemia virus reverse transcriptase (MMLV-RTase, 200 units, Gibco BRL, Gaithersburg, MD) in a buffer of 20 mM Tris–HCl, pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin, 10 mM dithiothreitol (DTT), and 0.5 mM deoxynucleotides to generate cDNAs using random hexamer (50 ng, Gibco BRL) at 37°C for 60 min. The RT reaction mixture was heated at 94°C for 5 min to inactivate MMLV-RTase.

Three cycles of PCR for E-cadherin mRNA from 3A(tPA-30-1) cells were carried out with reverse transcribed cDNA, 0.1 μM specific primers, and Vent DNA polymerase (New England Biolabs, Beverly, MA) in a buffer of 10 mM KCl, 20 mM Tris–HCl, pH 8.8, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% triton X-100, and 0.15 mM deoxynucleotide phosphates using the Iwaki thermal sequencer TSR-300 (Iwaki Glass, Tokyo). Each cycle consisted of (1) denaturation for 1 min at 94°C, (2) annealing for 1 min at 55°C, and (3) extension for 1 min at 72°C. Additionally, 23 cycles of PCR for E-cadherin mRNA with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA from 3A(tPA-30-1) cells as an internal standard were done in the same way. Also 23 cycles of PCR for V-cadherin mRNA with GAPDH mRNA from HUV-EC-C cells were done in the same way.

The oligodeoxynucleotides of specific primers in PCR (Fig. 1) were synthesized according to the published information (cDNA for E-cadherin [19], V-

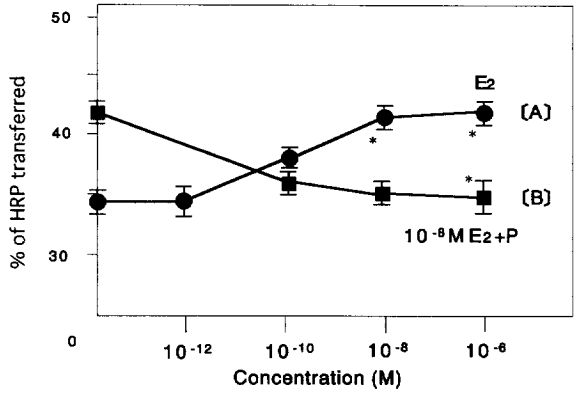

Fig. 1. Dose–response curve showing the effect of sex steroids on endothelial cell barrier properties (ECBP) of HUV-EC-C cells. HUV-EC-C cells on the filter in a chemotaxicell were incubated for 1 h in the serum-free and phenol red-free conventional medium with following sex steroids in [A] and [B]. Thirty minutes after addition of horseradish peroxidase (HRP) to the upper compartment, the quantity of HRP transferred to the lower compartment was determined. Data are the mean ± SD of six determinations. *$p < 0.05$ vs. controls. (A) Dose–response curve showing the effect of estradiol on ECBP, with increasing concentrations of 10^{-12} M to 10^{-6} M estradiol. E_2, estradiol. (B) Dose–response curve showing the effect of progesterone on ECBP altered by 10^{-8} M estradiol, with 10^{-8} M estradiol plus increasing progesterone concentrations up to 10^{-6} M. P, progesterone.

cadherin [20] and GAPDH [21]) as follows (extracellular domain, EC); sense primer for E-cadherin mRNA, 5′-CCATCAGCTGCCCAGAAAAT-3′ (in exon 4/EC 1); antisense primer for E-cadherin mRNA, 5′-TTGGATGACACAGCGTGAGA-3′ (in exon 6/EC 1); sense primer for V-cadherin mRNA, 5′-TGGAACCAGATGCACATTGA-3′ (in EC 1); antisense primer for V-cadherin mRNA, 5′-AAGCTGGAAGGAGTCTCCAG-3′ (in EC 1); sense primer for GAPDH mRNA, 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′ (in exon 2); antisense primer for GAPDH mRNA, 5′-CATGTGGGCCATGAGGTCCACCAC-3′ (in exon 8). The introns of V-cadherin gene have not yet been identified. Therefore, the position in EC 1 of the sense primer for V-cadherin mRNA was set in a position similar to that of the sense primer for E-cadherin mRNA. The antisense primer for E-cadherin mRNA involves the amino acid sequence 'SHAVSS'. However, V-cadherin does not possess 'SHAVSS'. Therefore, the position of the antisense primer for V-cadherin mRNA was simply set in a position similar to that of the antisense primer for E-cadherin mRNA.

Southern blot analysis for quantities of E- and V-cadherin mRNA expression

PCR products were applied to 1.2% agarose gel, and electrophoresis was performed at 50–100 V. PCR products were capillary-transferred to an Immobilon transfer membrane (Millipore, Bedford, MA) for 16 h. The membrane was dried at 80°C for 30 min, and UV-irradiated to tightly fix PCR products. PCR products on the membrane were prehybridized in a 1 M buffer of NaCl, 50 mM Tris–HCl, pH 7.6, and 1% sodium dodecyl sulfate (SDS) at 42°C for 1 h. They were then hybridized in the same solution at 65°C overnight with the biotinylated oligodeoxynucleotide probes synthesized from the sequences of cadherins and GAPDH cDNAs between the specific primers. Specific bands hybridized with the biotinylated probes were detected with Plex Luminescent Kits (Millipore), and an X-ray film was exposed on the membrane at room temperature for 10 min. The quantification of Southern blot was carried out with Bio Image (Millipore, Ann Arbor, MI). The intensity of specific bands was standardized with that of GAPDH mRNA.

Inhibition of ECBP by the antisense oligodeoxynucleotides (AS-ODN) for V-cadherin mRNA

The phosphorothioate sense oligodeoxynucleotides (S-ODN) and AS-ODN for V-cadherin mRNA were synthesized according to the published information (the sequences of 15 mer-upstream from the first codon of V-cadherin cDNA [20]) as follows: S-ODN, 5′-GATGCAGAGGCTATG-3′; AS-ODN, 5′-CATAGCCTCTGCATC-3′. On the other hand, non-specific oligodeoxynucleotides (NS-ODN) for V-cadherin mRNA were synthesized at random as follows: NS-ODN, 5′-GATGCAGAGGCTATG-3′.

The chemotaxicells before the experiment were prepared as described in the measure of ECBP. The three ODNs with increasing concentrations (0.5–50 μM) were added to the medium in the prepared chemotaxicells, and incubated for various times (15–360 min). Then horseradish peroxidase was added to the upper compartment after changing the medium, and the assay of ECBP was accomplished as described in the measure of ECBP.

Western blot analysis for V-cadherin

Tissues (wet weight: 10–20 mg) were homogenized in WB–HB buffer (10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.5% triton X-100 and 0.2 mM phenylmethyl sulfonyl fluoride) with a Polytron homogenizer (Kinematics, Luzern). The protein concentration of samples was measured by the method of Bradford [22]. Each sample (25 μl) containing 25 μg of protein was added to 25 μl of a sample buffer (12.5 mM Tris–HCl, pH 6.8, 2% glycerol, 0.4% SDS and 1.25% 2-mercaptoethanol) and analyzed by 7.5% SDS-PAGE under non-reducing conditions. The gel was transferred to a nitrocellulose membrane (Hybond ECL Western; Amersham, Arlington Heights, IL). The membrane was blocked with 5% milk (from dehydrate) in a blocking buffer (20 mM Tris–HCl, pH 7.6, 137 mM NaCl and 0.1% tween 20), incubated with mouse anti-human V-cadherin antibody (1:1000) (Transduction, Lexington, KY), washed, and then incubated with peroxidase-linked species-specific whole antibody, anti-mouse immunoglobulin from sheep (1:2000) (Amersham, Buckinghamshire). Specific bands were detected with ECL chemiluminescence reagent (Amersham, Arlington Heights, IL), and X-ray film was exposed on the membrane at room temperature for 10 min.

Statistics

The levels of E- and V-cadherin mRNAs and the indexes of cell aggregation and peroxidase permeability were measured in six experiments. Statistical analysis was performed with Student's *t*-test (for Figs 1, 3, 6 and 8) and one-way ANOVA (for Figs 2, 4, 7 and 9); differences were considered significant when $p < 0.05$ and tendentious when $p < 0.1$.

RESULTS

In the dose–response curve showing the effect of estradiol on endothelial cell barrier properties (ECBP) of HUV-EC-C cells, the quantity of horseradish peroxidase (HRP) passed from the top chamber to the bottom chamber was 100% when no endothelial barrier was seen in a chemotaxicell. The baseline (untreated) figure for HUV-EC-C cells was

32%. Estradiol increased the quantity dose-dependently up to 10^{-8} M in 1 h (Fig. 1[A]). On the other hand, progesterone at 10^{-6} M reversed the ratio increased by estradiol in 1 h (Fig. 1[B]). Thus the concentration of 10^{-8} M estradiol or 10^{-6} M progesterone was used for the following experiment. In the time course for the effect of sex steroids on ECBP of HUV-EC-C cells, estradiol significantly ($p < 0.05$) increased the quantity with the peak between 1 and 2 h, which decreased by 3 h, but progesterone alone did not demonstrate any effect on it (Fig. 2). Progesterone significantly ($p < 0.05$) reversed the ratio increased by estradiol (Fig. 2).

In the dose–response curve (Fig. 3) and time course (Fig. 4) for the effect of sex steroids on the cell aggregation index of 3A(tPA-30-1) and HUV-EC-C cells, neither estradiol nor progesterone demonstrated any effect on the activity of cell aggregation.

PCR templates were prepared from reverse transcribed total RNA (100 μg) of HUV-EC-C cells as follows: 1/8 ×, 0.375 μg total RNA reverse transcribed (RNA-RT); 1/4 ×, 0.75 μg RNA-RT; 1/2 ×, 1.5 μg RNA-RT; 1 ×, 3 μg RNA-RT (the standard amount of RNA-RT in this assay); 2 ×, 6 μg RNA-RT and 4 ×, 12 μg RNA-RT. The signal intensity curve for V-cadherin mRNA levels ranging from 1/8× to 2× of RNA-RT of HUV-EC-C cells by RT-PCR-Southern blot analysis (SBA) was linear (Fig. 5). The signal intensity curve for E-cadherin mRNA levels ranging from 1/8× to 2× of RNA-RT of 3A(tPA-30-1) cells by RT-PCR-SBA was also linear (data not shown). Therefore, the semiquantative level of the mRNA expression was thought to be reliable.

The level of the mRNA expressions without treatment was assigned as one arbitrary unit/GAPDH mRNA (AU/GAPDH mRNA). In the dose–response

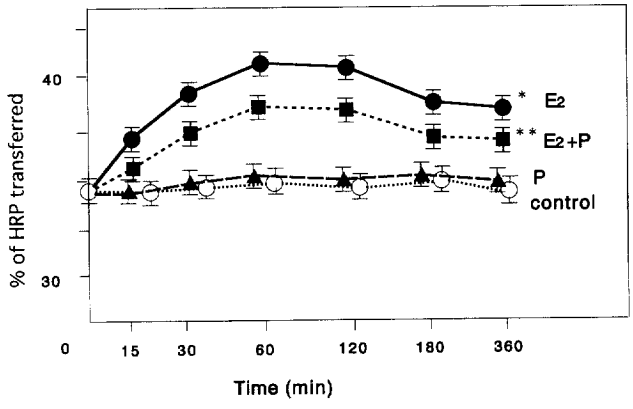

Fig. 2. Time course for the effect of sex steroids on ECBP of HUV-EC-C cells. HUV-EC-C cells were incubated with the same medium as for Fig. 1 with 10^{-8} M estradiol, 10^{-8} M estradiol plus 10^{-6} M progesterone, and 10^{-6} M progesterone for 6 h. The quantity was determined as described for Fig. 1. Data are the mean ± SD of six determinations. *$p < 0.05$ vs. controls; **$p < 0.1$ vs. treated with only E_2.

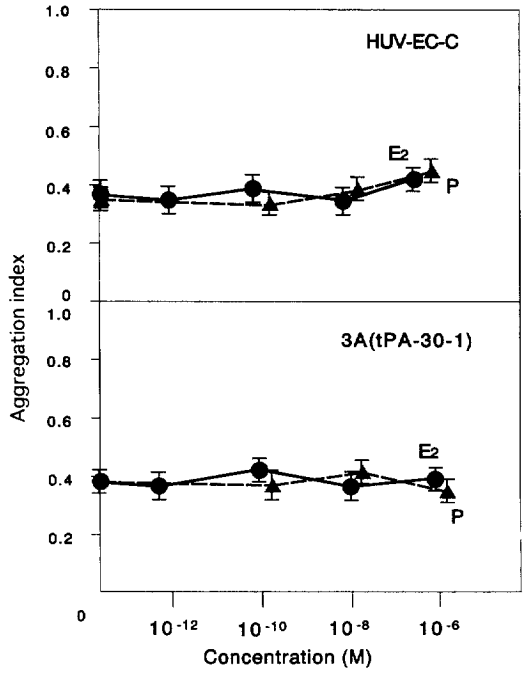

Fig. 3. Dose–response curve showing the effect of sex steroids on cell aggregation index of 3A(tPA-30-1) and HUV-EC-C cells. The cells in each plate were incubated in the serum-free and phenol red-free conventional medium with increasing concentrations of 10^{-12} to 10^{-6} M estradiol and 10^{-10} M to 10^{-6} M progesterone for 6 h. After the cells were rocked for 60 min, the number of cell clusters and single cells was counted using a phase-difference microscope for aggregation index. Data are the mean ± SD of six determinations.

curve showing the effect of estradiol on the level of V-cadherin mRNA in HUV-EC-C cells, estradiol decreased the level of V-cadherin mRNA dose-dependently up to 10^{-8} M in 1 h (Fig. 6[A]). On the other hand, progesterone at 10^{-6} M reversed the level decreased by estradiol in 1 h (Fig. 6[B]). The concentration of 10^{-8} M estradiol or 10^{-6} M progesterone was used for the following experiment. In the time course for the effect of sex steroids on the level of V-cadherin and its mRNA in HUV-EC-C cells, estradiol significantly ($p < 0.05$) decreased their levels by 1 h, which returned to the initial level in 6 h. However, progesterone alone did not demonstrate any effect on them (Figs 7 and 8). Progesterone significantly ($p < 0.05$) reversed the level decreased by estradiol (Figs 7 and 8).

In the dose–response curve and time course for the effect of sex steroids on the level of E-cadherin mRNA in 3A(tPA-30-1) cells, neither estradiol nor progesterone demonstrated any effect (Fig. 6[C] and Fig. 7).

Furthermore, to demonstrate the role of V-cadherin on ECBP directly, the HUV-EC-C cells in a chemotaxicell were treated with three phosphorothioate antisense (AS-ODN), sense (S-ODN) and non-specific oligodeoxynucleotides (NS-ODN) for V-cadherin mRNA. In the dose–response curve showing the

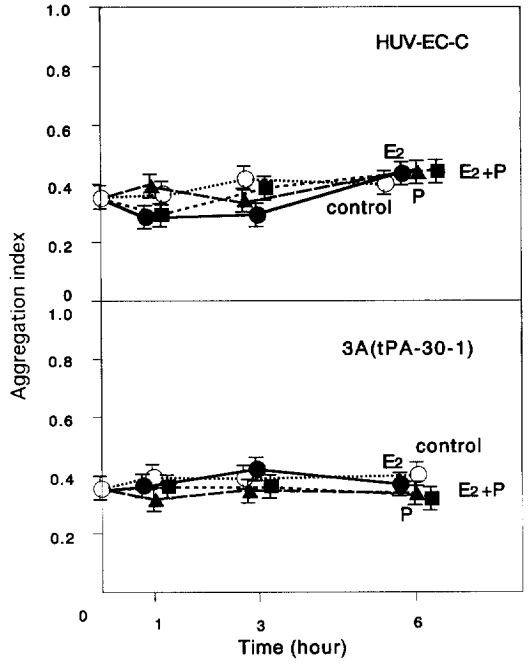

Fig. 4. Time course for the effect of sex steroids on cell aggregation index of 3A(tPA-30-1) and HUV-EC-C cells. The cells were incubated with the same medium as for Fig. 3 with 10^{-8} M estradiol, 10^{-8} M estradiol plus 10^{-6} M progesterone, and 10^{-6} M progesterone for 6 h. The quantity was determined as described for Fig. 3. Data are the mean ± SD of six determinations.

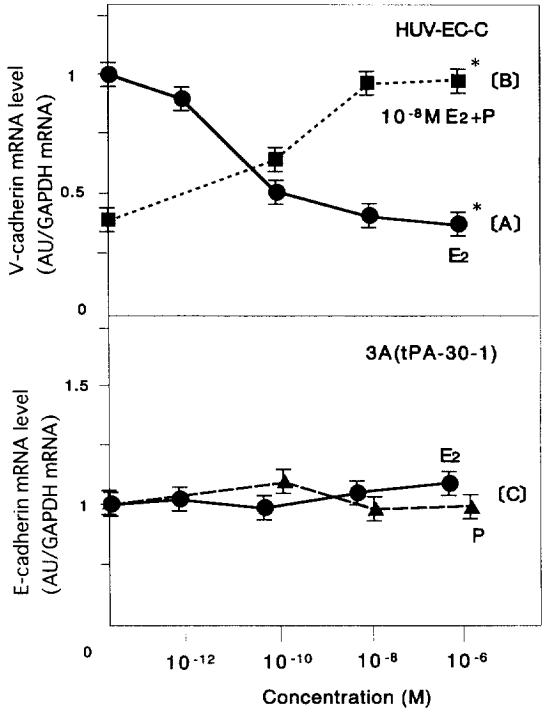

Fig. 6. Dose–response curve showing the effect of sex steroids on E-cadherin and V-cadherin mRNA expressions. The cells in each plate were incubated for 1 h with the same medium as for Fig. 3 with following sex steroids in [A], [B] and [C]. The quantity was determined as described for Fig. 5. Data are the mean ± SD of six determinations. *$p < 0.05$ vs. controls. [A] Dose–response curve showing the effect of estradiol on V-cadherin mRNA expression of HUV-EC-C cells, with increasing estradiol concentrations up to 10^{-6} M. [B] Dose–response curve showing the effect of progesterone on V-cadherin mRNA expression suppressed by 10^{-8} M estradiol of HUV-EC-C cells, with 10^{-8} M estradiol plus increasing progesterone concentrations up to 10^{-6} M. [C] Dose–response curve showing the effect of sex steroids on E-cadherin mRNA expression of 3A(tPA-30-1) cells, with increasing estradiol or progesterone concentrations up to 10^{-6} M.

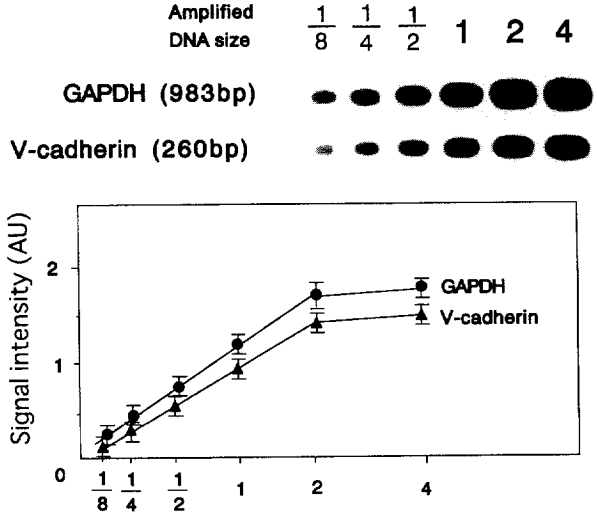

Fig. 5. Signal intensity curve for V-cadherin mRNA level in a series of reverse transcribed-total RNA of HUV-EC-C cells by reverse transcription–polymerase chain reaction–Southern blot analysis (RT-PCR-SBA). RT was carried out on total RNA (100 μg) isolated from HUV-EC-C cells. Then PCR-SBA was performed on a series of reverse transcribed-total RNA (3 μg) ranging from 1/8× to 4×. The representative Southern blots for the expressions of V-cadherin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs are shown. The level of mRNA expressions in the cells was assigned as one arbitrary unit (AU). Data are the mean ± SD of six determinations.

effect of AS-ODN, S-ODN and NS-ODN on ECBP of HUV-EC-C cells, the quantity was increased by AS-ODN dose-dependently from 1 to 25 μM, and by S-ODN and NS-ODN dose-dependently from 25 to 100 μM for 1 h (Fig. 9). Thus the concentration of 5 μM AS-, S- and NS-ODNs was used for the following experiment. In the time course for the effect of the three ODNs on ECBP of HUV-EC-C cells, AS-ODN significantly ($p < 0.05$) increased ECBP and decreased V-cadherin expression in 1 h, but S-ODN and NS-ODN did not demonstrate any effect on them (Figs 10 and 11).

DISCUSSION

The role of cadherins for conversion, rearrangement and separation between epithelium and non-epithelium in body morphogenesis can be demonstrated from the following. The function of E-cadherin is switched on with the conversion of non-

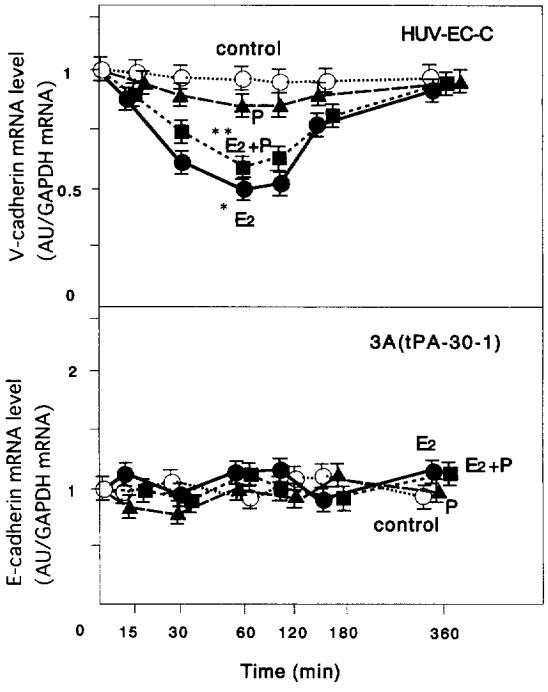

Fig. 7. Time course for the effect of sex steroids on E-cadherin mRNA expression of 3A(tPA-30-1) cells and V-cadherin mRNA expression of HUV-EC-C cells. The cells in each plate were incubated for 1 h with the same medium as for Fig. 3 with 10^{-8} M estradiol, 10^{-8} M estradiol plus 10^{-6} M progesterone, and 10^{-6} M progesterone for 6 h. The quantity was determined as described for Fig. 5. Data are the mean ± SD of six determinations. *$p < 0.05$ vs. controls; **$p < 0.1$ vs. treated with only E_2.

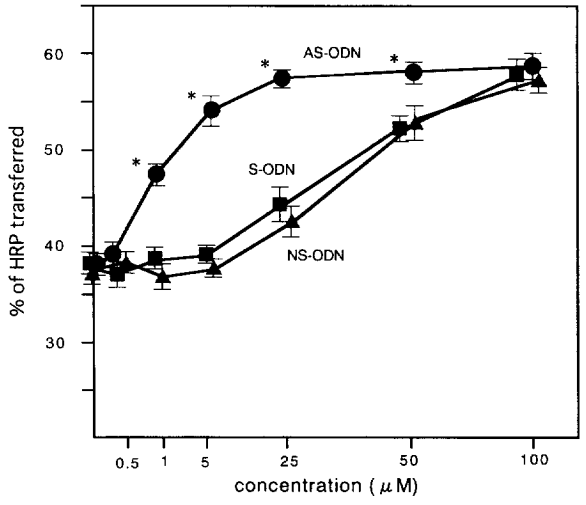

Fig. 9. Dose–response curve showing the effect of antisense (AS-ODN), sense (S-ODN) and non-specific oligodeoxynucleotides (NS-ODN) for V-cadherin mRNA on ECBP of HUV-EC-C cells. HUV-EC-C cells on the filter in a chemotaxicell were incubated for 1 h in the serum-free and phenol red-free conventional medium with increasing concentrations of 0.5 μM to 100 μM AS-, S- and NS-ODNs. The quantity was determined as described for Fig. 1. Data are the mean ± SD of six determinations. *$p < 0.05$ vs. NS-ODN.

epithelium to epithelium in oocytes in compaction [23]. Ectodermal cells are separated from the primitive streak as the ectodermal germ layer and invaginate to form the intra-embryonic mesodermal germ layer, in which E-cadherin expression is switched off and N-cadherin expression is on [23]. Ectodermal cells are then separated from the ectodermal germ layer and invaginate to form the neural tube, in which again E-cadherin expression is switched off and N-cadherin expression is on [24]. In the formation of the otic vesicle and Wolffian duct,

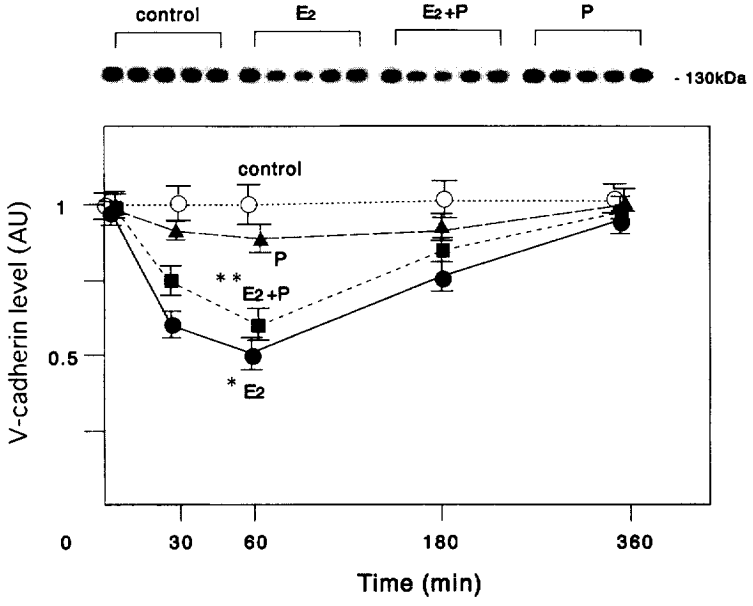

Fig. 8. Time course for the effect of sex steroids on V-cadherin expression of HUV-EC-C cells. The cells in each plate were incubated for 1 h with the same medium as for Fig. 3 with 10^{-8} M estradiol, 10^{-8} M estradiol plus 10^{-6} M progesterone, and 10^{-6} M progesterone for 6 h. Western blot analysis was carried out as described in Methods. Data are the mean ± SD of three determinations. AU, arbitrary unit. *$p < 0.05$ vs. controls; **$p < 0.1$ vs. treated with only E_2.

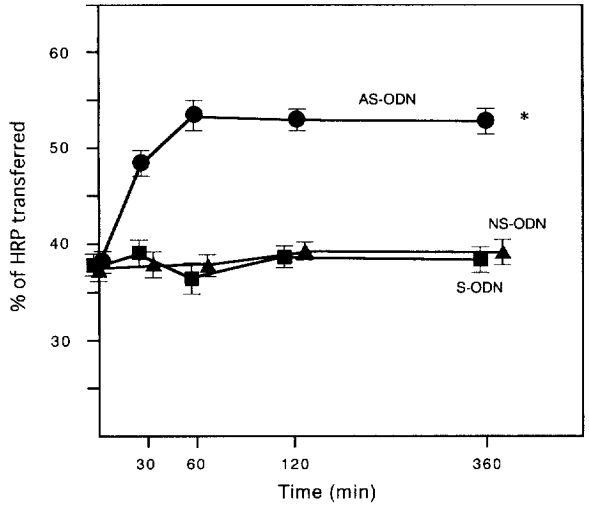

Fig. 10. Time course for the effect of AS-ODN, S-ODN and NS-ODN for V-cadherin mRNA on ECBP of HUV-EC-C cells. HUV-EC-C cells were incubated with the same medium as for Fig. 8 with 5 μM AS-, S- and NS-ODNs for 6 h. The quantity was determined as described for Fig. 1. Data are the mean ± SD of six determinations. *$p < 0.05$ vs. NS-ODN.

the conversion of E-cadherin expression to N-cadherin expression occurs [24]. Ectodermal cells nearby the optic vesicle form the lens placode, which invaginate to form the lens vesicle, in which the conversion of P-cadherin expression to N-cadherin expression occurs [24]. Renal vesicle developed from the metanephric tissue cup is rearranged with excretory tubules, resulting in the conversion of N-cadherin expression to E-cadherin expression [24]. These are representative phenomena of body morphogenesis, therefore adhesion molecules including cadherins might be associated with almost all steps of morphogenesis.

On the other hand, V-cadherin, a 130–135 KDa glycoprotein, conserves five repetition sequences which consist of the extracellular domain in the N-terminus as in other cadherins, but not the amino acid sequence 'HAV' in the first extracellular domain specifically related to homophilic cell-to-cell adhesion [20]. Specific expression of V-cadherin was seen in endothelial cells of human umbilical vein and placenta [25]. A role of V-cadherin, consisting of interendothelial junctions, was demonstrated *in vitro* as a dynamic process, namely vessel permeability [16].

In the present study, the vessel permeability and cell aggregation potential related to cadherins in 3A(tPA-30-1) as trophoblastic cells and in HUV-EC-C cells as endothelial cells were analyzed under the influence of sex steroids. 3A(tPA-30-1) cells expressed relatively low E-cadherin mRNA, but not V-cadherin mRNA. The present result is consistent with the reduction of E-cadherin expression in cytotrophoblasts during the differentiation into syncytiotrophoblasts [26]. Therefore, the trophoblastic cell line 3A(tPA-30-1) cell is considered not to be well representative of the syncytiotrophoblast. However, in present study, sex steroids did not alter the expression of E-cadherin mRNA, and the cell aggregation as the index of cell adhesion potential in 3A(tPA-30-1) cells. This suggests that it is useful to demonstrate no steroidal regulation of cell aggregation associated with E-cadherin expression in trophoblastic cells as a negative phenomenon.

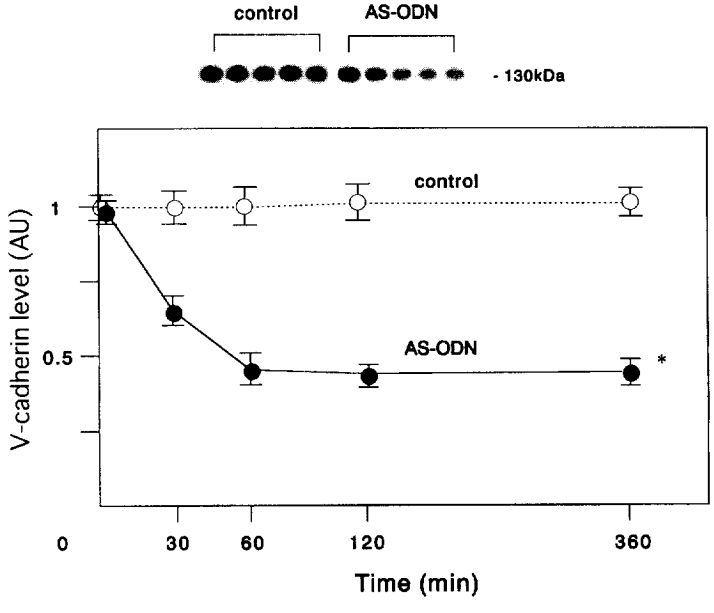

Fig. 11. Time course for the effect of AS-ODN for V-cadherin expression of HUV-EC-C cells. HUV-EC-C cells were incubated with the same medium as for Fig. 8 with 5 μM AS-ODN for 6 h. Western blot analysis was carried out as described in Methods. Data are the mean ± SD of three determinations. AU, arbitrary unit. *$p < 0.05$ vs. controls.

On the other hand, HUV-EC-C cells expressed V-cadherin and its mRNA, but not E-cadherin mRNA. In addition, estrogen transiently decreased the expression of V-cadherin and its mRNA and increased vessel permeability, while progesterone reversed the estrogen-induced events in HUV-EC-C cells. Furthermore, A-ODN for V-cadherin mRNA specifically increased vessel permeability and decreased V-cadherin expression in HUV-EC-C cells. Therefore, sex steroids play a role in placental development and function related to V-cadherin, plausibly via vessel permeability for the utilization of placental products.

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