Epidermal growth factor binding sites in human pituitary macroadenomas

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Abstract

The number of epidermal growth factor (EGF) binding sites was determined by competitive binding assays in a series of 46 pituitary macroadenomas. A single concentration of ^{125}I-EGF (1 nM) was used for all experiments. In four cases, a displacement curve was obtained by adding increasing concentrations of cold EGF, and Scatchard analysis showed the presence of two classes of EGF binding sites, with K_{d1}=0·62 ± 0·23 nM and K_{d2}=53·8 ± 8·2 nM for the high- and low-affinity binding sites respectively. The distribution of EGF binding sites was studied in 42 cases by a single-point assay, in the presence and in the absence of a 100-fold cold EGF excess. A non-parametric distribution of EGF binding sites was observed (median 10·2 fmol/mg membrane protein, range 0·0–332·0). EGF-receptor positivity, defined as EGF binding ≥10·0 fmol/mg protein, was observed in 23 samples (54·8%), especially in prolactinomas (76·5%, P<0·05 vs other tumors taken together) and in gonadotrope adenomas (62·5%). EGF binding was higher in invasive than in non-invasive adenomas (median: 12·8 vs 0·0 fmol/mg membrane protein, P=0·047), and especially in adenomas invading the sphenoid sinus (median 26·7 fmol/mg membrane protein, P=0·008 vs other adenomas). EGF binding also tended to increase with the grade of supra/extrasellar extension according to Wilson (P=0·15). Sex steroid receptors (SSRs) were simultaneously determined in both cytosolic and nuclear fractions of 31 pituitary adenomas. Estrogen and progesterone receptors were determined by an enzyme-linked immunoassay and androgen receptors by a competitive binding assay with [^{3}H]methyltrienolone. No correlation could be found between EGF binding and either the gender and gonadal status of the patients, or the expression of SSRs by the adenomas. We conclude that the EGF family of growth factors may play a role in the evolution of a significant subset of human pituitary adenomas, especially in their invasiveness, and that a high EGF binding capacity may represent an additional marker of aggressiveness for these tumors. Sex steroids do not appear to have a significant role in the regulation of EGF binding *in vivo* in these tumors.

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Introduction

Pituitary tumors are frequent benign neoplasia, accounting for about 10% of symptomatic endocranial neoplasms. In addition, autoptic studies indicate that silent pituitary adenomas – mostly microadenomas – are present in up to 10–20% of the general population (Molitch & Russell 1990). Therefore, attempts to identify factors which can promote or inhibit the clinical expression of these tumors – i.e. the ability to produce pituitary hormones in excess, leading to a specific syndrome of hormone hypersecretion, and the increase in proliferation rate and invasiveness, leading to neurological manifestations – are of special importance for the understanding of the pathogenesis of these tumors, and for the development of new therapeutic strategies (von Mehren & Weiner 1996, Huang & Heimbrook 1997). Pituitary tumors are mostly monoclonal in origin (Herman *et al.* 1990), indicating that primary genetic alterations can determine the clonal expansion of abnormal cells. However, few such abnormalities have been identified in these tumors (for review see Shimon & Melmed 1997). On the other hand, pituitary tumor cell function and/or proliferation can be modulated by a variety of extracellular factors, including steroid hormones (Jaffrain-Rea *et al.* 1996), cytokines and growth factors (for review see Shimon & Melmed 1997).

The epidermal growth factor (EGF) family of growth factors is able to modulate the proliferation, secretion and differentiation of most endocrine glands (for review see Fisher & Lakshman 1990). EGF is normally present in the

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pituitary, where it appears to be produced by subpopulations of all hormone-producing cell types, and especially by gonadotropes and thyrotropes (Kasselberg *et al.* 1985, Mouihate *et al.* 1996). The epidermal growth factor-receptor (EGF-R) is also normally expressed by anterior pituitary cells (Fan & Childs 1995), with EGF binding sites being localized mainly to lactotrope and somatotrope cells (Chabot *et al.* 1986). The EGF-R has an intrinsic tyrosine kinase activity, and inhibition of tyrosine kinase activities has recently been shown to decrease [^{3}H]thymidine uptake in primary cultures of human pituitary adenomas (Jones *et al.* 1997). EGF can modulate the secretion of pituitary hormones in both normal (Ikeda *et al.* 1984, Miyake *et al.* 1985, Childs *et al.* 1991) and tumor pituitary cells (Schonbrunn *et al.* 1980, Aanestad *et al.* 1993). Transforming growth factor-α (TGF-α), a member of the EGF family, which can thus also act through the EGF-R on target cells, has been detected in the normal pituitary gland (Kobrin *et al.* 1987, Ezzat *et al.* 1995, Fan & Childs 1995), and has been implicated in pituitary tumorigenesis (Finley & Ramsdell 1994).

There is recent evidence that the EGF-R mRNA is expressed by a significant number of human pituitary adenomas (LeRiche *et al.* 1996), and the expression of the corresponding protein was detected by immunohistochemical studies (Chaidarun *et al.* 1994*a*, LeRiche *et al.* 1996). Surprisingly, the ability of EGF to bind pituitary adenoma cells has not been documented (Birman *et al.* 1987). In addition, although both EGF and EGF-R can be regulated by sex steroids in hormone-dependent tissues (Mukku & Stancel 1985, Fisher & Lakshmanan 1990, Mouihate & Lestage 1995), no data are available concerning possible relationships between sex steroids and the EGF/EGF-R system in human pituitary tumors. The aim of this study was to investigate further the presence of EGF binding sites in a representative series of pituitary tumors and, if present, to look for possible correlations between EGF binding and the bio-clinical characteristics of the patients and tumors, including the expression of cellular receptors for sex steroids.

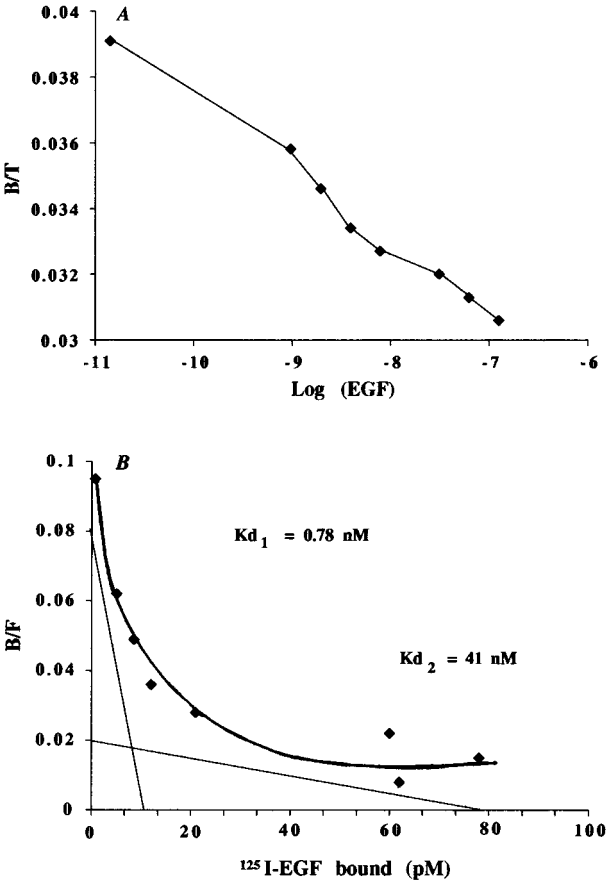

Figure 1 (*A*) An example of the displacement curve obtained in a case of null cell pituitary macroadenoma by incubating a single concentration of ^{125}I-EGF (1 nM) with increasing concentrations of cold EGF. (*B*) Analysis of the displacement curve according to Scatchard indicates the presence of two types of EGF binding sites, one with a high affinity (K_{d1} 0·78 nM) and one with a low affinity (K_{d2} 41 nM) binding site.

Materials and Methods

Patients and tumors

Forty-six patients with pituitary macroadenomas (diameter ≥1·0 cm) were operated on for medical reasons by either a transsphenoidal (n=40) or a transcranial (n=6) approach. There were 26 men (mean age: 45·5 ± 13·3 years) and 20 women (mean age: 47·3 ± 13·6 years). Amenorrhea-galactorrhea was present in 4 women, and typical clinical features of acromegaly in 9 cases. Neurological symptoms were shown by 24 patients, with 19 out of the 24 presenting with visual field defects. Nine patients had recurrent adenomas. Pre-operative RIA measurements of plasma prolactin (PRL), growth hormone (GH), thyroid-stimulating hormone (TSH), free thyroxine (FT_4), adrenocorticotropin (ACTH), cortisol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone and 17β-estradiol were carried out in all cases using available commercial kits. Hypogonadism in men was defined by plasma testosterone less than 2·5 ng/ml. Tumor volume was evaluated by pre-operative computerized tomodensitometry (TDM) and/or nuclear magnetic resonance imaging (NMR) in all cases. Thirty tumors were defined as invasive according to pre-operative radiological criteria and/or to intra-operative surgical findings, and twelve were non-invasive. The degree of supra/extrasellar extension was defined according to Wilson's criteria (Wilson 1984). Immunohistochemical examination of pituitary tumors was performed on paraffin-embedded tissue sections with polyclonal rabbit antibodies (anti-PRL, anti-GH, anti-FSH, anti-LH, anti-TSH, anti-ACTH;

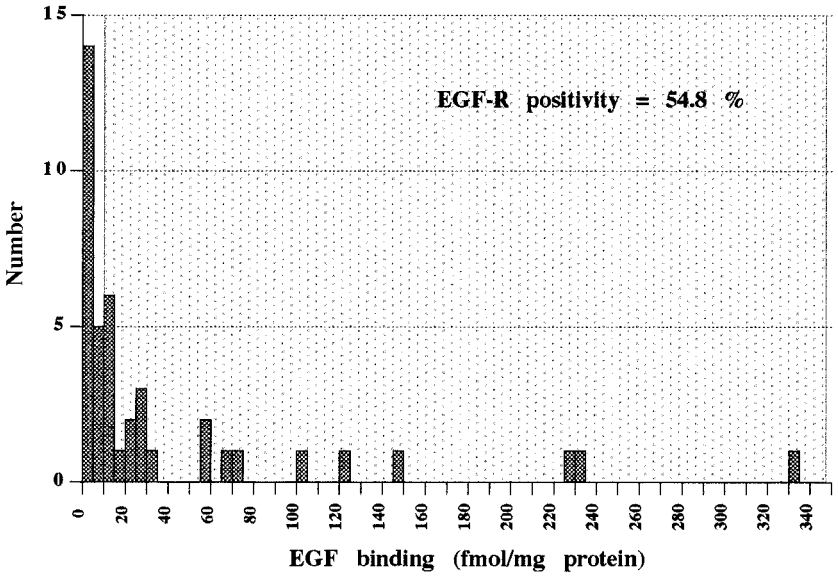

Figure 2 Distribution of EGF binding sites according to a single-point assay in a series of 42 human pituitary macroadenomas. Median value: 10·2 fmol/mg membrane protein (range 0–332). EGF-R positivity was defined by EGF binding values ≥10 fmol/mg protein and is represented by the dotted area.

Orthodiagnostic Systems, Raritan, NJ, USA), using the streptavidin-biotin detection system. Immunohistochemical data were lacking in one case. Tumors were finally classified as: prolactinomas (n=17), gonadotrope adenomas (n=9), null cell adenomas (n=8), pure GH-secreting adenomas (n=7), mixed GH/PRL-secreting adenomas (n=3), silent ACTH-secreting adenoma (n=1), including nine recurrent adenomas consisting of prolactinomas (n=2), null cell adenomas (n=2), pure GH-secreting adenomas (n=2), mixed GH/PRL-secreting adenomas (n=2), and gonadotrope adenomas (n=1).

Tissue preparation

Frozen tumor samples were homogenized in 2 ml TED buffer (0·01 M Tris–HCl, 0·001 M EDTA, 0·001 M dithiothreitol, 0·01 M sodium molybdate, 10% glycerol, pH 7·4 at 25 °C) and subsequently centrifuged at 800 ***g*** (ALC 4227 R refrigerated centrifuge) for 10 min to obtain cytoplasmic and nuclear fractions. Cytosolic fractions and nuclear extracts were obtained as previously described (Jaffrain-Rea *et al.* 1996). The cytoplasmic fraction was then ultracentrifuged at 30 000 ***g*** for 45 min to obtain the membrane pellet, which was resuspended in 2·5 ml 0·5 M $MgCl_2$ and incubated for 2 min at 25 °C for dissociating endogenous ligand from EGF-R. After the addition of 7·5 ml cold TE buffer (0·01 M Tris–HCl, 0·001 M EDTA, pH 7·4 at 25 °C), the membranes were ultracentrifuged at 30 000 ***g*** for 30 min. The final pellets were then re-suspended in TE buffer (final protein concentration 0·1 to 0·7 mg/ml). Protein concentration was determined according to Bradford (1976).

EGF binding experiments

All EGF binding experiments were carried out in triplicate with a single 1 nM concentration of ^{125}I-EGF (specific activity 150–200 mCi/mg, New England Nuclear, Dupont de Nemours, France), according to a modified published method (Lubrano *et al.* 1993). In 4 cases, including 2 null cell, 1 prolactinoma, and 1 pure GH-secreting adenoma, increasing concentrations of cold EGF (0·0156–15 nM) were added to 1 nM ^{125}I-EGF in order to obtain a displacement curve, and the non-linear Scatchard plots were analyzed on a personal computer using the LIGAND software according to the two-site model (Munson & Rodbard 1980). In most cases, a single-point saturation assay was obtained by determining EGF binding in the presence or absence of a 100-fold cold EGF concentration. In all cases, the incubation was performed at 25 °C for 60 min and, at the end of the incubation period, the EGF/EGF-R complexes were precipitated by polyethylene glycol. The radioactivity was counted in a Packard gamma-counter with an efficiency ratio of 48%. In agreement with most studies based on radioligand assays for EGF-R determination (for review see Klijn *et al.* 1992), EGF-R positivity was defined by EGF binding values ≥10 fmol/mg protein.

Table 1 Distribution of EGF binding sites in a series of 42 human pituitary macroadenomas according to patients' and tumors' characteristics, except hormone cell type

	n	Median (fmol/mg protein)	Range (fmol/mg protein)	Positivity
Gender/gonadal status				
Men	23	12·3	0–233	56·5%
Hypogonadal	11	10·0	0–148	6/11
Eugonadal	9	12·3	0–233	5/9
Women	19	10·3	0–332	52·6%
Amenorrhea/menopause	14	9·0	0–332	7/14
Eugonadal	5	10·3	0–123	3/5
Tumor recurrence				
No	33	12·0	0–332	57·5%
Yes	9	5·0	0–101	44·4%
Tumor volume				
Intrasellar	9	4·8	0–21·9	22·2%
Extrasellar	33	12·5	0–332	60·6%
Tumor invasivity				
Non-invasive	12	0·0	0–71·2	41·7%
Invasive	30	12·8[a]	0–332	63·3%

[a]P=0·047 vs non-invasive adenomas.

Cellular receptors for sex steroids

Cytosolic receptors for estrogens (ERs), progesterone (PgRs) and androgens (ARs) were studied in all cases, and the corresponding nuclear receptors were studied in 31 cases, according to previously published methods (Jaffrain-Rea *et al.* 1996). In brief, ERs and PgRs were determined by an enzyme-linked immunoassay using commercially available specific monoclonal antibodies (Abbott, Chicago, IL, USA), and ARs were determined by competitive binding with [^{3}H]methyltrienolone (R1881), in the presence of triamcinolone acetonide to avoid significant binding of methyltrienolone to the PgR (Ekman *et al.* 1982). Positivity for sex steroid receptors (SSRs) was defined by cytosolic concentrations $\geq$3 fmol/mg protein and/or nuclear concentrations $\geq$20 fmol/mg DNA.

Statistical analysis

Unless otherwise specified, results are expressed as median values and range. Only EGF binding values obtained by the single-point saturation assay were considered for statistical comparison of subgroups. Distribution of positive cases for EGF binding were compared by the Chi-square test, adequately modified by Yates correction if necessary. Because of the non-parametric distribution of EGF-binding values, bivariate analysis was performed by the Mann-Whitney U-test and multivariate analysis by the Kruskall-Wallis test. Correlations between the concentration of EGF binding sites and the concentrations of SSRs were studied by both linear regression and the non-parametric Spearman correlation test. Statistical analysis was supported by the Statview 4·0 software for MacIntosh.

Results

Scatchard analysis of EGF binding

Figure 1 shows the non-linear displacement curve of ^{125}I-EGF obtained in a sample of null cell pituitary tumor (panel A) and its resolution into a two-sites binding model according to Scatchard analysis (panel B). Similar results were obtained in all cases studied, indicating the presence of both high- and low-affinity binding sites in these tumors, with a K_{d1}=0·62 ± 0·23 nM for the high affinity site and a K_{d2}=53·8 ± 8·2 nM for the low affinity site (means ± S.D.). Similar results were also obtained in positive control samples from human benign prostatic hyperplasia (data not shown).

EGF binding according to the single-point assay

Analysis of 42 pituitary macroadenomas revealed a highly variable, non-parametric distribution of EGF binding sites, ranging from 0 to 332·0 fmol/mg protein, with a median value of 10·2 fmol/mg protein (Fig. 2). EGF-R positivity ($\geq$10 fmol/mg protein) was present in 23 cases (54·8%). Patients' gender and gonadal status were found to have no influence on EGF binding (Table 1).

Distribution of EGF binding sites according to recurrence, tumor volume and invasivity

As shown in Table 1, no statistical difference was found between recurrent and non-recurrent tumors. In contrast, EGF binding was higher in invasive adenomas than in non-invasive adenomas (P=0·047) and, accordingly, EGF-R positivity tended to be higher in the former group (63·3% vs 33·3%, χ^2=3·11, P<0·1). In particular, positivity for EGF-R was present in 90% of the tumors invading the sphenoidal sinus (P<0·02 vs other tumors) and EGF binding was significantly higher in this group than in other tumors (median 26·7 vs 4·9 fmol/mg protein, P=0·009) (Fig. 3, panel A).

EGF binding also tended to increase with the degree of supra/extrasellar extension (P=0·15) (Fig. 3, panel B). High values of EGF binding (≥30 fmol/mg protein) were significantly more frequent in C-D-E than in O-A-B adenomas defined according to Wilson (1984) (38·9% vs 8·8%, χ^2=3·94, P<0·05).

Distribution of EGF binding sites in hormone-producing cell types

EGF binding sites were mainly detected in prolactinomas (EGF-R positivity 13/17=76·5%) and in gonadotrope pituitary adenomas (EGF-R positivity 5/8=62·5%) (Table 2). Overall, EGF-R positivity was more frequent in prolactinomas than in non-prolactinoma tumors (χ^2=5·43, P<0·05) and EGF binding values tended to be higher in the former group (median 21·9 vs 5·0 fmol/mg protein, P=0·06). When only non-invasive tumors were considered, EGF positivity was observed in 2/3 gonadotrope adenomas, 1/3 prolactinomas, 1/3 pure GH-secreting adenomas, but not in null cell adenomas (0/4).

Patients' gender and dopamine-agonist therapy had no influence on EGF binding in the prolactinoma group (data not shown).

Correlation between EGF binding and expression of SSRs in pituitary tumors

The distribution of EGF binding in pituitary adenomas according to expression of SSRs is summarized in Table 3. No correlation was found between EGF binding values and either the cytosolic or nuclear concentrations of ERs, PgRs and ARs (r<0·15 in all cases). There were also no positive or negative associations between cytosolic, nuclear or total ERs, PgRs and ARs and EGF-R positivity.

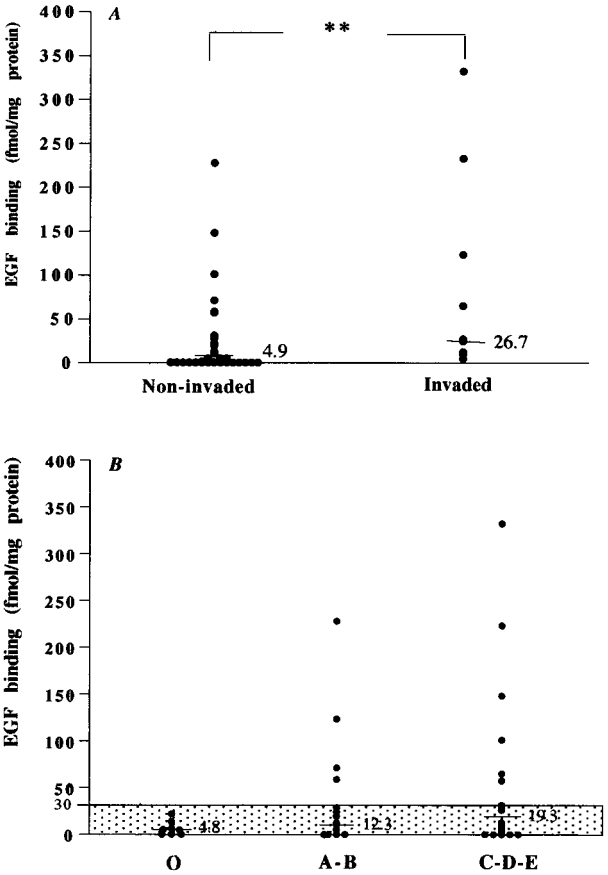

Figure 3 (*A*) Distribution of EGF binding sites in pituitary macroadenomas invading or not the sphenoid sinus. Median values are indicated for each group, and were significantly higher in adenomas invading the sphenoid sinus than in other adenomas, as indicated by ** (P<0·02). EGF-R positivity was significantly more frequent in adenomas invading the sphenoid sinus than in other adenomas (90·0% vs 43·7%, P<0·02). (*B*) Distribution of EGF binding sites in pituitary adenomas classified according to Wilson's criteria. EGF binding tended to increase with the grade of supra/laterosellar extension (P=0·15). High EGF binding values (≥30 fmol/mg protein) were more frequent in C-D-E than in O-A-B adenomas (38·9% vs 8·8%, P<0·05).

Discussion

To our knowledge, this is the first report showing the presence of EGF binding sites in a series of human pituitary macroadenomas. The presence of EGF binding sites is consistent with the presence of EGF-R mRNA and EGF-R protein detected by immunohistochemistry in a significant subset of these tumors (Chaidarun *et al.* 1994*a*, LeRiche *et al.* 1996). The reasons why EGF-binding was previously detected in the normal human pituitary gland but not in human pituitary tumors are unclear (Birman *et al.* 1987). In this study, two classes of EGF binding sites were shown by Scatchard analysis, with respective high and low affinities, which may reflect the capacity of EGF-R to present allosteric modifications–especially dimerization – following ligand binding (King & Cuatrecasas 1982, Boni-Schnetzler & Pilch 1987). This is

Table 2 Distribution of EGF binding sites in a series of 41 human pituitary macroadenomas according to hormone cell type

	n	Invasivity	Median (fmol/mg protein)	Range (fmol/mg protein)	Positivity
Tumor					
FSH-secreting	8	5/8	20·6	0–332	5/8
GH/PRL-secreting	3	3/3	6·5	0–101	2/3
GH-secreting	6	3/6	5·0	0–59	1/6
Null cell	6	2/6	0	0–65	1/5
ACTH	1	1/1	19·9	—	1/1
All non-PRL	24	58·3%	3·0	0–332	41·7%
PRL-secreting	17	82·3%	21·9[a]	0–233	76·5%[b]

[a]$P=0·063$ vs non-prolactinoma tumors (non-significant trend); [b]$P<0·05$ vs non-prolactinoma tumors.

in agreement with previous reports on human tumors of various origins (Nicholson *et al.* 1988, Lubrano *et al.* 1993), although other studies have reported the presence of a single class of high affinity binding sites (Yoshida *et al.* 1993), depending on experimental conditions and on the concentration of EGF binding sites themselves (Bolufer *et al.* 1990). The determination of the binding capacity for the high affinity binding site appears to be poorly influenced by the choice of the radioligand assay, and simplified methods, such as two-point assays (Nicholson *et al.* 1988) or single-point saturation assays (Etienne *et al.* 1990, Petrangeli *et al.* 1995), have been developed to allow easier evaluation of EGF-R status for clinical purposes. Because of the limited amount of material available in most pituitary tumors – microadenomas had to be excluded from this study because the concentrations of total membrane protein were too low to be suitable for the radioligand assay – a single-point assay was chosen for the study of EGF-R distribution.

EGF binding sites in the normal pituitary are usually detected in somatotropes and lactotropes (Chabot *et al.* 1986), but the expression of EGF-R by pituitary adenomas is less defined. EGF-R protein immunoreactivity was found to be almost limited to non-functioning adenomas, but gonadotrope adenomas and prolactinomas were poorly represented in these series (Chaidarun *et al.* 1994*a*). In contrast, using both immunohistochemical methods and reverse transcriptase (RT)-PCR analysis, a variable expression of EGF-R and its mRNA has been reported in all types of secreting and non-secreting tumors, with an overexpression in recurrent somatotrope adenomas (LeRiche *et al.* 1996). Data from the present study further argue for an ubiquitous, although highly variable, expression of the EGF-R in pituitary adenomas. It is difficult to determine whether the high levels of EGF binding detected in prolactinomas reflect an intrinsic characteristic of these tumors, since this result could be partially biased by the high percentage of invasive tumors in this subgroup.

Table 3 Distribution of EGF binding sites in a series of 31 human pituitary macroadenomas according to the expression of sex steroid receptors (SSRs). ERs and PgRs were determined by an enzyme-linked immunoassay (ELISA) with monoclonal antibodies (Abbott), and ARs were determined by a binding assay with [^{3}H]R1881. Both cytosolic and nuclear receptors were determined in these cases, and SSRs positivity was defined by either cytosolic concentrations ≥ 3 fmol/mg protein or nuclear concentrations ≥ 20 fmol/mg DNA respectively. The number of EGF-R positive samples is indicated within parentheses for each subgroup.

	n	EGF-R positivity (≥ 10 fmol/mg protein)	EGF binding (fmol/mg protein)	
			Median	Range
Tumor				
ER positive	21	61·9% (13)	13·1	0–233
ER negative	10	60·0% (6)	25·0	0–332
PgR positive	15	53·3% (8)	10·0	0–233
PgR negative	16	68·7% (11)	16·2	0–332
AR positive	25	60·0% (15)	16·2	0–233
AR negative	6	66·7% (4)	35·5	0–332

In fact, EGF binding was found to be significantly higher in invasive than in non-invasive pituitary adenomas, especially in those invading the sphenoid sinus, and tended to increase with tumor volume. High values of EGF binding ($\geq$30 fmol/mg protein) were observed mainly in tumors with a huge supra/laterosellar extension (C-D-E grading according to Wilson 1984), contrasting with the low levels ($\leq$22 fmol/mg protein) observed in intrasellar adenomas. Thus, a high EGF-R expression appears to represent a marker of aggressiveness in a significant subset of pituitary tumors, as reported in a variety of human neoplasia (Smith *et al.* 1989, Toi *et al.* 1990, Lund-Johansen *et al.* 1990, Yoshida *et al.* 1993) and increased EGF-R expression is likely to be a late event in pituitary tumorigenesis.

The mechanisms by which the EGF family of growth factors may promote the proliferation and the invasiveness of pituitary adenoma cells are unclear, since they have multiple and variable effects on pituitary cells, depending on the hormone cell type and on the malignancy of the cells. For instance, EGF inhibits the proliferation of GH/PRL secreting cell strains while inducing their differentiation into lactotropes (Schonbrunn *et al.* 1980, Felix *et al.* 1995). On the other hand, EGF stimulates [^{3}H]thymidine incorporation by sheep pituitary cells while increasing the percentage of undifferentiated cells (Chaidarun *et al.* 1994*b*) and enhances the proliferation of human non-functioning pituitary tumor cells *in vitro* (Chaidarun *et al.* 1994*a*). EGF also stimulates both hormone secretion and cell proliferation in pituitary corticotropes (Childs *et al.* 1991, 1995). EGF and EGF-R were found to be co-expressed in non-functioning pituitary adenomas (Chaidarun *et al.* 1994*a*), suggesting that autocrine mechanisms involving the EGF/EGF-R system are present in this subgroup. TGF-α immunoreactivity has also been detected in most pituitary adenomas, independent of their cell type (Ezzat *et al.* 1995), and may be especially important for the promotion of PRL/GH-secreting tumors (Finley & Ramsdell 1994). Taken together, these data indicate that the EGF/TGF-α system may be of special importance in the pathogenesis of human pituitary tumors.

Significant correlations have been made between the expression of EGF-R and SSRs in various tumors. In breast cancer, a negative correlation exists between ER/PgR and EGF-R expression (Klijn *et al.* 1992), and ER and EGF-R are considered as positive and negative prognostic markers respectively. In this study, EGF binding was found to be independent of SSRs expression in pituitary adenomas. In particular, EGF binding was similar in ER-positive and ER-negative tumors. Thus, although the expression of both ER and EGF-R tends to increase with tumor volume and invasiveness in pituitary adenomas (Jaffrain-Rea *et al.* 1996), the overexpression of ER and EGF-R appears as independent prognostic markers. On the other hand, both EGF secretion and EGF-R expression are regulated by various factors including sex steroids (for review see Carpenter & Wahl 1990). EGF binding is generally higher in male than in female tissues, including the normal pituitary gland (Birman *et al.* 1987), reflecting EGF-R induction by androgens. The expression of both SSRs (McGinnis *et al.* 1981, Handa *et al.* 1986) and EGF-R (Armstrong & Childs 1997*a*) also vary throughout the estrous cycle, reflecting cyclic variations in estradiol concentrations (Armstrong & Childs 1997*b*). In contrast to previous data from our laboratory, which strongly supported the influence of the gonadal environment on SSRs expression by human pituitary adenomas (Jaffrain-Rea *et al.* 1996), patients' gender and gonadal status at the time of surgery had no significant influence on EGF binding in this series. Because of the reduced number of subjects included in each subgroup, these results do not rule out the presence of regulating effects of sex steroids on EGF-R expression in these tumors, but suggest that such effects, if present, are not of major physiopathological importance *in vivo*.

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