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Effect of Centchroman (67/20) and 78/224 on nucleic acid and protein biosynthesis during implantation in rats

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Abstract. The biosynthesis of nucleic acids and proteins was studied in rat uterus by following the incorporation of [^{3}H]-thymidine, [^{3}H]-uridineand[^{14}C]-leucinein control and pregnant rats in the presence and absence of two anti-implantation drugs. One of the drugs, 78/224 caused a significant increase in incorporation whereas the other drug, Centchroman, caused an inhibition in incorporation of all the three precursors. The implications of these changes in the light of estrogenicity, agonist and antagonist actions of anti-estrogens have been analysed. The importance of homeostatic mechanisms involved in nucleic acids and proteins for the maintenance of constant internal milieu for blastocyst attachment has been discussed.

Keywords. Nucleic acid and protein synthesis; implantation; Centchroman.

Introduction

Implantation of rat blastocyst with the uterine endometrium is an inextricable phenomenon brought about by an interaction of the two steroidal hormones estrogen and progesterone with the target tissues. The mechanism by which the requisite changes are brought about is not known. When RNA extracted from the uteri of estrogen (E) stimulated rats was administered to E deprived ovariectomised rats, the resultant morphological changes in treated uteri were similar to those that occur following the administration of E (Segal *et al.,* 1965; Nishikawa, 1978; Lejeune *et al.,* 1982). Therefore, in the process of implantation, nidatory E may first promote RNA synthesis in the endometrium and then exhibit its action through the medium of RNA thus synthesised. Thus, a precise amount of nucleic acid and protein synthesis is an essential need of implantation. Any deviation from it leads to anti-implantation and anti-fertility activities.

Interestingly, both anti-estrogenic and estrogenic agents alter the preparatory changes necessary for implantation. Two anti-implantation agents synthesised in Central Drug Research Institute, Lucknow *viz.,* 78/224 (l,2-diethyl-1-3-bis-(*p*-methoxyphenyl)-1-propene) (Prakash *et al.,* 1980) a highly estrogenic compound and Centchroman-*Trans* (2-2-dimethyl-3-phenyl-4 (*p*- (*ß*-pyrrolidinoethoxy)phenyl) 7-methoxy chroman hydrochloride) (Kamboj *et al.,* 1977) an anti-estrogen, were found to arrest pregnancy but their mode of action was not very clear. An alteration in the activity of lysosomal enzymes acid-DNase, acid-RNase and acid-proteinase activity,

Abbreviations used: E, Estrogen; PCA, perchloric acid.

due to the administration of these 2 drugs was recorded (unpublished data) but significant changes in the levels of DNA, RNA and proteins were not seen. Therefore, it was of interest to study the synthesis of nucleic acid and proteins in the rat uterus during days 2, 5 and 6 of pregnancy under conditions of drug treatment.

Materials and methods

The radioactive precursors, thymidine (methyl-T), (45 mCi/m mol), [^{3}H]-uridine (12·7 mCi/m mol) and [^{14}C]-leucine (41·85 mCi/m mol), were obtained from Bhabha Atomic Research Centre, Bombay.

Virgin female albino rats (Wistar strain) bred in the colony of our Institute (weight 125 ± 5 gm) were kept in uniform animal husbandry conditions in air-conditioned quarters. Females were caged overnight with proven males in 3:1 ratio and next morning their vaginal smears were examined. The detection of sperm was used to compute the first day of pregnancy. Implantation and inter-implantation sites were differentiated on the 6th day of pregnancy by an intravenous injection of 1 ml of 1 % solution of pontamine Blue by a 27 gauge needle 1 h before sacrificing. Animals were administered orally drug no. 78/224 at the dose of 0·25 mg/rat for 5 days and the other drug Centchroman at the dose of 0·05 mg/rat. Rats were sacrificed by decapitation and uteri were immediately taken out, blotted, weighed, chopped into pieces and then processed. In each group 6 rats were undertaken.

[^3H] -*Thymidine incorporation*

The incorporation of the precursor into DNA was studied by the method of Ledford *et al.* (1978) with slight modifications and DNA extraction carried out by the method of Burton (1956). [^{3}H]-Thymidine (10 μCi) was incubated with pieces of each uterus in Kreb's Ringer Bicarbonate buffer at 37°C in a metabolic shaker for 1 h. Incubation was stopped by chilling. Each tissue sample was then washed thrice with cold buffer. Tissues were homogenized in an all glass homogenizer in Tris buffer; pH 7·4, at 0°C.

Homogenates were centrifuged at 800 *g* for 10 min in cold and the supernatant was discarded. The pellets were extracted with 1 ml of 0·6 N cold perchloric acid (PCA) and then washed consecutively with 2·0ml of absolute alcohol, 2:1 mixture of chloroform; methanol and then with cold solvent ether. The pellets were then dissolved in 3 ml of 0·2 N NaOH and incubated at 37°C for 16 h and then once again 1·0ml of cold 0·6 N PCA was added. Pellets were treated twice with 0·2 N PCA at 70°C for 10 min and then centrifuged. The supernatant was taken to represent the DNA digest. Aliquots were taken for radioactivity and A_{260} measurements. Calf thymus DNA (Sigma) was used as Standard.

[^3H] - *Uridine incorporation into RNA*

This was carried out as described by Singh and Roy (1979).

[^{14}C] - *Leucine incorporation into protein*

This was done following the procedure described by Roy *et al.* (1978).

Protein content was measured by Lowry's method (1951).

Results

The results obtained on the synthesis of DNA, RNA and protein are given in tables 1,2, and 3. The tables clearly show that the DNA synthesis on the 5th day of pregnancy *i.e.*, on the day of implantation was 5·3 fold more than the second day of pregnancy ($P < 0·01$). The rate of DNA synthesis in the implantation sites was 3·3 fold more than in the inter-implantation sites ($P < 0·01$). The synthesis of DNA on 5th day of

Table 1. Effect of Centchroman and 78/224 on DNA synthesis in rat uterus during implantation.

S. No.	Group	[^{3}H]-Thymidine incorporation (cpm/μg DNA)
1.	Intact control	2·01 ± 0·02
2.	2nd day of pregnancy	23·72 ± 1·60
3.	5th day of pregnancy	126·51 ± 12·34
4.	6th day of pregnancy (implantation site)	146·20 ± 2·0753
5.	6th day of pregnancy (inter-implantation site)	43·80 ± 0·9173
6.	5th day of pregnancy (78/224 treated)	158·09 ± 5·71
7.	5th day of pregnancy (Centchroman treated)	52·25 ± 9·13

Values are mean of 6 animals ± S.E.
Data were analysed through Student's *'t'* test and *'f'* test.
1 *vs* 2, $P < 0·01$; 1 *vs* 3, $P < 0·01$; 2 *vs* 3, $P < 0·01$; 4 *vs* 5, $P < 0·01$; 3 *vs* 6, $P < 0·05$; 3 *vs* 7, $P < 0·01$.

Table 2. Effect of Centchroman and 78/224 on RNA synthesis in rat uterus during implantation.

S. No.	Group	[^{3}H]-Uridine incorporation (cpm/μg RNA)
1.	Intact control	2·35 ± 0·01
2.	2nd day of pregnancy	4·15 ± 0·0509
3.	5th day of pregnancy	15·01 ± 1·2281
4.	6th day of pregnancy (implantation site)	25·95 ± 1·746
5.	6th day of pregnancy (inter-implantation site)	8·10 ± 0·7179
6.	5th day of pregnancy (78/224 treated)	43·43 ± 6·8199
7.	5th day of pregnancy (Centchroman treated)	3·28 ± 1·5502

Values are mean of 6 animals ± S.E.
Data were analysed through Student's *'t'* test and *'f'* test.
1 *vs* 2, $P < 0·01$; 1 *vs* 3, $P < 0·01$; 2 *vs* 3, $P < 0·01$; 4 *vs* 5, $P < 0·01$; 3 *vs* 6, . $P < 0·01$; 3 *vs* 7, $P < 0·01$.

Table 3. Effect of Centchroman and 78/224 on protein synthesis in rat uterus during implantaion.

S. No.	Group	[^{14}C]-Leucine incorporation (cpm/mg protein)
S. No.	Group	[^{14}C]-Leucine incorporation (cpm/mg protein)
1.	Intact control	6·66 ± 1·23
2.	2nd day of pregnancy	29·83 ± 1·92
3.	5th day of pregnancy	123·80 ± 13·7827
4.	6th day of pregnancy (implantation site)	179·98 ± 12·2684
5.	6th day of pregnancy (inter-implantation site)	124·22 ± 5·6333
6.	5th day of pregnancy (78/224 treated)	159·28 ± 1·544
7.	5th day of pregnancy (Centochroman-treated)	47·28 ± 2·3088

Values are mean of 6 animals ± S.E.
Data were analysed through Student's *'t'* test and *'f'* test.
1 *vs* 2, $P < 0{\cdot}01$; 1 *vs* 3, $P < 0{\cdot}01$; 2 *vs* 3, $P < 0{\cdot}01$; 4 *vs* 5, $P < 0{\cdot}01$; 3 *vs* 6, $P < 0{\cdot}05$; 3 *vs* 7, $P < 0{\cdot}01$.

pregnancy after treatment with the estrogenic drug (18/224) was 6·7 fold and 1·2 fold more than the values obtained on the 2nd and 5th day of normal pregnancy, respectively *(*$P < 0{\cdot}05$). In the case of Centchroman treated rats the rate of synthesis was slightly more than that obtained on the second day of pregnancy but decreased by more than two fold when compared to the values obtained on the fifth day of normal pregnancy.

RNA synthesis on the 5th day of pregnancy was 3·6 fold more than that obtained on the second day *(*$P < 0{\cdot}01$), while synthesis on the 6th day in the implantation sites was 3·2 fold more than in inter-implantation sites *(*$P < 0{\cdot}01$). The RNA synthesis of estrogenic drug (18/224) treated rats was 2·9 fold and 10·5 fold more than the values obtained on the 5th day and 2nd day of normal pregnancy, respectively *(*$P < 0{\cdot}01$). The RNA synthesis on the 5th day of pregnancy treated with Centchroman was 5-fold less than that seen with the normal 5th day of pregnancy.

Protein synthesis on the 5th day of pregnancy was 4-fold more than on the second day of pregnancy. An increase of 1·4 fold was observed in the implantation sites compared to the inter-implantation sites. In propene treated rats, protein synthesis was 1·25 fold more than that seen on 5th day of pregnancy. After Centchroman treatment the rate of protein synthesis was 2·5 fold lower than that of 5th day of normal pregnancy. The data were analysed by Student's *'t'* test and *'f'* test and found to be significant at 1 % level.

Discussion

Estrogenic and antiestrogenic drugs are good probes for studies on hormonal involvement on implantation. Some workers have suggested that rat uterine RNA synthesis is due to the alkyl amino ethoxy chain present in antiestrogenic drugs (Waters

et al., 1983; Jordan *et al.,* 1981; Jordan, 1982; Jordan and Gosden, 1982, 1983). A similar side chain is present in tamoxifen, thioxifene and also in our compound Centchroman. A *cis-trans* isomerism is very common in many of the non-steroidal antiestrogens. Interestingly, the *cis*-isomers possess estrogenic while *trans* isomers show anti-estrogenic response. The present compound Centchroman also shows *cis-trans* isomerism.

For most of the period the uterus remains in refractory state and it does not permit theblastocyst to implant. Sensitivity is brought about by the action of steroidal hormones (Sutherland and Murphy, 1982).

Throughout the preimplantation period, mouse uterine glands are active secretory structures similar to other exocrine cells. Thus, the uterine gland of mouse must be considered a source of uterine fluid proteins at the time of implantation. These uterine proteins help in the attachment of blastocyst. The inability of blastocyst attachment with the uterine endometrium due to anti-implantation agents could be due to altered level of synthesis of nucleic acids and proteins which is an essential requirement for the implantation process (Randall and Enders, 1981).

Antiestrogens have been found to show both agonist and antagonist properties. Roy and Datta (1976) and Durrani *et al.* (1979) have observed that Centchroman exerts a very weak estrogenic action in the absence of E, whereas in the presence of potent E, it exerts a relatively strong anti-estrogenic action. Thus, the decreased synthesis of nucleic acid and protein in the presence of Centchroman might be due to its antagonistic action. The observations of the present work are comparable to that of Reid and Heald (1971). The above decrease may alter the epithelial morphology which may be a factor in its post-coital contraceptive action (Poteat, 1981).

The increase in DNA synthesis due to the action of estrogens, similar to the present findings, has been observed by many workers (Shelesnyak and Tie, 1963; Miller *et al.,* 1968; Mohla *et al.,* 1970; Heald and O'Grady, 1970; Reid and Heald, 1971; Ledford *et al*.,1978; Harris and Gorski, 1978). An increase in the synthesis of nucleic acids and proteins by 78/224 might be due to an altered internal milieu in uterine luminal secretions and in uterine epithelial cells which become hostile for attachment of blastocyst. The present work is also in agreement with the estrogenic nature of drugs as reported earlier by Prakash and Roy (1980,1981,1982). Thus, from the present work the importance of homeostatic mechanisms involved in nucleic acids and protein metabolism, an essential pre-requisite for implantation process is well evident. Both increase and decrease in the synthetic and degradative processes disturb the uterine internal milieu which leads to anti-implantation and anti-fertility activities.

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References

Burton, K. (1956) *Biochem. J.,* **62,** 315.

Durrani, S., Agarwal, A. K., Saxena, R., Setty, B. S, Gupta, R. C, Ray, S. and Anand, N. (1979) *J. Steroid Biochem.,* **11,** 67.

Glasser, S. R. and McCormack, S. A. (1979) *Endocrinology,* **104,** 1112.
Harris, J. and Gorski, J. (1978) *Endocrinology,* **103,** 240.
Heald, P. J. and O'Grady, J. E. (1970) *Biochem. J.,* **117,** 65.
Jordan, V. C, Haldemann, B. and Allen, K. E. (1981) *Endocrinology,* **108,** 1353.
Jordan, V. C. (1982) *Endocrinology,* **110,** 1281.
Jordan, V. C. and Gosden, B. (1982) *Mol. Cell. Endocrinol.,* **27,** 291.
Jordan, V. C. and Gosden, B. (1983) *J. Steroid Biochem.,* **19,** 1249.
Kamboj, V. P., Setty, B. S., Chandra, H. and Kar,. A. B. (1977) *Indian J. Exp. Biol.,* **15,** 1144.
Ledford, B. E., Rankin, J. C. and Froble, V. L. (1978) *Biol Reprod.,* **18,** 506.
Lejeune, B., Puissant, F., Camus, M. and Leroy, F. (1982) *J. Endocrinol.,* **93,** 397.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.,* **193,** 265.
Miller, B. G., Owen, W. H. and Emmens, C. W. (1968) *J. Endocrinol.,* **42,** 351.
Mohla, S., Prasad, M. R. N. and Dass, C. M. S. (1970) *Endocrinology,* **87,** 383.
Nishikawa, M. (1978) *Folia Endocrinol. Jpn.,* **54,** 692.
Poteat, W. L. (1981) *Experientia,* **37,** 84.
Prakash, A. O., Ghosh, M., Tewari, S. C., Rastogi, S. N. and Roy, S. K. (1980) *Indian J. Exp. Biol.,* **18,** 557.
Prakash, A. O. and Roy, S. K. (1981) *Indian J. Exp. Biol.,* **19,** 1179.
Prakash, A. O. and Roy, S. K. (1982) *Indian J. Exp. Biol.,* **20,** 253.
Randall, G. and Enders, A. C. (1981) *Anat. Rec.,* **199,** 109.
Reid, R. J. and Heald, P. J. (1971) *J. Reprod. Fertil.,* **27,** 73.
Roy, S. K. (Jr), Mukherji, S. R. and Roy, S. K. (1978) *Proc. Soc. Exp. Biol. Med.,* **158,** 260.
Roy, S. N. and Datta, J. K. (1976) *Contraception,* **13,** 597.
Segal, S. J., Davidson, O. W. and Wada, K. (1965) *Proc. Natl. Acad. Sci. USA,* **54,** 782.
Shelesnyak, M. C. and Tie, L. (1963) *Acta Endocrinol.,* **42,** 465.
Singh, R. K. and Roy, S. K. (1979) *J. Biosci.,* **1,** 135.
Sutherland, R. L., and Murphy, L. C. (1982) *Mol. Cell. Endocrinol.,* **25,** 5.
Waters, A. P., Wakeling, A. E. and Knowler, J. T. (1983) *J. Steroid Biochem.,* **18,** 7.