Endothelin-2 induces oviductal contraction via endothelin receptor subtype A in rats

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Abstract

Proper function of the oviduct is critical to reproductive success with regulated contraction and relaxation facilitating transportation of the germ cells to the site of fertilization. Endothelin-2 (EDN2) is a potent vasoconstrictor produced by granulosa cells of the preovulatory follicle at the time of ovulation; however, whether this gonadotropin surge-induced peptide played a role in facilitating germ cell transportation by inducing oviductal contraction was unknown. The objectives of these experiments were (1) to determine whether the endothelin receptor system was present in the oviduct, (2) to test the hypothesis that EDN2 induces oviductal contraction via a specific endothelin receptor subtype, (3) to determine, as a possible alternate source of the ligand, whether mRNA for EDN2 was expressed in cumulus–oocyte complexes (COCs) within the oviduct, and (4) to determine whether EDN2 could overcome prostaglandin E2 (PGE2)-induced oviducal relaxation. Microarray and real-time PCR analysis indicated that mRNA for both the endothelin receptor subtypes (ETA and ETB) was present in the oviduct, whereas immunohistochemical examination revealed that ETA protein was the dominant isoform, present in the luminal epithelial cells of the oviduct. Real-time PCR analysis demonstrated that mRNA for EDN2 was expressed in COCs after ovulation. Isometric tension analysis indicated that EDN2 was a potent oviductal constrictor and that the contractile effect of EDN2 was mediated by the ETA and not the ETB receptor subtype. The oviductal contraction induced by EDN2 also reversed oviductal relaxation induced by PGE2. In summary, ETA receptor–specific EDN2-induced contraction as a facilitator of oviductal function suggests a novel pathway involved in germ cell transport and hence mammalian fertility.

Introduction

For successful fertilization, the female germ cell (the oocyte), along with its surrounding cumulus cells, must be transported from the ovary to the ampulla region of the oviduct. Initially, through the action of beating cilia, the cumulus–oocyte complex (COC) is transported from the confines of the ovarian bursa to the oviduct proper (Garcia-Pascual et al. 1996, Riveles et al. 2004, Wessel et al. 2004). Waves of oviductal contraction and relaxation then facilitate transport to the site of fertilization, the lower portion of the ampulla (Garcia-Pascual et al. 1996, Talbot et al. 2003). Several hormones have been identified that can affect oviductal contractility, including oxytocin, catecholamines (Kotwica et al. 2003), angiotensin II, prostaglandins (PGs), and endothelin–1 (Sakamoto et al. 2001, Wijayagunawardane et al. 2001a,b), however, the precise physiological mechanism regulating oviductal function and gamete transportation remains unclear.

We recently reported that endothelin–2 (EDN2), a vasoactive peptide produced by granulosa cells of preovulatory follicles, was a requisite for successful ovulation (Ko et al. 2006). EDN2 is one of the three 21 amino acid endothelin isoforms (EDN1, EDN2, and EDN3), which bind to two (ETA and ETB) known receptors (Masaki 2004). Upon considering (1) the vasoactive action of EDN2 (Yanagisawa et al. 1988), (2) that granulosa cell-produced EDN2 may be released with the COC and follicular fluid at the time of ovulation, (3) that the COC itself may be a source of EDN2, and (4) the contractile properties of the oviduct, we hypothesized that granulosa cell or COC-produced EDN2 binding to a specific endothelin receptor subtype in the oviduct would induce oviductal contraction, which in conjunction with regulated oviductal relaxation, would facilitate transportation of the gametes through the oviduct.

To investigate this hypothesis, our first objective was to determine whether the endothelin receptor system was present in the oviduct at the time of ovulation. Following
confirmation of specific endothelin binding sites in the oviduct, our next objectives were (1) to evaluate COCs as a potential source of post-ovulatory EDN2 production, (2) to determine whether the oviduct was responsive to EDN2 and if so, (3) to determine through which endothelin receptor subtype this response was generated. Since we hypothesized that EDN2 would induce oviductal contraction, we utilized an isometric tension recorder (Guo et al. 2003, Ko et al. 2006) and small sections of the oviduct to directly determine contractility of this tissue. However, transportation of the gamete to the site of fertilization involves coordinated waves of oviductal contraction and relaxation and hence, in our final objective, we evaluated whether the contractile action of EDN2 could overcome the dilation or relaxation induced by PGE2. In summary, the present study was undertaken to determine whether EDN2 played a role in contraction of the oviduct and therefore gamete transportation. The identification of a novel role for EDN2 in oviductal function and hence fertility will provide valuable knowledge that will increase our understanding of the factors regulating reproductive success.

Materials and Methods

Reagents

Pregnant mare’s serum gonadotropin (PMSG), human chorionic gonadotropin (hCG), and PGE2 were purchased from Sigma. Tezosantan was a gift from ACTELION Pharmaceuticals Ltd (Allschwil, Switzerland). EDN2 was purchased from the American Peptide Company, Inc. (Sunnyvale, CA, USA), real-time PCR reagents were purchased from Applied Biosystems (Foster City, CA, USA) and the antibodies for immunohistochemistry were purchased from Abcam, Inc. (Cambridge, MA, USA).

Animals and tissue collection

The University of Kentucky Animal Care and Use Committee approved all animal procedures. Immature female Sprague–Dawley rats were purchased from Harlan Inc. (Harlan, IN, USA). Animals were maintained in a ratio of 14 h light:10 h darkness and given a continuous supply of chow and water. Follicular development and ovulation were induced with PMSG (0 h), 48 h after PMSG administration, and 12 or 20 h after hCG injection (n=3 animals/time point). Oviducts were dissected from each ovary, and granulosa cells and COCs were isolated after follicular puncture of ovaries or dissection of the oviduct (hCG+20 h), as described previously (Jo et al. 2004, Ko et al. 2006). Briefly, with the aid of a dissecting microscope, preovulatory follicles were punctured with a 26 gauge needle to release granulosa cells and COCs. Approximately, 35–50 COCs per ovary were then collected using a 20 µl pipette and pooled for each animal. Ovulated COCs (10–20 per oviduct) were obtained by visualizing their location within the oviduct, making a small tear in the oviduct wall and gently pressing the oviduct to facilitate their release and collection. Ovulated COCs were also pooled for each animal. All samples were snap-frozen and stored at −80 °C for later isolation of total RNA. Total RNA was extracted with TRIzol (Invitrogen Corp.) according to the manufacturer’s directions and the integrity verified by visualization of distinct 18S and 28S rRNA bands after ethidium bromide staining in an agarose gel. cDNA was reverse transcribed from each sample of total RNA as described previously (Ko et al. 2006) and real-time PCR performed using the Applied Biosystems 7300 detection system (Applied Biosystems) to determine expression levels of mRNA for EDN2, ETA, ETB, and L19 (as the endogenous control). cDNA for EDN2 was amplified using TaqMan Universal PCR Master Mix (Applied Biosystems) and the following gene-specific primers and probes: EDN2-F 5'-CTC CTG GCT TGA CAA GGA ATG-3'; EDN2-R 5'-GCT GTC TGT CCC GCA GTG TT-3' and EDN2-Probe 5'-56-FAM/TCT GCC ACC TGG ACA TCA TCT GGG T/56-TAMSp/-3'. cDNA for ETA, ETB and L19 was amplified using SYBR Green PCR Master Mix (Applied Biosystems) using the following gene-specific primers and probes: ETA-F 5'-CCC TTC GAC CCC CTA ATT TG-3'; ETA-R 5'-TTT TTG TCT GCT GTG GGC AAT A-3'; ETB-F 5'-TGC ATG AGA AAT GGT CCC AAT-3' and ETB-R 5'-TGT CGA TGA TGA TGT GTA GCA GAT-3'; L19-F 5'-CCT GAA GGT CAA AGG GAA TGT G-3' and L19-R 5'-GTC TGC CTT CAG CTT GTG GAT-3'. cDNA generated from the appropriate cells from each of the three animals per time point was included in microarray analysis.

Microarray analysis

To facilitate analysis of gene expression profiles within the oviduct during gonadotropin-primed follicular development and ovulation, an addition was made to our previously described rat ovarian gene expression database (Jo et al. 2004). Briefly, rats were killed before follicular development was induced with PMSG (0 h), 12 or 48 h after PMSG administration, and 6 or 12 h after hCG injection (n=5 animals/time point). Oviducts were dissected from each ovary and processed for later extraction of total RNA. Microarray analysis was then performed using the Affymetrix Rat 230A and 230B oligonucleotide array sets (Affymetrix; DNA Microarray Core Facility, University of Kentucky, Lexington, KY, USA) and incorporated into a database, as described previously (Jo et al. 2004).

Real-time PCR analysis

Real-time PCR analysis was performed to (1) confirm the microarray gene expression profiles for ETA and ETB and (2) to determine whether mRNA for EDN2 was expressed in COCs after ovulation. Rats were killed before follicular development was induced with PMSG (0 h), 48 h after PMSG administration, and 12 or 20 h after hCG injection (n=3 animals/time point). Oviducts were dissected from each ovary, and granulosa cells and COCs were isolated after follicular puncture of ovaries or dissection of the oviduct (hCG+20 h), as described previously (Jo et al. 2004, Ko et al. 2006). Briefly, with the aid of a dissecting microscope, preovulatory follicles were punctured with a 26 gauge needle to release granulosa cells and COCs. Approximately, 35–50 COCs per ovary were then collected using a 20 µl pipette and pooled for each animal. Ovulated COCs (10–20 per oviduct) were obtained by visualizing their location within the oviduct, making a small tear in the oviduct wall and gently pressing the oviduct to facilitate their release and collection. Ovulated COCs were also pooled for each animal. All samples were snap-frozen and stored at −80 °C for later isolation of total RNA. Total RNA was extracted with TRIzol (Invitrogen Corp.) according to the manufacturer’s directions and the integrity verified by visualization of distinct 18S and 28S rRNA bands after ethidium bromide staining in an agarose gel. cDNA was reverse transcribed from each sample of total RNA as described previously (Ko et al. 2006) and real-time PCR performed using the Applied Biosystems 7300 detection system (Applied Biosystems) to determine expression levels of mRNA for EDN2, ETA, ETB, and L19 (as the endogenous control). cDNA for EDN2 was amplified using TaqMan Universal PCR Master Mix (Applied Biosystems) and the following gene-specific primers and probes: EDN2-F 5'-CTC CTG GCT TGA CAA GGA ATG-3'; EDN2-R 5'-GCT GTC TGT CCC GCA GTG TT-3' and EDN2-Probe 5'-56-FAM/TCT GCC ACC TGG ACA TCA TCT GGG T/56-TAMSp/-3'. cDNA for ETA, ETB and L19 was amplified using SYBR Green PCR Master Mix (Applied Biosystems) using the following gene-specific primers: ETA-F 5'-CCC TTC GAC CCC CTA ATT TG-3'; ETA-R 5'-TTT TTG TCT GCT GTG GGC AAT A-3'; ETB-F 5'-TGC ATG AGA AAT GGT CCC AAT-3' and ETB-R 5'-TGT CGA TGA TGA TGT GTA GCA GAT-3'; L19-F 5'-CCT GAA GGT CAA AGG GAA TGT G-3' and L19-R 5'-GTC TGC CTT CAG CTT GTG GAT-3'. cDNA generated from the appropriate cells from each of the three animals per time point was included in
each reaction and the analysis was replicated 2–3 times on different days. DEPC-treated water was used to replace cDNA and act as a negative control for each analysis. The relative amount of transcript was calculated by the ΔΔCT method (Livak & Schmittgen 2001) and normalized to L19.

**Immunohistochemistry**

Ovarian sections obtained from rats euthanized at 12 h after treatment with hCG were cut to 10 μm on a cryostat (Vibratome, St Louis, MO, USA) and mounted on Super-frost/Plus Microscope slides (VWR, West Chester, PA, USA). Immediately after mounting, sections were fixed with 4% paraformaldehyde and immunostaining was performed using the VECTASTAIN Elite ABC kit (Vector Laboratories, Burlingame, CA, USA). Slides were incubated in 0.3% H2O2 in methanol to destroy endogenous peroxidase activity, then blocked and incubated at 4°C overnight with an antibody (ab) to the endothelin receptor subtype A (ETA, ab1919) diluted 1:1000 or subtype B (ETB, ab1923) diluted 1:600. The antigen was detected with the peroxidase substrate diaminobenzidine tetrahydrochloride and nickel chloride. Signal was visualized on an Olympus BX51 microscope equipped with a digital camera.

**Isometric tension analysis**

Rats were euthanized at 10 h after treatment with hCG and the oviducts isolated. A section of the oviduct close to the bursal opening was dissected from the oviduct and tied, via silk threads, to tissue holder electrodes attached to a force transducer. The sections of oviduct were maintained in Kreb’s solution and a base-line depolarization was generated in response to K+, as described previously (Guo et al. 2003). The response to EDN2 was determined after a 15 min pretreatment of the oviductal sections with the antagonists or vehicle (PBS) and analyzed relative to the K+-induced depolarization of each oviductal section. A preliminary investigation was conducted to determine the minimal effective dose of each compound (data not shown). Treatments included 1 μM EDN2, 100 μg/ml tezosentan (a dual endothelin receptor antagonist), 50 nM BQ123 (an ETA antagonist), 50 nM BQ788 (an ETB antagonist), and 300 μM PGE2.

**Statistical analysis**

Data sets were first tested for homogeneity of variance. If heterogeneity was detected, data were log-(base 10)

![Graph](image)

**Figure 1** Expression of mRNA for ETA and ETB in the oviduct during gonadotropin-primed follicular development and ovulation. Immature rats were treated with 10 IU PMSG to induce follicular development and with 10 IU hCG 48 h later to synchronize ovulation. Animals were killed for tissue collection before PMSG treatment, at 12 and 48 h after PMSG and at 6 and 12 h after hCG (n=5 per time point). Relative levels of mRNA were obtained by microarray analysis as described by Jo et al. (2004).

**Figure 2** Pre- and post-ovulatory expression of mRNA for ETA and ETB in the oviduct. Immature rats were treated with 10 IU PMSG to induce follicular development and with 10 IU hCG 48 h later to synchronize ovulation. Animals were sacrificed for tissue collection before PMSG treatment, at 48 h after PMSG and at 12 and 20 h after hCG (n=3 per time point). Relative levels of mRNA were obtained by real-time PCR. Within a panel, values with different superscripts differ (P<0.05).
transformed before statistical analysis. Non-transformed data are depicted in all the figures. One-way ANOVA using SigmaStat 3.5 (Systat Software, Inc. Point Richmond, CA, USA) was used to determine differences in expression of mRNA levels. If differences were detected, Tukey’s test was used to determine which means differed. The Student’s t-test was used to determine the differences in the isometric tension analysis.

Results

Expression of mRNA and protein for $ET_A$ and $ET_B$ in the oviduct

Microarray analysis was performed to determine the relative level of expression of mRNA for the two endothelin receptor subtypes in the oviduct during the ovulatory cascade. Analysis revealed that mRNA encoding both of the endothelin receptor subtypes ($ET_A$ and $ET_B$) was detectable in the oviduct (Fig. 1). Levels of mRNA encoding $ET_A$ remained stable throughout gonadotropin-primed follicular development, whereas levels of mRNA for $ET_B$ gradually increased after treatment with hCG.

![Figure 3](image1.png)  
**Figure 3** Immunohistochemical expression of $ET_A$ (A and B) and $ET_B$ (C and D) in the oviduct at ovulation. Immature 23–25 day old rats were treated with 10 IU PMSG to induce follicular development and with 10 IU hCG 48 h later to synchronize ovulation. Animals were killed at 12 h after hCG for tissue collection. Asterisks indicate the absence of $ET_A$ protein expression in the ampulla region of the oviduct. SM, smooth muscle; Epi, epithelial cells.

![Figure 4](image2.png)  
**Figure 4** Expression of mRNA for EDN1 and EDN2 in the ovary and oviduct during gonadotropin-primed follicular development and ovulation. Immature rats were treated with 10 IU PMSG to induce follicular development and with 10 IU hCG 48 h later to synchronize ovulation. Animals were killed for tissue collection before PMSG treatment, at 12 and 48 h after PMSG and at 6 and 12 h after hCG ($n=5$ per time point). Relative levels of mRNA were obtained by microarray analysis as described by Jo et al. (2004).
to induce the ovulatory cascade. However, by 12 h after hCG, the relative level of expression of mRNA for ET<sub>A</sub> and ET<sub>B</sub> within the oviduct appeared similar.

Real-time PCR was then performed to (1) confirm the microarray expression profiles and (2) extend the analysis to encompass a time when COCs were present within the oviduct. Consistent with the results from microarray analysis, no dramatic changes in expression of mRNA for ET<sub>A</sub> or ET<sub>B</sub> were observed (Fig. 2). A small, transient decrease in expression of mRNA for ET<sub>A</sub> was observed at 48 h after PMSG (P<0.05) and levels of mRNA for ET<sub>B</sub> were increased ~1.75-fold at 12 and 20 h after hCG (P<0.05) when compared with earlier time points. Analysis of mRNA data was therefore inconclusive in establishing whether a specific endothelin receptor subtype mediated the hypothesized EDN2-induced oviductal contraction. In contrast, immunohistochemical analysis of ET<sub>A</sub> and ET<sub>B</sub> protein in sections of oviduct obtained at 12 h after hCG revealed both spatial and endothelin subtype-specific differences in endothelin receptor expression (Fig. 3). Strong staining of ET<sub>A</sub> protein was observed in the luminal epithelium of the isthmus but not in the ampulla portion of the oviduct at 12 h after hCG (Fig. 3A and B). In contrast, ET<sub>B</sub> protein was weak or absent in both the ampulla and isthmus of the oviduct at 12 h after hCG.

Expression of mRNA for EDN2 in granulosa cells and COCs

Analysis of mRNA expression patterns obtained by micro-array analysis for EDN1 and EDN2 in the oviduct versus granulosa cells of the ovary support our hypothesis that granulosa cell-derived EDN2 induces oviductal contraction. Expression of mRNA for EDN1 remains at basal levels in both the oviduct and granulosa cells throughout the ovulatory cascade, whereas mRNA for EDN2 is rapidly induced in granulosa cells by hCG as the ovulatory stimulus (Fig. 4). Real-time PCR analysis for EDN2 mRNA in granulosa cells confirmed the results obtained by microarray analysis (Fig. 5). Real-time PCR was then performed to determine whether mRNA for EDN2 in granulosa cells was detectable in COCs at 12 h after hCG, when mRNA for EDN2 in granulosa cells is abundant, and 8 h later when ovulation is complete and the COCs are within the oviduct proper. Consistent with the expression of mRNA for EDN2 in granulosa cells collected at 12 h after hCG, expression of mRNA for EDN2 in COCs was increased dramatically at this time point (P<0.05; Fig. 6). Interestingly, mRNA for EDN2 in COCs collected at 20 h after hCG was ~11-fold higher than in COCs collected at 48 h after PMSG, suggesting that the COC may be a source of EDN2 when within the oviduct itself.

Effect of EDN2 on contractility of the oviduct

We previously reported that granulosa cell-produced EDN2 was a requisite for successful ovulation (Ko et al. 2006); however, whether EDN2 produced by granulosa cells at ovulation or by COCs within the oviduct could affect oviductal constriction remained to be fully investigated. Hence, isometric tension analysis was performed to determine whether EDN2 induced a functional contraction of the oviduct. After pretreatment with PBS (vehicle) for 15 min, treatment with 1 µM EDN2 induced a potent contraction of the oviductal sections and almost abolished spontaneous contractions (P<0.05; Fig. 7A and B). The contractile effect of EDN2 was inhibited by pretreatment of
the ovarian sections with 100 μg/ml of the dual endothelin receptor antagonist, tezosentan (Fig. 7C and D).

Receptor-specific effect of EDN2 on contractility of the oviduct
Isometric measurement was then performed to determine the specific endothelin receptor subtype mediating EDN2-induced oviductal contraction. When oviductal sections were pretreated with 50 nM of the ET\textsubscript{B} antagonist BQ788, treatment with 1 μM EDN2 induced a potent contraction ($P<0.05$; Fig. 8A and B). In contrast, when oviductal sections were pretreated with an equimolar concentration of the ET\textsubscript{A} antagonist BQ123, treatment with 1 μM EDN2 was not effective in inducing oviductal contraction ($P>0.05$; Fig. 8C and D). The ability of BQ123, but not BQ788 to inhibit EDN2-induced oviductal contraction indicates signaling through ET\textsubscript{A}, consistent with the abundant expression of ET\textsubscript{A} protein in the luminal epithelium that was observed by immunohistochemical analysis of sections of oviduct collected at 12 h after hCG (Fig. 3).

Effect of EDN2 on PGE\textsubscript{2}-induced dilation of the oviduct
Coordinated waves of oviductal contraction and relaxation facilitates transportation of the germ cells within the oviduct and isometric tension analysis was performed to determine...
whether EDN2 could overcome the oviductal relaxation induced in response to PGE2. Treatment of oviductal sections with 300 μM PGE2 reduced the basal tension of the sections and almost abolished the spontaneous contractions ($P<0.05$; Fig. 9A and B). However, subsequent treatment with EDN2 overcame PGE2-induced relaxation ($P<0.05$).

**Discussion**

The experiments described herein demonstrate that the oviduct is responsive to EDN2 as a contractile stimulus. Immunohistochemical and isometric contraction data provide experimental evidence that the contractile response is mediated via the receptor subtype ET$_A$. Levels of mRNA for EDN2 are increased dramatically in granulosa cells (Ko et al. 2006) and COCs of the periovulatory follicle prior to ovulation and are maintained above preovulatory levels in COCs within the oviduct. It is therefore likely that EDN2-induced contraction plays a role in the regulation of gamete transport within the oviduct; however, the relative contribution of granulosa cell and/or COC produced EDN2 in oviductal function remains to be determined.

The concept of ovarian hormones regulating or modifying oviductal transportation is not new. For example, estradiol and progesterone are well established as antagonistic
regulators of ova movement toward the uterus (Ortiz et al. 1979, Fuentealba et al. 1988). However, EDN2 appears novel as a follicular produced peptide that is critical for ovulation, has its mRNA induced by hCG within hours of oocyte release (Ko et al. 2006) and is therefore presumably released with the follicular fluid at the time of ovulation. On the other hand, COCs within the oviduct expressed levels of mRNA for EDN2 that were greater than those observed in preovulatory follicles not exposed to hCG as the ovulatory stimulus. The large difference in relative levels of mRNA observed in granulosa cells at 12 h after hCG and in COCs at 20 h after hCG suggests the possibility of more than one role for EDN2 in the regulation of oviductal contractility.

The role(s) of gonadotropin surge-induced EDN2 at ovulation appear, however, to be independent of the actions of endothelins during the earlier stages of follicular growth and development. In the rat, expression of mRNA for EDN1 remains at a basal level throughout the ovulatory cascade, whereas mRNA for EDN2 remains at a basal level until 2–4 h prior to ovulation, at which time it is increased in a dramatic, yet transient manner (Ko et al. 2006). Immunoreactive EDN1 and EDN2 are also detectable within the preovulatory follicle of women (Kamada et al. 1993, Haq et al. 1996, Sudik et al. 1996); however, whether the gonadotropin surge induces a similar preovulatory increase in EDN2 that was not detected with the sampling schedules utilized in these studies is unknown. However, the dramatic induction of mRNA for only EDN2 immediately prior to ovulation in the rat is suggestive of an independent function of this isoform. However, a role for EDN1 in oviductal contractility cannot be discounted, as treatment of oviductal sections collected from women in the follicular phase of the menstrual cycle with EDN1 will induce contractions (Sakamoto et al. 2001). However, this may be a non-specific effect as endothelin receptors bind to both EDN1 and EDN2 (reviewed in Masaki et al. 1999).

In this study, analysis of mRNA expression levels for ETA and ETB throughout gonadotropin-primed follicular development and ovulation did not identify ETA as the functional endothelin receptor subtype present in the oviduct. This was somewhat surprising considering the strong protein signal for ETA when sections of oviduct collected at 12 h after hCG were examined by immunohistochemistry. Previously, Iwai et al. (1993) analyzed mRNA levels of ETA and ETB by in situ hybridization in the ovaries and oviducts of mature rats at random stages of the estrous cycle. However, differences appear in the regional and cellular location of mRNA versus protein for ETA between their study and ours. We observed strong staining of ETA protein in the luminal epithelium in regions of the oviduct with a thick musculature, whereas staining was weak or absent in the ampulla, the thinner muscled section of the oviduct where fertilization occurs. This is consistent with the expected contractile nature of these different regions of the oviduct; however, the precise signaling pathway between ETA-expressing luminal epithelial cells and the adjacent musculature remains to be fully investigated. Although analysis of regional differences in mRNA levels was not the focus of the study by Iwai et al. (1993), they detected a low level of expression of mRNA for ETA in the smooth muscle layer rather than the epithelium of a thinly muscled section, presumably the ampulla. Expression of mRNA in other areas of the oviduct was not reported which may have influenced their conclusion regarding overall cellular distribution. Taken together with the isometric tension results, it appears clear that EDN2-induced oviductal contraction is mediated by activation of ETA. Since both contractile and

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**Figure 9** Effect of EDN2 on PGE2-induced relaxation of the oviduct. Immature 23–25 day old rats were treated with 10 IU PMSG to induce follicular development and with 10 IU hCG 48 h later to synchronize ovulation. Animals were killed at 10 h after hCG for tissue collection. (A) Representative isometric tension recording after treatment of oviductal sections with 300 μM PGE2 then 1 μM EDN2. (B) Force percentage of EDN2 induced oviductal contraction after pretreatment of the tissue with PGE2, relative to K+ -induced depolarization. Data are the means ± S.E.M. of five isometric recordings. Values with different superscripts differ significantly (P<0.05).
relaxant forces are required to facilitate gamete transportation through the oviduct (Garcia-Pascual et al. 1996, Talbot et al. 2003), it was not unexpected that the oviductal relaxation induced by treatment with PGE2 was completely overcome by subsequent treatment with EDN2. Overall, this is consistent with the physiology regulating oocyte transportation prior to fertilization.

In our experiments, sections of the oviduct were obtained close to the bursal opening, a region of the oviduct that should be exposed to granulosa cell-produced EDN2 released from follicles at ovulation and, hence, effective in regulating contraction of at least the upper portions of the oviduct proper. The oviducts were also collected ~4 h prior to the expected time of ovulation, before any endogenous EDN2 would impose its contractile effect on the oviduct. Although likely that granulosa cell and/or COC-produced EDN2 is facilitating oviductal contraction, the complete mechanism(s) regulating transportation from the ovary to the uterus is yet to be fully elucidated. Whether granulosa cells of the COC continue to produce EDN2 as the oocyte continues its passage though the oviduct to further regulate passage in a local manner remains to be determined, although mRNA levels denote this as a possibility. In conclusion, granulosa cell and/or COC-derived EDN2 acting via ETα receptors in the oviduct is suggested as a novel regulator of oviductal contractility and hence gamete transportation in the oviduct.

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