SHORT COMMUNICATION

THE DUAL EFFECT OF CALCIUM ON AROMATIZATION BY CULTURED HUMAN TROPHOBLAST

ZEEV HOCHBERG, TOVA BICK, RINA PERLMAN, JOSEPH M. BRANDES and DAVID BARZILAI

Division of Pediatric Endocrinology, Endocrine Institute and Department of Obstetrics and Gynecology, Rambam Medical Center and Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel

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Summary—To study the effect of calcium ion on aromatization of an androgenic precursor to estradiol by the placenta, cultured term trophoblasts were used as a model system. Secretion of estradiol into the culture medium was regarded as indicating aromatization, since cells cultured with no androgenic precursors produced only insignificant amounts of estradiol. EGTA, verapamil and ionophore A23187 inhibited aromatization, while trifluoperazine, an inhibitor of the calcium-calmodulin complex, interfered with the stimulatory effect of cyclic AMP on aromatization. We conclude that calcium ion has an essential role in the aromatization of 4-androstene-3,17-dione to estradiol. The calcium-calmodulin complex is required for activation of aromatase by cyclic AMP. However, when flooded with calcium by ionophore A23187, the trophoblast is unable to effectively buffer calcium, and aromatization is inhibited.

INTRODUCTION

The biosynthesis of estradiol by the placenta requires an androgenic precursor, provided in vivo from fetal and maternal sources. The rate-limiting enzyme of estradiol synthesis is the aromatase complex on which regulatory mechanisms exert their action [1]. The well-described mediator of this regulation is cyclic AMP which, by way of phosphorylation-dephosphorylation [2], stimulates aromatization [3-5]. The development of a reliable primary monolayer cell-culture system of human term trophoblasts [5, 6] has enabled us to study regulatory mechanisms of estradiol secretion. This report describes the results of our experiments on the role of calcium ion in aromatization of 4-androstene-3,17-dione (androstenedione) to estradiol.

EXPERIMENTAL

Materials

Tissue culture medium, Waymouth MB 752/1, was purchased from Bio-Lab, Jerusalem, Israel. Trypsin, deoxyribonuclease, androstenedione, ionophore A23187, trifluoperazine and verapamil were purchased from the Sigma Chemical Company, St Louis, Miss., U.S.A. [3H]Estradiol was purchased from New England Nuclear, Boston, Mass., U.S.A.; anti-estradiol antibody from Bio-Yeda, Rehovot, Israel, and reference preparations from Makor, Israel.

Culture procedure

Cells were dispersed from term human placentae as previously described [5, 6]. One million trophoblast cells were plated in each 30-mm Petri dish and incubated for 48 h in Waymouth's MB 752/1 medium containing 0.812 mM calcium and enriched with 10% autologous cord serum and antibiotics. The cells were then preincubated for 2 h in fresh medium. After another medium change, the test substance was added. Unless otherwise specified, the culture medium was Waymouth MB 752/1 and contained 0.812 mM calcium and 25 μg/ml androstenedione as a precursor for aromatization to estradiol. Ionophore A23187 was dissolved in 1% ethanol, and control dishes in these experiments accordingly contained 1% ethanol. Ethanol produced no significant effect on estradiol secretion when compared to untreated cells.

Experimental incubations were carried out for 4 h in quintuplicates. Estradiol in the medium was determined by radioimmunoassay as previously described [5].

RESULTS

Enrichment of the medium with androstenedione produced a 10-fold increase in the amount of estradiol secreted...
The results presented in this report establish calcium ion as an essential factor in the aromatization of androgenic precursors to estradiol by the trophoblast in vitro. Deprivation of calcium either by EGTA chelation of calcium from the medium or by blocking calcium channels with verapamil interfered with estradiol synthesis. These effects were not the result of cell destruction, as shown by measurements of lactic dehydrogenase. Similar dependence on calcium has been described for the synthesis of cortisol by the adrenal in vitro. When calcium was chelated by EGTA, the adrenal failed to respond to ACTH [7].

Enhanced penetration of calcium from the medium into the cells was also inhibitory with respect to estradiol secretion. This effect was not altered by trifluoperazine, the blocking agent of calcium-calmodulin complex. Trifluoperazine, however, blocked the stimulatory effect of dibutyryl cyclic AMP on aromatization [5]. These effects can be summarized in the following postulate: calcium ion is an important factor in the aromatization of androstenedione to estradiol. By formation of a calcium-calmodulin complex, it is required for activation of aromatase by cyclic AMP. However, when flooded with calcium by ionophore A23187, the trophoblast is unable to effectively buffer calcium, and aromatization is inhibited.

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REFERENCES


