Vol. 201, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1994 Pages 143-148

ENDOGENOUS NITRIC OXIDE MODULATES ENDOTHELIN-1 INDUCED CONTRACTION OF BOVINE OVIDUCT

M. Rosselli,[1,*] B. Imthurn,[1] E. Macas,[1] P.J. Keller,[1] and R.K. Dubey[2]

[1]Department of Obstetrics and Gynecology, Clinic of Endocrinology, University Hospital Zürich, Zürich, Switzerland

[2]Department of Medicine, Division of Clinical Pharmacology, University Hospital Basel, Basel, Switzerland

Received March 23, 1994

Summary. We recently reported that oviduct epithelial cells in culture produce endothelin (ET) and postulated that ET could play an important role in the contraction of the oviduct. In the present study using an organ chamber myograph system, we evaluated the contractile effects of ET-1 on bovine oviduct ampullary-isthmus segments. Additionally, the possibility whether the basal release/synthesis of nitric-oxide (NO) and/or prostacyclin modulates the contractile effect of ET-1 was investigated. ET-1 (10^{-10} to 10^{-7} M) contracted oviduct rings in a concentration dependent manner ($P<0.05$). ET-1 (10^{-7} M) induced contractions were significantly enhanced in presence of nitric oxide synthase inhibitor N-nitro-L-arginine-methyl-ester (10^{-4} M), but remained unaltered in the presence of indomethacin (10^{-5} M), an inhibitor for prostacyclin synthesis. In conclusion, ET-1 induced contraction of the oviduct is reduced/modulated by endogenous basal release/synthesis of NO.

The oviduct importantly contributes to the reproductive process by controlling the transport of gametes and embryos. Smooth muscle cell contraction and relaxation, as well as ciliary beats of the oviduct-epithelia cells are essentially involved in regulating this process. Although several agents, such as catecholamine, prostaglandin (1) and peptides like vasoactive-intestinal peptide (2) are known to contract or relax the oviduct. However, the role of factors such as ET and NO in regulating the conduit function of the oviduct has not been studied.

*Corresponding author. *FAX:41.1.255 44 39.*

0006-291X/94 $5.00

ET, a 21 amino acid peptide, is a potent vasoconstrictor, originally isolated from the conditioned media of cultured endothelial cells (3). ET are a family of peptides, present in three isoforms (ET-1,-2,-3) and encoded by three distinct ET-related genes (4). In addition to vascular endothelial cells, several other cell types are known to produce ET, for example endometrial stromal and glandular epithelial cells (5). Large amounts of ET have also been reported in the human follicular fluid (6) and in human seminal plasma (7). Furthermore, production of ET by bovine oviduct epithelial cells was recently reported by us (8). ET stimulates rhythmic contractions in uterine horn of adult rats in vitro (9) and hence, could potentially also be involved in the contraction of the oviduct.

ET-1 is known to induce its effects through two receptor subtypes (ET-A, ET-B; 10, 11). The contractile effects of ET are largely mediated through ET-A receptor and increases intracellular calcium (12). On the other hand, ET-1 has also been shown to induce transient relaxations, by releasing potent endothelium-derived vasodilatatory substances such as prostacyclin and endothelium-derived relaxing factor (13), now identified as NO (14). NO is a labile, diffusible molecule, released by several cell types, including endothelium (15,16), ovarian cells (17) and macrophages (18). In blood vessels, NO relaxes smooth muscle cells by activating soluble guanylate cyclase within the vascular smooth muscle cells, which in turn generates cyclic guanosine monophosphate, followed by a decrease in intracellular calcium. NO is synthesized enzymatically by both constitutive and/or inducible form of NO-synthase, through the oxidation of the guanidine nitrogen atom of L-arginine, a process that can be antagonized by substituted L-arginine analogues, like N-nitro-L-arginine-methyl-ester (L-NAME , 19,20) .

Based on our observation that bovine oviduct epithelial cells produce ET (8), and the fact that ET is present in the follicular fluid as well as in the seminal plasma (6,7), we postulate that ET could be involved in the contraction of the oviduct and play an important role in the transport of gametes and embryos. In this study we, evaluate the in vitro effects of ET-1 on the contraction of oviduct isthmus rings. Moreover, considering that in the blood vessels, endogenous NO and prostacyclin reduces/modulates the contractile effect of ET, their role in regulating ET-induced contraction of the oviduct was also investigated.

Materials and Methods

Oviducts were obtained from young non-pregnant cyclic cows, sacrificed at the local abattoir and placed immediately in cold Hank's balanced salt solution (Sigma, Basel,

Switzerland). Oviducts containing corresponding ovaries with an hemorrhagic corpus luteum were used for the study. The ampullary-isthmus area was dissected away from the surrounding tissue, cut into 0.4 cm rings and then transferred into modified Krebs-Ringer's solution (mM: NaCl 118; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; $KHPO_4$ 1.2; $NaHCO_3$ 25.0; EDTA 0.026; Glucose 11.1). Two tungsten wires (30μm and 80 μm) connected to a myograph system, were passed through the lumen of the oviduct, which were then suspended in organ chamber containing 25 ml Krebs-Ringer's solution at 37°C, in presence of a mixture of 95% O_2, 5% CO_2.
After 45 min of pre-equilibration, the oviduct rings were stretched to a constant optimal tension of 3.5 ± 0.01 grams (g). To test the contractile response of the oviduct ring, in a comultive concentration acetylcholine (10^{-7} M-10^{-4} M, Sigma) or 100 m M KCl were added to the organ chamber and the response registered. After a washout period of 30 min. the effect of ET-1 (10^{-10} M-10^{-7} M) (Novabiochem, Läufingen, Switzerland) was tested by adding cumulative concentrations of ET-1 to the organ chambers. The maximal contraction obtained was recorded as tension in g. In parallel experiments, rings from the same oviduct were incubated for 45 min. with, N-nitro-L-arginine methyl ester (L-NAME 10^{-4} M, Sigma) an inhibitor of nitric oxide synthesis or with indomethacin (10^{-5} M) a prostacyclin synthesis inhibitor or with L-NAME (10^{-4} M) plus indomethacin (10^{-5} M); subsequently ET-1 (10^{-7} M) was added to the organ chambers. Each series of experiments were repeated 3 times using oviducts obtained from three different animals. Results are expressed as mean ± standard deviation (S.D.). Data were analyzed using Student-t-test; $p<0.05$ was considered statistically significant.

Results

General: under 3.5 g tension, all oviduct preparations exhibited a uniform rhythmic spontaneous contraction activity. Under these stretch conditions the addition of cumulative concentrations of acetylcholine (10^{-7}-10^{-4} M) contracted the oviducts in a concentration dependent manner (Fig. 1a). KCl (100 mM) significantly relaxed the optimally stretched oviduct from 3.5±0.01 to 2.85±0.006 g. No significant differences

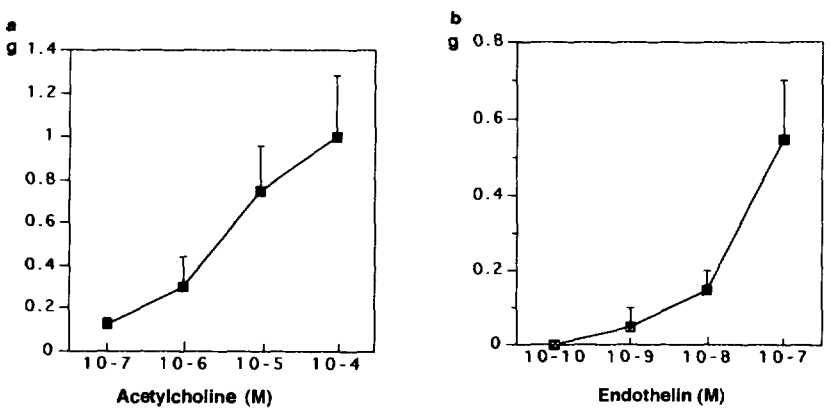


Figure 1. Contraction of bovine oviduct isthmus rings in response to acetylcholine (10^{-7}-10^{-4} M; Fig. 1a) and endothelin-1 (10^{-10}-10^{-7} M; Fig. 1b). Acetylcholine as well as endothelin-1 contracted the oviduct in a concentration dependent manner ($p<0.05$). Data are mean ± S.D. from 3 experiments.

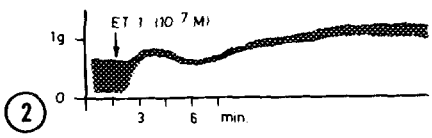
were observed in the induction of contraction and relaxation between oviducts obtained from different cows.

Effect of ET-1: ET-1 added in cumulative concentrations (10^{-10}-10^{-7} M) contracted the oviduct in a concentration-dependent manner ($p<0.05$; Fig 1b). Furthermore, ET-1 significantly decreased the spontaneous oscillation amplitude of the oviduct rings in a transient/reversible fashion (Fig. 2).

In the presence of L-NAME (10^{-4} M), ET-1 induced contraction was enhanced by 188 ±19% ($p<0.05$), but remained unaltered in the presence of indomethacin (10^{-5} M; Fig. 3). ET-1 induced contractions of the oviduct rings, preincubated with L-NAME plus indomethacin, were comparable to those obtained in the presence of L-NAME (Fig. 3). Furthermore in presence of L-NAME, but not indomethacin, the capability of ET-1 to decrease spontaneous oscillation was almost abolished (data not shown). Neither L-NAME nor indomethacin had any effect on the basal tone and spontaneous oscillations of the oviduct rings.

Discussion

Contraction and relaxation of smooth muscle cells in the oviduct are the major factors involved in promoting and regulating the transport of gametes and embryos. Which autocrine or paracrine factors regulate this contractile response are still unclear. We have recently shown that cultured bovine oviduct epithelial cells



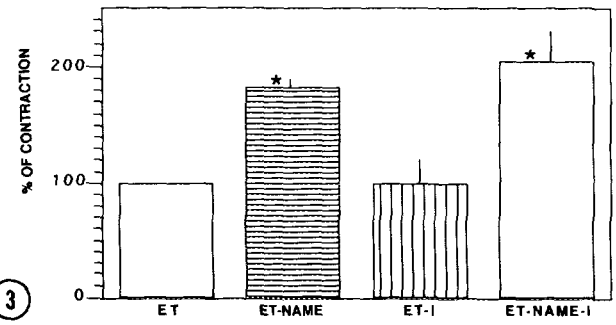


Figure 2. Representative myograph tracing showing the contractile effect of endothelin-1 (10^{-7} M) on the bovine oviduct isthmus ring segment. Endothelin-1 induced contraction of the oviduct, as well as markedly reduced the spontaneous oscillations. Similar results were obtained in four independent experiments.

Figure 3. Effect of nitric oxide synthase inhibitor (N-nitro-L-arginine methyl ester 10^{-4} M; NAME) and prostaglandin synthesis inhibitor indomethacin (10^{-5} M; I) on endothelin-1 (10^{-7}M; ET) induced contraction of the oviduct. Presence of NAME but not indomethacin significantly (*) increased endothelin-1 induced contraction of the oviduct ring. The data are presented as percentage of the contraction observed in response to endothelin-1 (10^{-7} M) which was considered as 100%. Values are mean ± S.D. from three independent experiments.

produce ET (8), a 21 amino acid peptide, first isolated from conditioned media of cultured endothelial cells and known to contract vascular as well as uterine smooth muscle cells. (3,9). Based on our finding and the fact that large amounts of ET are present in the follicular fluid (5) as well as in the seminal plasma (7), we hypothesize that ET could play an important role in the contractility of the oviduct. Our observation that ET-1 significantly contracts the oviduct in a concentration-dependent manner, greatly supports this hypothesis and suggests that ET-1 may play a physiological role by contributing to the rhythmic contraction of the oviduct.

ET exerts its effect via two receptor subtypes, ET-A and ET-B. ET-1 contracts smooth muscle cells by increasing intracellular calcium (10,12), a process which is mediated through the ET-A receptor. Via ET-B receptors, ET-1 stimulates transient release of relaxing factors such as NO and prostacyclin (11,13). The finding that ET-1 reduced the spontaneous oscillation of the oviduct in a transient fashion, in absence but not in the presence of L-NAME, suggests that this might be due to an ET-1 stimulated release of relaxing factors, potentially NO, by oviduct epithelial cells. This effect may involve changes in intracellular calcium, since NO is known to reduce intracellular calcium (19). Calcium antagonist as well as calcium depleted bathing solutions have been shown to reduce spontaneous oscillations in the oviduct (21). Considering that ET-1 induces contraction of the oviduct and transiently/reversibly reduces the spontaneous oscillation (possibly by generating NO), suggests that both ET-A and ET-B receptor may be present and involved in mediating this effect, which needs to be further investigated.

The finding that L-NAME an inhibitor of NO-synthase significantly enhanced ET-1 induced contraction of the oviduct, provides indirect evidence that there is basal release of NO in the oviduct, sufficient enough to reduce and/or modulate the contractile effects of ET-1. Since in contrast to L-NAME, preincubation of the oviduct with indomethacin did not alter basal tone nor the ET-1 induced contraction, strongly suggests that within the oviduct, endogenous NO, but not prostacyclin importantly modulates the contractile effect of ET-1. This is further supported by our observation that the increase in ET-1 induced contraction were comparable in oviduct rings treated with L-NAME and L-NAME plus indomethacin.

In summary our results provide the first evidence that ET-1 contracts bovine oviduct rings and transiently reduces the spontaneous oscillation. Basal release of NO occurs within the oviduct; endogenous NO but not prostacyclin reduces/

modulates ET-1 induced contraction of the oviduct. Hence, a balanced synthesis of endogenous ET-1 and NO may importantly contribute to the physiologic contraction or relaxation of the oviduct in an autocrine/paracrine fashion and potentially contribute to the transport function of the oviduct. Finally, altered release of endogenous ET-1 and/or NO under certain pathological conditions such as endometriosis or inflammatory processes induced by infections such as chlamydia, may result in abnormal contraction or relaxation of the oviduct and consequently lead to infertility or ectopic pregnancy.

References

1 Harper, M.J. (1988) In: The physiology of Reproduction. Knobil and Neil 103-134. Raven Press
2 Helm, G., Ekman, R., Owman, C. (1987) Int.J.Fertil. 32, 467-471
3 Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki,T. (1988) Nature 332, 411-415
4 Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K., Masaki, T. (1989) Proc. Natl. Acad. Sci. 86, 2863-2867
5 Economos, K., Mac Donald, P.C., Casey, M.L. (1992) J. Clin. Endocrinol. Metab. 74, 14-19
6 Schiff, E., Alcalay, M., Peleg, E., Rosenthal, T., Mashiach, S., Dor, J. (1993) Gynecol. Obstet. Invest. 36, 12-14
7 Casey, M.L., Byrd, W., Mac Donald, P.C. (1992) J. Clin. Endocrinol. Metab. 74, 223-225
8 Rosselli, M., Imthurn, B., Macas, E., Keller, P.J. (1994) J. Reprod. Fertil. (in press)
9 Kozuka, M., Ito, T., Hirose, S., Takahshi, K., Hagiwara, H. (1989) Biochem. Biophys. Res. Commun. 159, 317-323
10 Arai,H., Hori, S., Aramori,I., Ohkubo,H., Nakanishi,S. (1990) Nature 348, 730-732
11 Sakuri,T.,Yanagisawa, M., Takawa,Y., Miyazaki, H., Kimura S., Goto, K., Masaki, T. (1990) Nature 348, 732-735
12 Goto,K., Kasuya, Y., Matsuki, N., Takuwa, Y., Kurihara, H.,Ishikawa, T., Kimura, S., Yanagisawa, M., Masaki, T. (1989) Proc. Natl.Acad. Sci. (1989) 86, 3915-3918
13 De Nucci, G., Thomas, R., D'Orleans-Juste, P., Antunes, E., Walder, C., Warner, T.D,, Vane, J.R. (1988) Proc. Natl. Acad. Sci. 85, 9797-9800
14 Palmer, R.M., Ferrige, A.G., Moncada, S. (1987) Nature 327, 524-526
15 Cocks, T.M., Angus, J.A., Cambbell, J.H., Campbell, G.R. (1985) J. Cell. Physiol. 123, 310-320
16 Furchgott, R.F., Zawadkzi, J.V. (1980). Nature 288, 373-376
17 Ellman, C., Corbett, J.A. Misko, T.P., Beckerman, K.P. (1993) J. Clin. Invest. 92, 3053-3056
18 Drapier, J.C. Wietzerbin, J. Hibbs, J.B. (1988) Eur. J. Immunol. 18, 1587-1592
19 Moncada, S., Palmer, R.M., Higgs, E.A. (1991) Pharmacol. Rev. 43, 109-142
20 Förstermann, U., Schmidt, H.H.H.W., Pollock, J.S., Sheng, H., Mitchell, J.A., Warner, T.D., Nakane, M., Murad, F. (1991) Biochem. Pharmacol. 42, 1849-1857
21 Isla,M., Costa, G., Garcia-Pascual, A., Triguero, D., Garcia-Sacristan, A. (1989) J.Reprod. Fertil. 85, 79-87