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ORIGINAL ARTICLE

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Anatomical variation of the oestrogen receptor in normal myometrium

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Abstract Tissue from 20 surgically resected uteri was examined in order to test the hypothesis that the oestrogen receptor content of the myometrium may not be uniform throughout the uterus. All patients were premenopausal, had no history of any exogenous steroid hormone use and were in the proliferative phase of the menstrual cycle. Material was taken from the subserosal, midmyometrical and subendometrial regions of both the fundal and lower uterine segments. Care was taken to remove all endometrium. Suitable samples were analysed by radioimmunoassay (RIA) for oestrogen receptors. An adjacent block from each area was fixed in formalin and stained immunocytochemically for oestrogen receptors. The results of the RIA show a significantly higher receptor content in the subendometrial region than in either the midmyometrial or the subserosal region. No significant difference was demonstrated between the numbers of receptors in the fundus and in the lower uterine segments. The differences were also well demonstrated by immunocytochemistry, according to which 83% of all cells counted in the subendometrial region were positive, while only 61% of cells in the middle and 47% of those in the subserosal region were positive.

Key words Immunocytochemistry · Myometrium · Oestrogen receptors · Radioimmunoassay · Uterus

Introduction

In recent years the accurate localisation and quantification of various hormone-specific receptors has become valuable in the identification and prognostic assessment of many disease states [4, 26]. Groups of hormone-specific proteins or receptors that bind the reproductive steroid hormone oestrogen preferentially [6, 10, 17] have been isolated in both breast and uterine tissue [15, 29]. The interaction between oestrogen and its specific receptor produces a conformational change at the midreceptor region, unmasking high-affinity DNA-binding sites with subsequent DNA activation [7] and, ultimately, the synthesis of new proteins. Thus, under the influence of oestrogen, increases in uterine size and endometrial proliferation are achieved [2]. This oestrogen-receptor complex interaction also produces an increased affinity for subsequent oestrogen binding, as well as the activation of progesterone-specific receptor production [25].

Multiple studies involving the reproductive steroid hormone receptor for oestrogen have been conducted in uterine tissue, in an attempt to elucidate the relationships between the phase of the endometrial cycle, the level of circulating oestrogen and the quantities of myometrial oestrogen receptor [21, 23]. Variations between the receptor levels of normal and diseased uterine tissue have also been investigated [13, 15, 16, 24, 27, 31]. In most of the studies involving myometrial tissue a basic assumption is made that the distribution of the oestrogen receptor is uniform throughout the depth of the myometrium.

This study was undertaken to determine whether this assumption holds true.

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Materials and methods

Samples of uterine tissue were obtained from 20 patients undergoing hysterectomy for either menorrhagia or dysmenorrhoea. The patients ranged in age from 35 to 45 years and had no history of exogenous reproductive steroid hormone administration. At the time of surgery all patients were calculated to be in the proliferative phase of the endometrial cycle. Uteri were sectioned in the sagittal plane within 10 min of surgical removal from the patients. Two parallel transmural blocks of tissue were dissected from both the fundus and the lower segment of each uterus. One block from each region of six suitable uteri was further subdivided into three equal portions following careful resection of the endometrium and serosa. These blocks were labelled subendometrial, middle and subserosal before being snap-frozen in liquid nitrogen. This tissue was subsequently stored in a −70° C freezer until analysed by ra-

dioimmunoassay (RIA). The remaining blocks of fundal and lower segment tissue, with the endometrium and serosa intact, were used for routine histology and immunohistochemistry, following fixation in 10% formalin and processing to wax.

Wax sections 1 μm thick stained with haematoxylin and eosin (HE) were used to confirm both tissue normality and the endometrial cycle phase. The anatomical position of dissection (fundal or lower segment) was also confirmed histologically. The sections were viewed by standard bright-field microscopy.

The oestrogen receptor content of the myometrium was determined by RIA using the single saturation point assay method [12]. The tritiated steroid [2,4,6,7-^{3}H]oestradiol (102 Ci/mmol), obtained from Amersham International Laboratories (Amersham, Bucks., England) was used at a final concentration of 6 nmol. The concentration of oestradiol was measured by an exchange procedure in which the unbound hormone was removed using dextran-coated charcoal [5]. The binding data were analysed by Scatchard analysis [22] and expressed as ferntomoles per milligram of cytosolic protein. This protein was quantified by the method of Lowry et al. [11], bovine serum album being used as standard.

Immunocytochemical staining for oestrogen receptors in the paraffin-embedded tissue was performed by the avidin-biotin technique. The technique, described in detail elsewhere [8], can be summarised as follows: blocked dewaxed sections were microwaved in citrate buffer (pH 6.0) in a 600-W household microwave oven at 75% power for two 10-min periods to effect antigen retrieval. Sections were successively treated with (1) normal rabbit serum (1:20 dilution) and then (2) the monoclonal oestrogen receptor antibody (Dako-ER M7047 1:50 dilution), after which (3) a biotinylated rabbit anti-mouse was applied. (4) Streptavidin at a 1:500 dilution was applied prior to (5) diaminobenzidine-H_2O_2 (DAB). Each antibody application was followed by a 3-min wash in TRIS buffer (0.01 M TRIS-HCL, 1.5 mM ethylenediaminetetraacetic acid, pH 7.4). The sections were then lightly counterstained with haematoxylin, dehydrated and mounted. Sections were viewed and photographed by Hoffman modulation contrast microscopy [20].

For counting purposes, each section was subdivided into subendometrial, midmyometrial and subserosal regions. The total cell populations of 120 oil immersion fields were counted in each region where any cell showing evidence of the DAB reaction product was considered to be positive. A quantitative assessment of the percentage of nuclear staining of positive cells was conducted on a random sample of tissues from 12 of the 20 patients, in which 1000 positive nuclei in each region were examined. Nuclear staining was divided into three broad categories. First, those nuclei in which less than one third of the nucleus was obscured by reaction product. Secondly, those in which between one third and two thirds of the nucleus was stained, and finally, those with greater than two-thirds staining.

Results

Bright-field microscopy of the HE-stained sections confirmed that the dissected blocks of tissue were taken from the fundal and lower segment regions, that all uteri were in the proliferative phase of the endometrial cycle [3] and that the tissue was histologically normal. In all uteri the HE sections of the subserosal tissue appeared less cellular than those of the subendometrial tissue (Fig. 1).

Table 1 shows the results for the receptor content of the myometrium, as determined by RIA. The oestrogen receptor content of the subendometrial portion of the myometrium is shown to be statistically significantly greater than that of the middle and subserosal regions (Student's *T*-test [1], $P<0.01$). No significant difference is demonstrated between the receptor contents of the middle and subserosal regions. The receptor content of

Table 1 Myometrial concentration (fmol/mg)[a] of oestradiol receptors as determined by radioimmunoassay (*M* middle, *SE* subendometrial, *SS* subserosal regions)

Patient	SE (n=20)	M (n=20)	SS (n=20)	Fundus (n=30)	Lower segment (n=30)
1	25.69	13.56	8.03	15.70	15.81
2	11.92	10.50	4.63	8.51	10.84
3	44.43	16.43	13.03	25.71	23.55
4	43.51	10.50	10.08	22.41	20.58
5	20.98	6.86	5.59	12.63	9.65
6	43.29	26.19	15.63	32.57	24.16
Mean	31.64	14.01	9.50	19.59	17.43
SD	13.99	6.78	4.28	8.95	6.31
SEM	0.44	0.48	0.45	0.46	0.36

[a] Subendometrial region significantly different from subserosal and middle regions; no significant difference between fundus and lower segment regions

Fig. 1 Haematoxylin and eosin (HE) stained section of **a** subendometrial myometrium and **b** subserosal myometrium. Note the marked difference in cellularity between the two regions. ×135; *bar* 100 μm

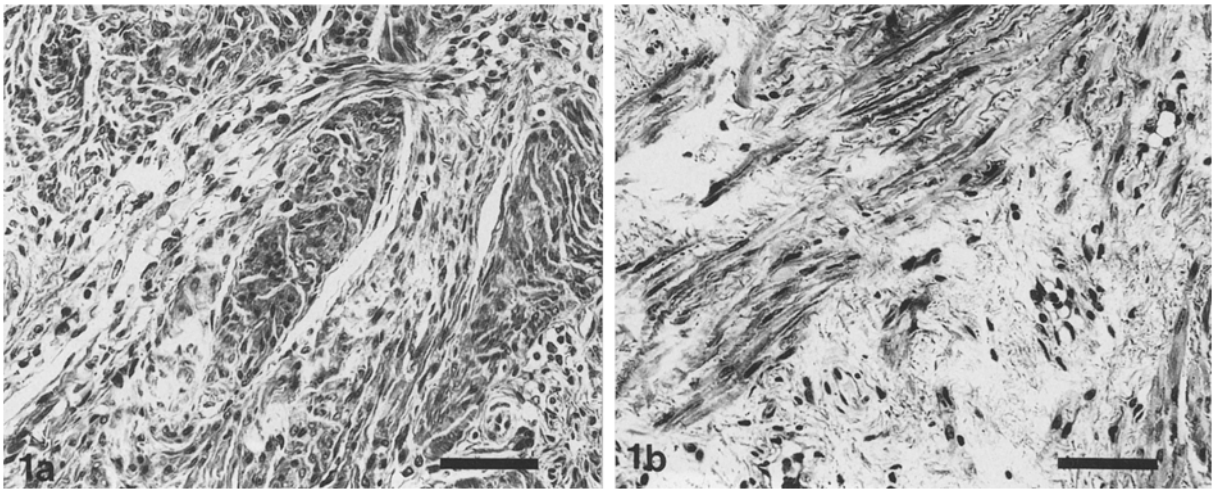

Table 2 Oestrogen receptor staining[a] of fundal region (*Tot cells/HPF* total cells counted per high-power field, *Pos/HPF* total oestrogen-receptor-positive cells counted per high-power field)

Area	Subendometrial		Midmyometrial		Subserosal	
	Tot cells/ HPF (n=20)	Pos/ HPF (n=20)	Tot cells/ HPF (n=20)	Pos/ PHF (n=20)	Tot cells/ HPF (n=20)	Pos/ PHF (n=20)
Mean	111.70	88.15	60.55	39.70	36.75	16.70
Max.	133	110	91	67	55	33
Min.	83	68	46	20	11	7
SD	12.66	12.06	13.51	15.25	10.61	7.38
SEM	0.11	0.11	0.14	0.22	0.38	0.29

[a] Positive cells per high-power field in the subendometrial region significantly more numerous than in subserosal and midmyometrial regions

Table 3 Oestrogen receptor staining[a] of lower segment region

Area	Subendometrial		Midmyometrial		Subserosal	
	Tot cells/ HPF (n=15)	Pos/ HPF (n=15)	Tot cells/ HPF (n=15)	Pos/ HPF (n=15)	Tot cells/ HPF (n=15)	Pos/ HPF (n=15)
Mean	105.27	94.13	55.53	32.40	37.53	18.67
Max.	140	136	79	49	47	37
Min.	71	62	38	17	27	7
SD	20.80	24.37	11.66	8.77	6.29	7.83
SEM	0.20	0.20	0.26	0.21	0.27	0.17

[a] Positive cells per high power field in the subendometrial region significantly more numerous than in subserosal and midmyometrial regions

the fundal and lower uterine segments is also shown in Table 1. No significant difference is discernible between these two segments.

In immunohistochemically positive cells, the DAB reaction product is limited to the nucleus, with no evidence of cytoplasmic or background staining. The nuclei of the uterine blood vessels to not stain for the oestrogen receptor. Variations in myometrial cellularity, expressed as total cells per high-power field, are presented in Tables 2 and 3. Of the three regions, the subendometrial region proves to be the most cellular, confirming the qualitative assessment of the HE sections. There are significant differences between the numbers of receptor-positive cells in the three transmural myometrial regions (analysis of variance test, $P<0.0001$). Almost 83% of all cells counted in the subendometrial region were positive, while only 61% and 47% of cells in the middle and subserosal regions were positive. No statistically significant difference in positivity is discernible between the regions of the fundus and lower uterine segment (Tables 2, 3). The percentage nuclear staining of the positive cells varied greatly. In the subendometrial region the entire nucleus was often obscured by reaction product (Fig. 2), while in the middle and subserosal regions, nuclear staining was often circumferential or unilateral (Fig. 2).

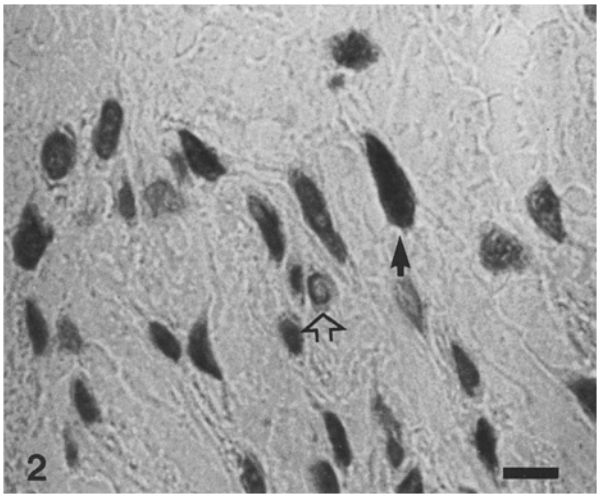

Fig. 2 Section of midmyometrium showing nuclear localisat in oestradiol receptors. *Closed arrow* indicates full staining of the nucleus, while *open arrow* indicates a circumferentially stained nucleus. ×775; bar 10 μm

Quantitative assessment of the percentage nuclear staining reveals that over 60% of nuclei within the subendometrial region were greater than two-thirds stained, while in the subserosal region an almost equivalent proportion of nuclei are less than one third stained (Table 4).

Discussion

In recent years the biochemistry of the oestrogen receptor has been more clearly elucidated [7, 14]. It is now known that the receptor is a purely nuclear resident [9, 18, 30], which is only loosely bound to the nucleus when in the unoccupied state. Any disruption of the cell, such as that produced by RIA techniques, causes the oestro-

Table 4 Percentage nuclear staining of normal myometrium in proliferative phase

Area	Subendometrial (n=12)			Midmyometrial (n=12)			Subserosal (n=12)		
% Stain	<33%	33% to 66%	>66%	<33%	33% to 66%	>66%	<33%	33% to 66%	>66%
Mean	8.92	29.00	62.08	19.92	62.17	17.92	64.25	29.75	6.00
Max.	31	47	85	41	74	32	86	47	18
Min.	3	11	41	8	49	7	49	12	1
SD	7.53	10.51	13.43	10.18	7.79	8.49	10.91	9.48	4.95
SEM	0.84	0.36	0.22	0.51	0.13	0.47	0.17	0.32	0.83
% Area	8.92	29.00	62.08	19.92	62.17	17.92	64.25	29.75	6.00

gen receptor to leak out into the cytoplasm. The cytosolic receptor fraction measured by RIA is thus an extraction artefact, and the value of such figures is of minimal use in the determination of total actual quantities of oestrogen receptors. However, the figures can be useful for investigating trends, provided that all the samples are treated in precisely the same manner and are quantified at the same time. As evidenced by the results obtained in this study, a difference does exist insofar as there is an increase in the myometrial receptor content of the subendometrium compared with that of the subserosa. These results, and the nuclear nature of the receptor may provide a partial explanation for the large standard deviations obtained by previous researchers into myometrial oestrogen receptor content [13, 21].

Immunocytochemistry, in comparison with RIA, provides both a more accurate and a visual method for localisation of the oestrogen receptor [19]. As noted by others, the oestrogen receptor is confined to the nucleus of positive cells [9, 18, 30]. Tsibris [28] postulated a steep concentration gradient in the oestrogen receptor levels from the fundal to the cervical regions of the human uterus, suggesting that this gradient persists throughout the menstrual cycle. We, like Press [19], have not been able to demonstrate such a gradient. However, from the immunological cell counts obtained for positivity, it appears that there are transmural differences in the concentration gradient of the oestrogen receptor in the human myometrium.

For the purposes of this study Hoffman modulation contrast microscopy was used. This form of microscopy eliminates tissue variations in the intensity of staining in terms of light reflection, as well as offering a 3D-type picture of the cells, with enhancement of the visualisation of DAB [20]. Being an operator-independent system, it is an accurate form of microscopy for quantification purposes, as it allows for repeatability. A number of scoring systems are available that take intensity of staining into account [14]; however, many of these systems, although accurate and repeatable, are cumbersome and time-consuming. In this study the form of microscopy used has eliminated certain variables, making the scoring of nuclear staining simple and easier to interpret. In general, the differences in the percentage nuclear staining seen using this method reflect the pattern of oestrogen positivity within the myometrium. The subendometrial region not only has a higher percentage of positive cells; the cells also show greater amounts of reaction product, compared with those in the subserosal region.

Whether the differences noted in the transmural myometrial receptor concentration and nuclear staining percentages are due to varying levels of receptor production, or to sensitivity, remains unclear. This study will be repeated to examine the remaining components of the endometrial cycle. One of the most striking features of this study is its confirmation of the need for standard sampling techniques in order to ensure experimental repeatability and the avoidance of error due to intratissue variation.

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