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The role of catecholamines in estradiol and progesterone secretion by cultured explants and cells of human term placentae

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Abstract. The effects of physiological concentrations of the native catecholamines norepinephrine and epinephrine upon term placental hormonal function were examined by measuring estradiol and progesterone secretion by organ and cell culture systems. Results show that, in explants, both catecholamines caused a significant increase in the secretion of both sex steroids, $p < 0.05$. Estradiol secretion was blocked by the alpha and beta adrenergic receptors antagonists, phenoxybenzamine and propranolol, respectively, $p < 0.05$. Norepinephrine but not epinephrine dependent progesterone secretion was blocked by propranolol. In cells, epinephrine stimulated cyclic AMP generation and caused a 30% increase in estradiol secretion, $p < 0.05$. Both were abrogated by propranolol. Norepinephrine increased secretion by 25%, $p < 0.05$. This was inhibited by yohimbin and prazosin, alpha-1 and -2 receptors antagonists, respectively. In conclusion, the placenta in vitro is a target organ for catecholamines. The marked response of the explant system as compared with the marginal response of the cell culture system indicates that cell to cell contact/communication is required for full expression of catecholamine effect.

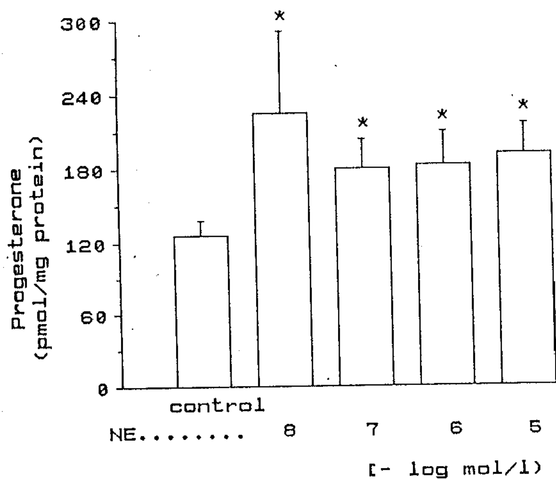
During pregnancy, levels of circulating catecholamines (CA) are 5-fold higher in the fetal compared with the maternal compartment (1). The transfer of maternal originating CA to the fetal circulation is limited by the efficient metabolism of placental catechol-O-methyl transferase and monoamine oxidase enzymes (2,3). It was reported that in certain high risk pregnancies (caused by hypertension and diabetes mellitus) which are accompanied by a heightened adrenergic activity, the activity of placental CA metabolic enzymes is low, which leads to elevated levels of circulating CA (4–6). The effects of circulating CA on the maternal (7) and fetal organism have been well documented (8), but their role in the placenta is largely unknown.

Since the placenta contains adrenergic receptors (9, 10), it is a likely target organ for these potent biogenic amines. β-adrenergic agents were reported to stimulate P4 secretion by placental explants (11). However, the effect of naturally occurring CA, epinephrine (EPI), and norepinephrine (NE) on placental function have not been addressed.

The aims of the study were to determine the effect of natural CA on placental steroidogenesis, and to identify the sites of CA binding by using specific CA antagonists.

Materials and Methods

Waymouth MB 752/1 Medium and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Biological



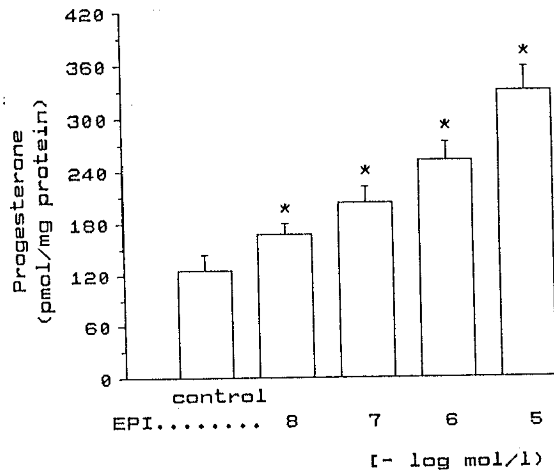


Fig. 1.
Effect of various concentrations of norepinephrine (NE) (panel A), and epinephrine (EPI) (panel B) upon placental explants progesterone secretion. * $p < 0.05$, mean ± SEM, N = 3 separate placentae.

Industries (Beit Haemek, Israel). Norepinephrine and epinephrine were purchased from Sigma, MO. Estradiol-17β and P4 antiserum were purchased from Bio-Yeda (Rehovot, Israel). [^{3}H]6,7-estradiol, 45 Ci/mmol and [^{3}H]1,2,6,7,16,17-progesterone, 112 Ci/mmol were purchased from Nuclear Research Center (Negev, Israel). Catecholamine antagonists were purchased from Sigma, MO. Other chemicals were of the highest analytical quality available.

Explants

A total of 20 placentas were studied. Explants were prepared as previously described (12). Small fragments were dissected in sterile fashion from term placental tissue obtained from healthy women following normal deliveries. After rinsing in 0.9% NaCl to remove residual debris and blood, the tissue was rinsed in DMEM. Placental tissue was then explanted as described. Explants from each placenta, 6-10 dishes/per test dose or control, were placed in DMEM which contained 10% autologous cord serum and 1% antibiotic solution (penicillin 10 000 U, streptomycin 10 µg, and Fungizone® 10 000 U). Incubations were carried out at 37°C in an atmosphere of 95% air and 5% CO_2. The incubations was stopped by placing dishes on ice. The media was removed and saved at −20°C for radioimmunoassay. The tissue was saved for protein analysis.

Cell culture

Placental trophoblast monolayer cultures were prepared as previously described (13). After trypsin dispersion, one million cells were plated in each 35-mm Petri dish in Waymouth's MB 752/1 containing 10% autologous cord serum for 24 h. Following that cells were washed for 5 min with Earle's balanced salt solution, which removed non-attached cellular debris and red blood cells. Subsequently, the cells were incubated with various concentrations of the test substrate for 4 h. These conditions were found to be optimal for the cell culture system (data not shown). After the addition of 8 µmol/l delta 4-androstene-3,17-dione as precursor, E_2 secretion was interpreted as the combined activity of aromatase and 17-hydroxysteroid dehydrogenase (3). Experiments were terminated by removal of the media and storage at −20°C until assayed.

We have previously demonstrated that the careful trypsinization leaves the cells responsive to insulin, dibutyryl cyclic AMP (13, 14), and growth hormone (15). Morphological examination revealed that most dispersed cells were cytotrophoblasts and continued to secrete neoformed estradiol for several days (data not shown).

Radioimmunoassay for E_2, P4, and cyclic AMP was carried out as previously reported, (16, 17). Protein analysis was carried out according to (18).

Statistical analysis was carried out by one-way ANOVA and Student's *t*-test. Significance was defined as $p < 0.05$. In each experiment, the medium with 10% cord serum was assayed and the hormone concentration was subtracted from each data point.

Results

Effect of CA on explants E_2 and P4 secretion

In placental explants, NE at 10−10 000 nmol/l concentrations caused a significant effect on P4 secretion (Fig. 1, panel A). Maximal effect was seen with

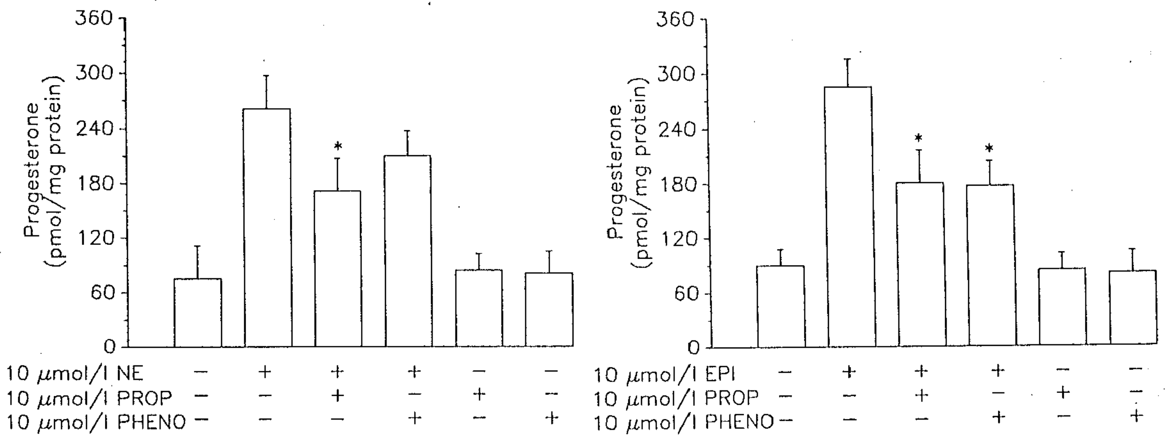


Fig. 2.
The effect of catecholamine receptor blockers upon norepinephrine (NE) (panel A) and epinephrine (EPI) (panel B) dependent progesterone secretion by placental explants. Mean ± SEM, * $p < 0.05$ vs NE and EPI alone, N = 3 separate placentae. PROP: propranolol; PHENO: phenoxybenzamine.

a physiological dose of 10 nmol/l. In the case of EPI, the effect was dose-dependent. The effect was already significant with 10 nmol/l (Fig. 1, panel B). Further experiments with pharmacological blockers were performed at this concentration. The stimulatory effect of NE on P4 secretion was attenuated by the alpha blocker, phenoxybenzamine, but not by the beta blocker, propranolol (Fig. 2, panel A). In contrast, both blockers attenuated P4 stimulation by epinephrine (Fig. 2, panel B). When tested alone, these antagonists did not exert any significant effect upon P4 secretion.

In the same explant system, the effect of NE and EPI upon E_2 secretion was stimulatory and dose-dependent (Fig. 3, panel A and B). While NE and EPI in a physiological concentration of 10 nmol/l concentration produced significant stimulation, maximal secretion was observed at 10 µmol/l concentration. Addition of both propranolol and phenoxybenzamine significantly suppressed the CA-

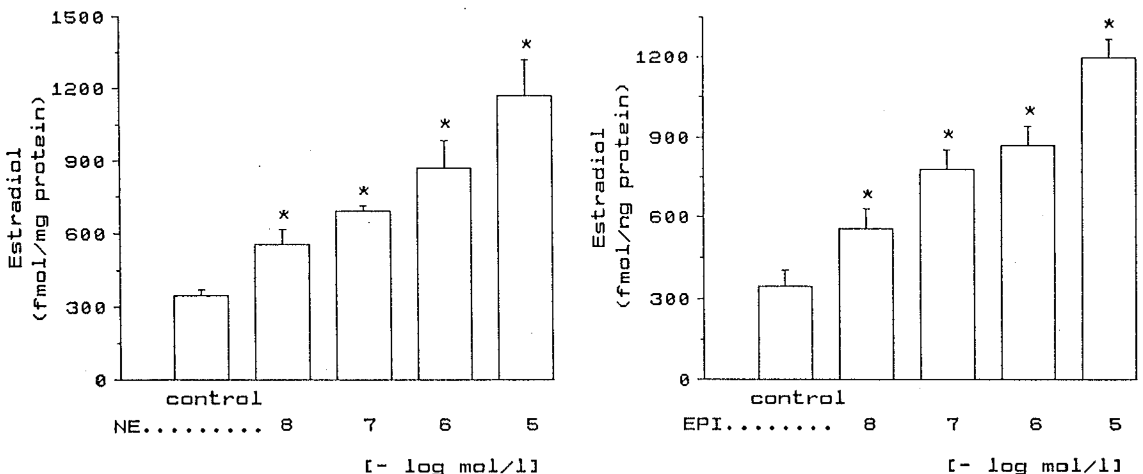


Fig. 3.
The effect of various concentrations of norepinephrine (NE) (panel A), and epinephrine (EPI) (panel B) upon placental explants E_2 secretion. * $p < 0.05$, mean ± SEM, N = 4 separate placentae.

Table 1.
Effect of CA with or without adrenergic receptors blockers upon isolated term placental cells E_2 secretion and total cAMP (secreted and cell content). Data are expressed as mean ± SEM% change from untreated controls.

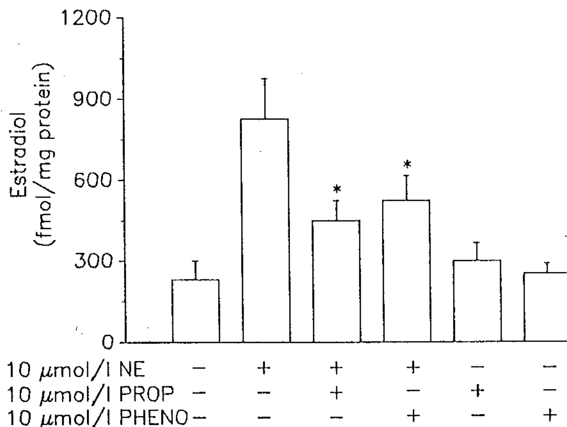
	E_2	cAMP
Control	100 ± 11	100 ± 3
NE		
1 nmol/l	115 ± 8	ND
10 nmol/l	130 ± 6[a]	103 ± 7
100 nmol/l	120 ± 7[a]	ND
10 nmol/l + 100 nmol/l YOH	105 ± 4[c]	ND
10 nmol/l + 100 nmol/l PRAZ	107 ± 6[c]	ND
EPI		
1 nmol/l	125 ± 10[a]	ND
10 nmol/l	122 ± 7[a]	275 ± 8[a]
100 nmol/l	112 ± 6	ND
10 nmol/l + 10 μmol/l PROP	103 ± 5[b]	104 ± 5[b]

Isolated cells were incubated for 4 h with the estrogen precursor, 8 μmol/l delta-4-androstenedione-3,14-dione in the presence of CA or specific inhibitors. At the end of the experiment the media were collected and examined for E_2 secretion and for cAMP total content in the cells and the media. Results represent data obtained in a total of 10 placentae.
PROP: propranolol, beta blockers.
YOH: yohimbin, alpha-2 blocker.
PRAZ: prazosin, alpha-1 blocker. ND: not determined.
a: $p < 0.05$, compared with controls.
b: $p < 0.05$, compared with 10 nmol/l EPI alone.
c: $p < 0.05$, compared with 10 nmol/l NE alone.

induced effect, whereas the adrenergic blockes alone did not affect E_2 secretion (Fig. 4, panels A and B).

Effect of CA on dispersed placental cells' E_2 and cAMP secretion

In order to further elucidate the mechanisms by which CA stimulate E_2 secretion, we incubated dispersed term placental cells with an E_2 precursor, androstenedione. This enabled closer observation of the effect of CA on the two enzyme systems of androgen metabolism, aromatase and 17-hydroxysteroid dehydrogenase. Results are reported in Table 1. Both NE and EPI caused a small (25–30%), but statistically significant increase ($p < 0.05$) in E_2 secretion after 4 h of incubation. In order further to elucidate the mechanism of CA action, we measured cyclic AMP concentration in the combined medium and disrupted cells. At 10 nmol/l concentration, EPI, but not NE, caused a significant increase in total cAMP content. Accordingly, propranolol interfered with the EPI-dependent E_2 and cAMP, but not the NE-dependent E_2 secretion. To study the mechanism of the NE-stimulated E_2 secretion, we utilized alpha-1 and -2 adrenergic blockers. The addition of yohimbin, a specific alpha-2 receptor blocker and prazosin, a specific alpha-1 blocker inhibited the effect of NE.



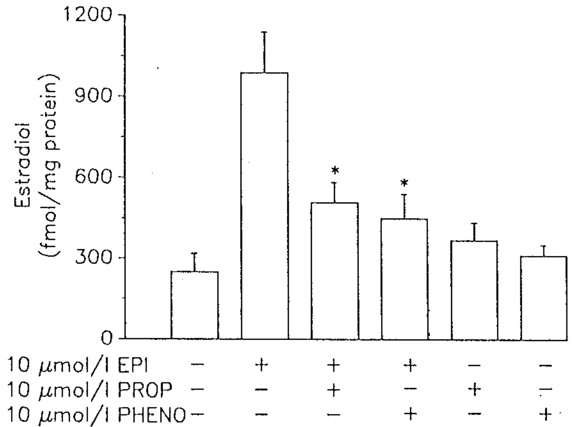


Fig. 4.
The effect of catecholamine receptor blockers upon norepinephrine (NE) (panel A) and epinephrine (EPI) (panel B) dependent E_2 secretion by placental explants. * $p < 0.05$ vs NE and EPI alone, mean ± SEM, N = 3 separate placentae. PROP: propranolol; PHENO: phenoxybenzamine.

Discussion

To study the potential role of the adrenergic alpha and beta receptors found in placental tissue, we have utilized two model systems. The explant tissue culture enables the investigation of the de-novo steroidogenesis, whereas the cell culture system which is enriched with an androgen precursor is useful for studies on the regulation of the aromatase and 17-hydroxysteroid dehydrogenase enzymes. In a previous study we have demonstrated the dependency of E_2 secretion on the androgen precursor, delta-4-androstenedione,3,14-dione (13). This implies that under these experimental conditions the cell culture system is a measure of the combined regulation of these two enzymes. Aromatase is believed to be the hormonally regulated step in E_2 biosynthesis (13, 19).

The data presented in this report indicates that naturally occurring CA, EPI and NE exert a significant stimulatory effect upon placental steroidogenesis at term. In the explant system, the CA effect on both P4 and E_2 secretion was marked, confirming previous investigations (11). Recently, beta 2 mimetic agents, terbutaline and isoproterenol, were shown to stimulate P4 secretion by explants. As expected, propranolol blocked their effect completely (20). In contrast, in the cell culture system, the effect of CA on E_2 biosynthesis was only marginally significant.

The CA exerted their effects on steroidogenesis through their beta and alpha-2 receptors, and to some extent through the alpha-1 receptor. Epinephrine induced its effects mainly through the beta receptor: cyclic AMP was generated and its effects were blunted by propranolol. We now report the stimulatory effect of EPI on P4 secretion. The antagonists to alpha-1 and -2 receptors utilized in this study confirm that the stimulatory effect of CA is exerted through both types of alpha receptors. Through the use of antagonists, with their differential function in the explants de-novo synthesis and in the cell culture system with its specific enzymes activity the following can be concluded: placental aromatase and 17-hydroxysteroid dehydrogenase activity was enhanced by EPI and was beta receptor dependent. The effect of NE was alpha-1 and -2 receptor dependent. These results are similar to those previously reported on catecholestrogens where 2-hydroxy estrone stimulated E_2 and P4 secretion by placental explants. This secretion was blocked by both alpha and beta adrenergic blockers (12).

In context with our previous reports on the placental effects of glucose regulating hormones and stress counterregulators, CA testing followed the general pattern, (13, 16, 17). In response to stress, the counterregulatory hormones induced a rise in placental hormones. The role of sex steroids in combatting stress remains to be explored.

Acknowledgments

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