J. steroid Biochem. Vol. 36, Nos 1/2, pp. 43–55, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

SPATIAL AND MOLECULAR ASPECTS OF ESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION IN HUMAN UTERI AND UTERINE CARCINOMAS

GÜNTER VOLLMER,* MARGIT KNIEWE, UTE MEYN, LARA TUCHEL, HANS ARNHOLDT[1] and RUDOLF KNUPPEN

Institut für Biochemische Endokrinologie and [1]Pathologisches Institut Medizinische Universität, Ratzeburger Allee 160, D-2400 Lübeck, F.R.G.

(*Received* 29 *March* 1989; *received for publication* 16 *January* 1990)

Summary—The expression of steroid hormone receptors as molecules reflecting processes of development and differentiation in the human uterine tissue was analysed in a spatial distinct fashion: tissue samples were excised at the fundus and at different, spatially distinct positions of the uterus. They were analysed for concentrations of cytosolic estrogen and progesterone receptors in supernatants from frozen sections using an isoelectric focusing technique. The spatial and molecular distinct, qualitative and quantitative pattern of their expression in the human uterus and uterine adenocarcinomas were studied by sectioning tissue sample from the functionalis through the basalis of the endometrium until reaching deep myometrial parts of the tissue: (1) Specific spatial patterns of estrogen and progesterone receptor levels were detectable throughout the menstrual cycle. (2) For proliferative endometrium from the functionalis to the basalis of the endometrium, the content of both cytosolic receptor species increased up to 6-fold. (3) Differences detectable were less pronounced in the myometrial part of the tissue. (4) Differences of steroid receptor concentrations measured in the endometrium at different uterine positions were highest between fundus and corpus of the endometrium. (5) Maximal differences were detectable around ovulation. (6) After secretory transformation of the organ, specific patterns were still detectable, however quantitative differences were less pronounced. (7) Additionally, quantitative differences measurable were accompanied by variations of molecular properties of the progesterone receptor as demonstrated in an isoelectric focusing gel. (8) In endometrial adenocarcinomas, not only significant quantitative alterations in steroid receptor content were measured, but also a significantly changed spatial pattern of receptor concentrations, also a change of the molecular properties of the progesterone receptor was resolved if these tumor parameters were compared to those detected in the normal tissue of the same organ surrounding the tumor.

INTRODUCTION

The human uterus is an organ exposed to a periodically changing hormonal environment [1, 2]. In response to the hormonal exposure the concentrations of estrogen (ER) and progesterone receptors (PR) undergo characteristic variations throughout the menstrual cycle. With ligand binding assays these changes have been resolved in normal uterine tissue [3–6] and in uteri bearing endometrial carcinoma [7–9]. After the availability of specific monoclonal antibodies against the ER [10] and the PR [11] the quantitative alterations in receptor content could be attached to the precise cellular localization, stromal or epithelial, of the altered receptor species, both in the normal uterine tissue dependent on the respective stage of the menstrual cycle [12–15] and in the endometrium after its neoplastic transformation [16, 17]. Concerning spatial aspects of receptor expression only comments on receptor distribution between endometrium and myometrium or on differences between receptor concentrations of functionalis and basalis of the endometrium were made [12, 13].

Like others [18] with homogenated endometrial tissue we tried to establish the specific spatial distribution of quantative levels of ER and PR dependent on a given stage of the menstrual cycle and dependent on spatially distinct positions of the uterus. For comparison the resolved pattern of ER and PR expression in the endometrium were compared to those detected in the myometrium and in the endometrial adenocarcinoma as well as to transversally sectioned tissue specimens. The observations were validated by comparative histological and enzymatic examinations and by studying alterations of the PR at molecular levels, which can be done if the receptor content is quantitated by isoelectric focusing.

*To whom correspondence should be addressed.

MATERIAL AND METHODS

Tissues and preparation of the tissue

Uterine tissue of 18 women with an apparently normal menstrual cycle (13 proliferative specimens, one specimen originating from a uterus immediately after ovulation and four secretory specimens) and 9 cases of endometrial adenocarcinomas were included in this study. Uteri were brought to the department of pathology immediately after hysterectomy performed for different reasons e.g. descensus uteri, endometrial carcinomas as well as carcinoma *in situ* of the squamous epithelium of the cervix.

To study spatial aspects of uterine development, samples to be analysed were excised from as many different uterine positions as possible. In normal uteri samples from the midfundus within the uterine cavity, samples from the midcorpus uteri, a sample originating from a peripheral position of the corpus quite close to the cervix and in some cases a specimen from the uterus near the tube were taken. Uterine positions where the specimen originated are illustrated in Fig. 1. The samples containing both endometrium and myometrium were fixed to a piece of cork by deep freezing in liquid nitrogen with the myometrial part attached to the cork [19].

In cases of endometrial adenocarcinoma a sample was excised out of the tumor and cut of adhering normal tissue as complete as possible. For comparative studies, if possibe, specimen of normal tissue originating from the same uterus were prepared as described above.

To obtain a spatial orientation within a given piece of tissue the sandwiches, prepared as described above, were sectioned from the luminar surface of the endometrium through the basalis of the endometrium until reaching deeper myometrial portions of the tissue. Within a given plane sections were collected alternating for ER and PR quantitations and in some cases for the quantitative determination of estradiol-17β hydroxysteroid dehydrogenase (17β-HSD) as well. As controls transversally sectioned tissue was used, meaning each section approx. contains the same tissue composition.

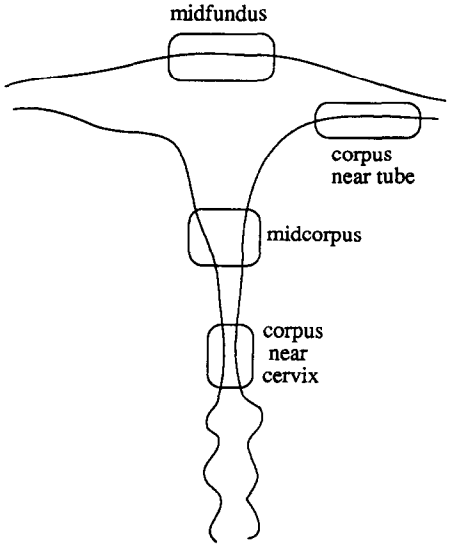

Fig. 1. In this figure the positions in the uterus where specimens were obtained are schematically illustrated.

Chemicals and buffers

Radiolabelled ligands [2,4,6,7-^{3}H]estradiol (85–110 Ci/mmol), [6,7-^{3}H]estradiol (40–60 Ci/mmol) and [^{3}H]ORG (16α-ethyl-21-hydroxy-19-nor[6,7-^{3}H]-pregn-4-ene-3,20-dione); (40–60 Ci/mmol) as well as unlabelled ORG 2058 were purchased from Amersham (Braunschweig, F.R.G.). Estradiol-17β, acrylamide, *N,N'*-methylenebisacrylamide (BIS) and inorganic buffer substances were from Merck (Darmstadt, F.R.G.). Ampholines were from LKB (Sweden). The buffer for all hormone receptor analyses was composed of 10 mM NaH_2PO_4, 10 mM $Na_2MoO_4 \cdot 2H_2O$, 10% glycerol (v/v), 1.5 mM EDTA, 1% monothioglycerol (v/v); pH = 7.4. The enzymatic analyses for 17β-HSD activity were performed in the a buffer composed of 5 mM Na_2HPO_4, 1 mM dithiothreitol, 10 mM $Na_2MoO_4 \cdot 2H_2O$ and 10% glycerol (v/v); pH = 8.5).

Preparation and incubation of sections for receptor determination

ER and PR quantitations and assays for 17β-HSD were performed as described recently [19–21]. For DNA determination insoluble cellular components and tissue ghosts were centrifuged (800 ***g***; 10 min; 0°C). Thereafter the tissue was washed with phosphate buffered saline once, centrifuged as described above, and DNA was extracted and determined using the diphenylamine reaction [22] with the modification of Richards[23].

For evaluation, IEF-gels were placed on diagrammatic paper. Then gels were cut into sample containing stripes and thereafter fractioned manually into 3 mm wide pieces. For this purpose we used a microtom knife spanning the entire width of the gel. In this way the results and pattern obtained for a single receptor species of a single specimen in various section planes were highly reproducible. To obtain quantitative results graphs of radioactivity vs slice number were drawn for each sample and the cumulative height of the points in a single peak attributable to receptor-bound hormone was measured above the diagramatic baseline. The amount of hormone receptor within this peak was calculated and the result expressed in fmol/mg supernatant protein or DNA.

Assay for estradiol-17β hydroxysteroid dehydrogenase activity

The method for the quantification of 17β-HSD-activity, an enzyme predominantly located in the mitochondrial and microsomal sub-fraction of the cells [24], and the kinetics of the enzyme reaction has

been described recently [21]. We proceeded as described earlier with modifications in the preparation of the tissue. The extracted, insoluble residue of the sections was carefully rinsed by three washes with phosphate buffered saline by intermediate centrifugation at 800 *g* at 4°C for 10 min. Thereafter cell particles were resuspended in 275 μl buffer supplemented additionally with 20 μM estradiol and 0.5 μCi [6,7-^{3}H]estradiol. The enzymatic reaction was started by the addition of 50 μl NAD (2 g/l). The incubation and separation of the estrogens was performed as already described. The loss during the experimental procedure was estimated directly by u.v. detection of the estrone added as carrier. For protein determination [25] the small remaining organic phase was evaporated and the complete residue solubilized in the presence of 0.3 M KOH.

Finally, as a control the first and the last section (8 μm thick) within a given section plane was stained for histology with a standard hemalum/eosin procedure.

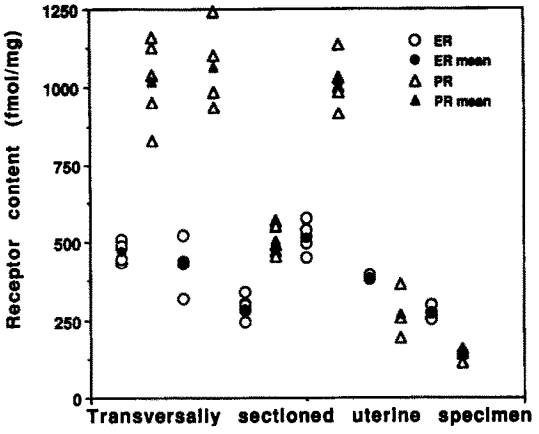

Fig. 2. Controls: as controls transversally sectioned tissue samples were used. This means each section comprises approx. the same amount of endometrial and myometrial tissue and therefore the receptor concentrations measured in the extracts of these sections should be independent of the section plane and approx. identical. The quantification of the concentrations of the ER (circles) and PR (triangles) for individual samples (open symbols) and the mean values (solid symbols) for transversally sectioned tissue is shown.

RESULTS

To study spatial aspects of uterine development we have examined uteri from premenopausal women with an apparently normal menstrual cycle undergoing hysterectomy. The results of these analyses were compared to those obtained by the analysis of nine uterine adenocarcinoma and their surrounding normal tissues. Each uterus was analysed at 2–6 independent uterine positions in 4–9 independent section planes. Although determinations within a given section plane were not performed in duplicate and therefore runaways could not be ruled out, the validity of the results and of the conclusions drawn is substantiated by more than 1200 analyses for ER and PR.

Controls

As controls transversally sectioned tissue samples were used. Typical analyses are illustrated in Fig. 2. For each specimen individual determinations and the mean values are plotted.

Endometrial/myometrial relation

Differences in receptor content between functionalis and basalis of the endometrium and the relation of the expressed receptor levels to those detected in the myometrium are more evident during the proliferation phase of the menstrual cycle than during the secretory phase. As illustrated in Fig. 3 and schematically summarized in Fig. 4 the cytosolic receptor content measured, typically increases from the luminar surface of the endometrium until approx. the basalis of the endometrium at the border of endometrial and myometrial parts of the tissue. In the myometrium the receptor content decreases, but is less pronounced than the increase in the endometrial part of the tissue. The described pattern for the endometrial part of the tissue is resolvable even if all experiments performed with endometrial tissue are summarized (Fig. 5). The average of all experiments shows that these patterns are more obvious for PR than for ER. In comparison to these mean values during the secretory phase of the menstrual cycle the observed pattern becomes less pronounced (Fig. 6; ER) or even irregular (Fig. 6; PR) as illustrated with a specimen originating from the early secretory phase of the menstrual cycle.

As the maximum we measured up to a 13-fold increase in PR concentration and about a 11-fold increase in ER concentration from the functionalis to the basalis of the endometrium. The maximal differences in the myometrial part of the tissue were in a single case 3-fold for ER and 2.3-fold for PR, however in general were below a 2-fold fluctuation. This means that the endometrial increase in receptor concentrations is much higher than the decrease in the myometrium. Additionally, these relative differences in receptor levels are dependent on the position of the uterus from which the tissue had been obtained. Again the measured differences are less pronounced or hardly detectable for tissue originating from the secretory phase of the menstrual cycle.

As described elsewhere [19] a variety of peak pattern can be resolved if analysing uterine, ovarian or mammary tissue for PR content by isoelectric focusing. With respect to changes in PR peak pattern in the endometrial/myometrial direction we were able to resolve three groups of cases: the first major group comprises samples which exhibit no change in the peak pattern (Fig. 7a). The PR in this group was mainly represented by a single peak. A second major group (Fig. 7b) is characterised by a complex peak pattern for PR in the endometrium (two or more

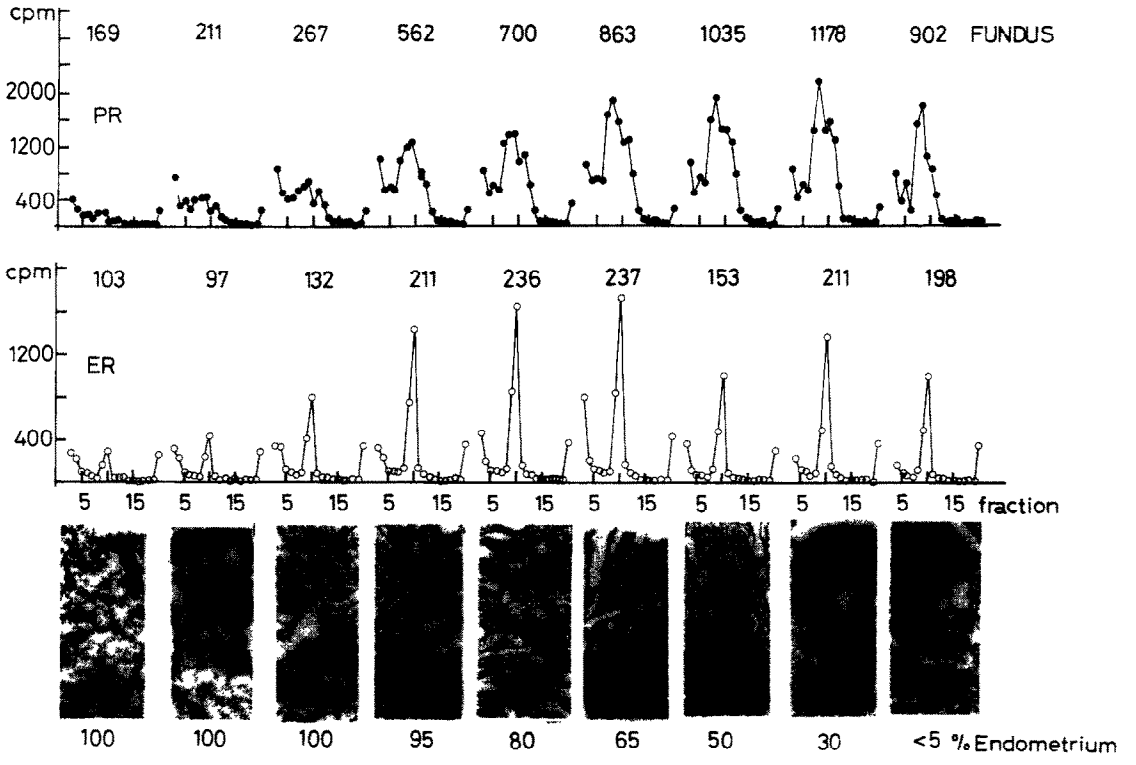

Fig. 3. The analysis of proliferative phase endometrium. The complexity of the analysis used is shown in this figure. A tissue specimen excised from a distinct uterine position, here from the fundus was sectioned from the luminar surface of the endometrium, starting on the left side of the graph through the basalis of the endometrium towards deep myometrium and analysed for ER and PR content. If the measured protein content is similar in each test tube the receptor analysis can be illustrated directly as a fractioned isoelectric focusing gel which for each individual fraction was counted for radioactivity. For all section planes the PR (upper panel), the ER (middle panel) and the hem aulaun/eosin stained control sections (lower panel) are shown. The receptor concentrations expressed in fmol/mg and the estimated composition of the tissue (% endometrial tissue) is included.

peaks or a double peak with shoulders) than in the myometrium (PR mainly represented by a single peak). In the third group, minor in occurrence, we found an alteration of the peak pattern from the functionalis of the endometrium to the basalis of the endometrium and a second change from the basalis of the endometrium to the myometrium.

Positional aspects

Quantitatively significant differences in receptor content at different uterine positions in a given section plane and therefore in the depth of the tissue are resolvable for both receptors (Figs 4 and 5). For the PR within a comparable section plane we measured up to 6-fold differences if the receptor concentrations measured in the fundus of the uterus are compared to those measured in the corpus of the identical organ. However, the data shown and discussed originate from specimens which have to be dated as proliferative tissues. More interesting in the sense of spatial differences are cases in which the organ is in the transition from the proliferative to the secretory phase of the menstrual cycle e.g. at a stage of differentiation when the hormonal environment changes immediately after ovulation. Uteri described by this stringent definition are rare in occurrance and therefore single cases have to be described.

As described above, a clear and pronounced pattern of expression of steroid hormone receptors is obvious during the proliferative phase of the menstrual cycle. Positional they become even more pronounced if the stage of the menstrual cycle is immediately after ovulation. This clearly can be demonstrated by the analysis of the uterus of a 28-year-old patient undergoing hysterectomy because of descensus uteri. For ER neither a pronounced pattern of receptor expression nor significant differences in quantities are detectable when fundus specimen, transversally sectioned fundus specimen and those obtained at the corpus region of the tissue were compared (Fig. 8a). If looking at PR levels (Fig. 8b), the spatial pattern characteristic for proliferative tissues was still detectable in the fundus specimen, while apparently most PR binding capacity of the corpus is undetectable. As a control the transversally sectioned fundus specimen is included.

Again the significant changes in receptor quantities are reflected by changes in the peak pattern of the PR. While there is a rather complex peak pattern detectable in the endometrium of the fundus (Fig. 9a),

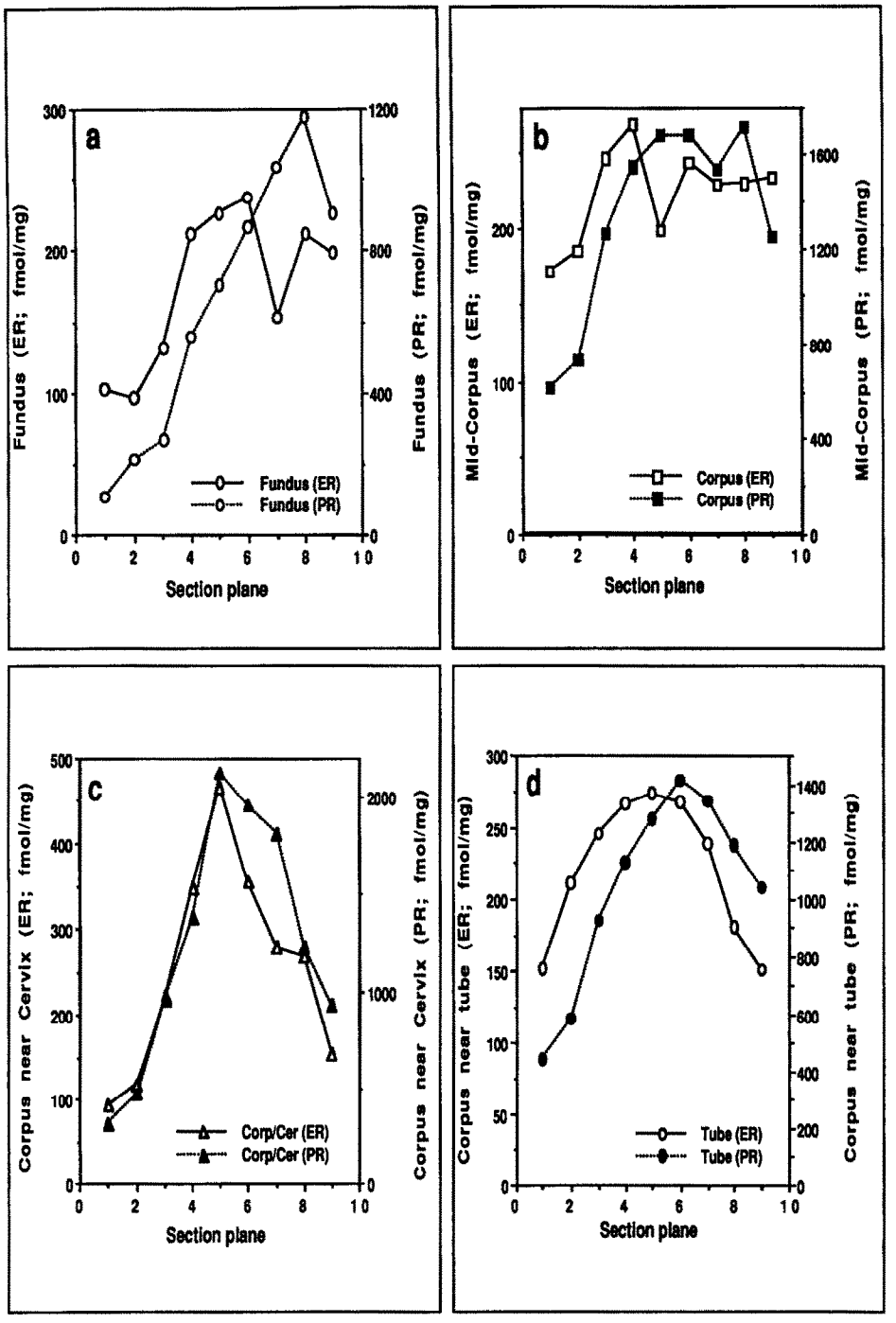

Fig. 4. Endometrial/myometrial relation: quantitative aspects in proliferative phase endometrium. To demonstrate endometrial/myometrial aspects of receptor expression quantitatively, data obtained from mid-proliferative uterine tissue are shown. The data are presented schematically as receptor content (fmol/mg) measured in a distinct section plane, starting from the luminar surface of the endometrium (first section plane) and proceeding until reaching deep myometrial portions of the uterus. Positions included are the fundus (a), the mid-corpus (b), the peripheral corpus near the cervix (c) and the uterus near the tube (d). The quantity of cytosolic receptor content increases many-fold if analysed from the functionalis of the tissue towards the basalis and decreases in the myometrium.

in the myometrium of the fundus (Fig. 9b) and in the corpus (Fig. 9c) only a single peak can be resolved.

To verify and to evidence the detected receptor pattern biologically we questioned for 17β-HSD activity. As can be delineated from Fig. 8c even for 17β-HSD activity a specific spatial pattern of expression can be resolved. Significantly, high activities are detectable in the endometrium while there is hardly

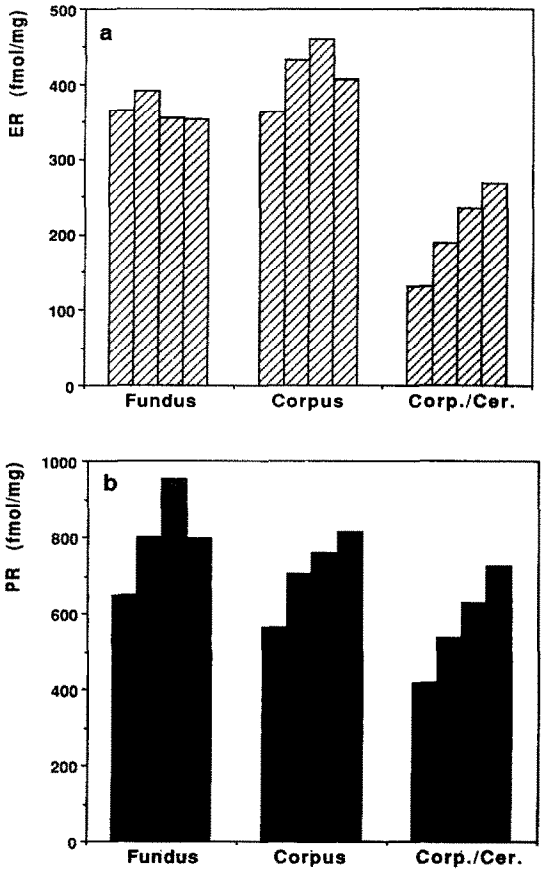

Fig. 5. Mean values of receptor content in endometrial section planes. The values for endometrial section planes of all normal specimen analysed (proliferative and secretory) were averaged and plotted. Data are expressed as fmol/mg.

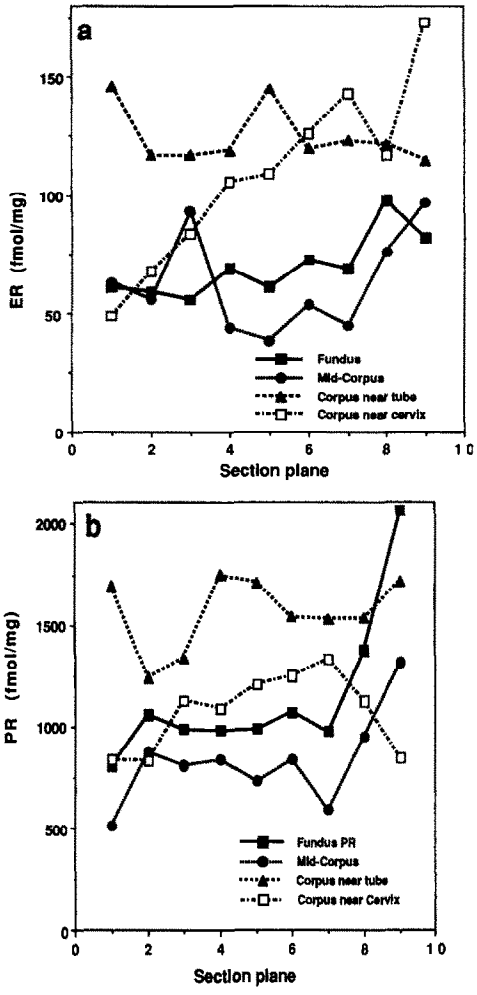

Fig. 6. Endometrial/myometrial relation: quantitative aspects in secretory phase endometrium. Here the data for an early secretory phase endometrium are shown. Again the uterus was analysed at different uterine positions (fundus, mid-corpus, corpus peripheral near cervix, and the uterus near the tube) in different section planes of the tissue starting at the functionalis of the endometrium and sectioning through the basalis until reaching myometrial parts of the tissue. In (a) the ER in (b) the PR is shown, data are expressed in fmol/mg depending on the analysed section plane.

any activity measurable in the myometrium. Positionally there are significant higher levels detectable in the corpus specimen than in the fundus area, although 17β-HSD activity in the endometrial part of the fundus is significantly higher than in the myometrium or in the transversally sectioned fundus specimen.

In other specimens originating from early secretory phase endometria usually the resolvable differences are less dramatic. In general, the receptor levels decrease if compared to those measured during the proliferative phase of the menstrual cycle. In the example shown, this finding in particular is evident for the ER (Fig. 10a). The pattern of receptor distribution becomes less pronounced or even irregular, as is demonstrated for PR (Fig. 10b). At stages later in the menstrual cycle the ER levels remain constant or slightly increase (Fig. 10c), while the measurable PR content again decreases (Fig. 10d). The resolved pattern becomes even more irregular and positional differences in receptor content at this late stage of the menstrual are by far less pronounced than those observed during the proliferative phase.

The problem of standardisation

To rule out the possibility that the analysed patterns of receptor expression are evoked by the standardisation of the data on protein, some experiments were additionally normalised to total DNA-content of the samples or for endometrial parts of the tissue to cell number/area as evaluated in the microscope. The relative differences of receptor concentrations were not significantly altered if referred to DNA content. However, the pattern of spatial expression of receptors vary slightly (figures not shown).

Endometrial adenocarcinomas

The applied method allows the parallel analysis of tumor and surrounding normal tissue in immediately adjacent tissue blocks. In general in the analysed specimen, which were mainly obtained from postmenopausal women, we made the following observations: in the normal part of this postmenopausal

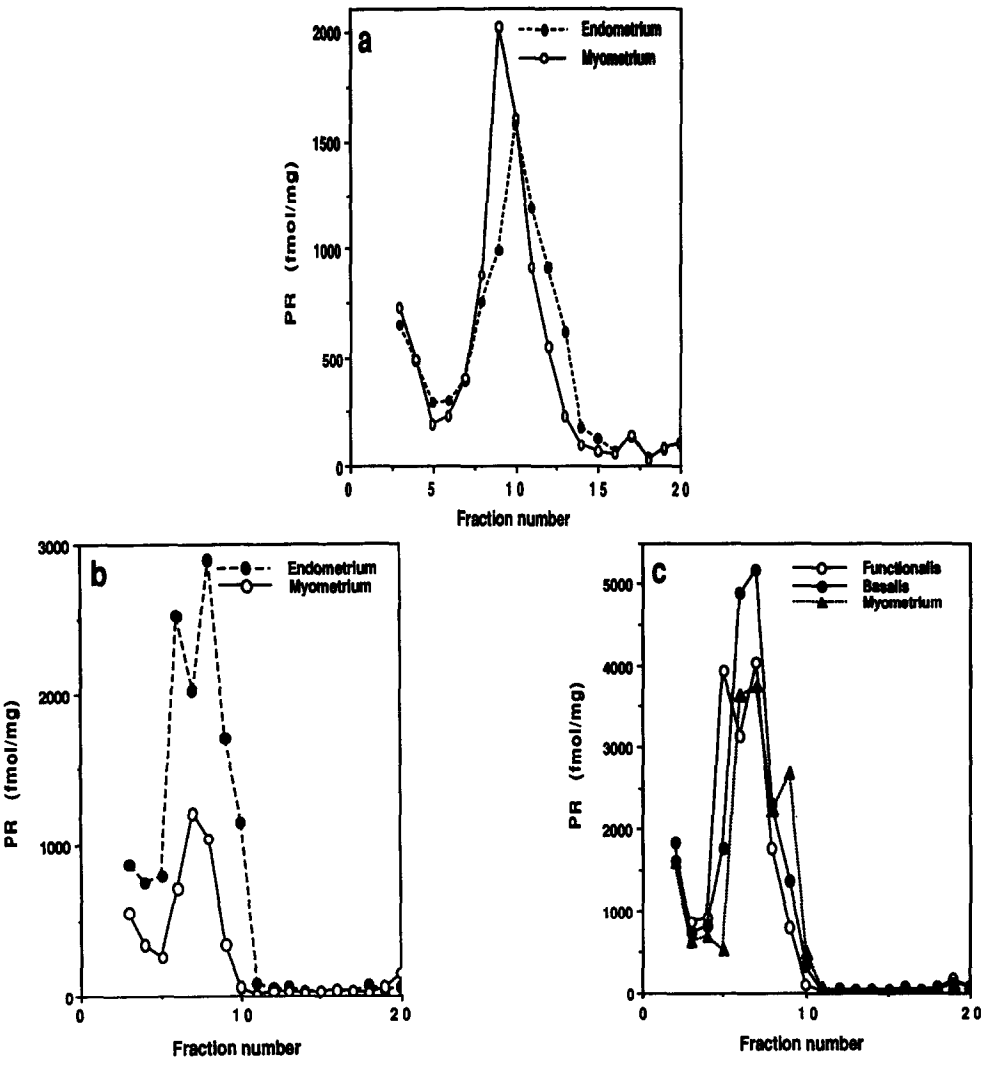

Fig. 7. Endometrial/myometrial relation: PR qualitative aspects. If steroid receptors are analysed by an isoelectric focusing procedure in a polyacrylamide gel they are concentrated according to their isoelectric points at distinct positions of the gel. If the gel is fractioned manually and the cpm measured in each fraction were plotted depending on the corresponding fraction number of the gel, as the final result a peak is obtained comprising all receptor bound, radiolabeled hormone. For the PR different peak patterns are obtained. Comparing these peak patterns in the endometrial/myometrial direction we were able to distinguish three classes of peak pattern. The first major class comprising approx 50% of the analysed uteri was characterized by a single peak for PR in the endometrium and in the myometrium (a), for the second major class of uteri analysed a complex peak pattern was resolved in the endometrium and a single peak in the myometrium (b). A single uterus has been analysed so far where the peak pattern changed from the functionalis (open circles) to the basalis (solid circles) and a second alteration was detectable in the myometrium (triangles).

tissue the spatial pattern of the distribution of cytosolic estrogen and progesterone receptors resembled that observed in proliferative uteri (Fig. 11), however, the differences in magnitude were not as prominent as observed in the normal proliferative tissue. As in 7 out of 9 uterine carcinomas in the case shown here the levels of both ER and PR were much lower in the tumor than in the surrounding normal tissue. In these cases the measured receptor content increases to deeper section planes reflecting decreasing parts of the tissue infiltrated by the tumor tissue (Fig. 11). In the noninvaded myometrium which surrounds the tumor, receptor levels are detectable which are comparable to those observed in the normal myometrium. The differences in PR-concentrations between normal and tumor tissue again are accompanied by alterations of qualitative PR peak pattern. Mainly we observed a shift from complex peak pattern in the normal tissue to a single peak in the tumor (Fig. 12), with one exception where the tumor was found to contain the most complex peak pattern.

Most striking were the profound differences in absolute receptor quantities in normal and tumor tissue. Regarding only the most superficial section plane, we had one specimen with the tumor exhibiting the higher receptor concentrations. Only in one case

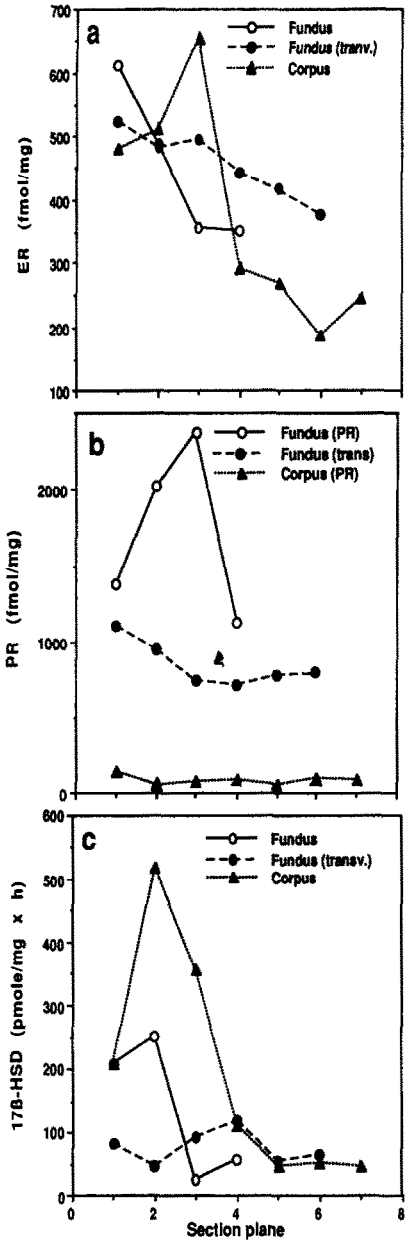

Fig. 8. Positional aspects: maximal quantitative differences. The maximal differences in receptor content between different uterine positions were measured after ovulation. Data are illustrated as receptor content per section plane. In (a) the ER measured in the fundus, a transversally sectioned fundus specimen and in the corpus is shown. Maximal differences between fundus and corpus were detected for PR (b), again a transversally sectioned fundus specimen is included as control. For verification the same sets of tissue sections have been analysed for estradiol-17β hydroxysteroid dehydrogenase (c), a progestin inducible enzyme.

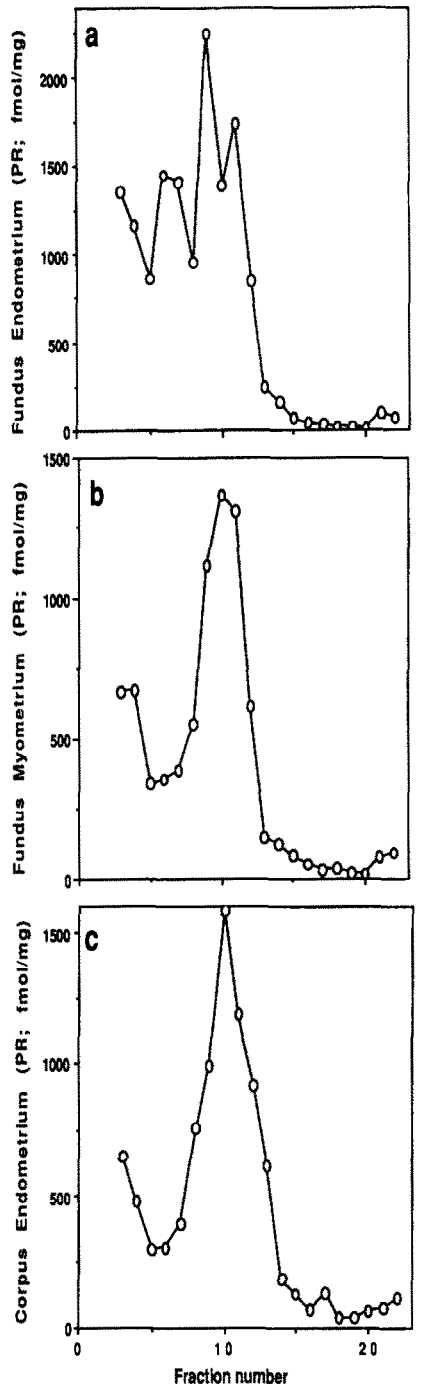

Fig. 9. Positional aspects: qualitative alterations of PR expression. The quantitative differences in PR content described in Fig. 7, which were detectable after ovulation were accompanied by significant qualitative alterations of the PR peak pattern. While the PR of the endometrium of the fundus is represented by a triple peak in the isoelectric focusing gel (a), in the myometrium at the same position (b) or in the corpus of the same organ only a single peak is detectable.

of a moderately differentiated carcinoma the receptor content of the normal tissue approx. equalled that measured in the tumor. All other carcinomas analysed exhibited a moderately or poorly differentiated morphology and were characterized by far higher receptor concentrations in the normal than in the tumor tissue (Table 1).

DISCUSSION

The periodically changing processes of development and differentiation in the adult uterine organ

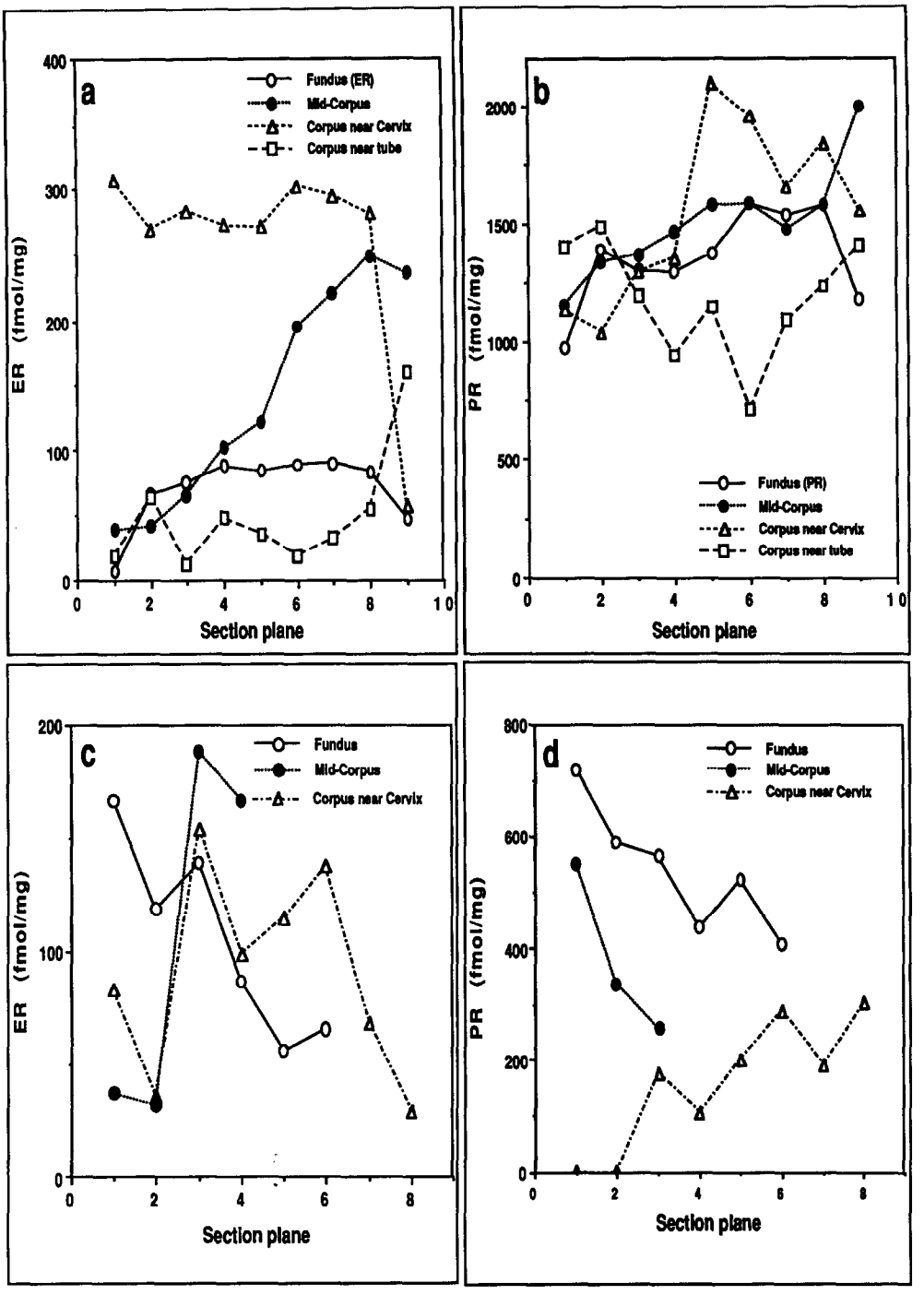

Fig. 10. Positional aspects: secretory phase endometrium. Again tissue samples were analysed from the functionalis of the endometrium through the basalis until reaching the myometrium. In general, the receptor quantities measured were significantly lower (a) especially for ER and the pattern resolved were less pronounced, as illustrated for PR (b) than during the proliferative phase of the menstrual cycle or even became irregular. At late secretory stages of the menstrual cycle ER content slightly increases again (c), while the measurable PR content decreases again (d).

were analysed to determine if spatial and temporal aspects of these processes can be resolved. Especially for proliferative endometrial tissue evidence can be provided that the quantitative expression of molecules, reflecting development and differentiation, in particularly, the expression of ER and PR, occurs in a high spatial order within this organ. Additionally, evidence is provided that especially for proliferative phase endometrium high differences in receptor concentrations can be detected if measured at different uterine positions. In contrast to Tsibris *et al.*[18] who detected a graded decline of concentrations of

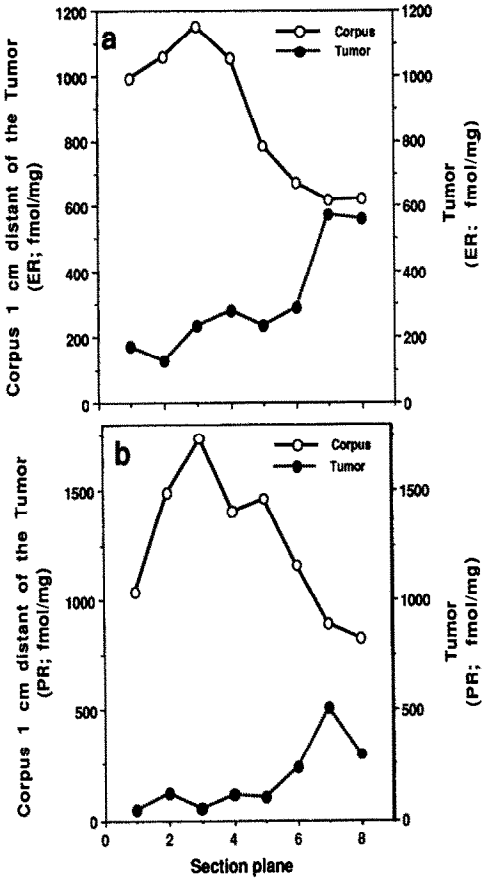

Fig. 11. Tumoranalysis: quantitative aspects of receptor expression. Completely differing quantitative patterns of receptor expression were detectable if analyses were performed in tumor or normal tissue of the identical organ. In deep myometrial section planes without tumor infiltration the ER content was similar to that of the normal tissue (a) while the PR content differed significantly (b).

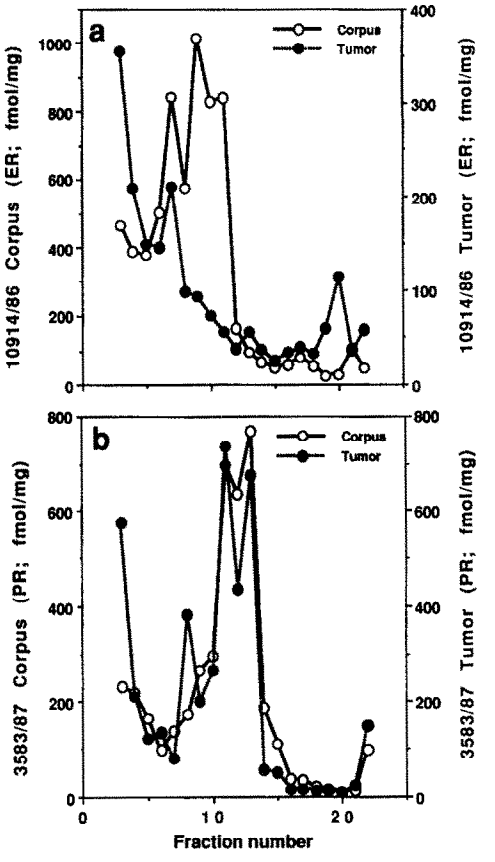

Fig. 12. Tumoranalysis: qualitative aspects of PR expression. Two crude classes of uteri bearing endometrial adenocarcinoma could be discriminated according to measured receptor quantities. The first class had significant lower receptor concentrations than the surrounding normal tissue. The second class was characterised by similar or even higher receptor concentrations in the tumor than in the normal tissue. In the first class usually in the tumor tissue only a single peak for PR was detectable, while the normal tissue exhibited the more complex PR peak pattern (a). In contrast, in the second class of tumor specimen the PR peak pattern of the tumor was as complex as that found in the normal tissue (b).

steroid hormone receptors from fundal regions towards cervical positions, we detected in particular for ER slight increases of ER concentrations from fundus to corpus regions with a significant drop of receptor concentrations towards cervical positions. Finally, for PR these quantitative patterns or resolvable positional differences are accompanied by specific variations at the molecular level, as is detectable in an IEF analysis of the PR. For ER these qualitative analyses could not be performed because for better evaluation the ER had been trypsinised prior to IEF-analysis.

Very complex changes especially on the level of PR expression are detectable qualitatively and

Table 1. Relation of steroid receptors in normal and tumor tissue of identical uteri

Case	ER Corpus (fmol/mg)	ER Tumor (fmol/mg)	ER Factor	PR Corpus (fmol/mg)	PR Tumor (fmol/mg)	PR Factor	Tumor differentiation
9757/86	1356	173	7.8	792	48	16.5	Moderate–poor
10914/86	954	59	16.2	462	269	1.7	Data not available
12207/86	369	50	7.4	—	—	—	Data not available
574/87	126	168	0.75	38	255	0.15	Moderate
1121/87	230	26	8.8	182	48	3.8	Moderate–high
3711/87	841	162	5.2	547	123	4.4	Moderate
11893/87	451	105	4.3	76	11	6.9	Poor
13651/87	354	107	3.3	385	28	13.8	Poor
14226/87	252	259	0.97	226	248	0.9	Moderate

This table summarizes the results obtained by the analysis of tumor specimen and the corresponding normal tissue of the identical organ. The results are expressed in fmol/mg protein. In addition a factor gives the relation of receptor content in normal and tumor tissue. The degree of the differentiation of the tumor is given.

quantitatively after ovulation when the tissue is in transition from the proliferative to the secretory phase endometrium. The quantitative pattern becomes far less pronounced, the significant reduction in receptor quantities found at this stage of the menstrual cycle for PR are accompanied by the reduction of a multi-peak pattern to a single peak (Fig. 9) as revealed by the IEF analysis.

Even in endometrial adenocarcinoma quantitative differences if compared to the surrounding normal tissue are found to exhibit significant alterations in the quantitative and qualitative pattern of receptor expression. Especially when those tumors with comparable receptor levels in the tumor and in the surrounding normal tissue are compared to those cases with high receptor concentrations in the normal and low levels in the tumor tissue.

We feel that our data are a completion to immunocytochemical data, which were obtained with tissue originating from the fundus uteri only. Immunocytochemical analyses of ER and PR of the myometrial receptor content detect no difference in the staining pattern of ER [12] and PR [13] throughout the menstrual cycle. A maximum staining intensity in this part of the tissue is described in the midproliferative phase [15]. In the myometrium we found slightly higher receptor quantities during the secretory phase. In the myometrium the PR is mainly represented by a single peak in the IEF analysis.

We detected the highest receptor concentrations and the most pronounced quantitative differences of their distribution in the late proliferative stage of the menstrual cycle. These quantitative findings are consistent with immunological findings since the highest percentage of labeled cells with the highest staining intensity were detectable at this particular stage by immunocytochemistry [12, 13, 15]. However, our data show significant quantitative differences in the relation of the functionalis and the basalis of the endometrium, while in this respect no comparable differences were detectable by immunological means.

During the secretory phase of the menstrual cycle immunocytochemical staining for ER disappears gradually [12, 15], this finding can now be validated by our quantitative data. The regulation of the PR obviously is much more complex in secretory endometrial tissue than is the ER. In glandular epithelium by PR immunocytochemistry high scores of labeled cells with a high staining intensity of the cells is detectable until postovulatory day three. In epithelial cells both the percentage of stained cells and the staining intensity rapidly decreased from postovulatory day 4 [13, 15, 26]. Conversely, by immunocytochemistry a high percentage of stromal cells in the functionalis of the endometrium [16] or the entire endometrium [13, 15, 26] is constitutively stained with a median staining intensity throughout the entire secretory phase. This finding suggests a different regulation of PR in stroma and epithelium. To us the question arises whether or not these functional differences are reflected by different peaks of the PR in an IEF analyses. Our findings provide evidence for this suggestion since, in the case of the early postovulatory endometrium described by us, we find proliferative phase-like receptor concentrations at the fundus location associated with a complex peak pattern for the PR. On the other hand the down regulated levels in the corpus position were associated with a single peak of the PR. In the myometrial part of the tissue the PR is also represented by a single peak and again in immunocytochemical analyses PR in myometrium seems not to be down regulated during the secretory phase of the menstrual cycle [13, 15, 26].

Whether the histochemical findings as well as quantitative and qualitative differences in PR content described here can be correlated to the specific A and B isoforms of the PR [27] which can be discriminated immunologically [28, 29] or to specific phosphorylation events [30–32] described for the human PR remains to be elucidated. First evidence for molecular changes of PR after transition from proliferative to secretory endometrium was provided by Western blot analyses [33]. They found a high molecular weight triplet and a low molecular duplet of PR in proliferative endometrium which was reduced to a high molecular weight duplet and low molecular singulet in the secretory endometrium.

With receptor concentrations as a tool we were able to discriminate two crude classes of endometrial adenocarcinomas. The first class had quite similar or even higher receptor levels compared to the surrounding normal tissue. The second class was characterised by significantly decreased receptor concentrations. It was conspicuous that the significantly different receptor concentrations were reflected by different PR peak pattern. High PR levels showed a complex peak pattern, in tumors with low PR concentrations only a single peak could be detected. To correlate these PR results to histological parameters [34] hyperplasias within the tumor [35] or to a possible clinical relevance [17] much more cases of endometrial carcinomas have to be studied.

With this study we were able to demonstrate spatial and temporal differing pattern of the expression of concentrations of ER and PR, meaning that mechanisms may exist that create an unequal pattern of the distribution of molecules reflecting differentiation, not only on a quantitative but also on a qualitative level. The question remains open if by those mechanisms e.g. implantation sites can be specified biochemically. However, first evidence exists that this might be the case because in rat uterine tissue it has been shown that implantation sites are characterised by significantly different cytosolic and nuclear ER and PR levels as is the surrounding uterine tissue bearing no oocyte [36].

Acknowledgements—This paper includes parts of the dissertation of Lara Tuchel. It was supported by the Deutsche Forschungsgemeinschaft SFB 232/A1.

REFERENCES

1. Brenner R. M. and West N. B.: Hormonal regulation of the reproductive tract in female mammals. *A. Rev. Physiol.* **37** (1975) 273–302.
2. Katzenellenbogen B. S.: Dynamics of steroid receptor action. *A. Rev. Physiol.* **42** (1980) 17–35.
3. Flickinger G. L., Elsner C., Illington D. V., Muechler E. K. and Mikhail G.: Estrogen and progesterone receptors in the female genital tract of human and monkeys. *Ann. N.Y. Acad. Sci.* **286** (1977) 180–189.
4. Levy C., Robel P., Gautray J. P., DeBrux J., Verma U., Descomps B. and Baulieu E. E.: Estradiol and progesterone receptors in human endometrium: Normal and abnormal menstrual cycles and early pregnancy. *Ann. J. Obstet. Gynec.* **136** 646–651.
5. Sanborn B. M., Kuo K. S. and Held B.: Estrogen and progestogen binding site concentrations in human endometrium and cervix throughout the menstrual cycle and in tissue from women taking oral contraceptive. *J. Steroid Biochem.* **9** (1978) 951–955.
6. Bayard F., Damilano S., Robel P. and Baulieu E. E.: Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium. *J. Clin. Endocr. Metab.* **46** 635–648.
7. Spona J., Ulm R., Bieglmayer C. and Husslein P.: Hormone serum levels and hormone receptor content of endometria in women with normal menstrual cycles and patients bearing endometrial carcinoma. *Gynec. Obstet. Invest.* **10** (1979) 71–80.
8. Martin P. M., Rolland P. H., Gammerre M., Serment H. and Toga M.: Estradiol and progesterone receptors in normal and neoplastic endometrium: correlation between receptors, histopathological examinations and clinical responses under progestin therapy. *Int. J. Cancer* **23** (1979) 321–329.
9. Cao Z. Y., Eppenberger U., Roos W., Torhorst J. and Almendral A.: Cytosol estrogen and progesterone receptor levels in normal and pathological tissue of endometrium, endocervical mucosa, and cervical vaginal portion. *Arch. Gynec.* **233** (1983) 109–119.
10. Greene G. L., Nolan C., Engler J. P. and Jensen E. V.: Monoclonal antibodies to human estrogen receptor. *Proc. Natn. Acad. Sci. U.S.A.* **77** (1980) 5115–5119.
11. Perrot-Applanat M., Logeat F., Groyer-Picard M. T. and Milgrom E.: Immunocytochemical study to mammalian progesterone receptor using monoclonal antibodies. *Endocrinology* **116** (1985) 1473–1484.
12. Press M. F., Nousek-Goebl N., King W. J., Herbst A. L. and Greene G. F.: Immunohistochemical assessment of estrogen receptor distribution in human endometrium throughout the menstrual cycle. *Lab. Invest.* **51** (1984) 495–503.
13. Press M. F. and Greene G. L.: Localization of progesterone receptor with monoclonal antibodies to the human progestin receptor. *Endocrinology* **122** (1988) 1165–1175.
14. Charpin C., Martin P. M., Lavant M. N., Pourreau-Schneider N. and Toga M.: Estrogen receptor immunocytochemical assay (ER-ICA) in human endometrium. *Int. J. Gynec. Path.* **5** (1986) 119–131.
15. Lessey B. A., Killam A. P., Metzger D. A., Haney A. F., Greene G. L. and McCarthy K. S. Jr: Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J. Clin. Endocr. Metab.* **67** (1988) 334–340.
16. Bergeron C., Ferenczy A. Toft D. O. and Shyamala G.: Immunocytochemical study of progesterone receptors in hyperplastic and neoplastic tissues. *Cancer Res.* **48** (1988) 6132–6136.
17. Zaino R. J., Clarke C. L., Mortel R. and Satyaswaaroop P. G.: Heterogeneity of progesterone receptor distribution in human endometrial adenocarcinoma. *Cancer Res.* **48** (1988) 1889–1895.
18. Tsibris J. C. M., Fort F. L., Cazenave C. R., Cantor B., Bordawil W. A., Notelovitz M. and Spellacy W. N.: The uneven distribution of estrogen and progesterone receptors in human endometrium. *J. Steroid Biochem.* **14** (1981) 997–1003.
19. Vollmer G., Bindewald I., Meyn Y., Wünsche W. O., Arnholdt H. and Kuppen R.: An improved micromethod for the determination of biochemically active estrogen and progesterone receptors in parallel to comparative histological examination of a single piece of tissue. *J. Steroid Biochem.* **31** (1988) 899–907.
20. Vollmer G., Gerdes J. and Knuppen R.: Quantification of estrogen- and progesterone receptors from cryostat sectioned human tumor tissue: Comparison of ligand binding and immunocytochemical assays. *J. Steroid Biochem.* **33** (1989) 41–47.
21. Vollmer G., Wünsche W. O. and Knuppen R.: Quantitative determination of oestradiol-17β hydroxysteroid dehydrogenase: increased sensitivity By HPLC separation of hormones permits the measurement of enzyme activity in cryostat sections. *J. Clin. Chem. Clin. Biochem.* **26** (1988) 453–458.
22. Burton K.: A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62** (1956) 315–322.
23. Richards G. M.: Modifications of the diphenylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. *Analyt. Biochem.* **57** (1974) 369–376.
24. Pollow K., Lübbert H., Boqoui E., Kreutzer G., Jeske R. and Pollow B.: Studies on 17-hydroxy steroid dehydrogenase in human endometrium and endometrial carcinomas. *Acta Endocr.* **79** (1975) 134–145.
25. Bradford M. M.: A rapid and sensitive method for the determination of microgram quantities utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72** (1976) 248–254.
26. Garcia E., Bouchard P., DeBrux J., Berdale J., Frydman G., Schaison G., Milgrom E. and Perrot-Applanat M.: Use of immunocytochemistry of progesterone and estrogen receptors for endometrial dating. *J. Clin. Endocr. Metab.* **67** (1988) 80–87.
27. Horwitz K. B., Wei L. L. and Francis M. D.: Structural analysis of progesterone receptors. *J. Steroid Biochem.* **24** (1986) 109–117.
28. Estes P. A., Suba E. J., Lawler-Havner J., Elashry-Stowers D., Wei L. L., Toft D. O., Sullivan W. P., Horwitz K. B. and Edwards D. P.: Immunologic analysis of human breast cancer progesterone receptors: 1. Immunopurification of transformed receptors and production of monoclonal antibodies. *Biochemistry* **26** (1987) 6250–6262.
29. Wei L. L., Sheridan P. L., Kreft N. L., Francis M. D., Toft D. O., Edwards D. P. and Horwitz K. B.: Immunologic analysis of human breast cancer progesterone receptors: 2. Structure, phosphorylation and processing. *Biochemistry* **26** (1987) 6262–6272.
30. Garcia T., Jung-Testos I. and Baulieu E. E.: Tightly bound nuclear progesterone receptor is not phosphorylated in a primary chick oviduct cultures. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 7573–7577.
31. Horwitz K. B., Francis M. D. and Wei L. L.: Hormone dependent covalent modification and processing of human progesterone receptors in the nucleus. *DNA* **4** (1985) 451–460.
32. Rao K. V., Peralta W. D., Greene G. L. and Fox C. F.: Cellular progesterone receptor phosphorylation in response to ligands activating protein kinases. *Biochem. Biophys. Res. Commun.* **146** (1987) 1357–1365.

33. Feil P. D., Clarke C. L. and Satyaswaroop P. G.: Progestin-mediated changes in progesterone receptor forms in the normal human endometrium. *Endocrinology* **123** (1988) 2506–2513.
34. Zaino R. J., Satyaswaroop P. G. and Mortel R.: The relationship of histologic and histochemical parameters to progesterone receptor status in endometrial adenocarcinomas. *Gynec. Oncol.* **16** (1983) 196–208.
35. Deligdish L. and Holinka C. F.: Progesterone receptors in two groups of endometrial carcinoma. *Cancer* **57** (1986) 1385–1388.
36. DeHertogh R., Ekka E., Vanderheyden I. and Glorieux B.: Estrogen and progesterone receptors in the implantation sites and interembryonic segments of rat uterus endometrium and myometrium. *Endocrinology* **119** (1986) 2506–2513.