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CELLULAR AND SUBCELLULAR LOCALIZATION OF [^{3}H]-PROGESTERONE AND ITS METABOLITES IN RAT UTERUS STUDIED BY AUTORADIOGRAPHY

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SUMMARY

The cellular and subcellular localization of radioactivity after the injection of [^{3}H]-progesterone in rat uterus was studied at 5, 15 and 60 min, using dry-autoradiography. A clear pattern of radioactivity distribution was observed. In all animals studied, without and with estrogen pretreatment, radioactivity was concentrated most heavily in nuclei of muscle cells, with less marked nuclear concentration in stromal cells. Nuclei of the luminal and glandular epithelium remained unlabeled.

INTRODUCTION

EARLIER attempts to utilize autoradiography for the identification of progestin binding sites in rat [1] and mouse [2] uterus have failed to reveal a distinct cellular or subcellular pattern. Recently John and Rogers [3] re-investigated the distribution of progesterone and stated that binding sites exist at low concentrations and that these sites are distributed between nucleus and cytoplasm in approximately equal proportions. Biochemical data suggest the existence of intracellular binding protein in the rat [4–6]. Specific binding proteins for progesterone have been demonstrated more clearly in the chick oviduct [7], in the guinea pig [8] and in the rabbit and human uterus [9]. The autoradiographic demonstration of progestin binding sites appears to be difficult because of rapid metabolism [10] and possible translocation artifacts during tissue preparation. Dry-mount autoradiography excludes or minimizes such artifacts and its utility has been demonstrated in the localization of estradiol, androgen and corticosterone [11–14]. Recently, a nuclear concentration of progestin has been demonstrated in guinea pig uterus [15]. Therefore, the present study was undertaken to find out specific cellular and subcellular sites for progestin localization in rat uterus.

MATERIAL AND METHODS

Three groups of Sprague–Dawley rats were used: 24-day intact, 2 months old ovariectomized, and 2 months old ovariectomized and adrenalectomized. Two rats of each group were injected with 1 μg per day of estradiol-17β in sesame oil or with the vehicle only. [1,2-^{3}H]-progesterone, specific activity 33·5 Ci/mmol or [1,2,6,7-^{3}H]-progesterone, specific activity 81·1 Ci/mmol, dissolved in isotonic saline, was injected subcutaneously at a dose of 1 μg/100 g body wt. Animals were decapitated at 5 min, 15 min, or 1 h after the injection. Pieces of uterus and diaphragm, as control, were excised, placed on a tissue holder and frozen in liquefied propane. Two μm sections were cut in a Wide Range Cryostat (Harris Mfg. Co., Cambridge, Mass.) and freeze-dried in a Cryo-Pump (Thermovac In-

dustries, Copiague, Long Island, N.Y.). The freeze-dried, unfixed and unembedded sections were dry-mounted on photographic emulsion (Kodak NTB 3) coated slides and exposed at − 15°C for a period of 7–12 months. After exposure the autoradiograms were photographically processed and stained with methylgreen-pyronin. The technique has been described in detail[16].

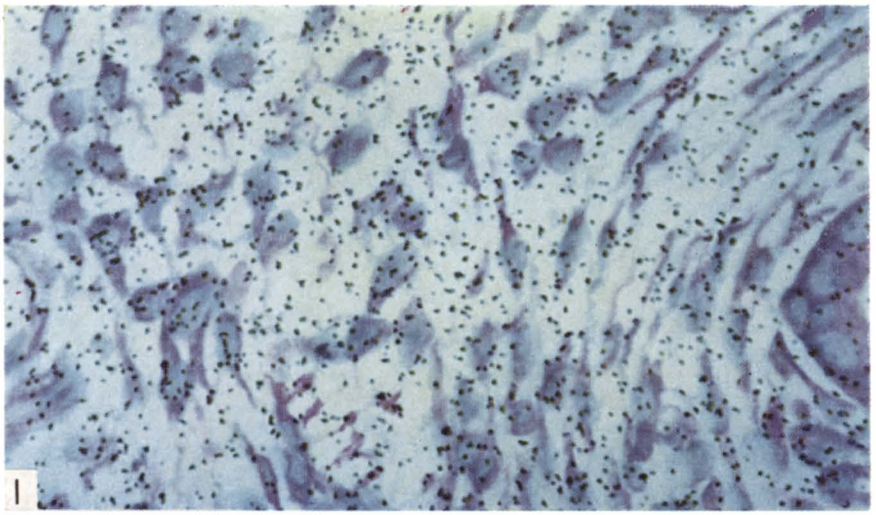
RESULTS

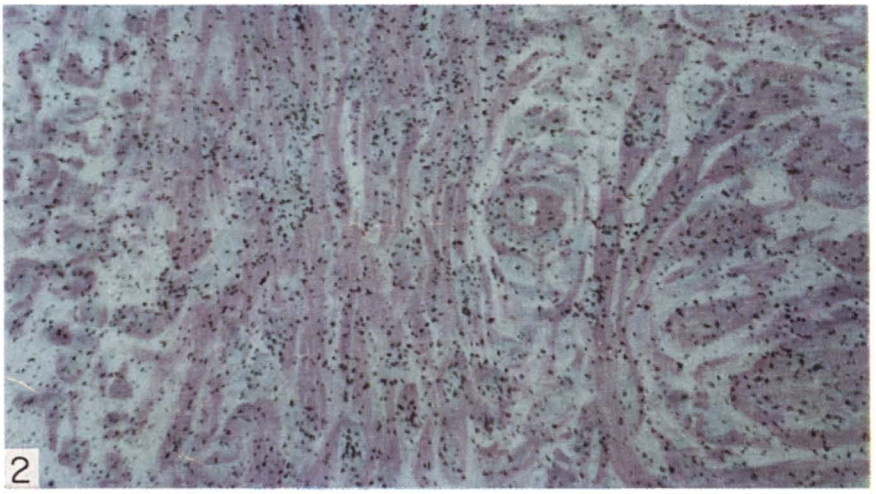
In all animals studied at 5 min, 15 min and 1 h after the injection of ^{3}H-progesterone, radioactivity was observed in all uterine tissues and extracellular space. Although radioactivity existed in the different uterine structures (Figs. 1–4), nuclear concentration was most distinct in muscle cells (Figs. 1–4), with maximal differential accumulation 15 min after the injection. Nuclei of stromal cells showed weak labeling when compared to the muscle cells which, on the average, concentrated and retained 3–5 times more radioactivity. Thus, at shorter exposure times, nuclear concentration of radioactivity was only apparent in muscle cells and not in other cell types of the uterus. In the luminal epithelium (Fig. 3) and glandular epithelium (Fig. 1) no nuclear concentration was observed at all time intervals in immature and adult animals. In the zona vasculosa, between the inner circular and outer longitudinal muscle layer, little or no radioactivity was retained in the perivascular cells (Fig. 2). Polymorph neutrophiles did not concentrate radioactivity. After pretreatment with estradiol, both in the immature intact and adult ovariectomized animals, increased nuclear concentration of radioactivity was observed in muscle cells as well as stromal cells, but no accumulation of radioactivity could be observed in luminal and glandular epithelium. The effect of estrogen stimulation was conspicuous by an increased uptake of radioactivity in stromal cells. In ovariectomized animals, in which adrenals have been removed, increased nuclear labeling was observed in these structures. The results are summarized in Table 1 as a semi-quantitative evaluation of the autoradiograms. The 'non-target' tissue diaphragm did not show selective subcellular accumulation although diffuse distribution of radioactivity was observed.

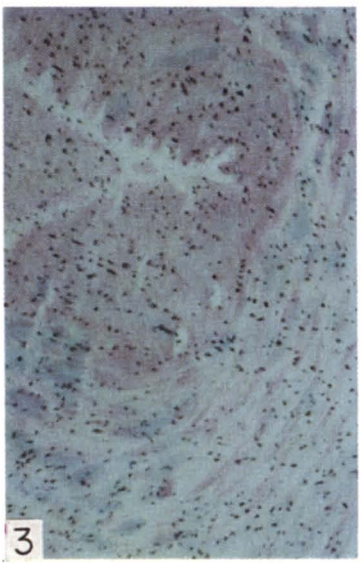
DISCUSSION

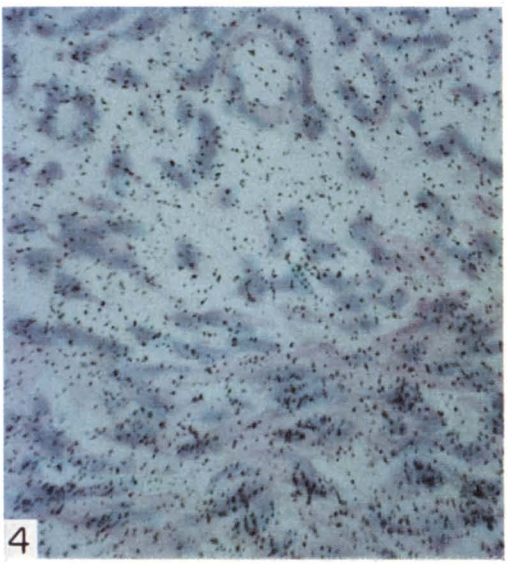
After the injection of [^{3}H]-progesterone, a distinct cellular and subcellular distribution of radioactivity is obtained in rat uterus, using dry-autoradiography. These results contrast to those published by Rogers *et al.*[1] and Rogers and John[3], who did not find selective concentration of radioactivity, neither in the different uterine tissues nor in subcellular compartments. As discussed earlier[16], these differences are likely to be attributable to differences in technique. However, a nuclear 'receptor'-progestin complex has not yet been demonstrated in the rat. This apparent lack of specific compartmental concentration,

Figs. 1–4. Autoradiograms of rat uterus, obtained 1 h after injection of ^{3}H-progesterone. The rats were castrated 3 days prior to the experiment. Concentration of radioactivity is visibly most pronounced in nuclei of muscle cells (Figs. 2 and 3). Radioactivity, less than in muscle cells, is also concentrated in nuclei of substantia propria cells (Fig. 1), while in nuclei of the glandular (Fig. 1) and the luminal (Fig. 3) epithelium, no such concentration of radioactivity can be demonstrated. Some radioactivity exists in cytoplasm and extracellular space. Stained with methylgreen-pyronin for DNA and RNA: nuclei blue, cytoplasm red. 2 μm; magnification × 890 (Fig. 1), × 480 (Figs. 2–4). Exposure times: 350 days (Fig. 1), 210 days (Figs. 2 and 3), 252 days (Fig. 4).









(Facing page 478)

Table 1. Nuclear concentration of radioactivity in rat uterine tissues after subcutaneous injection of [^{3}H]-progesterone

Treatment	Time after injection (min)	Luminal epithelium	Stromal cells	Glandular epithelium	Uterine muscle cells
OV	5	0	(+)	0	+
OV+E	5	0	+	0	++
OV+Ad+E	5	0	+	0	++/+++
OV	15	0	(+)	0	+/++
OV+E	15	0	+	0	++/+++
OV+Ad+E	15	0	+	0	+++
OV	60	0	(+)	0	+
OV+E	60	0	+	0	+
OV+Ad+E	60	0	+	0	++

Adult Holtzman rats 2 months old. OV = ovariectomy; OV + Ad = ovariectomy and adrenalectomy; E = Estradiol-17β injection 1 μg in 0·2 ml sesame oil daily for 3 days. The degree of intensity of silver grains is expressed by an arbitrary scale: where 0 is equal to or only little more than background; (+) = 2 to 3 times background; and +, ++, and +++ designate low, medium, and high intensity respectively.

contrary to estrogen, may find some explanation from our autoradiographic results. In the biochemical experiments, different uterine tissues with different binding affinities are contained in the homogenate which may obscure the selective binding of uterine muscle, clearly demonstrable in our autoradiograms. Evidence for a comparable selective cellular and subcellular distribution could be obtained with biochemical techniques, provided muscle and endometrium can be separated satisfactorily and are studied individually.

The chemical nature of the selectively concentrated radioactive label has not been identified in our experiments. In subcellular fractions of rat uteri, Trams *et al.*[5] reported that 20 min after injection most of the radioactivity corresponds to progesterone whereas after 3 h the major portion is a metabolite. Accordingly, the nuclear radioactivity in our experiments may be progesterone or, perhaps, more likely one of its metabolites, 5α-dihydroprogesterone[10].

The distribution in the rat uterus of progesterone and/or its metabolites differs from the distribution of [^{3}H]-estradiol as demonstrated earlier[17]. It has been shown by dry-autoradiography, in essential agreement with biochemical data, that in the immature intact and mature castrated rat [^{3}H]-estradiol concentrates in nuclei of luminal and glandular epithelium, substantia propria cells, and muscle cells as well. Under pretreatment with progesterone, [^{3}H]-estradiol shows a different distribution with no or diminished nuclear binding in the luminal epithelium[18, 19], but high concentration in glandular epithelium and certain areas of the substantia propria cells[19]. No such drastic changes appear to occur in muscle cells.

While there is an apparent 'receptor shift' for estrogen after progesterone pretreatment, regarding different uterine tissues, no such qualitative change was seen for progestin distribution after estrogen pretreatment. After estradiol pretreatment, the capacity to concentrate [^{3}H]-progesterone and/or metabolites of it appears to be increased in nuclei of muscle cells and substantia propria cells, the same tissues that show, however, lesser affinity without pretreatment. This latter observation is in agreement with biochemical data[6, 10]. Perivascular cells of the intermuscular layer did not show concentration of radioactivity after [^{3}H]-progesterone administration. This is in contrast to our observations with [^{3}H]-estradiol which shows a distinct nuclear labeling of these perivascular cells. The

localization of estradiol in these cells may be related to the characteristic early estrogen effects of vascularization and water imbibition.

The localization of progestin in nuclei of stromal cells may be correlated to changes that occur in these cells under the influence of progesterone, which lead to decidualization. Effects of progesterone on uterine muscle are also well known and have been reported to be in part synergistic with, and in part antagonistic to estrogen[20]. As in the stromal cells, the nuclear concentration of 'progestin' in muscle cells suggests a genomic effect, which, in view of the high binding affinity in the rat deserves further investigation.

While there is no or only little radioactivity visible in nuclei of luminal and glandular epithelium, progestin effects on these cells apparently do exist. Progesterone pretreatment of ovariectomized–adrenalectomized rats for at least 12 h abolished the response of the uterine luminal epithelium to estradiol with respect to mitotic activity and nucleolar enlargement[18]. The alteration of mitotic response to estradiol by progesterone pretreatment was earlier reported for the mouse uterus by Martin and Finn[21]. In the glandular epithelium, progesterone prevented the estrogenic induction of mitotic activity, but not the stimulation of [^{3}H]-uridine uptake[18]. The question remains to be answered: how does progestin act on luminal and glandular cells without the typical nuclear concentration and binding known for other steroid target tissues? The effect could be an indirect one, through, for instance, the progestogenic induction of tropic hormone secretion, with subsequent action of, perhaps, LH on these cells. The possibility must be considered that receptor interaction may occur without lasting and detectable binding and accumulation of specific binding proteins in nuclei of target cells, existing as another mode of steroid-target tissue interaction. Considering the autoradiographic cellular distribution of estrogens and progestins in the uterus, it is apparent that the same cell can be chemically addressed by different hormones. This has been suggested from other autoradiographic studies of the pituitary and brain for estradiol and androgen[14].

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