Endocrine (1995) 3, 251-254

Modulatory effects of neurohypophysary origin hormones on placental hormone secretion at term

E.R. Barnea[1], T. Maruo[2] & R. Schurtz-Swirsky[3,4]

[1]CAMcare Health, Three Cooper Plaza, Suite 104, Camden, New Jersey, USA; [2]Department of Obstetrics and Gynecology, Kobe University School of Medicine, Kobe 650, Japan; [3]Feto-Placental Endocrinology Unit, Rappaport Institute, Technion Institute, Haifa 34601, Israel; [4]Current address: Division of Research, Nahariah Hospital, Nahariah, Israel

Oxytocin (OT) and arginine-vasopressin (AVP) are two major neurohypophysary nonapeptides that have important roles during pregnancy and labor. Using the explant model at term, we have examined the effect of physiologic concentrations of both nonapeptides on placental hormone secretion. We found that OT had a dose-dependent inhibitory effect on P (2–3-fold. $P<0.05$) and hCG (2-fold, $P<0.05$) while increasing E_2 secretion (150–180%, $P<0.05$) into the media. The effect of OT on P secretion was receptor-dependent since it was abolished by addition of CAP-450 into the media. AVP increased E_2 secretion up to 2-fold. <0.05) while not having a consistent effect on P secretion. In conclusion, OT and to a lesser degree AVP, have significant modulatory effects on placental hormone secretion. Whether the resulting locally increased E_2/P secretion ratio favors progression of labor remains to be shown.

Keywords: Oxytocin; arginine-vasopressin; placenta; steroids; human chorionic gonadotropin

Introduction

The circulating neurohypophyseal peptides, oxytocin (OT) and arginine-vasopressin (AVP), have important roles during pregnancy and labor. These nonapeptides are detected in fetal circulation from the tenth week of pregnancy and rise with advancing gestation until term. In the maternal circulation OT pattern of secretion is episodic (Fuchs *et al.*, 1991). Further increases may be present in the umbilical cord during labor (Chard *et al.*, 1971; Liggins, 1983) or levels remain unchanged (Pochard & Lutz-Bucher, 1986; Oosterban & Swaab, 1989). OT acts on specific receptors present in myometrium and decidua (Fuchs *et al.*, 1982). Increases in umbilical cord and amniotic fluid AVP levels were also reported during labor and fetal distress (Oosterban & Swaab, 1989).

Sensitivity of OT to the myometrium increases 8-fold between 20–39 weeks of gestation. AVP also induces contractions by acting on specific OT and AVP receptors present in myometrium, but they do not increase with gestational age, nor do they change during labor (Ivanisevic *et al.*, 1989). OT receptors are sensitive to changes in levels of sex-steroids: E_2 acts as a promoter, while P has an opposite effect (Makino *et al.*, 1983). It was previously shown that in the rabbit circulating levels of estrogens increase while that of P decrease close to labor (Fuchs & Fuchs, 1984). Similar changes were noted in the sex steroid levels present in amniotic fluid of women (Romero *et al.*, 1988). Whether OT and AVP are involved in causing this effect is not known. We have recently reported that both OT and AVP had a direct effect on the first trimester placenta *in vitro* (Tal *et al.*, 1991). In the present study the effect of physiologic concentrations of OT and AVP was examined on placental explants hormone secretion at term. Here we report that both nonapeptides exert a significant modulatory effect on placental hormones secretion.

Results

Effect of OT on hormone secretion

Figure 1 shows a significant (2–3-fold) dose dependent inhibitory effect of OT 5–25 μU/ml on placental explants P secretion after 24 h in culture in the physiologic range of concentrations, $P<0.05$. Significant inhibition was noted already at 5 μU/ml, while at 1 μU no effect was noted. No differences in the response to the compound were noted whether the placentas were from patients in labor (SVD) or following elective caesarean section (CS) not in labor. Figure 2 shows that addition of the OT receptor antagonist- CAP 450,

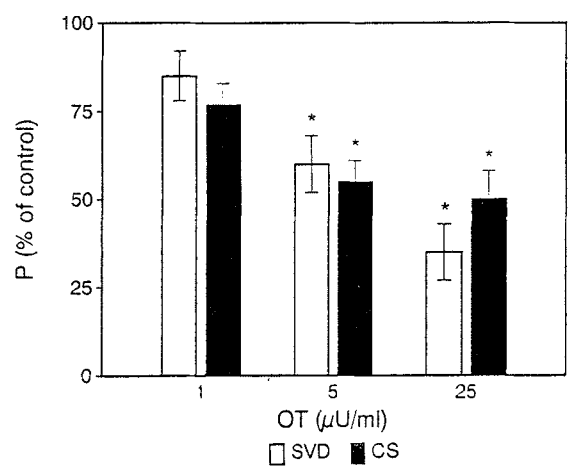

Figure 1 Dose dependent inhibitory effect of OT on P secretion by placental explants derived from SVD or CS deliveries following 24 h of incubation. Data is expressed as mean ± SEM per cent change from controls. The inhibition was similar with SVD and CS cases. *$P<0.05$ *vs* controls ($n=7$).

Correspondence: E.R. Barnea
[4]Current address: Division of Research, Nahariah Hospital, Nahariah, Israel
Received 14 April 1994; accepted 10 November 1994

10 μg/ml abolished the 25 μU/ml OT induced inhibitory effect while antagonist itself had no effect on P secretion. Figure 3 shows the dose dependent stimulatory effect of OT on placental explants E_2 secretion into the media. Maximal effects were noted with 25 μU/ml. The effect at 4 h was only mild, not being significant (data not shown). The effect was similar with placentas from SVD and CS patients. Table 1 shows that hCG secretion was significantly inhibited by addition of 25 uU OT/ml, $P<0.05$.

Effect of AVP on hormone secretion

AVP exerted a biphasic stimulatory effect on E_2 secretion by placental explants after 24 h of culture. This was maximal at 100 μU/ml an 183 ± 15% increase compared to controls, while at 1000 μU/ml (a level rarely seen *in vivo*) the stimulatory effect was somewhat lower: 165 ± 14%, $P<0.05$ (Table 2). The effect of AVP on P secretion was not consistent where in four of seven placentas an inhibitory effect was noted (data not shown). With respect to both steroids no differences in the effect were noted in placentas from SVD and CS patients.

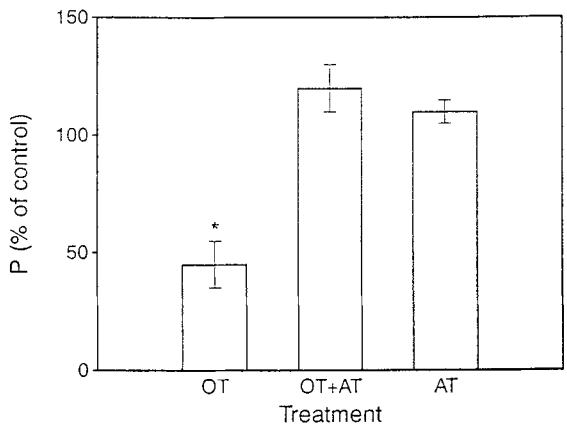

Figure 2 Abolition of 25 μU/ml OT induced effect on P secretion by placental explants, following co-incubation for 24 h with 10 μg/ml CAP 450 (AT), an OT receptor antagonist. The antagonist itself did not affect the hormone secretion. Data is expressed as mean ± SEM. *$P<0.05$ *vs* AT and OT + AT ($n=3$).

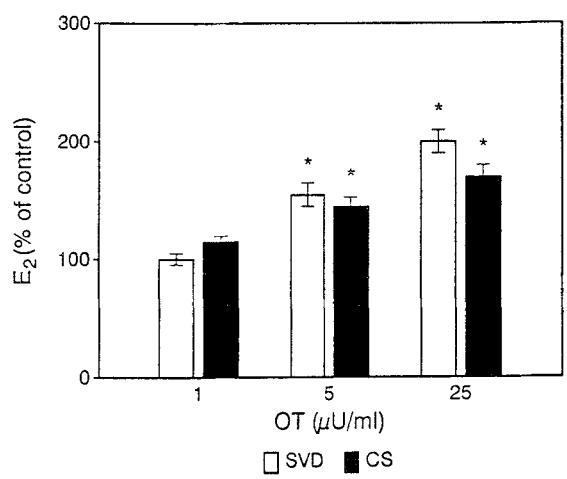

Figure 3 Dose dependent stimulatory effect of OT on E_2 secretion by placental explants following exposure for 24 h. The effect was similar with SVD and CS explants. Data is expressed as mean ± SEM. *$P<0.05$ *vs* controls ($n=6$).

Discussion

The precise events that take place during human labor are not known now though OT specifically has been shown to have an important, if not initiating at least promoting role. Therefore in the present report we have examined whether neurohypophysary hormones of fetal or maternal origin could be involved in regulating placental hormone secretion through a direct action. In the present study significant effects of physiologic concentrations of neurohypophysary peptides, OT and AVP on placental explants hormone secretion was shown at term. Addition of OT to explants affected steroid secretion as evidenced by increased E_2 and decreased P secretion into the media.

Placental sensitivity to OT action was similar irrespective whether explants were from laboring (SVD) or nonlaboring (CS) patients suggesting that changes in the metabolic and endocrine milieu present in labor does not modify the response to the nonapeptide. Any OT that may have been present in the tissue due to labor which was not tightly bound was likely to be removed by extensive washing of the explants prior to treatment.

The specificity of OT action was shown since in the presence of an OT antagonist the OT induced effect on P secretion was abolished. This is in accord with our previous data on hCG secretion in the first trimester where OT antagonist blocked OT induced hCG stimulation when given in short pulses in superfusion (Tal *et al.*, 1991). The specificity of this OT antagonist was reported previously where it was shown to block OT induced action on the myometrium *in vivo* (Akerlund *et al.*, 1987). Further present results show that OT has a gestational age dependent effect on hCG secretion stimulatory in the first trimester and inhibitory at term. We and others have reported such varying effects at different gestational ages (Barnea *et al.*, 1993; Ren & Braunstein, 1994). Previously hCG was shown to have several paracrine roles on steroid metabolism and secretion and recently specific receptors were described locally (reviewed in Licht *et al.*, 1994).

Table 1 Inhibitory effect of OT on hCG secretion by placental explants at term

Compound vehicle	*100%*
OT 10 uU	78 ± 20
OT 25 uU	47 ± 11*

Data is expressed as mean ± SEM % change compared to controls. *$P<0.05$ *vs* vehicle alone.

Table 2 Stimulatory effect of various AVP concentrations on E_2 secretion by placental explants at term

Compund vehicle	*E_2 100%*
AVP 10 μU/ml	110 ± 12
AVP 100 μU/ml	185 ± 18*
AVP 1000 μU/ml	165 ± 14*

Data is expressed as per cent change mean ± SEM. *$P<0.05$ compared to controls and 100 μU/ml AVP.

Others have also reported that OT has a direct effect on the placenta *in vitro*. In perfusion experiments at term OT stimulated the secretion of proopiomelanocortin peptides and increased prostaglandin secretion in the perfused placenta (Margioris *et al.*, 1988). The data herein presented and that previously reported strongly suggest that this action of OT is exerted through a specific binding site, which characteristics remain to be further defined.

Earlier investigators have shown that the placenta contains OT like compounds (Mitzutani *et al.*, 1982). This was detected in spite of oxytocinase, an oxytocin metabolizing enzyme, in both the placenta and the maternal circulation (Mizutani & Tomoda, 1992). These observations suggest that OT may have in addition to its well recognized endocrine effect on the myometrium and decidua also a local effect on the placenta. We hypothesize that the resulting locally increased E_2/P ratio may help in the process of labor creating an environment which supports decidual phospholipase activation, leading to prostaglandin release (Casey *et al.*, 1983; Fuchs & Fuchs, 1984) and promote myometrial contractility. Evidence for a local increased E_2/P ratio in the fetal compartment, i.e., amniotic fluid in humans was recently demonstrated (Romero *et al.*, 1988).

In its action on the placenta AVP appeared to complement that of OT with respect to E_2 while having no consistent effects on P secretion. In case of AVP the presence of lysipressin, a vasopressin-like substance previously documented in the mammalian placenta (Rouille *et al.*, 1988) is suggestive of a local as well as endocrine effect, as was reported by us in the superfusion model in the first trimester placenta (Tal *et al.*, 1991). As for the endocrine effect of AVP the previously shown increase in circulating AVP with fetal acidosis and rupture of membranes is illustrative and suggest that under these circumstances it may aid in labor promotion (Pochard & Lutz-Bucher, 1986). Furthermore AVP was also shown to bind to specific myometrial receptors aiding in promotion of labor (Maggi *et al.*, 1990).

The results herein add to the body of knowledge generated by this model on the effect of several ligands i.e. growth hormone, prolactin, catechols, ACTH and others as reported by us and others (reviewed in Barnea *et al.*, 1986, 1989b). This points to the maintained responsivity of the placenta to endocrine stimuli, confirming the value of the explant model for short term experiments.

In conclusion, neurohyposary peptides modulate placental hormone secretion, whether the resulting local increase in E_2/P ratio and the decrease in hCG are involved in labor remains to be demonstrated.

Materials and methods

Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Beit Haemek (Israel). OT and AVP were purchased from Sigma (St. Louis, MO). OT receptor antagonist CAP-450 was received as a gift from Dr Per Melin, Ferring (Sweden). The kits for measuring E_2 and P were purchased from DPC (Los Angeles, CA). The kits for measurements of hCG MAIA clone were purchased from Serono (Israel).

Placental material

A total of 11 placentae were studied. Five were obtained from term pregnancies following elective caesarian section not in labor (CS), while the remainder were obtained following spontaneous vaginal delivery at term (SVD). Placental tissue fragments were randomly selected, removed, and rinsed extensively in large volumes of cold 0.9% NaCl until clear of blood.

Explant cultures

The method of explant cultures was previously reported (Barnea *et al.*, 1989a). Briefly, the placental tissue was washed in DMEM plus 2% antibiotics (penicillin 10 000 U/ml, streptomycin 10 µl/ml, and amphotericin-B 25 µg/ml). Approximately 100 mg wet weight explants were prepared and incubated in 2 ml of DMEM plus 1% antibiotic solution and 0.5% bovine serum albumin with the test compounds or vehicle alone for 24 h in an atmosphere of 95% air and 5% CO_2. Each treatment group or vehicle-treated explants were run in 6–10 replicates per placenta. At the end of the incubation period, explants were removed and stored at −20°C until assay.

Explant viability was established by the linear increase in sex steroids and hCG secretion for the first 48 h of culture, progressive glucose consumption, and by vital staining with hematoxylin-eosin (Barnea & Fakih, 1985). Progressive increase in the activity of glucose 6-phosphate dehydrogenase (EC 1.1.49) using a commercial kit from Sigma Chemical Co St Louis, MO, USA). Unaltered pH in the media during incubations. In preliminary experiments the effect of fetal calf serum (FCS) 10% (Beit Haemek, Israel) and BSA 0.5% were compared. Under both culture conditions the levels of steroids increased linearly. However, that containing the 10% FCS led to a significantly higher P secretion compared to those with BSA. Since both types of medias yielded results with similar trends and FCS is an undefined, in further experiments 0.5% BSA was used (Barnea *et al.*, 1989b). Under these control conditions basal levels for P were 20–60 ng/mg protein, E_2 15–70 pg/mg protein and hCG 50–200 mIU/mg protein.

Assays

Steroid measurement in the media was carried out as previously reported (Barnea *et al.*, 1989b). Intra-assay variability for P and E_2 was 11% and 10%, respectively. Measurement of hCG in the media was described (Barnea *et al.*, 1993), intraassay variability was 1.7%. Protein content of explants was measured by method of Lowry *et al.* (1951). Statistical analysis was performed using one way ANOVA and Student's *t* test, with $P < 0.05$ considered to be statistically significant. Data is expressed as mean ± SEM per cent change from controls, and are representative of three or more placentae with similar results.

References

Akerlund, M., Stromberg, P., Hauksson, A., Andersen, L.F., Lyndrup, J., Trojnar, J. & Melin, P. (1987). *Br. J. Obstet. Gynaecol.*, **94,** 1040–1044.

Barnea, E.R. & Fakih, H. (1985). *Steroids*, **45,** 427–432.

Barnea, E.R., Fares, F. & Kol, S. (1989a). *Placenta*, **10,** 37–43.

Barnea, E.R., Perlman, R., Fakih, H., Bick, T., Kol, S. & Hochberg, Z. (1989b). *Acta Endocrinol.*, **121,** 767–772.

Barnea, E.R., Lavy, G., Fakih, H. & Decherney, A.H. (1986). *Placenta*, **7,** 307–313.

Barnea, E.R., Neubrun, D. & Shurz-Swirsky, R. (1993). *Hum. Reprod.*, **8,** 858–862.

Casey, M.L., Winkel, C.A., Porter, J.C. & MacDonald, P.C. (1983). *Clin. Perinatol.*, **10,** 709–713.

Chard, T., Hudson, C.N., Edwards, C.R.W. & Boyd, N.R.H. (1971). *Nature*, **234,** 352–254.

Fuchs, A.R. & Fuchs, F. (1984). *Br. J. Obstet. Gynaecol.*, **91,** 948–953.

Fuchs, A.R., Fuchs, F., Husslein, P., Soloff, M.S. & Formstrom, M.J. (1982). *Science*, **215,** 1396–1398.

Fuchs, A.R., Romero, R., Keefe, D., Para, M., Oyarzun, E. & Behnke, E. (1991). *Am. J. Obstet. Gynecol.*, **165,** 1515–1523.

Ivanisevic, M., Behrens, O., Helmer, H., Demarest, K. & Fuchs, A.R. (1989). *Am. J. Obstet. Gynecol.*, **161,** 1637–1641.

Licht, P., Lei, Z.M., Rao, Ch.V. & Merz, W.E. (1994). *Implantation and Early Pregnancy in Humans.* Barnea, E.R., Check, J.H., Grudzinskas, J.G. & Maruo, T. (eds). Parthenon Press: Carnforth. pp. 235–247.

Liggins, C.G. (1983). *Clin. Obstet. Gynecol.*, **26,** 47–53.

Lowry, O.H., Rosebrough, N.H., Farr, A.L. & Randall, R.J. (1951). *J. Biol. Chem.*, **193,** 265–270.

Maggi, M., Del Carlo, P., Fantoni, G., Giannini, S., Torrisi, C., Casparis, D., Massi, G. & Serio, M. (1990). *J. Clin. Endocrinol. Metab.*, **70,** 1142–1154.

Makino, T., Nakazawa, K., Ishii, K., Haginiwa, I., Nakayama, A. & Iizuka, R. (1983). *Endocrinol. Jpn.*, **30,** 389–392.

Margioris, A.N., Grino, M., Protos, P., Gold, P.W. & Chrousos, G.P. (1988). *J. Clin. Endocrinol. Metab.*, **66,** 922–927.

Mizutani, S., Hayakawa, H., Akiyama, H., Sakura, H., Yoshino, M., Oya, M. & Kawashima, Y. (1982). *Clin. Biochem.*, **15,** 141–146.

Mizutani, S. & Tomoda, Y. (1992). *Seminar Reprod. Endocrinol.*, **10,** 146–154.

Oosterbaan, H.P. & Swaab, D.F. (1989). *Early Human Develop.*, **19,** 253–258.

Pochard, J.L. & Lutz-Bucher, B. (1986). *Acta Paediatr. Scand.*, **75,** 774–778.

Ren, S.G. & Braunstein, G.D. (1994). *Implantation and Early Pregnancy in Humans.* Barnea, E.R., Check, J.H., Grudzinskas, J.G. & Maruo, T. (eds). Parthenon Press: Carnforth. pp. 247–270.

Romero, R., Severia, B., Mazor, M., Wu, Y. & Benveniste, R. (1988). *Am. J. Obstet. Gynecol.*, **159,** 659–660.

Rouille, Y., Chauvet, M.T., Chauvet, J., Acher, R. & Hadley, M.P. (1988). *J. Comp. Endocrinol.*, **71,** 475–483.

Tal, J., Kaplan, M., Sharf, M. & Barnea, E.R. (1991). *Human Reprod.*, **6,** 766–769.