The effect of 17ß-estradiol and endothelin 1 on prostacyclin and thromboxane production in human endothelial cell cultures

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Summary: The effect was investigated of endothelin 1 and 17β-estradiol on prostacyclin and thromboxane A_2 production (determined as the stable metabolites 6-keto-$PGF1_\alpha$ and thromboxane B_2, respectively) in endothelial cell cultures obtained from veins of human umbilical cord.

There was a statistically significant increase of 6-keto-$PGF1_\alpha$ production with endothelin 1 at concentrations of 10^{-8} and 10^{-9}M [9.5 ± 1.1% and 7.2 ± 0.9%, ($\bar{x}$ ± SD, n = 5), respectively] compared to basal production. In contrast, 17β-estradiol alone (10^{-6} and 10^{-8} M) had no effect. In the presence of 17β-estradiol (10^{-8}M) the stimulating effect of endothelin 1 (10^{-8} and 10^{-9}M) on 6-keto-$PGF1_\alpha$ production was further enhanced to 60.0 ± 22.5% and 39.5 ± 22.1%, respectively, compared to basal values.

With respect to thromboxane B_2, no change in its production was observed by the addition of endothelin 1 and 17β-estradiol, alone or in combination in the concentrations mentioned above.

These results indicate that 17β-estradiol potentiates the effects of endothelin 1 on prostacyclin production in human endothelial cells.

Key words: Human umbilical cord; 17β-estradiol; Endothelin 1; Prostacyclin; Thromboxane A_2.

INTRODUCTION

The endothelins are very potent vasoconstrictive substances. Endothelin 1, which is produced by endothelial cells has in addition to its constrictory properties a stimulating effect on the production of vasodilatory substances such as prostacyclin, endothelial derived relaxing factor (EDRF) and atrial natriuretic peptide (ANP). This can be interpreted as a counterbalance to vasoconstriction ([1-3]).

By means of dopplersonographic methods it has been shown that the natural estrogen, 17β-estradiol, induces vasodilatation ([4, 5]). The mechanism(s) by which this occurs is not quite clear yet. In *in vivo* studies there was an increase of prostacyclin metabolites in urine after estradiol application but in *in vitro* investigations estradiol had no effect on prostacyclin production ([6, 7]).

The purpose of the present investigation was to evaluate the effect of estra-

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diol and endothelin 1 on prostacyclin production in human umbilical cord endothelial cells.

MATERIALS AND METHODS

Human umbilical cords were obtained after normal vaginal deliveries. Cell dispersion were prepared by treatment with collagenase (0.1%) in phosphate buffer (pH 7.4) for 10 min at room temp., placed in culture dishes and allowed to replicate to confluence in Dulbecco's Modified Eagle Medium/Nutrient Mix F12 (1:5, v/v) that contained fetal calf serum (10% v/v), endothelial growth factor (20 µg/ml), heparin (50 µg/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml). Cell cultures were maintained in an atmosphere of CO_2 (5%) in air.

Tissue culture media and supplies, antibiotics and fetal calf serum were purchased from Gibco, Eggenstein, Germany. Endothelial cell growth factor was purchased from Boehringer, Mannheim, Germany.

Assay procedures.

The experiments were initiated in duplicates by the addition of 17β-estradiol (dissolved in ethanol and or endothelin (dissolved in 0.9% NaCl-solution) to confluent cell cultures (passages 2-3) to give a final concentration of 10^{-8} and 10^{-9}M for endothelin 1 and 10^{-6} and 10^{-8} M for 17β-estradiol in the culture medium. The amount of ethanol was < 0.2% (v/v). Incubations were performed for 24 h. All experimental values were corrected for blanks in samples that were treated with solvent mixture alone. At the end of the incubation period the culture medium was collected and 6-keto-$PGF1_\alpha$ and thromboxane B_2 were determined directly according to the method of Dray (8). The sensitivity of the 6-keto-$PGF1_\alpha$ assay was 160 pg/ml and the inter- and intra-assay coefficient of variation were 8.4 and 6.2%, respectively. The sensitivity of the thromboxane B_2 assay was 52 pg/ml and the inter- and intra-assay coefficient of variation were 7.4 and 4.9%, respectively.

Statistical analyses were conducted by the use of the unpaired Student t test.

RESULTS

Estradiol alone had no influence on the prostacyclin production in all concentrations tested compared to the basal value. Endothelin 1 alone changed the prostacyclin production in endothelial cell cultures from human umbilical cord veins in both concentrations (Tab 1, Fig. 1). The increase was 9.5 ± 1.1 % and 7.2 ± 0.9% at a concentration of 10^{-8} M and 10^{-9} M, respectively. The addition of estradiol (10^{-6} M) to both endothelin concentrations did not change the prostacyclin production statistically significant. In contrast, addition of the lower dosage estradiol (10^{-8} M) showed a statistically significant increase of the prostacyclin production of both endothelin concentrations. With the endothelin concentration of 10^{-8} M an increase of 60.0 ± 22.5%, with 10^{-9}M an increase of 39.5 ± 22.1% was observed.

Table 1. — *6-keto-$PGF1_\alpha$ and thromboxane B_2 production in cell cultures from human umbilical cord vein after addition of different concentrations of endothelin 1 (ET1) and 17β-estradiol (E2) alone or in combination (pg/ml culture medium, $\bar{x} \pm SD$, $n = 5$).*

	6-keto-$PGF1_\alpha$ (pg/ml)	Thromboxane B_2 (pg/ml)
Control	454± 99	379±20
ET1 (10^{-8} M)	465±103*	388±25
ET1 (10^{-9} M)	466±123*	399±22
E2 (10^{-6} M)	456±101	375±23
E2 (10^{-8} M)	452± 98	381±32
ET1 + E2 ($10^{-8}/10^{-6}$ M)	466± 51	393±19
ET1 + E2 ($10^{-8}/10^{-8}$ M)	1033±102**	401±27
ET1 + E2 ($10^{-9}/10^{-6}$ M)	453±113	388±31
ET1+ E2 ($10^{-9}/10^{-8}$ M)	903±120**	396±28

* $p < 0.05$; ** $p < 0.01$.

As shown in figure 1 the thromboxane B_2 production in endothelial cell cultures was not affected by endothelin 1 and estradiol, alone or in combination.

DISCUSSION

The mechanism by which 17β-estradiol induces vasodilatation in vivo is not known but it is possible that prostaglandins are

involved. In *in vitro* studies with endothelial cell cultures 17β-estradiol alone did not stimulate prostacyclin production

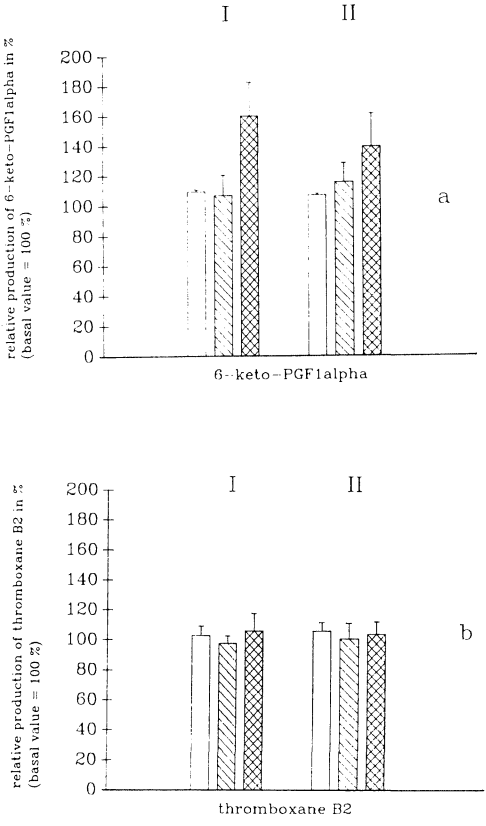

Fig. 1. — Relative production of 6-keto-PGF1$_\alpha$ (a) and thromboxane B_2 (b), expressed as percentages of the basal values, in endothelial cell cultures from human umbilical cord vein after addition of endothelin 1 (ET1) and 17β-estradiol (E2) alone or in combination ($\bar{x} \pm SD$, $n = 5$).

Concentrations in I:

- 10^{-8}M ET1
- 10^{-8}M ET1 + 10^{-6}M E2
- 10^{-8}M ET1 + 10^{-8}M E2

Concentrations in II:

- 10^{-9}M ET1
- 10^{-9}M ET1 + 10^{-6}M E2
- 10^{-9}M ET1 + 10^{-8}M E2

($^{7, 9, 10}$). On the other hand, endothelin 1 has been shown to increase prostacyclin synthesis in cell cultures of different species and it has been hypothesized that this may due to activation of phospholipase A_2, an increase of intracellular Ca^{2+} or by a G-protein mediated mechanism ($^{11\text{-}13}$).

We found that 17β-estradiol potentiated the effect of endothelin 1 on prostacyclin production in human umbilical cord endothelial cells. The stimulation of prostacyclin synthesis was greatest at a concentration of 10^{-8} M for both endothelin 1 and estradiol. The mechanism by which this occurs is still unknown. The vasodilatory effect of 17β-estradiol that has been observed in *in vivo* experiments ($^{4, 5}$) may be due to a synergistic action of different substances. As shown, a possible way could be the combined effect of estradiol and endothelin 1.

REFERENCES

1) Wright C. E. and Fozard J. R.: "Vasodilatation is a prominent feature of the haemodynamic response to endothelin in anaesthetised spontaneously hypertensive rats". *Eur. J. Pharmacol.*, 1988, *155*, 201.
2) Nucci (de) G., Thomas R., D'Orleans-Juste P., Antunes E., Walder T. D. and Vane J. R.: "Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by a release of prostacyclin and endothelium derived relaxing factor". *Proc. Natl. Acad. Sci. USA*, 1988, *85*, 9797-9800.
3) Fukuda Y., Hirata Y., Yoshimi H., Kojima T., Kobayashi Y., Yanagisawa M. *et al.*: "Endothelin is a potent secretagogue for atrial natriuretic peptide in cultured rat atrial mycocytes". *Biochem. Biophys. Res. Commun.*, 1988, *155*, 167-172.
4) Bourne T., Hillard T. C., Whitehead M. I., Crook D. and Campell S.: "Oestrogens, arterial status and post menopausal women". *Lancet*, 1990, *II*, 1470-1471.
5) Sarrell P.: "Effect of ovarian steroids on the cardiovascular system". In: 'The circulation in the female: from the cradle to the grave'. (J. Ginsberg, ed.) Parthenon Publishing, Camforth U.K., 1989, p. 117-141.
6) Foidart J. M., Dombrowicz N. and de Lignieres B.: "Percutaneous administration of 17β-estradiol and oral administration of

Premarin have different effects on urinary excretion of prostacyclin metabolites and serum high density lipoproteins". 6. Internat. Congr. Menopause, Bangkok, (Oct. 29 - Nov. 2) 1990, Abstr. no. 215.
7) Mück A. O., Seeger H., Korte K., Dartsch P. C. and Lippert T. H.: "Natural and synthetic estrogens and prostacyclin production in human endothelial cells from umbilical cord and leg veins". *Prostaglandins*, in press.
8) Dray F.: "Iodinated derivatives as tracers for eicosanoid radioimmunoassays". *Methods in Enzymology*, 1982, *86*, 297-306.
9) Witter F. R. and Di Blasi M. C.: "Effect of steroid hormones on arachidonic acid metabolites of endothelial cells". *Obstet. Gynecol.*, 1984, *63*, 747-751.
10) Corvazier E., Dupuy E., Dosne A. M. and Maclouf J.: "Minimal effect of estrogens on endothelial cell growth and production of prostacyclin". *Thrombosis Res.*, 1984, *34*, 303-310.
11) Filep J. G., Battistini B., Cote Y. P., Beaudoin A. R. and Sirois P.: "Endothelin-1 induces prostacyclin release from bovine aortic endothelial cells". *Biochem. Biophys. Res. Commun.*, 1991, *177*, 171-176.
12) Kato K., Sawada S., Toyoda T., Kobayashi K., Shirai K., Yamamoto K. *et al.*: "Influence of endothelin on human platelet aggregation and prostacyclin generation from human vascular endothelial cells". *Jpn. Circ. J.*, 1992, *56*, 422-431.
13) Rakugi H., Nakamaru M., Tabuchi Y., Nagano M., Mikami H., Ogihara T.: "Endothelin stimulates the release of prostacyclin from rat mesenteric arteries". *Biochem. Biophys. Res. Commun.*, 1989, *160*, 924-928.

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