Eur J Clin Chem Clin Biochem 1996; 34:23–30 © 1996 by Walter de Gruyter · Berlin · New York

Analysis of the In Vitro Secretory Activity of Human Pituitary Adenomas: Modification of Corticotropin Release from Adenoma Tissue Explant Cultures by Addition of a Human Plasma Ultrafiltrate Bioactive Fraction

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Summary: The lack of control of tumour behaviour is manifested in different ways, depending primarily on the type of tumour. This results in numerous problems of tumour diagnosis and therapy. In the case of "benign" tumours, like pituitary adenomas, in vitro studies are often used for evaluation of the tumour. The use of tissue explant cultures of human pituitary adenomas and the comparison of the feature of cultured tumours with their behaviour in vivo showed that corticotropin is released not only from the tumours associated with *Cushing*'s disease, but also from clinically non-functioning tumours. Hence, it was supposed that the release of corticotropin in vivo from non-secreting tumours is probably under the influence of certain neuroendocrine and/or systemic humoral factors. To test this possibility, samples of 22 tumours were cultured in plain culture medium or in the presence of the "human plasma ultrafiltrate bioactive fraction" (tentatively termed as TBP) prepared by anion-exchange chromatography. In the presence of TBP the release of corticotropin was strongly inhibited in adenomas showing relatively high spontaneous secreting activity in vitro (> 200 ng/l in 24 hours), while immunohistochemistry of these tumours indicated accumulation of corticotropin inside the cells. In contrast, TBP stimulated corticotropin release from tumours that showed relatively low basic corticotropin release (< 200 ng/l in 24 hours), with no obvious change in cellular corticotropin immunoreactivity. Such a dual activity of TBP was not observed for 8 samples of adenomas cultured in the presence of surrounding pituitary tissue, probably because TBP did not affect corticotropin secretion by the normal pituitary cells (as indicated by immunohistochemistry). From these results, it appears that TBP could be one of the humoral factors involved in the regulation of corticotropin release from pituitary adenoma tissue. Its possible involvement in the regulation of corticotropin release from normal pituitary tissue, however, is uncertain.

Introduction

By measuring blood hormone concentration, pituitary adenomas are classified in vivo as "non-secreting" (clinically non-functioning tumours) or as hormone-secreting adenomas which cause recognised clinical disturbances (galactorrhoea and oligomenorrhoea, acromegaly, *Cushing*'s disease, etc.) (1–3). The use of in vitro cultures of pituitary adenomas and comparison of the features of the cultured tumours with their behaviour in vivo revealed further variability of morphological features and hormone secreting activity (4–8). This in vitro analysis of the characteristics of pituitary tumours also provides a basis for research on the activity of factors that might be involved in the regulation of tumour behaviour (9, 10).

However, it is not known whether adenoma cells are subject to control by the neuroendocrine system. The nature of factors that might be involved in the regulation of hormone secreting activity of pituitary adenomas is

also unknown. Thus, tumours associated with clinical evidence of hormone excess show high hormone secreting activity if cultured in vitro, but clinically "non-functioning" tumours also display a low level of hormone secreting activity in vitro (4, 6). Most of clinically "non-functioning" tumours, null cell adenomas and oncocytomas secret follitropin and lutropin in vitro, but occasional tumours (like normal pituitary gland) release somatotropin, prolactin and corticotropin, as well (6). Similarly, we have noticed that in pituitary adenomas cultured in vitro for 24 hours as tissue explant cultures (intact samples of the tumourous tissue), the release of corticotropin does not correspond to the hormonal activity of the tumours in vivo (11).

Hence, it seems that the secretory activity of pituitary tumours may be influenced by systemic regulatory mechanisms, probably mediated by hypothalamic as well as by certain humoral factors. To test this possibility and to further evaluate the hormone secreting activity of pituitary tumours, their sensitivity to the "bioactive fraction of the human plasma ultrafiltrate", tentatively termed as TBP[1]), (12) was analysed in vitro.

Materials and Methods

Patients

Pituitary adenomas of 22 patients were analysed (tab. 1). Eleven patients (5 male and 6 female) 25–76 (52 ± 18) years old had clinically non-functioning tumours. All of them had visual disturbances and headaches, and preoperative CT imaging scans demonstrated a macroadenoma. Basal serum levels of hormones were in the reference range, except prolactin which was elevated in 5 patients (60.7 ± 20.9 µg/l) due to the stalk compression.

Five female patients, 29–40 (30 ± 5) years old, presented with the syndrome of galactorrhoea and amenorrhoea associated with elevated serum prolactin levels (185.2 ± 59.1 µg/l). Three men, 28–57 (42 ± 14) years of age, also had elevated serum prolactin levels (274.1 ± 36.4 µg/l). In these two groups of patients the serum corticotropin concentrations were not elevated. However, the corticotropin values were increased in the sera of 2 patients with manifested *Cushing*'s disease (one male and one female, 31 and 66 years old respectively).

All the patients underwent pituitary operation, and subsequent morphological evaluation of the tumours revealed 10 null-cell adenomas, 8 sparsely granulated "prolactin cell" adenomas, two corticotropin cell adenomas, one oncocytoma and one mixed densely granulated somatotropin- and sparsely granulated prolactin-cell adenoma. Samples of the tumours were cultured in vitro, and later analysis showed that 8 of them contained pituitary gland tissue, while the remaining 14 adenomas showed no indication of the presence of pituitary gland.

Hormone assay

Corticotropin was determined in sera and culture media by IRMA, using the kit of CIS bio international (France). Coefficients of variation were below 10% and the detection limit for corticotropin was 2 ng/l (reference plasma values were 10–75 ng/l). Background values of corticotropin determined for the plain culture media used as a control were < 2 ng/l.

[1]) TBP = Tumour Basic Protein

Morphological studies

A portion of surgically removed pituitary adenoma was fixed in 40 g/l buffered paraformaldehyde, dehydrated in graded ethanol, and embedded in paraffin. An identical fixation procedure was applied to the adenoma explants obtained after surgical removal of the tumour, which were also used for the in vitro studies. Paraffin sections of 4 to 6 µm thickness were stained with hematoxylineosin, *Mallory* 3-chrome and the periodic acid-*Schiff* technique. For the immunohistochemical detection of the pituitary hormones, the immunoperoxidase technique was used. Antisera for the following hormones were used: thyrotropin (diluted 1 : 500), prolactin (1 : 300), corticotropin (1 : 400), somatotropin (1:500), follitropin (1:150) and lutropin (1:700) (all by DAKO (Danmark)). Normal pituitary tissue obtained at the autopsy served as positive control. The specificity of each primary antiserum was previously verified by adsorption with its respective primary antigen. For the electron microscopic study, the portion of the adenoma tissue was fixed in buffered 40 g/l paraformaldehyde, processed in buffered 20 g/l osmium tetroxide, then embedded in Epon 812. Semithin sections, stained with toluidine blue, were examined to select the areas appropriate for the ultrastructural study. The ultrathin sections of 15 nm stained with uranyl-acetate and lead-citrate, were studied in an Opton-Zeiss electron microscope EM 9S-2.

Preparation of the bioactive fraction of the human plasma ultrafiltrate

For the preparation of the bioactive fraction of the human plasma ultrafiltrate (tentatively termed as TBP[1]) the pooled EDTA-plasma of 49 healthy, male (20–35 years old) blood donors (hepatitis and HIV negative) was used. A sample of 1 l was repeatedly filtered on an Amicon membrane ultrafiltration system using a 30 000 M_r cut-off membrane (Amicon, Ireland) under nitrogen pressure, in a stirred ultrafiltration cell. The eluant was further repeatedly applied to the same ultrafiltration system using a 3000 M_r cut-off membrane. The final retentate of 50 ml volume was lyophilised and 5 mg of the lyophilisate was used for further purification by anion exchange fast protein liquid chromatography (FPLC). Anion-exchange chromatography was performed on FPLC, Mod. LCC 500 (Pharmacia, Sweden) using a 1 ml Resource-Q-column (Pharmacia, Sweden) equilibrated with 10 mmol/l Tris-buffer (pH 8.0).

Lyophilised 3000–30 000 M_r plasma ultrafiltrate was dissolved in water (5 mg powder per 0.5 ml Tris H_2O) and applied to the column. Column elution was started with 5 ml of Tris-buffer, followed by a salt gradient of 0.5 ml NaCl in the same buffer within 30 min, increasing finally to 1 mol/l NaCl in 5 min (chromatogram in figure 1). Two ml of fractions were collected and biological activity of all the fractions was tested in vitro. According to its "immunomodulating" activity for cultured human lymphocytes (12), a particular fraction (No. 9-TBP; eluted after 12 ml) was chosen for further analysis. This was lyophilised and kept at + 4 °C. Further analyses of TBP composition (fig. 2) using SMART Mini-Q anion-exchanger sepharose (Pharmacia, Sweden) revealed that TBP behaves as a "double-peak" compound with a dominating sharp peak eluting at approximately 35% of solvent B (10 mmol/l Tris/HCl + 250 mmol/l NaCl). Due to lack of material, further purification and analysis of TBP have not yet been possible.

For experimental purposes the sample of TBP was dissolved in saline, filtered through a 500 M_r cut-off membrane using saline as elution liquid. The retentate was passed through a 0.22 µm filter (Millipore, USA) and aliquots were kept at −20 °C, until used in vitro at a concentration of 100 g/l plasma equivalent concentration (approximately 5 µg/l).

Adenoma tissue explant cultures

Pituitary adenoma tissue was collected in iced saline with penicillin and streptomycin at the time of operation and washed twice with the same solution. Afterwards, the weight of the wet tissue was measured under sterile conditions, and the specimen was dissected into equal pieces of approximately 4–6 mm^3 size. The weight of the tissue particles was measured again and the samples were

Tab. 1 Characteristics of patients and pituitary adenoma tissue explants used for in vitro analysis.

Patient – sex/age	Clinical findings of adenoma secretory activity	Pathological diagnosis of the operated tumour specimen	Serum corticotropin (ng/l)	Morphology of tissue explant cultures*
1. ♀/65	non-functioning	null-cell adenoma	12.2	chromophobic adenoma
2. ♂/65	non-functioning	null-cell adenoma	8.5	chromophobic adenoma
3. ♀/65	prolactinoma	sparsely granulated prolactinoma	12.3	chromophobic adenoma
4. ♀/28	prolactinoma	sparsely granulated prolactinoma	22.5	chromophobic adenoma
5. ♂/28	prolactinoma	sparsely granulated prolactinoma	19.9	chromophobic adenoma
6. ♀/38	non-functioning	null-cell adenoma	9.9	chromophobic adenoma
7. ♀/28	prolactinoma	sparsely granulated prolactinoma	12.9	chromophobic adenoma
8. ♀/70	non-functioning	null-cell adenoma	16.3	chromophobic adenoma
9. ♀/37	acromegaly	mixed densely granulated somatotropin cell adenoma and sparsely granulated prolactinoma	1.5	chromophobic adenoma
10. ♀/25	non-functioning	null-cell adenoma	16.5	chromophobic adenoma
11. ♂/33	non-functioning	null-cell adenoma	24.8	chromophobic adenoma
12. ♀/70	non-functioning	null-cell adenoma	28.1	chromophobic adenoma
13. ♂/76	non-functioning	oncocytoma	1.5	oncocytoma
14. ♀/51	non-functioning	null-cell adenoma	0.6	chromophobic adenoma
15. ♀/31	*Cushing*'s disease	corticotropin-cell adenoma associated with *Cushing*'s disease	228.6	chromophobic adenoma mixed with pituitary gland
16. ♂/57	prolactinoma	sparsely granulated prolactinoma	27.6	chromophobic adenoma mixed with pituitary gland
17. ♂/41	prolactinoma	sparsely granulated prolactinoma	7.0	chromophobic adenoma mixed with pituitary gland
18. ♂/37	non-functioning	null-cell adenoma	11.3	chromophobic adenoma mixed with pituitary gland
19. ♂/41	non-functioning	null-cell adenoma	22.1	chromophobic adenoma mixed with pituitary gland
20. ♂/66	*Cushing*'s disease	corticotropin cell adenoma associated with *Cushing*'s disease	221.0	chromophobic adenoma mixed with pituitary gland
21. ♀/40	prolactinoma	sparsely granulated prolactinoma	17.4	chromophobic adenoma mixed with pituitary gland
22. ♀/28	prolactinoma	sparsely granulated prolactinoma	31.7	chromophobic adenoma mixed with pituitary gland

* – there were no major differences of the tissue morphology observed between the experimental and the respective control adenoma tissue explant cultures

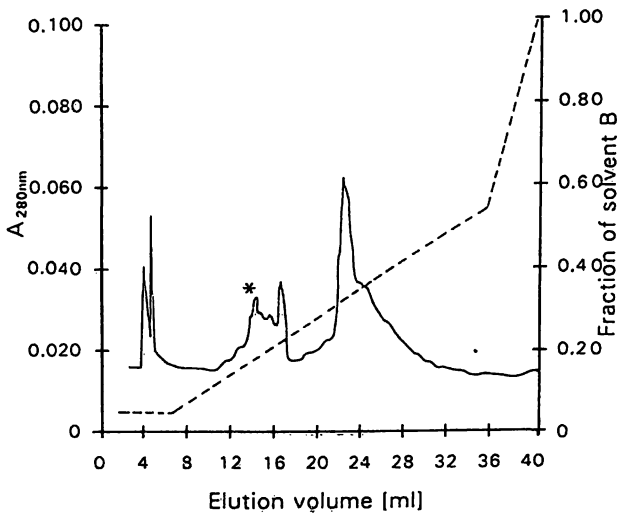

Fig. 1 Preparative anion-exchange liquid chromatography of the human plasma 3000–30 000 M_r membrane ultrafiltrate. Lyophilised 3000–30 000 M_r plasma ultrafiltrate was dissolved in water (5 mg powder/0.5 ml Tris H_2O-solvent A), applied to the column and eluted with 5 ml of Tris-buffer followed by a salt gradient of 0.5 ml NaCl in the same buffer (solvent B = solvent A + 1 mol/l NaCl) within 30 min, finally increasing to 1 mol/l NaCl in 5 min. * – Bioactive fraction (No. 9) used for the in vitro experiments; tentatively termed as TBP.

placed in plastic *Petri* dishes (Greiner, Germany) containing Dulbecco's Modified Eagle's Medium Ham's F-12 supplemented with fetal calf serum (volume fraction 0.1) and 1 mU/l penicillin and 1 mg/l streptomycin. The culture medium of the tissue explant cultures treated with TBP contained the sample of bioactive plasma ultrafiltrate fraction at 100 g/l plasma equivalent. The volume of the medium used for tissue explant cultures depended on the weight of adenoma tissue, thus the ratio was 2 ml medium per 10 mg tissue. Tissue explants were first incubated at 37 °C in humidified air containing 5% CO_2 for 1 hour without any treatment (for the comparison of the basic hormone secreting activity which appeared to be equal for equivalent samples of the same tumour) and then for 24 hours in the plain medium or in the medium containing TBP.

Afterwards, the medium was removed and stored at −20 °C until radioimmunoassay, while the tissue was fixed for morphological analysis as described. Simultaneously, the same amount of medium was incubated in the same way without any pituitary tissue, for determination of the corticotropin background.

Statistics

Differences between the absolute values of corticotropin determined in the culture media of the control and TBP incubated adenoma tissue explants were evaluated by the *Mann-Whitney*-U test. Modulation by TBP of the corticotropin secretory activity of the pituitary adenomas in relation to their basic hormone secretory activity was evaluated using the *Spearman* rank correlation.

Results

The values of corticotropin found in the culture media of adenomas that were surgically removed without pituitary gland tissue (as revealed by histology, tab. 1) then incubated in the plain medium or in the medium containing TBP are presented in figure 3 and in table 2. If the hormone values determined for the control media were relatively high (> 200 ng/l), addition of TBP to the culture medium resulted in a strong decrease of corticotropin concentration (P = 0.0008). In contrast, if the tumours showed a lower capacity for corticotropin secretion in vitro (< 200 ng/l), addition of TBP increased their corticotropin secreting activity (P = 0.0283). Thus, for the in vitro high corticotropin secreting adenomas the inhibition of the hormone release ranged from 47–99%, while the stimulated hormone release observed for the low corticotropin secreting tumours was 162–2370% of the respective basic control hormone secreting activity. Furthermore, while immunohistochemistry of the control "high corticotropin secreting" tumours revealed weak corticotropin positivity in only one adenoma tissue explant culture, corticotropin positivity was observed for almost all those adenoma explants incubated with TBP, for which inhibition of the corticotropin in the culture medium was noticed.

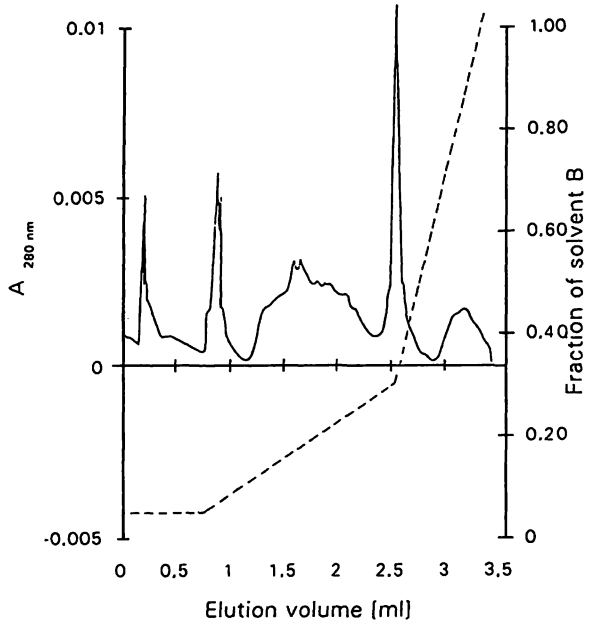

Fig. 2 Analytical anion-exchange liquid chromatography of the bioactive fraction of human plasma 3000–30 000 M_r membrane ultrafiltrate.
Lyophilised TBP (amount corresponding to approximately 50 ml of plasma) was dissolved in 25 µl of 10 mmol/l Tris/HCl buffer (pH 8) – solvent A, diluted 1 : 10 by H_2O and applied to SMART Mini-Q anion-exchanger Sepharose using a linear 250 mmol/l NaCl gradient in the same buffer (solvent B = solvent A + 250 mmol/l NaCl).

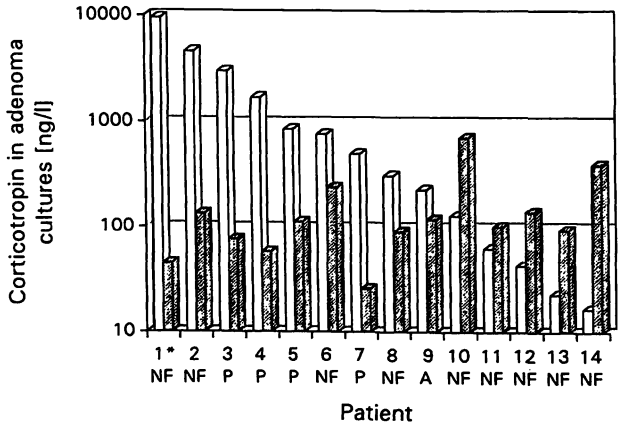

Fig. 3 The effects of TBP on corticotropin release in vitro from the pituitary adenomas incubated as tissue explants without pituitary gland tissue.
Effects of TBP (shaded bars) on corticotropin release in the culture medium (ng/l · d) are presented in comparison with the respective adenoma incubated in the plain medium only (opened bars).
* – patient number (according to the tab. 1) and clinical diagnosis of the respective adenoma secretory activity; NF – non-functioning, P – prolactinoma, A – acromegaly.

Such effects of TBP on the corticotropin secreting activity of the pituitary tumours in vitro were observed only for those tumours removed by surgery without pituitary gland tissue. If the samples of tissue explant cultures contained pituitary gland tissue in addition to adenomatous tissue (as revealed by histology, tab. 1) (fig. 4 and tab. 3), addition of TBP[1]) did not produce any regular and significant effect P > 0.1). Although high corticotropin concentrations (> 200 ng/l) were measured in the medium of all these cultures, a moderate decrease (approximately 50% or less) of the corticotropin concentration in the culture medium was noticed for three tissue explant cultures only. Moreover, for two other samples of this group TBP was even stimulatory (above 250% of the respective control value), in spite of the high corticotropin concentrations in the control culture medium. Immunohistochemistry of the control tissue explant cultures showed moderate to strong corticotropin positivity in four tumours. For all of these, addition of TBP to the culture medium decreased corticotropin positivity in the tumour cells, although there was no obvious change of the hormone concentration in the culture medium. Widespread corticotropin staining of the normal pituitary tissue was noticed in all the tissue explants, in the control samples, and in the TBP treated tissue explants.

Furthermore, there was a strong negative correlation (r = −0.92, P < 0.01) between the dependence of the biomodulating effects of TBP on the corticotropin secretory activity of the pituitary adenomas and their basic hormone secretory activity (fig. 5); such a correlation was not observed for the samples of pituitary adenomas incubated with the pituitary gland tissue (r = −0.14, P > 0.1). Thus, while TBP did not influence corticotropin release from the tissue explant cultures of the tumours mixed with the pituitary gland tissue, the dual effect of TBP on corticotropin secretion in vitro, i. e. inhibition of the secreting activity of "high corticotropin secreting" tumours and stimulation of corticotropin secretion of "low corticotropin secreting" tumours was

Tab. 2 Comparison of the corticotropin secretory activity and immunohistochemistry (corticotropin positivity) of the pituitary adenoma explants without normal pituitary gland tissue.

Patient – sex/age	Clinical findings of adenoma secretory activity	Control adenoma tissue explant culture corticotropin secretory activity (ng/l)	Relative values of adenoma tissue explant culture corticotropin secretory activity in presence of TBP (% of the respective control value)	Immunohistochemistry (corticotropin positivity) of the adenoma cells in the control adenoma tissue explant cultures	Immunohistochemistry (corticotropin positivity) of the adenoma cells in the tissue explants cultured in presence of TBP
1. ♀/65	non-functioning	9500.0	0.5	negative*	+
2. ♂/65	non-functioning	4619.0	2.9	negative	+++
3. ♀/27	prolactinoma	3000.0	2.6	+	++
4. ♀/28	prolactinoma	1698.0	3.4	negative	+
5. ♂/28	prolactinoma	857.0	13.2	negative	++
6. ♀/38	non-functioning	761.0	31.3	negative	++
7. ♀/28	prolactinoma	498.3	5.9	negative	negative
8. ♀/70	non-functioning	308.0	29.2	negative	negative
9. ♀/37	acromegaly	226.6	52.3	negative	not done
10. ♀/25	non-functioning	127.7	548.1	negative	negative
11. ♂/33	non-functioning	62.1	162.2	negative	negative
12. ♀/70	non-functioning	42.8	324.8	negative	negative
13. ♂/76	non-functioning	22.8	409.7	negative	netative
14. ♀/51	non-functioning	16.5	2369.7	negative	not done

* – negative no positive cells
\+ weak positivity (< 5% of the cells)
++ moderate staining (approximately 25% of the cells)
+++ strong staining (50–75% of the cells)

According to *Katznelson* et al. (13) tissue explants were cultured for 24 hours in the plain medium (control) or in the medium containing bioactive fraction (100 g/l plasma equivalent concentration) of the human plasma ultrafiltrate (TBP).

strong and regular, provided the adenoma tissue was removed without traces of the surrounding pituitary gland.

Discussion

The results obtained show that the human plasma ultrafiltrate bioactive fraction (TBP) contains potent factor(s) which regulate(s) secretion of corticotropin from the human pituitary adenoma tissue explants in vitro. The nature of the active component of TBP is not yet defined. However, it might be similar to the growth modifying factor found as a heat and acid resistant component in different tissues and culture media (12, 14, 15). Similar factors modifying tumour cell behaviour have been described under various names (depending on the source of preparation, method of purification and the bioassay used for determination of the activity) (16–21). The basic principle of the biological activity of TBP is still uncertain, but it appears to be a ubiquitous factor that modifies cellular activity. Due to its ability to pass through a M_r 30 000 cut-off membrane and inability to pass through a M_r 3000 cut-off membrane, the M_r of TBP should be within the range of numerous bioactive peptides and low M_r proteins (20, 22). TBP cannot be a non-specific very low M_r component (like hydrocortisone, cAMP, etc.) since the M_r of such factors is below 3000. Thus it is not yet known, whether TBP is a new bioactive plasma (and/or tissue) factor. Preliminary data indicate that TBP could also influence the reactivity of the human peripheral blood mononuclear cells to phytohaemagglutinin, stimulating the reactivity of poorly reactive cells and inhibiting the reactivity of highly reactive cells (12).

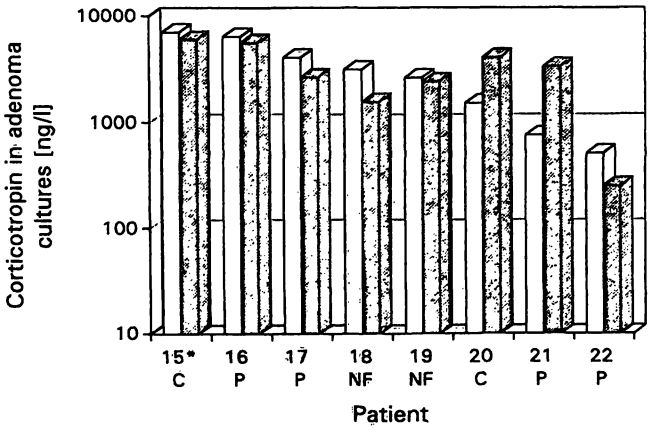

Fig. 4 The effects of TBP on corticotropin release in vitro from the pituitary adenomas incubated as tissue explants in the presence of pituitary gland tissue.
Effects of TBP (shaded bars) on corticotropin release in the culture medium (ng/l · d) are presented in comparison with the respective adenoma incubated in the plain medium only (opened bars).
* – patient number (according to the tab. 1) and clinical diagnosis of the respective adnoma secretory activity; NF – non-functioning, P – prolactinoma; C – *Cushing*'s disease.

Similarly, the results show that the effects of TBP on corticotropin release from the cultured pituitary adenomas depended on the basal corticotropin secreting activity of the adenomas. Thus, TBP inhibited (on average more than 90%) corticotropin release from the adenomas that exerted relatively high basic hormone release

Tab. 3 Comparison of the corticotropin secretory activity and immunohistochemistry (corticotropin positivity) of the pituitary adenoma explants with surrounding normal pituitary gland tissue.

Patient – sex/age	Clinical findings of adenoma secretory activity	Control adenoma tissue explant culture corticotropin secretory activity (ng/l)	Relative values of adenoma tissue explant culture corticotropin secretory activity in presence of TBP (% of the respective control value)	Immunohistochemistry (corticotropin positivity) of the adenoma cells in the control adenoma tissue explant cultures	Immunohistochemistry (corticotropin positivity) of the adenoma cells in the tissue explants cultured in presence of TBP	Immunohistochemistry (corticotropin positivity) of the pituitary cells in the control adenoma tissue explant cultures	Immunohistochemistry (corticotropin positivity) of the pituitary cells in the tissue explants cultured in presence of TBP
15. ♀/31	*Cushing*'s disease	7300.0	89.7	+++*	+	++++	++++
16. ♂/57	prolactinoma	6418.0	87.6	++	+	+++	+++
17. ♂/41	prolactinoma	4102.0	65.5	negative	negative	+++	+++
18. ♂/37	non-functioning	3146.0	49.9	negative	negative	+++	++++
19. ♂/41	non-functioning	2640.0	91.7	negative	not done	+++	+++
20. ♂/66	*Cushing*'s disease	1525.0	268.1	+++	negative	++++	++++
21. ♀/40	prolactinoma	761.0	440.3	negative	negative	++++	++++
22. ♀/28	prolactinoma	514.0	48.9	++	+	++++	+++

* – negative no positive cells
\+ weak positivity (< 5% of the cells)
\++ moderate staining (approximately 25% of the cells)
\+++ strong staining (50–75% of the cells)
\++++ widespread staining (> 75% of the cells)

According to *Katznelson* et al. (13) tissue explants were cultured for 24 hours in the plain medium (control) or in the medium containing bioactive fraction (100 g/l plasma equivalent concentration) of the human plasma ultrafiltrate (TBP).

(200 ng/l in 24 hours appeared to be the minimal released control corticotropin value which was inhibited). In contrast, TBP stimulated corticotropin release (on average up to four-fold) by the adenomas that exerted relatively low (< 200 ng/l in 24 hours) basic hormone release. Furthermore, almost opposite results were obtained for corticotropin immunohistochemistry and corticotropin concentration in the medium of the control and TBP treated "high corticotropin secreting" adenomas, indicating that TBP inhibition was actually prevented of the release of the hormone into the culture medium.

In tissue explant cultures of the "low corticotropin secreting" tumours, increase of corticotropin concentration in the culture medium was not associated with any obvious change of the cellular corticotropin immunoreactivity. Hence, either the TBP activity mechanism was different for the high and for the low corticotropin secreting (or releasing) adenomas, or immunohistochemistry could not reveal corticotropin in the "low corticotropin secreting" tumours.

Fig. 5 Dependence of the effects of TBP on the corticotropin secretory activity of the cultured pituitary adenomas in relation to their basic corticotropin secretion in vitro.
A – samples of the pituitary adenomas cultured without pituitary gland; B – samples of the pituitary adenomas cultured with surrounding pituitary gland tissue.
The relative effects of TBP (expressed as percentage of the hormonal activity measured for the same tumours incubated in the plain culture medium) were compared by using the *Spearman* rank correlation.

Such effects of TBP were not observed if the adenoma samples contained pituitary gland tissue. That was a surprising finding, since most of these samples showed a very high basic corticotropin secretion, so that a relatively strong inhibition by TBP could be expected. There are at least two possible explanations for this ineffectiveness of TBP. Thus, either TBP could not suppress corticotropin release from the pituitary gland in these tissue explant cultures, or it could not suppress corticotropin release from the adenoma tissue, due to interference by the tumour and pituitary gland corticotropin secretion.

In favour of the first possibility is the presence of high corticotropin in the culture media of the normal pituitary gland (23) and widespread immunohistochemical positivity to corticotropin in the normal pituitary surrounding adenoma tissue, which was not influenced by TBP treatment. The finding of decreased immunohistochemical positivity of the tumour cells after treatment with TBP, with no effect on corticotropin concentration in the culture medium of the adenomas incubated with the surrounding pituitary gland, could indicate that TBP has a different effect on normal pituitary and adenoma cells, i. e. stimulating the release of corticotropin from the tumour cells, but not affecting corticotropin metabolism in the normal cells. However, this possibility should be verified by further experimental studies.

The second possibility cannot be excluded, since it was not possible to distinguish release of corticotropin from the adenoma and corticotropin release from the surrounding pituitary gland tissue. Moreover, cell to cell communication is of high importance in the regulation of corticotropin secretion (24), and the integrity of the tumourous and surrounding pituitary tissue was preserved during incubation of these tissue explants.

On the other hand, it is possible that TBP influenced the hormonal activity of "multipotential pituitary cells" which mainly contain corticotropin and play an important role in the regulation of hormone secretion by the other pituitary cells (25).

Furthermore, post-surgical stress (removal of the pituitary tumour) as well as growth factor synthesis by the tumourous tissue in vitro modulate hormone secretion (26–28). It is possible that TBP (bioactive fraction of the human plasma ultrafiltrate) contains factor(s) involved in the stress reaction or paracrine regulation of the growth factor synthesis, thus indirectly affecting the hormone release, but these possibilities have to be further evaluated. Finally, it is not impossible that TBP, like some other bioactive peptides (29–35), is involved in regulation of adenylate cyclase activity and/or calcium metabolism, thereby acting as a regulator of the hormonal activity of the normal and tumourous pituitary tissue.

From the present results, however, it seems that TBP could be the humoral factor involved in the regulation corticotropin release from the pituitary adenoma tissue, but not in the regulation of corticotropin release from normal pituitary tissue. To verify this possibility, it will be necessary not only to analyse the nature of this bioactive component of the human plasma ultrafiltrate but also to use the advantages of the tissue explant culture method to evaluate the effects of TBP on the hormone release from the normal pituitary gland, as well as on the growth features of normal and tumour cells.

Acknowledgements

This study was supported by Croatian Ministry of Science and Technology, Austrian Science Foundation (FWF) and International Association for Cancer Research (AICR). The authors would like to express their sincere gratitude to Biomedica (Austria) and Mr. *Herbert Zakarias* for collaboration.

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Received January 23/May 29, 1995

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