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LOCAL STIMULATION OF PROSTAGLANDIN PRODUCTION BY CORTICOTROPIN-RELEASING HORMONE IN HUMAN FETAL MEMBRANES AND PLACENTA

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Corticotropin-releasing hormone is produced by the human placenta and fetal membranes, but its physiological significance is not established. We examined the possibility that CRH might affect prostaglandin output by these intra-uterine tissues. Primary cultures of amnion, chorion, decidua and placenta were established from tissue obtained from women at term elective cesarean section were maintained in the presence of increasing concentrations of synthetic hCRH. PG output at 48h was measured by radioimmunoassay. hCRH stimulated PGE2 output by amnion, chorion and placenta, but not by decidual tissue. $PGF_{2\alpha}$ output was stimulated in amnion, decidua and placenta but not chorion, whereas output of 13, 14-dihydro-15-keto $PGF_{2\alpha}$ was stimulated in all four tissues. We conclude that hCRH stimulates prostaglandin output by human placenta, decidua and the fetal membranes, raising the possibility of paracrine or autocrine interactions between CRH and prostaglandins in vivo.

Human corticotropin-releasing hormone of hypothalamic (1) and placental origin (2) ia a 41- amino acid peptide. hCRH mRNA has been detected in the placenta from 7 to 40 weeks of gestation (3). These observations are consistent with a progressive rise during gestation in the levels of hCRH peptide in maternal plasma (4-7) which correlate with h-CRH concentrations in placental tissue (7). Within the hypothalamic pituitary axis CRH plays a major role in stimulating adrenocorticotrophic hormone release (8) from the anterior pituitary. However, within the placenta the role of hCRH still remains to be defined. The human placenta contains biologically active ACTH (9), and CRH stimulates secretion of a peptide containing the ACTH sequence in a dose dependent manner from placental tissue in vitro (10, 11). However there is no information concerning the possibility that CRH may

ABBREVIATIONS: CRH, corticotropin-releasing hormone; PG, prostaglandin; hCRH, human CRH; ACTH, adrenocorticotropin hormone ; PGFM, 13,14-dihydro-15-keto $PGF_{2\alpha}$.

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stimulate other factors such as the prostaglandins known to be produced by the intrauterine tissues. An increase in prostaglandin production by the fetal membranes and placenta accompanies the onset of spontaneous labor in the women (12). In the absence of any consistent changes in peripheral plasma concentrations of either steroids or regulatory peptides, it is possible that locally produced steroids or peptides may modulate prostaglandin production and metabolism within the intrauterine tissues.

In the present study we examined whether the production of $PGF_{2\alpha}$ and of PGE2 by placental tissue, decidua and by the fetal membranes might be modulated by hCRH. We also determined the effects of hCRH on the output of 13, 14-dihydro-15 keto-$PGF_{2\alpha}$ the major metabolite of $PGF_{2\alpha}$ produced by intrauterine tissues (13). We report that during in vitro short term culture the production of $PGF_{2\alpha}$, PGE2 and PGFM by the fetal membranes and placenta are increased in a dose dependent fashion in response to exogenous hCRH. These findings raise the possibility that intrauterine paracrine regulation of PG production by CRH occurs within the human fetal membranes and placenta.

MATERIALS & METHODS

Collection and preparation of tissue

Amnion, chorion-decidua and placental tissue were collected at term elective cesarean section from women who had received no medication other than epidural anaesthesia. The length of gestation was 37-42 weeks. Within 15 min of delivery the amnion was peeled away from the chorion-decidua. The decidua was carefully dissected from the chorion and approximately 4g of each tissue was minced and rinsed twice in Hanks Buffered Saline Solution, (HBSS, Flow Laboratories, Inc., Virginia, USA), supplemented with 10% gentamycin, (Gibco Laboratories Inc., New York, USA). A single cell suspension of the tissues was prepared as previously described (14). The mean cell viability for all tissues was 87.4±9.8% and was not significantly different between tissues, as assessed by the trypan blue exclusion test. Cells were plated at 2-3x10^5 cells/well in 17mm 24-flat bottom well multidishes (Limbro, Flow Laboratories), in 1ml of culture medium per well, in the presence of 10% charcoal stripped fetal calf serum (14,15,16). The culture wells in which the cells were plated had been coated previously with Vitrogen (Collagen 100, Collagen Corporation, Real Laperriere Inc., Montreal, Canada). The cultures were maintained at 37C in a water saturated atmosphere containing 5% CO_2.

Modulation of Prostaglandin Output

Amnion, chorion, decidual and placenta cultures (n=4 tissues) were maintained for 48 hr in the presence of h-CRH (Bachem Inc., California, USA; 0-1000ng/ml; n=12 wells/treatment). Media was then collected and stored at -70C. Cell number was determined using a Coulter Counter (Coulter Electronics, U.S.A.)

Prostaglandin Radioimmunoassay

Concentrations of PGE2 and $PGF_{2\alpha}$ were determined by specific radioimmunoassay (17) in media collected from cultures of all tissue

types. For the PGE2 and $PGF_{2\alpha}$ assays the inter- and intra-assay coefficients of variation were less than 11% and 9% respectively, and the minimal detectable dose was greater than 8pg/ml.
Concentrations of PGFM were determined following diethyl ether extraction of the media from all tissue types (17). The combined inter and intra assay coefficients of variation for the PGFM assays were less tan 12%, and the minimal detectable dose was greater than 14pg/ml.

Data Analysis
Results are expressed as mean±SEM for averages of four separate experiments. The effects of hCRH on PG output were assessed by analysis of variance.

RESULTS

Modulation of placental and fetal membrane PGE2 output:

Following a 48 hr incubation in the presence of hCRH there was a significant increase in the concentration of PGE2 in media collected from amnion ($P<0.001$: 100ng/ml and 1000ng/ml), chorion ($P<0.001$: 10 and 100ng/ml) and placental ($P<0.001$: 10- 1000ng/ml) cell cultures compared to media taken from cells maintained in the absence of hCRH (Figure 1). There was no significant effect of hCRH on PGE2 production by decidual cells in culture (Figure 1).

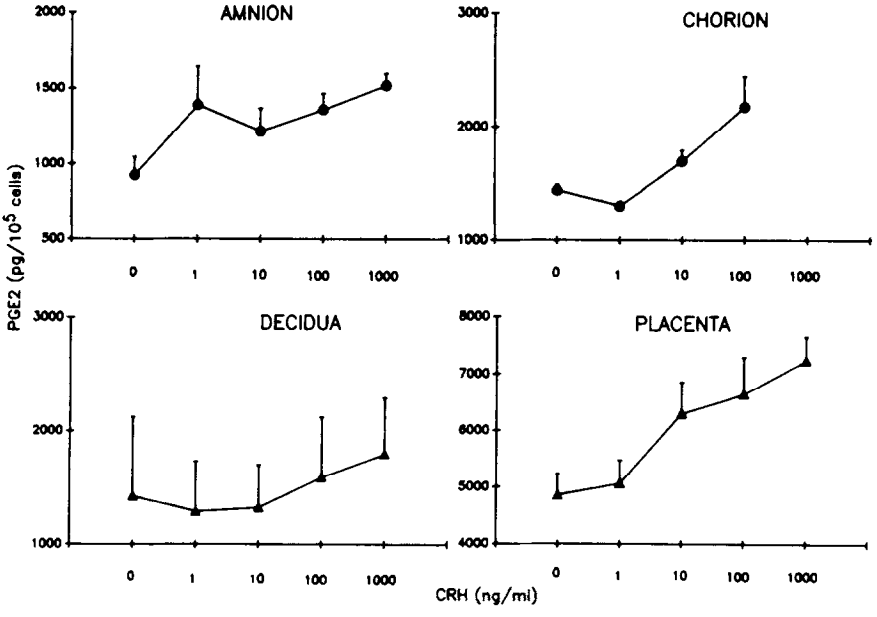

Figure 1: Concentration of PGE2 in media collected from amnion, chorion, decidual and placental cultures maintained in the presence of increasing concentrations of hCRH for 48 hours. Values are mean ± SEM for tissues from 4 patients.

Modulation of placental and fetal membrane $PGF_{2\alpha}$ output:

There was a significant increase in the concentration of $PGF_{2\alpha}$ in media collected from amnion and decidual cells following a 48 hr incubation in the presence of hCRH in concentrations ranging from 1-1000ng/ml ($P<0.001$; Figure 2). $PGF_{2\alpha}$ output from placental cells was significantly greater after addition of hCRH (1000ng/ml; $P<0.001$) than from cells maintained in the absence of hCRH (Figure 2). Concentrations of $PGF_{2\alpha}$ were undetectable in media collected from chorion cells following the 48hr culture period.

Modulation of placental and fetal membrane PGFM output:

Following a 48 hr incubation in the presence of hCRH at concentrations ranging from 10-1000ng/ml, there was a significant ($P<0.001$) increase in the output of PGFM from both amnion and placental cultures compared to untreated cells (Figure 3). In the presence of 100 and 1000ng/ml hCRH there was a significant ($P<0.001$) increase in the concentration of PGFM in media collected from decidual cells (Figure 3). The output of PGFM from chorion cells was significantly greater when cells were maintained for 48 hr in the presence of 1000ng/ml of hCRH compared to cells maintained in the absence of h-CRH ($P<0.001$; Figure 3).

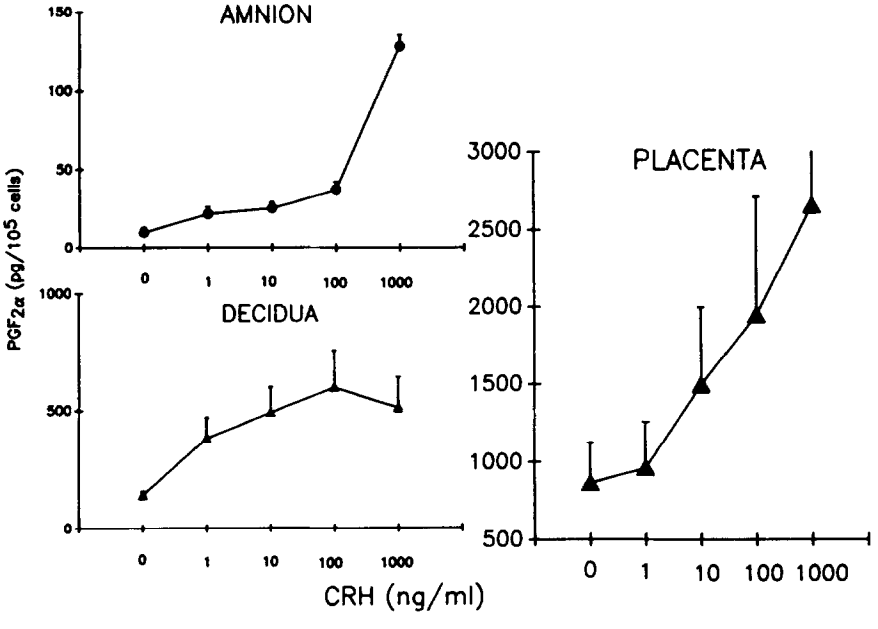

Figure 2: Concentration of $PGF_{2\alpha}$ in media collected from amnion, decidual and placental cultures maintained in the presence of 0, 1, 10, 100 and 1000ng/ml of hCRH. Concentrations of $PGF_{2\alpha}$ in media collected from chorion cells were below the detectability of the assay (<10pg/ml). Values are mean ±SEM for tissues from 4 patients.

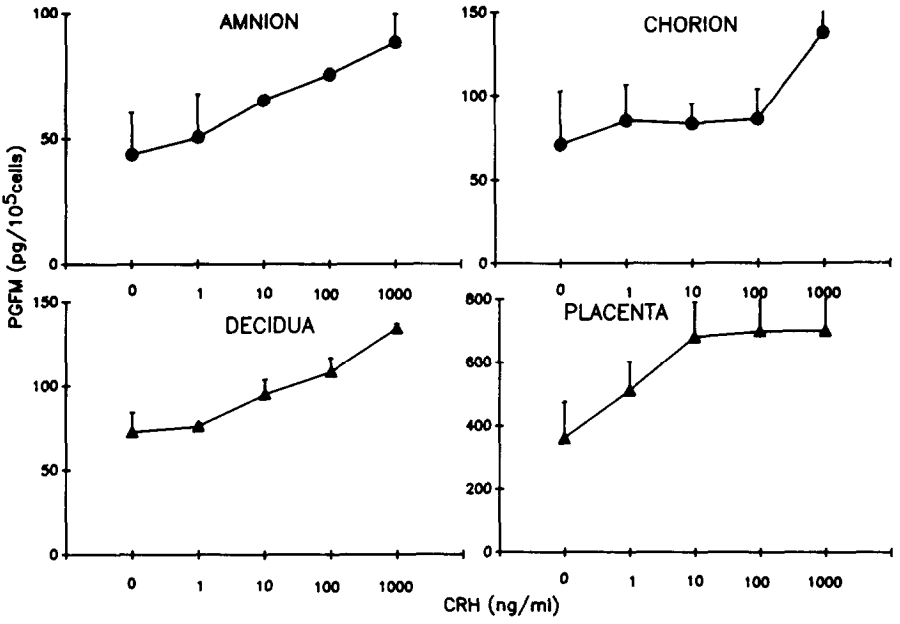

Figure 3: Concentration of PGFM in media collected from amnion, chorion, decidual and placental cultures maintained in the presence of increasing concentrations of hCRH for 48 hours. Values are mean ±SEM for tissues from 4 patients.

DISCUSSION

We have found that synthetic CRH stimulates increased output of PGE2, $PGF_{2\alpha}$ and PGFM by primary cultures of human fetal membranes, decidua and placenta in vitro. Significant effects were found within the range of CRH concentrations reported for human placenta (7,18), raising the possibility that these interactions may have physiological significance. Recently it has been reported that the human placenta, decidua and fetal membranes produce CRH during in vitro tissue culture (3,14,19). In placental tissue, CRH is produced by purified cytotrophoblast cell preparations (19). As yet the cell types responsible for prostaglandin and CRH production in the membranes and decidua remain to be determined, and it is not known whether these are the same or different. However, collectively these results suggest that CRH may influence PG production in vivo through either paracrine or autocrine mechanisms.

Prostaglandins generated within the fetal membranes and/or decidua and placenta are believed to play a pivotal role in the endocrine events leading to parturition. It has been well established that several factors including steroids and growth factors may stimulate PG output from decidua and fetal membranes (20). As no marked changes occur in the

peripheral concentrations of estrogen or progesterone in the plasma of women before labor, it has been suggested that local changes may occur in the production and/or action of these steroids on PG output within the tissues of the pregnant uterus (20). Similarly, cortisol is produced locally from cortisone (21) and cortisol sulphate (19,22) within the fetal membranes, although systemic concentrations in maternal and fetal plasma rise during the latter part of human pregnancy (23). Recently it has been shown that glucocorticoids stimulate PGE2 output by human amnion maintained in monolayer culture (24,25). In addition, it has been demonstrated that glucocorticoids stimulate CRH output from placental tissue and fetal membranes, and increases the levels of CRH mRNA in purified cytotrophoblasts maintained in vitro (19). The present results raise the further possibility that the stimulatory effect of glucocorticoids on PG output might be mediated, at least in part, through elevated CRH production.

The placenta also contains biologically active ACTH. In vitro, CRH-stimulated secretion of peptides containing the ACTH sequence in a dose-dependent manner (10,11). Both PGE2 and $PGF_{2\alpha}$ stimulate immunoreactive ACTH output by the placenta (10), but it remains to be established whether PGs mediate CRH effects on placental ACTH. Centrally, PGs stimulate ACTH release from the pituitary gland (26). It is likely that this effect is mediated by hypothalamic CRH (26). If a similar interaction occurs in the placenta and fetal membranes, it would set up the possibility of a positive feedback loop between CRH and PGs, which may contribute to the progressive rise in both plasma CRH (4-7) and ACTH (22) during human pregnancy. In turn, output of CRH and PGs may be driven, in part, by rising glucocorticoids (22).

In the present study, the output of PGE2, $PGF_{2\alpha}$ and PGFM was higher per cell, in the placenta than in the amnion, decidua or chorion. This could reflect an increased proportion of cells with PG synthesizing activity, rather than differences in enzyme activity or substrate availability per cell. PGs have been suggested as local mediators of intra-placental blood flow (27) and alterations in the patterns of eicosanoid production or metabolism occur at term in association with changes in utero-placental perfusion, for example in pre-eclampsia (27). Peripheral concentrations of CRH in maternal blood are elevated significantly over normal in patients with pregnancy -induced hypertension (6). The cause or effect component of this latter relationship is not yet apparent. However, our results suggest the possibility that CRH stimulation of local PG output in the placenta may

contribute to the pathophysiology of hypertensive disorders of pregnancy.

It is becoming apparent that a series of positive feedback loops involving glucocorticoids, CRH, ACTH and prostaglandins exist in the placenta, and perhaps in the fetal membranes. These control mechanisms are the opposite of feedback relationships at the level of the hypothalamus and pituitary.

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REFERENCES

1. Vale, W., Spiess, J., Rivier, C., Rivier, J. (1981) Science 213, 1394-1397.
2. Shibasaki, T., Odagin, E., Shisuma, K., Ling, N. (1982) J. Clin. Endocrinol. Metab. 55, 384-386.
3. Frim, D.M., Emanuel, R.L., Robinson, B.G., Smas, C.M., Adler, G.K., Majzoub, J.A. (1988) J. Clin. Invest. 82, 293-299.
4. Goland, R.S., Wardlaw, S.L., Stark, R.I., Brown, L.S., Frantz, A.G. (1986) J. Clin. Endocrinol. Metab. 63, 1199-1203.
5. Sasaki, A., Liotta, A.S., Luckey, M.M., Margioris, A.N., Suda, T., Krieger, D.T. (1984) J. Clin. Endocrinol. Metab. 59, 812-814.
6. Campbell, E.A., Linton, E.A., Wolfe, C.D.A., Scraggs, P.R., Jones, M.T., Lowry, P.J. (1987) J. Clin. Endocrinol. Metab. 64, 1054-1059.
7. Sasaki, A., Shinkawa, O., Margioris, A.N., Liotta, A.S., Sato, S., Murakami, O., Go, M., Shimizu, Y., Hanew, K., Yoshinaga, K. (1987) J. Clin. Endocrinol. Metab. 64, 224-229.
8. Plotsky, P.M., Bruhn, T.O., Vale, W. (1985) Endocrinol. 117, 323-329.
9. Liotta, A., Osathanonah, R., Ryan, K.J., Krieger, D.T. (1977) Endocrinol. 101, 1552-1558.
10. Perraglia, F., Sawchenko, P.E., Rivier, J., Vale, W. (1987) Nature (Lond.) 328, 717-719.
11. Margioris, A.N., Grino, M., Protos, P., Gold, P.W., Chrousos, G.P. (1988) J. Clin. Endocrinol. Metab. 66, 922-926.
12. Keirse, M.J.N.C. (1979) In Human Parturition (M.J.N.C. Keirse, A.B.M. Anderson, J.B. Gravenhorst, Eds.), pp.101. Leiden University Press, Leiden.
13. Okazaki, T., Casey, M.L., Okita, J.R., MacDonald, P.C., Johnston, J.M. (1981) Am. J. Obstet. Gynecol. 139, 373-381.
14. Jones, S.A., Brooks, A.N., Challis, J.R.G. (1989) J. Clin Endocrinol. Metab. (in press).
15. Challis J.R.G., Patrick, J.E., Cross, J., Workewych, J., Manchester, E., Power, S.G.A. (1981) Can. J. Physiol. Pharmacol. 59, 261-267.
16. Norman, L.J., Lye, S.J., Wlodek, M.E., Challis, J.R.G. (1985) Can. J. Physiol. Pharmacol. 63, 1398-1403.
17. Skinner, K.A., Challis, J.R.G. (1985) Am. J. Obstet. Gynecol. 151, 519-523.
18. Cunnah, D., Jessop, D.S., Besser, G.M., Rees, L.H. (1987) J. Endocrinol. 113, 123-131.

19. Robinson, B.G., Emanuel, R.L., Frim, D.M., Majzoub, J.A. (1988) Proc. Nat. Acad. Sci. USA 85, 5244-5248.
20. Challis, J.R.G., Olson, D.M. (1988) In The Physiology of Reproduction (E, Knobil, J. Neill, Eds.), pp.2177. Raven Press, New York, NY.
21. Tanswell, A.K., Worthington, D., Smith, B.T. (1977) J. Clin. Endocrinol. Metab. 45, 721-725.
22. Mathis, J., Johnston, J., MacDonald, P.C., Casey, M. (1983) J. Steroid Biochem. 18, 575-579.
23. Mitchell, M.D., Lytton, F.D., Varticovski, L., (1988) Bichem. Biophys. Res. Commun. 151, 137-141.
24. Potestio, F.A., Zaker, T., Olson, D.M. (1988) J. Clin. Endocrinol. Metab. (in press).
25. Hedge, G.A., (1977) Life Sciences 20, 17-33.
26. Walsh, S.W., Pavisi, V.M. (1986) Semin. Perinatol. 10, 334-355.