0022-1554/91/$3.30
The Journal of Histochemistry and Cytochemistry

Vol. 39, No. 6, pp. 823-828, 1991
Printed in U.S.A.

Original Article

Induction of Catechol-O-methyltransferase in the Luminal Epithelium of Rat Uterus by Progesterone

KATSUHIRO INOUE and CYRUS R. CREVELING[1]

Department of Oral Anatomy, School of Dentistry, Okayama University, Okayama 700, Japan (KI), and Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, Maryland 20892 (CRC).

Received for publication April 2, 1990 and in revised form November 15, 1990 and January 31, 1991; accepted February 1, 1991 (0A1941).

We performed light microscopic immunocytochemical observations of the localization of catechol-*O*-methyltransferase (COMT) in rat uterus, using a rabbit anti-rat serum specific for the soluble form of rat liver COMT, biotinylated goat anti-rabbit immunoglobulin, and peroxidase conjugated with streptavidin. In the non-pregnant rat, COMT was minimal but detectable in the uterine luminal and glandular epithelium, with greater amounts present in uteri from rats in estrus than those in diestrus. In early pregnancy a robust accumulation of COMT was observed in the luminal epithelium. To more precisely define both the timing and the factors contributing to the appearance of COMT, uteri were examined on Days 1–5 in pregnant and pseudopregnant rats. Accumulation of COMT in the luminal epithelium was observed by Day 3 in uteri from pregnant and pseudopregnant rats and by Day 4 in lactating post-partum rats. No immunostaining of COMT was observed in uteri from non-lactating post-partum rats. Ovariectomy on Day 0 or 1 but not on Day 2 of pregnancy prevented the appearance of COMT on Day 4. Progesterone treatment immediately after ovariectomy on Day 0 or 1 of pregnancy restored the COMT. (*J Histochem Cytochem 39:823–828, 1991*)

KEY WORDS: Progesterone; Rat; Uterus; Luminal epithelium; Pseudopregnancy; Immunocytochemistry; Peroxidase; Diaminobenzidine; Catechol-*O*-methyltransferase; Streptavidin.

Introduction

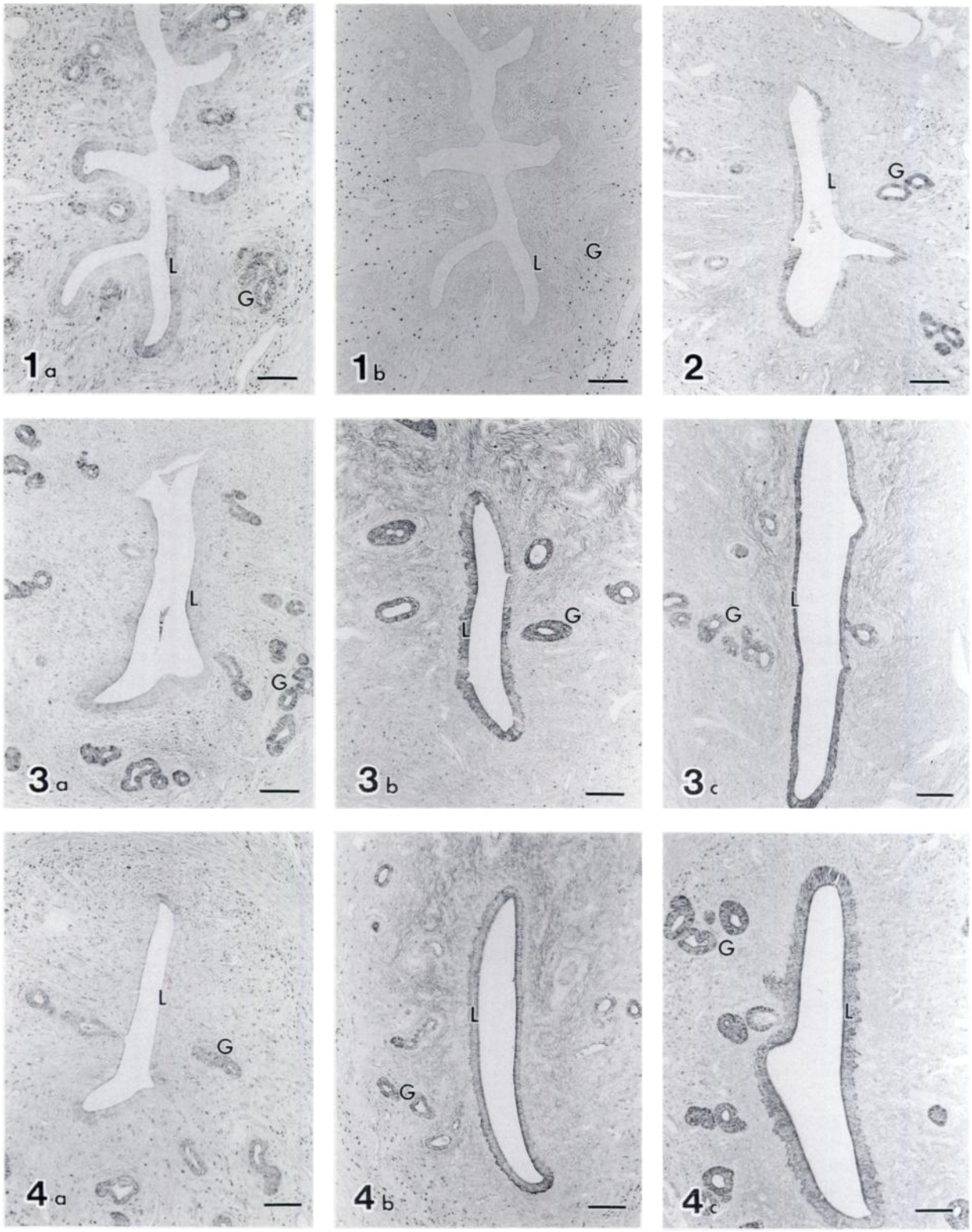
Catechol-*O*-methyltransferase (COMT; E.C. 2.1.1.6) catalyzes *O*-methylation and thereby inactivates a wide range of endogenous catechols. In the past, COMT has been thought to be an enzyme with a ubiquitous but nonspecific distribution. The purification of COMT and development of specific antiserum against COMT (Grossman et al., 1985) have permitted definition of the specific cellular distribution of this enzyme. The localization of COMT has been described in a series of immunohistochemical studies in several species (for reviews see Thakker and Creveling, 1990; Creveling, 1984; Creveling and Hartman, 1982; Inoue et al., 1977). In most tissues, the level of COMT activity reaches a characteristic level early in life and remains essentially constant throughout adult life (Goldstein et al., 1980). In the rat uterus, however, the level of COMT activity is clearly influenced by the hormonal status of the uterus (Inoue et al., 1980; Giles and Miller, 1967). Metabolic studies of norepinephrine in uterine slices suggest that progesterone has a direct stimulatory effect on the extraneuronal *O*-methylation of norepinephrine (Kennedy et al., 1984). In this regard, it is known that the levels of plasma progesterone increase in early pregnancy or pseudopregnancy (Horikoshi and Suzuki, 1974). Previously, we demonstrated that COMT is elevated in luminal epithelial cells in uteri from pregnant rats (Creveling, 1984; Inoue et al., 1980). These results suggest that induction of COMT in the uterus of rat may be related to conditions of progesterone dominance.

In the present study, we have attempted to provide evidence that progesterone dominance is the primary factor responsible for the induction of COMT in the luminal epithelium of rat. To explore this hypothesis, the immunocytochemical localization of COMT, using an antibody specific for the 23 KD form of soluble COMT from rat liver, was examined in uteri from pregnant, pseudopregnant, lactating and non-lactating post-partum rats, and ovariectomized pregnant rats with and without progesterone replacement. In uteri from pregnant and pseudopregnant rats, the increase in COMT was observed on Day 3, and in lactating post-partum rats on Day 4. In uteri from non-lactating post-partum rats COMT was not present on Day 3, 4, or 5. We further demonstrate that ovariectomy of pregnant rats on Day 0 or 1, but not on Day 2, of pregnancy prevents increase in COMT, while ovariectomy followed by progesterone replacement restores COMT. These results suggest that progesterone domination of the uterus is the primary factor in the accumulation of COMT in the luminal epithelium.

Materials and Methods

Virgin female rats of the Wistar strain, 10–20 weeks old, were used (Katayama Chemical Co., Tokyo, Japan). Rat chow and water were given ad libitum. Estrous cyclicity was determined by vaginal exfoliate cytology every morning. Only rats that had regular 4-day cycles were used. There were five to

[1] Correspondence to: Dr. C. R. Creveling, Building 8A, Room 1A-27, NIDDK, NIH, Bethesda, MD 20982.



Figures 1–8. Transverse sections of rat uterus showing immunocytochemical reaction of catechol-*O*-methyltransferase. Strong specific staining for COMT is seen in the luminal epithelium of Figures 3b, 3c, 4c, and 7. DAB background was limited to the myeloperoxidase activity of polymorphonuclear leukocytes. L, luminal epithelium; G, glandular epithelium of uterine gland. Bars = 100 μm.

Figure 1. (**a**) Nonpregnant uterus (estrus); (**b**) control section.

Figure 2. Non-pregnant uterus (diestrus).

Figure 3. The second (**a**), third (**b**), and fourth (**c**) day of pregnancy.

Figure 4. The second (**a**), third (**b**), and fourth (**c**) day of pseudopregnancy.

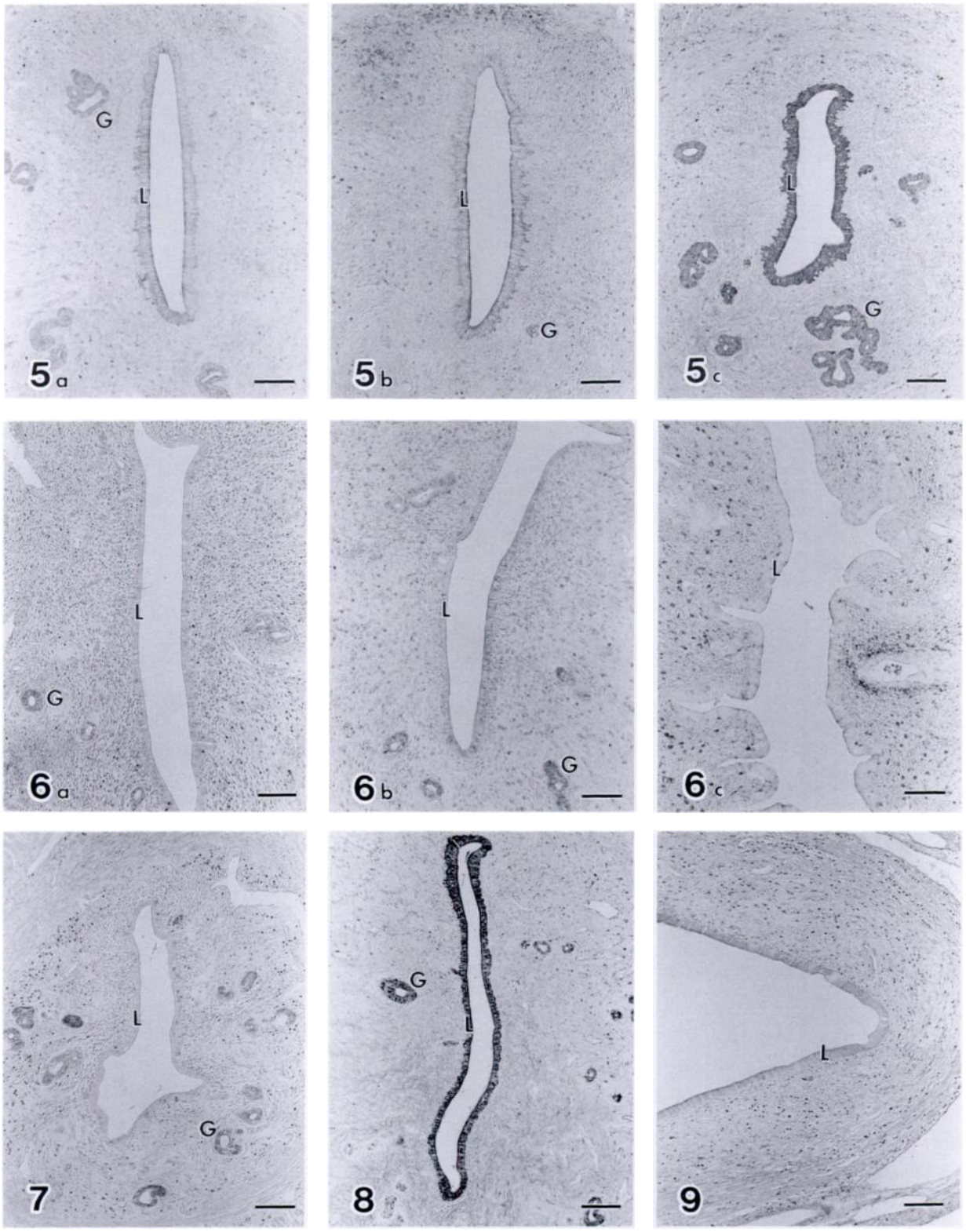


Figure 5. The second (**a**), third (**b**), and fourth (**c**) day post partum with neonate.

Figure 6. The second (**a**), third (**b**), and fourth (**c**) day post partum with removal of the neonate.

Figure 7. The fourth day of pregnancy with ovariectomy on Day 0 of pregnancy.

Figure 8. The fourth day of pregnancy with injection of progesterone after ovariectomy on Day 0 of pregnancy.

Figure 9. The fourth day of pregnancy with injection of estradiol after ovariectomy on Day 0 of pregnancy.

ten animals in each experimental group. Rats were kept in a constant-temperature room (25°C) in which the lighting schedule was 12 hr light (0600–1800 hr):12 hr dark (1800–0600 hr).

Uteri were obtained on Days 1–5 as described below from the following experimental groups: (a) Pregnant rats: female rats were placed with a male rat, mating confirmed the following morning by the presence of a vaginal plug or sperm, and this day designated as Day 0 of pregnancy. (b) Pseudopregnant rats: female rats were made pseudopregnant by mating with a vasectomized male rat. The morning a vaginal plug was identified was designated as Day 0 of pseudopregnancy. (c) Post-partum lactating rats: female rats were kept with 10–12 neonates. (d) Post-partum non-lactating rats: after parturition all neonates were removed from the mother within 4 hr. (e) Ovariectomized pregnant rats: pregnant rats were bilaterally ovariectomized under sodium pentobarbital anesthesia, using the dorsolateral approach, on Day 0, 1, or 2 of pregnancy. Progesterone was administered immediately after surgery by subcutaneous injection of 2 mg of progesterone (Nakari Chemical; Kyoto, Japan) dissolved in 0.2 ml of sesame oil. Controls received the vehicle only. Another group of pregnant rats, ovariectomized on Day 0 of pregnancy, were given 2 μg of β-estradiol (Sigma; St Louis, MO) dissolved in 0.2 ml of sesame oil, by subcutaneous injection immediately after surgery. Uteri from ovariectomized rats were examined on Day 4.

Tissue Preparation. Rats were anesthetized with sodium pentobarbital and perfused through the heart with 100 ml of periodate–lysine–paraformaldehyde (PLP) (McLean and Nakane, 1974). After perfusion, uteri were removed, cut into small pieces, and fixed in PLP at 4°C for 3 hr, dehydrated through graded alcohol, embedded in paraffin, sectioned at 5 μm, mounted on gelatin-coated slides, and dried overnight at 37°C.

Immunocytochemistry. Sections were deparaffinized with xylene, brought to water through graded alcohol, washed with distilled water, treated with 3% aqueous H_2O_2 for 5 min, washed with two changes of water for 10 min and in PBS, pH 7.2, for 5 min. Sections were then incubated for 30 min at room temperature with normal goat serum diluted 1:50 in PBS, washed in three changes of PBS for 15 min, incubated for 24 hr at 4°C with rabbit antiserum to rat liver COMT diluted 1:2000 in PBS, and washed three times for 15 min in PBS. Normal rabbit serum was used for control sections. Sections were then treated for 15 min at room temperature with biotinylated goat anti-rabbit immunoglobulin (Bio Genex; Dublin, Ireland), washed in PBS, treated for 15 min at room temperature with peroxidase-conjugated streptavidin (Bio Genex), and washed for 15 min in PBS. Development of the peroxidase reaction product was carried out by incubation of the sections for 5 min in 3,3'-diaminobenzidine (0.05%) in 0.05 M Tris buffer, pH 7.6, containing 0.01% H_2O_2 and then washed in water, dehydrated through graded alcohol, cleared with xylene, and mounted in a resinous mounting medium (Entellan; Merck, Darmstadt, FRG).

Results

In all the reactive cells the COMT-positive reaction product was present throughout their cytoplasm and was uniformly absent from their nuclei. A minimal but clearly COMT-positive reaction was observed in the luminal epithelium in uteri from non-pregnant rats during estrus and diestrus (Figures 1a and 2). The reaction appeared to be marginally greater during estrus. A more pronounced positive reaction was present in the glandular epithelium. Immunocytochemical control sections developed with normal rather than with COMT-specific rabbit serum did not show any reaction product in either the luminal or glandular epithelial cells. A representative example of the adjacent section from Figure 1a is shown in Figure 1b.

In uteri from pregnant and pseudopregnant rats a weakly positive reaction was observed in the luminal epithelium on Day 2 of pregnancy and pseudopregnancy (Figures 3a and 4a). The COMT-positive reaction appeared somewhat greater in the glandular epithelium on Day 2 in the uterus of pregnant as compared to pseudopregnant rats (Figures 3a and 4a). On Days 3 and 4 of pregnancy and pseudopregnancy, the luminal and glandular epithelia were distinctly COMT positive (Figures 3b, 3c, 4b, and 4c).

In uteri from lactating rats, a weak COMT-positive reaction was detectable in the luminal and glandular epithelium on Days 2 and 3 post partum (Figures 5a and 5b). By Day 4, however, the luminal and glandular epithelia were markedly COMT positive (Figure 5c). In uteri from post-partum non-lactating rats virtually no COMT-positive reaction could be detected in the luminal epithelium either on or after post-partum Day 1 (Figures 6a-6c). The glandular epithelium in this group, however, was weakly COMT positive (Figures 6a and 6b).

No COMT-positive reaction was observed on Day 4 of pregnancy in the luminal epithelium of rats that had been bilaterally ovariectomized on Day 0 or Day 1 of pregnancy (Figure 7). The glandular epithelium in these animals was weakly COMT positive (Figure 7). When bilateral ovariectomy was performed on Day 2 of pregnancy, the expected COMT-positive reaction was observed in the luminal and glandular epithelia on Day 4 of pregnancy (data not shown). Rats that had received progesterone immediately after bilateral ovariectomy on Day 0 or 1 of pregnancy showed a pronounced COMT-positive reaction in the luminal and glandular epithelia on Day 4 (Figure 8), whereas rats receiving β-estradiol showed no COMT-positive reaction (Figure 9).

Discussion

Our present evidence indicates that, in the rat, a very early response to the hormonal actions initiated by pregnancy or pseudopregnancy is an increase in the level of COMT in the luminal epithelium of the uterus. This increase is clearly evident on Day 3 and becomes marked by Days 4 and 5. The increase of COMT in the luminal epithelium appears to result from the early domination of the uterus by progesterone in both pregnant and pseudopregnant rats. A direct relationship between early progesterone dominance and the appearance of COMT in the luminal epithelium is shown by the marked accumulation of COMT seen after administration of progesterone to pregnant rats ovariectomized on Day 0 or 1 of pregnancy. Administration of β-estradiol after ovariectomy fails to elicit immunostaining for COMT. More significantly, without replacement with exogenous progesterone the accumulation of luminal COMT is absent. The early dependence of the COMT response on progesterone is clear, since ovariectomy on Day 2 of pregnancy fails to prevent the subsequent increase in luminal epithelial COMT.

The minimal level of immunopositive COMT observed in the luminal and glandular epithelium of the non-pregnant rat uterus is in agreement with the low levels of COMT activity obtained by biochemical measurements in non-pregnant rat uterus (Guldberg and Marsden, 1975; Giles and Miller, 1967; Wurtman et al., 1964) and in luminal epithelium from non-pregnant rat uterus (Creveling, 1984). Uterine COMT activity was greater during estrus (Giles and Miller, 1967). The minimal increase in luminal epithelial COMT

during estrus compared with the level during diestrus may reflect stimulation by antecedent hormonal interactions in preparation of the uterus for the expected fertilization.

It is suggested that the progesterone-induced increase in luminal COMT is an additional factor in the sequence of events occurring during early pregnancy and pseudopregnancy in the rat which is necessary for preparation of the uterus for decidua formation and successful implantation. It is important to note that the increase of COMT in the luminal epithelium occurs before the changes in the luminal epithelial surfaces that accompany blastocyst adhesion in the rat (Enders et al., 1980; Tachi et al., 1970). Although the function(s) of COMT in the luminal epithelium of the uterus is not clear, the biochemical properties of the enzyme are quite specific. Extensive studies have unequivocally demonstrated that COMT specifically catalyzes the *O*-methylation of catechols in conjunction with a single co-substrate, S-adenosylmethionine, as the methyl donor (for recent reviews see Thakker and Creveling, 1990; Kopin, 1985). It is not known whether the increase of COMT, and by implication the inactivation of endogenous catechol(s), are involved in the interaction of the luminal epithelial cells with the underlying stroma, with events related to the increasing vascularization and incipient endometrial capillary permeability (Psychoyos, 1973), or with events within the lumen (Lundkvist et al., 1979). In the lactating post-partum rat, the increase of COMT in the luminal epithelium appears to be delayed until Day 4 and is again a response to progesterone derived from a continued luteal function. The absence of COMT in the non-lactating post-partum rat is in agreement with the rapid drop of progesterone after removal of the litter reported by van der Schoot et al. (1978). The role of the increased level of COMT in the lactating post-partum rat and the significance of its absence in the non-lactating post-partum rat are unknown.

It is not surprising that the level of COMT in the uterus is modulated by the hormonal status of the animal, in view of the extensive evidence that ovarian and uterine hormones, in concert with pituitary hormones, act directly on the uterus (Finn, 1977; Psychoyos, 1973). Cell division in the epithelium of the uterus in mouse (Martin and Finn, 1968) and rat (Watson et al., 1975; Tachi et al., 1972), and the induction of many enzymes in the uterus, are under hormonal control (Mazumder et al., 1980; Alam et al., 1976; Parves et al., 1976). Progesterone-mediated effects include the stimulation of prostaglandin dehydrogenase activity (Alam et al., 1976; Nargis et al., 1976) and monoamine oxidase-A activity in rat uterus (Mazumder et al., 1980; Collins and Southgate, 1970). Progesterone also induces an increase in the extraneuronal *O*-methylation of norepinephrine in rabbit uterus (Kennedy et al., 1984). The increase in COMT may be related to changing levels of catecholamines accompanying vascular modifications in the pregnant and pseudopregnant uterus. Another consideration regarding the role of COMT comes from studies on the regulatory role of catecholestrogens in the estrous cycle (Ball et al., 1982) and the recent demonstration of catecholestrogen formation in mouse (Paria et al., 1990) and rabbit uterus (Chakraborty et al., 1990). The high affinity of COMT for catecholestrogens (Ball and Knuppen, 1980) compared with the affinity for catecholamines suggests that COMT may provide a mechanism to protect the cells of the uterus, and later the blastocyst, from the deleterious effects of local synthesis of catecholestrogens (Chakraborty et al., 1990). A similar functional role for COMT as a enzymatic barrier for the free diffusion of catechols has been implicated in many tissues (Creveling and Hartman, 1982).

Finally, it must be noted that there are subtle differences in the level of COMT between the glandular and luminal epithelium in the rat uterus. In general, the levels of COMT in the glandular epithelium observed in response to changes in progesterone dominance are quite similar to the changes seen in the luminal epithelium. This similarity is evident in the prominent increase in COMT by Day 4 in pregnant, pseudopregnant, and lactating post-partum rats, as well as in ovariectomized pregnant rats, after progesterone replacement. However, in pregnancy the increase in glandular COMT appears to precede the increase in the luminal epithelium. The level of COMT also appears to be moderately prominent in the ovariectomized pregnant rat without progesterone replacement and in the non-lactating post-partum rat. Furthermore, the level of COMT in the glandular epithelium in the non-pregnant rat is similar to, if not slightly greater than, the level of COMT in the luminal epithelium. Our results appear to be in agreement with the progesterone-dependent maintenance of the metrial gland reported by Martel et al. (1989) and with the stimulation of *O*-methylation by progesterone in the endometrium reported by Kennedy and de la Lande (1986). We suggest that the level of COMT in glandular epithelium in response to progesterone may be either superimposed on a basal constitutive level of COMT or the result of a differential response of more than one cell population in the glandular epithelium.

Literature Cited

Alam NA, Russell PT, Wilsontabor M, Moulton BC (1976): Progesterone and estrogen control of uterine prostaglandin dehydrogenase activity during deciduomal growth. Endocrinology 198:859

Ball P, Emons G, Knuppen R (1982): Importance of catecholestrogens in the regulation of the ovarian cycle. Arch Gynecol 231:315

Ball P, Knuppen R (1980): Catechol estrogens (2- and 4-hydroxyestrogens). Chemistry, biogenesis, metabolism, occurrence and physiological significance. J Clin Endocrinol Metab 34:736

Chakraborty C, Huet-Hudson YM, Dey SK (1990): Catecholestrogen synthesis and metabolism in the rabbit uterus during the periimplantation period. J Steroid Biochem 35:39

Collins GGS, Southgate J (1970): The effect of progesterone and oestradiol on rat uterine monoamine oxidase activity. Biochem J 117:38

Creveling CR (1984): The functional significance of the cellular localization of catechol-O-methyltransferase. In Usdin E, Kvetnansky R, Axelrod J, eds. Stress: the role of catecholamines and other neurotransmitters. New York, Gordon and Breach, 447

Creveling CR, Hartman BK (1982): Relationships between the cellular localization and the physiological function of catechol-O-methyltransferase. In Usdin E, Borchardt RT, Creveling CR, eds. Biochemistry of S-adenosylmethionine and related compounds. London, Macmillan, 479

Enders AC, Schlafke A, Welsh AO (1980): Trophoblastic and uterine luminal epithelial surfaces at the time of blastocyst adhesion in the rat. Am J Anat 159:59

Finn AF (1977): The implantation reaction. In Finn RM, ed. Biology of the uterus. New York, Plenum, 245

Giles RE, Miller JW (1967): The catechol-O-methyltransferase activity and endogenous catecholamine content of various tissues in the rat and the effect of administration of U-0521 (3',4'-dihydroxy-2-methylpropiophenone). J Pharmacol Exp Ther 158:189

Goldstein DJ, Weinshilboum RM, Dunnette JH, Creveling CR (1980): Developmental patterns of catechol-O-methyltransferase in genetically different rat strains. J Neurochem 34:153

Grossman MH, Creveling CR, Rybczynski R, Braverman M, Isersky C, Breakfield XO (1985): Soluble and particulate forms of rat catechol-O-methyltransferase distinguished by gel electrophoresis and immune fixation. J Neurochem 44:421

Guldberg HC, Marsden CA (1975): Catechol-O-methyltransferase: pharmacological aspects and physiological role. Pharmacol Rev 27:135

Horikoshi H, Suzuki Y (1974) On circulating sex steroids during the estrous cycle and the early pseudopregnancy in the rat with special reference to its luteal activation. Endocrinol Jpn 21:69

Inoue K, Tice LW, Creveling CR (1980): Immunocytochemical localization of catechol-O-methyltransferase in the pregnant rat uterus. Endocrinology 107:1833

Inoue K, Tice LW, Creveling CR (1977): Immunocytochemical localization of catechol-O-methyltransferase. In Usdin E, Weiner N, Youdin MBH, eds. Structure and function of monoamine enzymes, modern pharmacology-toxicology. Vol 10. New York, Basel, Marcel Dekker, 835

Kennedy JA, de la Lande IS (1986): Effect of progesterone on the metabolism of noradrenalin in rabbit uterine endometrium and myometrium. Naunyn Schmiedebergs Arch Pharmacol 333:368

Kennedy JA, de la Lande IS, Morris RG (1984): Effect of ovarian steroids on the metabolism of noradrenaline in rabbit uterus. Naunyn Schmiedebergs Arch Pharmacol 326:1322

Kopin IJ (1985): Catecholamine metabolism: basic aspects and clinical significance. Pharmacol Rev 37:334

Lundkvist O, Nilsson O, Bergstrom S (1979): Studies on the trophoblast-epithelial complex during decidual induction in rats. J Anat 154:211

Martel D, Monier MN, Roche D, De Feo VJ, Psychoyos A (1989): Hormonal dependence of the metrial gland: further studies on oestradiol and progesterone receptor levels in the rat. J Endocrinol 120:465

Martin L, Finn CA (1968): Hormonal regulation of cell division in epithelial and connective tissues of the mouse uterus. Endocrinology 41:363

Mazumder RC, Glover V, Sandler M (1980): Progesterone provokes a selective rise of monoamine oxidase A in the female genital tract. Biochem Pharmacol 29:1857

McLean IW, Nakane PK (1974): Periodate–lysine–paraformaldehyde fixative, a new fixative for immunoelectron microscopy. J Histochem Cytochem 22:1077

Nargis A, Russell P, Wilsontabor M, Moulton BC (1976): Progesterone and estrogen control of uterine prostaglandin dehydrogenase activity during deciduomal growth. Endocrinology 98:856

Paria BC, Chakraborty C, Dey SK (1990): Catechol estrogen formation in the mouse uterus and its role in implantation. Mol Cell Endocrinol 59:25

Parvez S, Raza–Bukhari A, Parvez H (1976): Sexual steroids and monoamine metabolism during gestation. Experientia 32:59

Psychoyos A (1973): Hormonal control of ovoimplantation. Vitamins Hormones 31:201

Tachi C, Tachi S, Lindner HR (1972): Modification by progesterone of oestradiol-induced cell proliferation, RNA synthesis and oestradiol distribution in the rat uterus. J Reprod Fertil 31:59

Tachi C, Tachi S, Lindner HR (1970): Ultrastructural features of blastocyst attachment and trophoblastic invasion in the rat. J Reprod Fertil 21:37

Thakker DR, Creveling CR (1990): O-Methylation. In Mulder GJ, ed. Conjugation reactions in drug metabolism. London, Taylor and Francis, 193

van der Schoot P, Lankhorst RR, de Roo JA, de Greef WJ (1978): Suckling stimulus, lactation, and suppression of ovulation in the rat. J Endocrinol 103:949

Watson J, Anderson FB, Alam M, O'Grady JE, Heald PJ (1975): Plasma hormones and pituitary luteinizing hormone in the rat during early stages of pregnancy and after postcoital treatment with tamoxifen. Endocrinology 65:7

Wurtman RJ, Axelrod J, Potter LT (1964): The disposition of catecholamines in the rat uterus and the effect of drugs and hormones. J Pharmacol Exp Ther 144:150