Effect of angiotensin II on ion transport across human Fallopian tube epithelial cells *in vitro*

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Angiotensin II type 1 receptors have been identified in Fallopian tube epithelia. Polarized confluent human Fallopian tube epithelial cell cultures were used under short-circuit conditions to study the actions of angiotensin II on electrogenic ion transport. The results demonstrate that angiotensin II increases baseline short-circuit current, implying a net transport of negatively charged ions from a basal to apical direction. This effect was inhibited by the selective angiotensin II type 1 receptor antagonist losartan. The effects of angiotensin II on short-circuit current were

rapid in onset, brief in duration, and although less than those achieved with ATP, similar in amplitude to those described for other epithelia with angiotensin II. These findings reflect a significant retention of function for these cells in monolayer culture. Immunohistochemistry using the antibody 6313/G2, which is directed against a specific sequence in the extracellular domain of the angiotensin II type 1 receptor, confirmed that the receptor was retained in cultured cells. The results indicate that angiotensin II plays a role in regulating the composition of Fallopian tube secretions.

Introduction

The Fallopian tube is the site of several important reproductive events, including gamete transport and conditioning, fertilization and pre-implantation embryonic development. Tubal fluid provides the appropriate milieu for these reproductive events, and has an ionic composition that varies during the ovarian cycle (David *et al.*, 1973) and differs from that of plasma (Lippes *et al.*, 1972; David *et al.*, 1973). Mechanisms responsible for the formation and composition of tubal fluid in animals (Brunton and Brinster 1971; Gott *et al.*, 1988; Dickens *et al.*, 1993) and humans (Dickens *et al.*, 1995; Downing *et al.*, 1997) implicate a role for ATP, acting via increases in intracellular calcium, in stimulating chloride secretion. Water then follows the osmotic gradient created by chloride transport. Despite this important finding, knowledge about the mechanisms responsible for the formation and composition of human tubal fluid remains limited and further studies are needed.

It is possible that agents other than nucleotides, such as angiotensin II, stimulate ion transport in the Fallopian tube. This peptide is known to act in a paracrine–autocrine manner to effect ion transport in a variety of epithelia, including those of the rat intestine (Cox *et al.*, 1987), rat cauda epididymidis (Wong *et al.*, 1989) and canine trachea (Tamaoki *et al.*, 1992). The presence of angiotensin II type 1

and 2 receptors was demonstrated in the human Fallopian tube, and angiotensin II was shown to stimulate increases in the ciliary beat frequency in the mucosa of Fallopian tube explants, and this stimulatory effect is inhibited by the selective angiotensin II type 1 receptor antagonist, losartan (Saridogan *et al.*, 1996). The actions of angiotensin II on electrogenic ion transport across polarized human Fallopian tube epithelial cell cultures, under short-circuit conditions, were studied to determine whether angiotensin II also regulates electrolyte and fluid transport in Fallopian tube epithelium.

Materials and Methods

Tissue collection

Fallopian tubes that were healthy in appearance were collected at various stages of reproductive life from women undergoing hysterectomy for benign conditions, after obtaining written consent and appropriate local ethical committee approval.

Patients were aged between 31 and 54 years (mean 47 ± 1.65 years). On average two cell cultures (range 1–4) from each patient were used for electrophysiological studies. Histological analysis of the endometrium revealed a proliferative pattern in four patients (eight cell cultures were grown from these patients) and a secretory pattern in three patients (ten cell cultures). In six patients, the histology revealed an atrophic or inactive pattern (ten cell cultures) of which hormonal studies confirmed that two were post-menopausal. Classification was not possible in three patients (four cell cultures).

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Fallopian tubes were collected into ice-cold minimum essential medium (MEM) with Earle's salts and L-glutamine and supplemented with 1.8 iu heparin ml⁻¹ (CP Pharmaceuticals, Wrexham), 50 µg streptomycin ml⁻¹ (Evans Medical, Leatherhead), 100 iu penicillin ml⁻¹ (Glaxo Laboratories, Stockley Park) and 10 mmol Hepes l⁻¹ (Life Technologies, Paisley) and processed immediately for cell culture.

Epithelial cell culture

The method used for Fallopian tube epithelial cell culture and characterization was similar to that described by Dickens *et al.* (1993) and Kervancioglu *et al.* (1994). Fallopian tubes were rinsed thoroughly in MEM with Earle's salts and L-glutamine and supplemented with heparin, streptomycin, penicillin and Hepes, and cut longitudinally along the antimesenteric border. Healthy 5 mm portions of mucosa were excised from the ampullary and fimbrial portions of both Fallopian tubes. The samples from each patient were pooled as there is no evidence to indicate that the basic mechanisms of electrolyte transport should differ between contralateral Fallopian tubes or along the length of an individual Fallopian tube *in vitro*. Tissue explants were digested in Ca²⁺- and Mg²⁺-free Hank's balanced salt solution (HBSS; Life Technologies) in tissue culture flasks containing 0.5% trypsin and 2.7% pancreatin (Sigma Chemical Company, Poole) for 1 h at 4°C followed by 1 h at room temperature. The enzyme medium was removed, MEM added and the culture flask shaken to release epithelial cells into suspension. The procedure was repeated and the suspensions centrifuged at 500 g for 5 min. The cells were washed in MEM and, after centrifugation, re-suspended in culture medium at a concentration of 10⁶ cells ml⁻¹.

The culture medium consisted of a 1:1 mix of Dulbecco's modified Eagle's medium and F12 Ham's nutrient mix (Life Technologies) with 50 µg streptomycin ml⁻¹ (Evans Medical), 100 iu penicillin ml⁻¹ (Glaxo Laboratories), 10% fetal bovine serum (Sigma) and 15 µg fungizone ml⁻¹ (Life Technologies). Cell suspensions (250 µl) were plated on to Snapwell clear polyester filters (12 mm in diameter, effective insert growth area 1 cm², 0.4 µm pore size) in six-well plates (Corning Costar, High Wycombe) and cultured at 37°C, under 5% CO₂:95% air in 100% humidity. Filters were previously coated with type IV collagen (Sigma) and sterilized by overnight exposure to UV light. Cell growth was observed periodically under a phase-contrast inverted microscope until confluence was attained and then cultures were processed for electrophysiological studies. The culture medium was changed after 24 h and thereafter every 48 h until confluence was attained. A total of 32 confluent Fallopian tube cell cultures were established from 16 patients and included in the study.

Immunohistochemistry

Angiotensin II type 1 receptor immunostaining using the monoclonal antibody 6313/G2, which is directed against a

specific sequence in the extracellular domain of the angiotensin II type 1 receptor (Barker *et al.*, 1993), was used to determine whether the angiotensin II type 1 receptor was retained in culture.

Batches of cell cultures grown on glass microscope slides under the same culture conditions as those used for electrophysiological studies and from the same patients were fixed in formalin between days 3 and 9 of culture. Cells were washed twice in PBS with 0.2% (v/v) Tween (polyoxyethylene sorbitol monolaurate; Sigma) before incubation with methanol containing 3% (v/v) H₂O₂ for 30 min to block endogenous peroxidase activity. Non-specific sites were blocked with normal rabbit immunoglobulin at 37°C for 1 h (0.2% Tween in PBS with 10% normal rabbit immunoglobulins and 2% (w/v) milk; Dako, High Wycombe). After overnight incubation with 6313/G2 antibody at 4°C, cells were washed with 0.2% (v/v) Tween PBS and then incubated with secondary antibody (1:300 dilution of rabbit anti-mouse IgM in 1% Tween PBS; Dako) for 1 h at room temperature. After washing with 0.2% (v/v) Tween PBS, cells were incubated with streptavidin-biotin complex (Dako) for 30 min at room temperature; 3,3'-diaminobenzidine tetrahydrochloride (Sigma) was used for visualization. Slides were counterstained with Gill's haematoxylin (Sigma) for 2 min, washed in tap water for 5 min, differentiated briefly in acid alcohol, rewashed in water, dehydrated in industrial methylated spirit (BDH, Lutterworth), cleared with xylene and mounted with DPX mountant. Human adrenal tissue was used as a positive control and the 6313/G2 antibody was omitted in negative controls.

Immunofluorescence for cytokeratin and vimentin.

Immunofluorescence was performed on parallel cultures grown on glass slides, with cytokeratin and vimentin antibodies, to determine the percentage of epithelial cells in cultures.

Cells were fixed in a fresh mixture of equal parts of methanol and acetone for 2 min and washed in Tris-buffered saline (TBS; 50 mmol Tris l⁻¹, 150 mmol NaCl l⁻¹, pH 7.6; Amersham International, Amersham). Non-specific sites were blocked with normal rabbit serum (Dako) at 1:5 dilution in TBS for 20 min. After a 60 min incubation with mouse monoclonal anticytokeratin or antivimentin antibodies (1:100 dilution in TBS; followed by washing for 5 min in TBS), the slides were incubated with fluorescein isothiocyanate (FITC)-conjugated antimouse IgG antiserum (1:20 dilution in TBS; Dako) for 30 min. Excess antibody was removed as above and the cells were examined by fluorescence microscopy. All experiments included control slides in which the primary antibody was omitted.

Electrophysiological studies

All test substances and inhibitors used were purchased from Sigma.

Trans-epithelial electrophysiological properties of cultured Fallopian tube cells were studied by mounting Snapwell inserts containing confluent monolayers of cells between two modified perspex Ussing half-chambers (Ussing and Zerahn, 1950). The principle of this electrophysiological assessment of ion transport is to eliminate any chemical gradient across the cell cultures by adding equal concentrations of Krebs–Henseleit solution to either side of the culture and to eliminate any electrical gradient by clamping the potential difference at zero volts. In this state, any detected current (the short-circuit current, SCC) is attributable to the active transport of ions. An assessment of the prior potential difference (the open-circuit potential difference) between the apical and basal aspects of the cell culture may be made before voltage clamping.

After mounting Snapwell inserts containing confluent monolayers of cells into the Ussing chambers, the cells were bathed on both sides with equal concentrations of modified Krebs–Henseleit solution with the following composition: 118 mmol NaCl l⁻¹, 25 mmol NaHCO₂ l⁻¹, 11.5 mmol glucose l⁻¹, 4.7 mmol KCl l⁻¹, 2.56 mmol MgCl₂ l⁻¹, 1.19 mmol KH₂PO₄ l⁻¹, 2.53 mmol CaCl₂ l⁻¹. The Krebs–Henseleit solution was gassed with 95% O₂:5% CO₂ and maintained at 37°C.

Before any electrical measurements and addition of test substances, the Krebs' solution bathing either side of the epithelial cell layer was changed several times to remove any cell debris and culture medium and the current allowed to reach a stable reading. At this point a record of the open-circuit potential difference (mV) was made. The tissue was then short-circuited with a DVC-1000 dual voltage high impedance clamp (World Precision Instruments, Sarasota, FL) and the SCC measured in $\mu\text{A cm}^{-2}$ and recorded continuously using a Linseis flat-bed four-channel recorder (Linseis, Cambridge). At this stage test substances of interest were added to the serosal or mucosal aspect of the cell cultures and the change in SCC was recorded graphically.

The voltage clamp was intermittently raised to 1 mV during experimentation and the change in current was used to calculate the resistance (Ω) using Ohm's law. Angiotensin II (0.1–10 $\mu\text{mol l}^{-1}$) was made up in Krebs' solution and added in small volumes to the serosal side of the polarized cell preparation.

Krebs' solution alone, at the volumes and concentrations used in this study, had no effect on the electrical properties of the cell cultures in control experiments over prolonged periods.

In each experiment in which a single test substance, such as angiotensin II or captopril, was used alone, the SCC before addition of hormone was taken as the basal control value. In experiments involving the further addition of ion-channel inhibitors and angiotensin-receptor antagonists, the stable SCC after the addition of these agents was taken as the control value, and compared with the angiotensin II-stimulated SCC. Other protocols are described below, but in every case each culture provided its own control values in this way.

Experiments

Nine cell cultures were randomly selected from seven patients to determine whether locally produced angiotensin II affects ion transport *in vitro*. The SCC of the cultures was recorded (control) and then the angiotensin-converting enzyme inhibitor captopril was added in concentrations of up to 10 $\mu\text{mol l}^{-1}$. The effect on the SCC was measured over 40 min. These cell cultures were then washed several times and the SCC (control) was allowed to stabilize. Angiotensin II (0.1–10 $\mu\text{mol l}^{-1}$) was added and the effect on the SCC recorded. These cell cultures were then discarded.

A further 23 cell cultures from nine patients were stimulated with angiotensin II alone. Of these, ten cell cultures from three patients that had shown a significant response to angiotensin II stimulation were then washed several times and the sodium-ion channel blocker amiloride (100 $\mu\text{mol l}^{-1}$; amiloride is an inhibitor of the Na⁺–H⁺ antiport) was added for 30 min. The SCC was allowed to stabilize (control); one further dose of angiotensin II was added and the effect noted. These cell cultures were then discarded.

The effect of chloride-ion channel blockade was tested in a similar way by preincubating four cell cultures from three patients with the chloride-channel inhibitor 4-acetamido-4' isothiocyanostilbene-2,2-disulphonic acid (SITS, 1 mmol l⁻¹; SITS is an inhibitor of the Cl⁻–HCO₃⁻ exchanger). These cell cultures were then discarded.

In the above experiments, each cell culture was tested with no more than one inhibitor and no more than two doses of angiotensin II.

Preliminary experiments using this approach demonstrated that two additions of angiotensin II with thorough washing in between caused little observable tachyphylaxis in the SCC response.

The receptor mediating the response to angiotensin II was determined by adding the selective angiotensin II type 1 receptor antagonist losartan (DUP, 753; Du Pont Merck, Wilmington, DE) to a fresh set of six cell cultures established from three patients that had previously demonstrated a significant response to angiotensin II stimulation. The SCC was allowed to stabilize (control) and the subsequent effect of angiotensin II on the SCC was recorded. The effect of the angiotensin II type 2 receptor antagonist PD123,319 was tested in a similar way using a different set of five fresh cell cultures from the same three patients. As a positive control, the effect of angiotensin II on the SCC in rat descending colon was assayed, using the method of Cox *et al.* (1987).

Results

Epithelial cell culture

Fallopian tube cell cultures were confluent after 4–9 days in culture (mean 6.2 ± 0.52 days). Cytokeratin and vimentin immunofluorescence established that > 95% of all cells in culture were epithelial in origin. Transmission electron microscopy demonstrated polarized cell growth on collagen-coated filters with the basal side in contact with

Fig. 1. Immunostaining for the angiotensin II type 1 receptor in Fallopian tube primary culture with the 6313/G2 antibody. The angiotensin II type 1 receptor is stained brown ($\times 200$). Scale bar represents $40\ \mu\text{m}$.

the collagen layer and the apical surfaces covered with microvilli. Fallopian tube cells on day 5 in culture are shown (Fig. 1). Positive angiotensin II type 1 receptor immunostaining demonstrates that the receptor is retained in Fallopian tube epithelial cells in culture (Fig. 1).

Electrophysiological studies

Baseline values. Cell cultures were excluded from the study if the basal resistance was $< 50\ \Omega$, if the resistance deteriorated significantly during experimentation, or if a stable reading of SCC could not be obtained. A total of 32 confluent Fallopian tube cell cultures was established from 16 patients and included in the study and their baseline SCC and resistance were $8.5 \pm 1.2\ \mu\text{A cm}^{-2}$ and $200 \pm 21\ \Omega$, respectively. The resistance of cell cultures ranged between 50 and $500\ \Omega$ and varied little during the course of the experiment, as demonstrated by the current that flowed in response to a planned increase in potential difference from 0 to 1 mV across cell cultures. The direction of current flow under short-circuit conditions implied active net transport of negatively-charged ions from a basal to apical direction. The average open-circuit potential difference (before voltage clamping) was $2.5 \pm 0.7\ \text{mV}$ with the apical side negative relative to the basal.

Collagen-coated Snapwell inserts alone contributed negligibly to the value of baseline electrical parameters of cell cultures as the resistance and potential difference across collagen-coated blank inserts on day 7 in culture were $4.9 \pm 1.6\ \Omega$ and $0.13 \pm 0.09\ \text{mV}$, respectively.

Effect of angiotensin II on baseline SCC of epithelial cell cultures

The addition of angiotensin II at $10\ \mu\text{mol l}^{-1}$ to the basal aspect of 32 confluent Fallopian tube cell cultures resulted in an increase in SCC, implying active transport of ions

(Table 1; $P < 0.001$, $n = 32$, Wilcoxon signed rank test). The response to angiotensin II was seen in 19 of the 32 cell cultures stimulated. The increase in SCC stimulated by angiotensin II commenced within 30–60 s, reached a peak within 60 s and returned to the baseline slowly. The whole response lasted 2–8 min and ranged in magnitude from 0.5 to $1.5\ \mu\text{A cm}^{-2}$, and the mean value for the 19 cultures that did respond was $0.9 \pm 0.1\ \mu\text{A cm}^{-2}$. Although these values were numerically small, clear peaks were obtained at high recording sensitivities owing to stable baseline SCC and a high signal-to-noise ratio. Lower concentrations of angiotensin II (1.0 and $0.1\ \mu\text{mol l}^{-1}$) produced smaller peaks of SCC that were difficult to quantify numerically.

Effect of ion transport inhibitors on the SCC response to angiotensin II

When angiotensin II-responsive cultures were washed, and then treated with amiloride, subsequent retreatment with angiotensin II gave enhanced responses (Table 2). Conversely, similar pretreatment of responding cell cultures with $1\ \text{mmol SITS l}^{-1}$ prevented the SCC response after angiotensin II stimulation, implicating Cl^- in the SCC response to angiotensin II (Table 2).

Effect of captopril on baseline SCC of epithelial cell cultures

The angiotensin-converting enzyme inhibitor captopril added to the apical or basal side of a random selection of confluent epithelial cell monolayers in concentrations of up to $10\ \mu\text{mol l}^{-1}$ had no effect on SCC (Table 2).

Effect of angiotensin II receptor antagonists on the SCC response to angiotensin II

The effect of angiotensin II receptor antagonists was assessed using fresh cultures established from three patients that had previously demonstrated a significant response to angiotensin II stimulation. The endometrial histology showed a proliferative, secretory and inactive pattern in these three patients. The effect of angiotensin II was inhibited by the angiotensin II type 1 receptor antagonist losartan (DUP, 753), but not by the angiotensin II type 2 receptor antagonist PD123, 319 (Table 1).

Thirteen cell cultures did not respond to angiotensin II stimulation. Differences or similarities between cultures that did and did not respond were determined by examining their quality as indicated by baseline resistance, potential difference, response to the known agonist ATP and the clinical characteristics of the patients from whom they originated. The resistance and potential difference of the 13 cultures that did not respond to angiotensin II stimulation were higher ($218 \pm 49\ \Omega$ and $3.7 \pm 1.2\ \text{mV}$, respectively) than those of the 19 cultures that did respond to angiotensin II ($187 \pm 14.5\ \Omega$ and $1.3 \pm 0.21\ \text{mV}$, respectively).

Furthermore, the characteristics and magnitude of the change in SCC of responding and non-responding cultures

Table 1. Effect of angiotensin II (AII) and AII receptor antagonists on short-circuit current (SCC) of cultured human Fallopian tube epithelial cells

	Number of cell cultures	SCC ($\mu\text{A cm}^{-2} \pm \text{SE}$)
Before AII (all samples)	32	8.5 ± 1.2
After AII (all samples)	32	$9.0 \pm 1.3^{**}$
Before AII (responders)	19	7.8 ± 1.8
After AII (responders)	19	$8.7 \pm 1.9^{**}$
PD alone	5	4.0 ± 1.1
PD + AII	5	$5.1 \pm 1.1^*$
DUP alone	6	4.3 ± 0.6
DUP + AII	6	4.5 ± 0.7

DUP: DUP, 753 (losartan) angiotensin II type 1 receptor antagonist; PD: PD123 319, angiotensin II type 2 receptor antagonist.

Asterisks indicate values that are significantly different: * $P < 0.05$; ** $P < 0.001$ (Wilcoxon signed rank).

Table 2. The effect of captopril and ion-channel blockers on short-circuit current (SCC) of cultured human Fallopian tube epithelial cells

	Number of cell cultures	SCC ($\mu\text{A cm}^{-2} \pm \text{SE}$)
SITS alone	4	5.25 ± 1.3
SITS + AII	4	5.25 ± 1.3
Amiloride alone	10	7.5 ± 0.9
Amiloride + AII	10	$9.2 \pm 1.1^*$
Before captopril	9	11.4 ± 3.8
After captopril	9	11.4 ± 3.8

All: Angiotensin II; Amiloride: inhibitor of the $\text{Na}^+ - \text{H}^+$ antiport; Captopril: angiotensin-converting enzyme inhibitor; SITS: inhibitor of the $\text{Cl}^- - \text{HCO}_3^-$ exchanger.

Asterisk indicates value significantly different from Amiloride alone (* $P < 0.01$; two tailed paired t test).

Table 3. Endometrial histology of patients from whom cultures responsive and non-responsive to angiotensin II (AII) were established

Endometrial histology	Number (%) of cell cultures responsive to AII	Number (%) of cell cultures non-responsive to AII
Proliferative	5 (26)	3 (23)
Secretory	6 (32)	4 (31)
Inactive-atrophic	7 (37)	3 (23)
Hyperplastic	0 (0)	2 (15)
Other	1 (5)	1 (8)

to the purinergic receptor agonist ATP were similar ($10.9 \pm 5.0 \mu\text{A cm}^{-2}$ and $12.9 \pm 2.0 \mu\text{A cm}^{-2}$, respectively), indicating electrophysiological responsiveness. Age and ovarian cycle stage did not seem to correlate with responsiveness of cell cultures to angiotensin II. The proportion of patients in the secretory and proliferative phases of the menstrual cycle were similar in responding and non-responding cultures (Table 3). The only difference of significance was that cultures that responded to angiotensin II stimulation grew to confluence (and were therefore experimented on) sooner (5.4 days) than those that did not respond (7.4 days).

The action of angiotensin II on rat colon was studied as a positive control for the Ussing apparatus. Angiotensin II at

$10 \mu\text{mol l}^{-1}$ produced a decrease in SCC of $6 \mu\text{A } 0.6 \text{ cm}^{-2}$ after 5 min, and the SCC remained well below the baseline even after 20 min. This effect was similar in characteristics and magnitude to that described by Cox *et al.* (1987).

Discussion

The Fallopian tube has an important role in gamete interaction and transport and early embryogenesis. Tubal fluid provides the optimum micro-environment for these processes, and varies in consistency and composition during the ovarian cycle (Lippes *et al.*, 1972; David *et al.*, 1973). The role of angiotensin II in electrolyte and fluid transport in a variety of tissues such as rabbit proximal renal

tubule (Schuster *et al.*, 1984), frog skin (McAfee and Locke, 1967), rat jejunum and colon (Cox *et al.*, 1987), rat cauda epididymidis (Wong *et al.*, 1989) and canine trachea (Tamaoki *et al.*, 1992) has already been established.

The polarized Fallopian tube epithelial cell preparation is a valuable tool for functional studies. In many respects, structure and function of the epithelial cells are maintained, and this retention of phenotype is also seen in the presence of the angiotensin II type 1 receptor (the present study). Although G-protein-coupled receptors are active at the cell surface, stimulation is followed by rapid internalization (Yang *et al.*, 1997; Hunyady, 1999) and, in many types of cell, most of the angiotensin II type 1 receptor is internalized (Vinson *et al.*, 1994). The distribution of receptor seen in these Fallopian tube epithelial cultures is similar to that described elsewhere (Saridogan *et al.*, 1996; Inwang *et al.*, 1997).

In the present study, angiotensin II increased baseline SCC in a direction that indicates electrogenic negative-ion transport in a basal to apical direction. This effect was rapid in onset (occurring within 60 s), brief in duration (2–8 min) and significant at angiotensin II concentrations of $10 \mu\text{mol l}^{-1}$.

Pre-treatment of cultures with the selective angiotensin II type 1 receptor antagonist losartan or the Cl^- – HCO_3^- co-transport blocker SITS reduced the SCC response to angiotensin II, implying a role for the angiotensin II type 1 receptor in electrogenic Cl^- transport in the Fallopian tube. However, pre-treatment of cultures with the angiotensin II type 2 receptor antagonist PD123,319 or the inhibitor of the Na^+ – H^+ antiport amiloride did not reduce the SCC response to angiotensin II. Surprisingly, amiloride enhanced the SCC response to angiotensin II stimulation, possibly by eliminating the contribution made by Na^+ absorptive currents to baseline SCC.

The magnitude and duration of the increased SCC response after angiotensin II stimulation in the Fallopian tube are relatively small compared with those occurring after ATP stimulation. However, the magnitude of the responses in the present study are not dissimilar to those described for other cultured monolayers in which a role for angiotensin II stimulation of epithelial transport has been postulated. For example, in studies on rat cauda epididymidis, the change in SCC after angiotensin II stimulation was 3.14 and $1.2 \mu\text{A cm}^{-2}$ on the basal and apical sides, respectively (Wong *et al.*, 1989), and in canine trachea, the maximum response to angiotensin II stimulation was $5 \mu\text{A cm}^{-2}$ (Tamaoki *et al.*, 1992). In contrast and as expected, SCC responses to angiotensin II from freshly prepared intact epithelia such as rat colon and jejunum (which is several cells thick, and of known polarity) are higher (Cox *et al.*, 1987). However, the use of cell monolayers has the advantage of providing a near pure population of cells, to which specific transport functions can be attributed. The currents produced by such monolayers are inevitably smaller, and the detection of submaximal changes is difficult.

The concentration of angiotensin II required to effect a significant change in SCC across cultures was high ($10 \mu\text{mol l}^{-1}$) compared with serum concentrations of angiotensin II. The maximum effects of angiotensin II in other electrolyte-transporting epithelia also occur at higher concentrations than occur in plasma (Cox *et al.*, 1987; Wong *et al.*, 1989; Tamaoki *et al.*, 1992).

Without accurate knowledge of the concentration of angiotensin II that is likely to reach the Fallopian tube epithelium, it is difficult to determine whether angiotensin II is physiologically a major factor in ion exchange in the Fallopian tube. The concentration of angiotensin II may be much higher in the Fallopian tube than it is in plasma, as the Fallopian tube concentrates hormones locally (Jansen 1984), and there may be various sources of this hormone. For example, at ovulation the rupturing follicle releases angiotensin II, which could accompany the ovum into the infundibulum of the Fallopian tube (Glorioso *et al.*, 1986; Delbaere *et al.*, 1996). In addition, the Fallopian tube may, like the uterus (and many other tissues), contain a discrete tissue renin–angiotensin system (Vinson *et al.*, 1995, 1997), although the absence of an effect of captopril on baseline SCC in these cultures indicates that, even if present *in vivo*, a tissue renin–angiotensin system is not sustained *in vitro*. It is also possible that, for example, any locally generated angiotensin II *in vitro* may be excessively diluted by the medium bathing the cultures on either side. Alternatively, local generation of angiotensin II in the Fallopian tube may be dependent upon an exogenous source of angiotensin-converting enzyme, such as spermatozoa, which contain a high concentration of this enzyme (Foresta *et al.*, 1987, 1991). Chan and Wong (1996) have proposed a similar hypothesis for the generation of angiotensin II by epithelial cells of the rat epididymis. The locally generated angiotensin II may then act in a paracrine–autocrine manner to effect electrolyte transport at the critical time.

In addition to stimulating Cl^- secretion, angiotensin increases ciliary motility in tracheal epithelium (Kobayashi *et al.*, 1990) and in the Fallopian tube (Saridogan *et al.*, 1996), and the present results indicate that angiotensin II may have a role in Cl^- transport in the Fallopian tube. Such a dual action has physiological relevance as both ciliary beat and the composition of intraluminal secretions affect transport of luminal contents, ciliary beat by direct propulsion and intraluminal secretions by affecting rheologic properties of mucosal surface fluid.

Not all cultures responded to angiotensin II stimulation in the present study. The quality of the non-responding cultures, in terms of resistance, potential difference and response to ATP, was not significantly different from that of those that responded to angiotensin II stimulation, discounting any potential effect resulting from differences in the quality of cell cultures. In addition, there was no apparent correlation between responsiveness of cell cultures to angiotensin II and the age and ovarian cycle stage of patients, which may at first seem surprising, considering the dependence of Fallopian tube physiology on steroid

hormones (Jansen, 1984; Mahmood *et al.*, 1998), and the cycle-related changes in angiotensin II type 1 receptor concentrations in the Fallopian tube (Saridogan *et al.*, 1996; Johnson *et al.*, 1998). However, cells in culture do not have the normal hormonal influences that are present *in vivo*, and the *in vivo* cyclic influence may not persist *in vitro*. Therefore, variation in responsiveness to angiotensin II may depend on *in vitro* events independent of the prior stage of the cycle, or other hormonal factors *in vivo*. Alternatively, the small sample size in the present study may have precluded the expected detection of subtle differences in responsiveness relating to clinical characteristics.

Cell cultures that responded to angiotensin II stimulation grew to confluence (and were therefore studied) earlier than those that did not respond. Such variation may be expected in primary culture of any type.

In summary, angiotensin II was shown to affect electrogenic ion transport across confluent polarized human Fallopian tube epithelial cell cultures via its type 1 receptor, carrying implications for our understanding of the regulation of sperm, ovum and zygote viability. However, further studies are needed to correlate responsiveness to angiotensin II with a quantitative assessment of angiotensin II type 1 receptor concentration and to establish whether responsiveness to angiotensin II is related to the age, or reproductive or hormonal status of the patient.

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