Eicosanoid production in human umbilical cord vessels: effect of 17β-estradiol

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Summary: This study was designed to investigate the effects of 17β-estradiol on prostanoid formation from exogenous arachidonic acid in homogenates of human umbilical cord vessels. The veins produced more prostanoids than the arteries and predominantly 6-keto-$PGF_{1\alpha}$. 17β-estradiol had no effect on the rate of production of prostanoids in either vessels. Thus, at least in our in vitro system, the regulation of the vascular tone by prostanoids seems not to be altered by the addition of 17β-estradiol.

Key words: Human umbilical cord; 17β-estradiol; Prostanoid metabolism.

INTRODUCTION

Prostanoids are mediators in numerous physiologic pathways: for example thromboxane A_2 (TxA_2) is a powerful vasoconstrictor and platelet aggregator, whereas prostacyclin (PGI_2) relaxes vascular smooth muscle and blocks platelet aggregation [1]. Plostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) play important parts in kidney blood flow and act as uterotonins in uterine tissues [2, 3]. Moreover, prostanoids are intimately involved in processes of inflammation [4]. The effects of exogenous estrogens on prostaglandin metabolism have been investigated in a variety of tissues. In endometrial cells in culture 17β-estradiol stimulates the production of $PGF_{2\alpha}$ and other prostanoids [5–10]. In endothelial cells of piglets there is an increase of PGE_2, $PGF_{2\alpha}$ and PGI_2 after treatment with 17β-estradiol [11]. On the contrary, after 5 minutes of treatment of human umbilical cord endothelial cells with 17β-estradiol, there was no effect on PGI_2 production observed [12]. With respect to a relationship of exogenous estradiol and cardiovascular disease our knowledge is incomplete. It has been proposed that the increase in cardiovascular disease in postmenopausal women is due to a reduction in endogenous estrogen production [13]. On the other hand, women on hormone replacement therapy have a significantly reduced risk of dying from ischaemic heart disease than women without therapy, and it has been suggested that this is mediated through beneficial changes in plasma lipid and lipoprotein metabolism [14, 15]. Moreover it has been hypothesized that estrogens will have a positive effect on the production of PGE_2 and PGI_2 within vascular endothelial cells. It has been shown that

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estrogens applied orally or percutaneously stimulate PGI_2 production ([16]).

In this study we evaluated the effects of 17β-estradiol on the conversion of arachidonic acid to prostanoids in homogenates of human umbilical cord vessels.

MATERIALS AND METHODS

Prostaglandins E_2, $F_{2\alpha}$, 6-keto-$F_{1\alpha}$ and thromboxane B_2 and antibodies were from Paesel, Frankfurt, FRG. Arachidonic acid, 17β-estradiol, reduced glutathion, tryptophan and hematin was purchased from Sigma, Munich, FRG. All other chemicals were of analytical grade from scientific supply houses. Human umbilical cords were obtained after normal vaginal deliveries. Arteries and veins were prepared on ice and homogenized in 5 ml sodiumphosphate buffer (50 mM, pH 7.4) with EDTA (2 mM) by means of a Ultraturrax homogenizer. The homogenate was centrifuged at 750 g for 10 minutes and the supernatant was used as enzyme source ("homogenate").

Assays were conducted by incubation of aliquots of the enzyme source (0.1 ml) with arachidonic acid (100 μM), L-tryptophan (4.2 mM), reduced glutathion (5.1 mM) and hematin (1.75 μM) at 37° C for 15 minutes in 0.2 ml total volume. 17β-estradiol was included in some samples, as indicated, in ethanol at a final concentration of 1 and 0.01 μM. The final ethanol concentration was 0.15% (vol/vol). The reaction was terminated by the addition of 0.1 ml of acetic acid (1N). Prostanoids that accumulated in the assay mixture were determined by radioimmunoassay (RIA) in duplicate. The tracers were prepared with chloramin-T according to the method of Dray ([17]). The cross-reactivities of the antisera were less than 1% except for the 6-keto-$PGF_{1\alpha}$ antibody which had a cross-reactivity with PGE_2 and $PGF_{2\alpha}$ of 6.2 and 7.5%, respectively.

Protein was quantified by the method of Lowry *et al.* ([18]) with bovine serum albumin as the standard. Statistical analyses were conducted by use of the U-test according to Mann and Whitney ([19]).

RESULTS

Tissue homogenates were prepared from 8 umbilical cord tissue specimens. After the addition of arachidonic acid (100 μM), there was an increase of the concentrations of prostanoids up to 15 minutes. Thereafter, we observed, at best only minor increases up to 24 h. The concentrations of the prostanoids determined in tissue homogenates of human umbilical arteries and veins is given in Table 1. The concentrations of the prostanoids were higher in the veins than in arteries. Umbilical cord tissue homogenates produced predominantly PGI_2 (as evidenced by the concentration of 6-keto-$PGF_{1\alpha}$) and PGE_2. There was only little TXB_2 measurable. In homogenates that contained no exogenous arachidonic acid the concentration of 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$ and TXB_2, that accumulated over a time period of 15 minutes was 60.29 ± 19.07, 3.42 ± 1.38, 1.92 ± 0.52 and 0.26 ± 0.03 ng/mg protein ($\bar{x} \pm SD$, $n = 8$), respectively, for the artery and 95.13 ± 38.74, 6.34 ± 2.21, 4.21 ± 1.34 and 0.47 ± 0.10 ng/mg protein ($\bar{x} \pm SD$, $n = 8$), respectively, for the vein.

The increase in prostanoid production in homogenates of human umbilical cord arteries and veins that have been treated with arachidonic acid alone and with 17β-estradiol at 2 different concentrations for

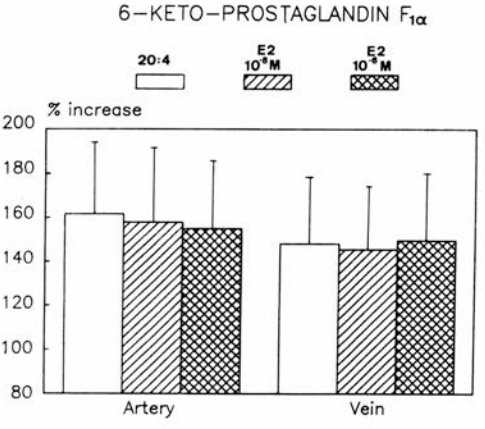

Fig. 1. — The percentage increase in the production of 6-keto-$PGF_{1\alpha}$ in homogenates of human umbilical cord arteries and veins that had been treated with arachidonic acid and 17β-estradiol at 2 different concentrations ($\bar{x} \pm SD$, $n = 8$).

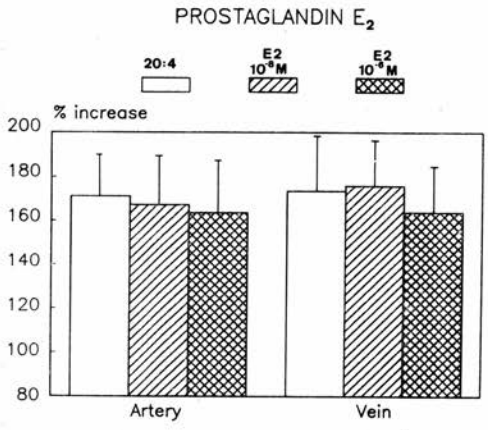

Fig. 2. — The percentage increase in the production of PGE_2 in homogenates of human umbilical cord arteries and vein that had been treated with arachidonic acid and 17β-estradiol at 2 different concentrations ($\bar{x} \pm SD$, $n = 8$).

15 minutes as illustrated in Figures 1-4. The percent increases over samples without estrogen were approximately 160% for 6-keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$. There were no significant differences between artery and vein tissue specimens. With respect to TXB_2 the percent increase was about 130% in the artery and 140% in the vein although not significantly different. Thus, 17β-estradiol had no significant effect on the production of prostanoids in umbilical vessels.

DISCUSSION

The regulations of blood pressure are physiological events which occur in humans as a result of a series of complex biochemical interactions. These interactions depend, in part, in the production of prostanoids within the blood vessels and are influenced by a variety of hormones including steroids. In vascular tissues it has been shown that glucocorticosteroids and testosterone inhibit PGI_2 production ([20, 21]), whereas estradiol has a stimulatory effect ([22]).

We found that after the addition of arachidonic acid (100 μM), human umbilical cord vessels produced PGE_2, $PGF_{2\alpha}$, TxB_2 and, predominantly, 6-keto-$PGF_{1\alpha}$ (the main hydrolysis product of PGI_2).

The production of these prostanoids in venous tissues was about 2 fold higher than in the arteries (Table 1). The reverse seems to be true for the nonpregnant state ([23]). Various tissues in human pregnancy are known to synthesize a variety of prostanoids ([24]).

With respect to the percentage increase in prostanoid production in arteries and veins, 17β-estradiol had no effect compared with samples that contained only arachidonic acid (Figs. 1-4). Moreover, there was no change in the ratios of different prostanoids, e.g. 6-keto-$PGF_{1\alpha}$ and PGE_2. These are the two major products within umbilical tissues which have opposing actions on umbilical tone ([25]). In addition, we found no imbalance in the ratio of TXB_2 to 6-keto-$PGF_{1\alpha}$ that favors TXB_2, and thus vasoconstriction, as has been proposed in preeclampsia and other hypertensive states ([26]).

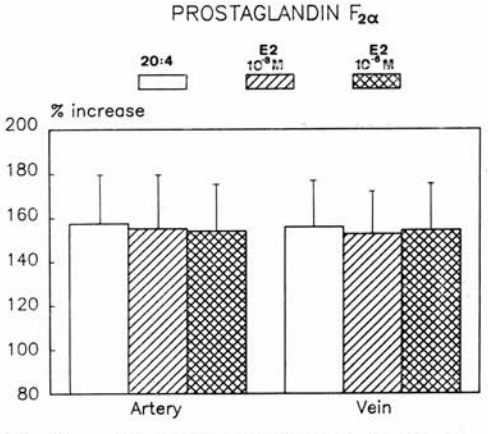

Fig. 3. — The percentage increase in the production of $PGF_{2\alpha}$ in homogenates of human umbilical cord arteries and veins that had been treated with arachidonic acid and 17 β-estradiol at 2 different concentrations ($\bar{x} \pm SD$, $n = 8$).

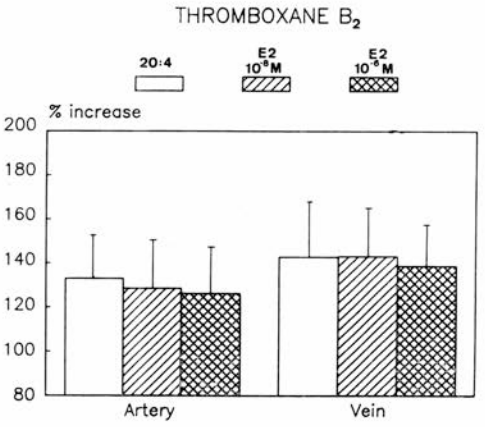

Fig. 4. — The percentage increase in the production of TXB_2 in homogenates of human umbilical cord arteries and veins that had been treated with arachidonic acid and 17 β-estradiol at 2 different concentrations ($\bar{x}$ SD, n = 8).

In our in vitro system we did not find stimulation of 6-keto-$PGF_{1\alpha}$ with 17β-estradiol. Others have reported on the vasodilatory properties of estradiol that are probably mediated by an increase in PGI_2 production ([22]). Our results with the natural estrogen 17β-estradiol do, of course, not rule out possible effects of metabolites of 17β-estradiol that may be formed during the incubation period. It is known that because of differences in chemical composition, in metabolism, in affinity for receptors and in site of action, estrogens or their metabolites may have different effects on the vascular tone in humans ([27]).

Table 1. – *The concentration of prostanoids in human umbilical cord vessels (ng/mg protein, $\bar{x} \pm SD$, $n = 8$).*

Prostanoid	Artery	Vein
6-keto-$PGF_{1\alpha}$	103.78±40.25	153.10±79.79
PGE_2	5.66± 1.78	10.75± 3.76
$PGF_{2\alpha}$	2.92± 1.06	6.66± 2.68
TxB_2	0.33± 0.04	0.64± 0.21

However, in preliminary experiments we did not find a decrease of the concentration of 17β-estradiol in the homogenate (unpublished observation). One reason why we did not see an effect of 17β-estradiol on prostanoid production in human umbilical vessels may be that this tissue is already primed by the endogenous amounts of estrogens that are circulating in pregnancy. Further studies are needed including nonpregnant vascular tissues and different groups of estrogens to evaluate the possible effects on prostanoid formation, and thereby regulation of vascular tone.

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