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Oestrogen and testosteron effects on hormone secretion and cell morphology of human pituitary tumours

An in vitro analysis

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Summary. Estradurin® (a polymer of 17-β-oestradiol) has an inhibitory effect on the synthesis/secretion of PRL. This effect is assumed to be caused by the strong inhibitory effect of the drug on phosphatases. With increasing time of low doses of Estradurin® (0.001 μg/ml) in vitro, the inhibitory effect on PRL secretion was overcome and the synthesis/secretion of PRL increased. The secretion of GH was unaffected in all concentrations (0.001–0.1 μg/ml) except 1 μg/ml. A stimulatory effect of 17-β-oestradiol (0.01 μg/ml) on the synthesis/secretion of PRL was suggested by the tissue from one post-pregnancy pituitary.

Short time organ cultures from prolactinomas or tumors with a concomitant secretion of GH and PRL show no changes of GH or PRL secretion following incubation with (0.001–0.1 μg/ml) of 17-β-oestradiol. However, electron microscopy of 17-β-oestradiol incubated specimens revealed an increased number of lysosomes and inclusion bodies in the cell cytoplasm.

Incubation with testosterone (0.001–1 μg/ml) caused inhibition of PRL synthesis/secretion in two of three prolactinomas. In GH secreting tumours testosterone did not effect PRL or GH secretion in vitro when compared with controls. The ultrastructural analysis of specimens in which a decrease of PRL synthesis/secretion had occurred showed myelin figures in the cell cytoplasm and accumulations of amorphous electron dense inclusion bodies.

Key words: Pituitary tumours – In vitro – Receptors – Morphology – Gonadotropins

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Adrenal and gonadal steroids may affect pituitary function indirectly via their effects on hypothalamus or by a direct action of these hormones on the pituitary gland (Strumpf et al. 1975; Mahesh and McPherson 1977). Most studies on direct gonadotropin effects on the pituitary gland have been performed on animal material, normal or neoplastic. The data by Matsukara et al. (1977) on pituitary hormones in human tumours indicate a possible role of steroids in altering receptor activity in the control of hormone secretion. It is therefore important to study human tissue directly. In vitro techniques using human pituitary material, either as dispersed cells (Mashiter et al. 1977; Adams et al. 1979) or as organ cultures (Batzdorf et al. 1971; Peillon et al. 1975; Goddyer et al. 1977; Skyler et al. 1977; Anniko et al. 1980) (review: Tixier-Vidal and Farquhar 1975), are particularly suitable, since they exclude extrapituitary regulatory mechanisms.

The present investigation has used the organ culture technique in the study of oestrogen and testosterone effects on growth hormone (GH) and prolactin (PRL) secretion of human GH and PRL secreting tumours in correlation with cell morphology following incubation.

Material and methods

Material

Pituitary tumour tissue was obtained from seven patients with pituitary adenomas (Table 1). Three tumours secreted PRL, two secreted GH and one had a concomitant secretion of GH and PRL. Tissue from one post pregnancy pituitary was also obtained. Preoperative endocrine insufficiencies are shown in Table 1.

Table 1. Table illustrating the patients subjected to surgery

Patient no.	Age/sex	Type of tumour	GH[a]		PRL[b]		Endocrine insufficiency
			In vivo	In vitro[c]	In vivo	In vitro[c]	
1[d]	25 F	PRL	<12	3,400±260	560	103±15	LH, FSH
2	34 M	PRL	<12	29±9	1,600	16,150±2000	LH, FSH
3[d]	35 F	PRL	<12	11,900±1520	300	81±16	LH, FSH
4	35 M	GH	1,700	3,500±360	14	67±11	0
5[d]	55 F	GH+PRL	530	239,500±28 300	130	750±94	LH, FSH
6	37 M	GH	10,000	395,000±65 000	11	191±26	0
7	29 F	Post pregnancy hyperplasia	80	6,500±3 500	2	40±11	0

[a] Normal range in serum: <433 pmol/l
[b] Normal range in serum: <25 μg/l
[c] Secretion of hormone during 48 h
[d] Preoperative bromocriptine therapy: case no 1–12.5 mg/d for 6 months, case no 3–12.5 mg/d for 9 months, case no 15–10 mg/d for 3 months

Methods

Organ culture. The present organ culture technique has been described in detail (Anniko et al. 1980). In brief: After excision of the tumour which has been excised in fragments it is placed in Dulbecco's phosphate buffered saline and transferred to the organ culture laboratory. Within 30 min after removal the tumour is divided into small fragments with a diameter of less than 1 mm. Organ culture is performed in Neuman and Tytell's serumless medium supplemented with 15% fetal calf serum and 1% L-glutamine. Antibiotics or antibiotics are not used. Only one specimen is kept in each organ culture disk.

Organ culture was performed in air atmosphere supplemented with 5% CO_2. The organ culture medium was renewed every second day. All specimens were cultured $1–2 \times 48$ h to allow an estimation of the basal secretion of GH and PRL. Both hormones were analyzed in all samples.GH was analyzed by a commercial RIA-Kit, Phadebas hGH Prist, and PRL according to Bio-Data.

Every experiment included 6–12 organ cultures. The number of controls was approximately 10–15 for each pituitary tumour. Basal secretion was considered to be the final concentration reached in the culture medium after the first 48 h in vitro.

Incubation with oestradiol. Two different solutions were used. First: Estradurin® (LEO Corporation, Sweden) which is a polymer of 17-β-oestradiol with a slow hydrolysis and soluble in water. Second: pure 17-β-oestradiol dissolved directly in the culture medium. Both substances were used in the concentrations 1.0, 0.1, 0.01 and 0.001 µg/ml. Incubation were usually for 2×48 h, but sometimes extended to $3–6 \times 48$ h.

Incubation with testosterone. Sustanon® (Organon Corporation) was used. This drug is a combination of four different types of testosterone esters: testosteroni propinas, testosteroni phenylpropionas, testosteroni isocapronas and testosteroni decanoas in the ratios 0.3:0.6:0.6:1. Incubation was performed with the same concentrations and using the same procedure as described for oestradiol.

Morphological processing. At the end of the experiment the specimens were fixed in a solution of 3% glutaraldehyde in 0.133 M sodium phosphate buffer and prepared for light and electron microscopy. C.f. Anniko et al. (1980).

Results

General considerations

A difference in hormone synthesis/ secretion varied between different tumours in vitro (Table 1). The basal secretion of GH generally decreased in organ cultures but occasionally kept at a constant level during the first 1–2 weeks in vitro. The synthesis/secretion of GH in individual specimens varied from 20 to 20,000 pmol/1/48 h per culture dish (PRL secreting tumours) to 2,500–1,200,000 pmol/1/48 h per culture dish (GH secreting tumours). In contrast, PRL secretion increased with time in vitro, both in prolactinomas and tissue from acromegalic patients. The concentration of PRL obtained in individual culture dishes after 48 h in culture varied from 90 µg/l to 30,000 µg/l.

The diagrams for the experimental series are presented as representative individual specimens because correlations are made with the morphological state of the tissue. The general outlines for the secretion of GH and PRL in vitro in each case/group/subgroup is commented on in the text.

1. Incubations with oestradiol

Estradurin®

a) Hormonal secretion. In *PRL secreting tumours* a very pronounced inhibition of PRL secretion occurred using 1 μg/ml. Concentrations of 0.1, 0.01 and occasionally 0.001 μg/ml also lowered the PRL secretion when compared with controls (Figs. 1 and 2). The lowest concentration of 0.001 μg/ml frequently caused only a temporary inhibition of PRL secretion (Fig. 3).

Specimens from the three patients with prolactinomas (cases no 1, 2 and 3) also secreted GH into the culture medium (12–3,500 pmol/1/48 h). The secretion of GH was unaffected by the incubation with Estradurin® except in the highest concentration, 1 μg/ml, which depressed GH secretion.

In tumour tissue from patients with *acromegaly* (cases nos. 4 and 6) incubation with 0.001–1 μg/ml did not affect the secretion of GH when compared with controls (Fig. 4). However, the secretion of PRL, which occurs in these tumours in low amounts, was lowered by 1 μg/ml, but not following exposure to lower concentrations (Fig. 5).

b) Morphology. At the light microscopical level an accumulation of hormone-like inclusions and vesiculation of cell cytoplasm occurred after 4–6 days of in vitro culture using the concentration 1 μg/ml (Figs. 6 and 7). In lower concentrations (0.1 and 0.01 μg/ml) only an increased number of hormone-like inclusion bodies were observed.

The ultrastructural analysis following incubation with 1, 0.01 and occasionally 0.001 μg/ml revealed a large number of myelin figures in the cell cytoplasm, an increased number of lysosomes, accumulations of amorphous

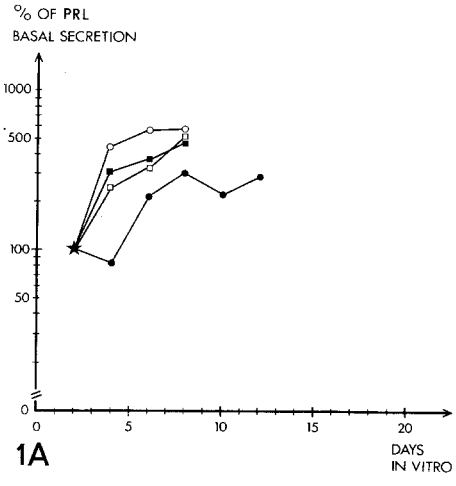
Fig. 1. **A** Diagram showing the secretion of PRL in controls. In all cases the secretion of PRL increases with time in vitro. (■ case no 1; ● case no 4; □ case no 3; ○ case no 5) **B** Diagram showing the secretion of GH in control organ cultures. In general, the secretion of GH decreases with time in vitro as represented by two specimens from case no 4 (●). In only one case (no 5; ■) of the total material an initial increase of GH secretion occurred in organ culture

Fig. 2. Diagram showing the secretion of PRL (case no 2) after incubation with Estradurin®: ●, 1 μg/ml. ○, 0.1 μg/ml. ■, 0.01 μg/ml. □, 0.001 μg/ml. ▲, controls

Fig. 3. Diagram showing the secretion of PRL (case no 1) after incubation with Estradurin®: ●, 1 μg/ml. ○, 0.1 μg/ml. ■, 0.01 μg/ml. ▲, controls. The dotted line illustrates the incubation period. Exposure to 0.01 μg/ml of Estradurin® causes an initial decrease of PRL secretion which later is overcome and reaches rather normal values after 8 days in vitro

Fig. 4. Diagram showing that in explants from tumour no 4 the incubation with Estradurin does not influence the secretion of GH. ▲, control. ●, 1 μg/ml. ○, 0.1 μg/ml. ■, 0.01 μg/ml. Exposure to 0.01 μg/ml can possibly act stimulatory on the secretion of GH. However, this was found only in 2/7 organ culture explants

Fig. 5. Diagram showing the secretion of PRL (case no 4) after exposure to Estradurin: ▲, control. ●, 1 μg/ml, ○, 0.1 μg/ml. ■, 0.01 μg/ml


% OF PRL
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1000
500
100
50
0
0
5
10
15
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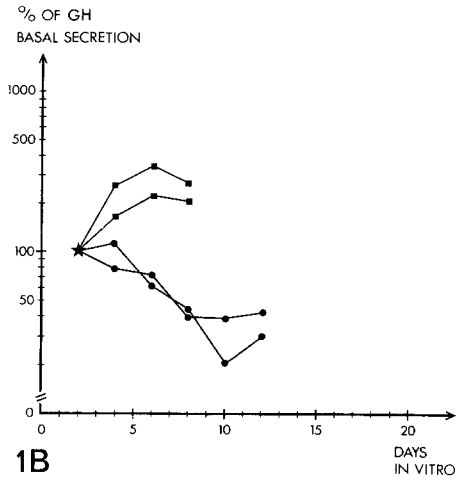
DAYS
IN VITRO

1A


% OF GH
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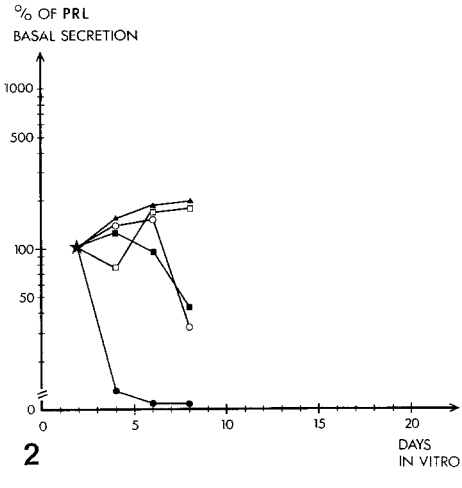
1B


% OF PRL
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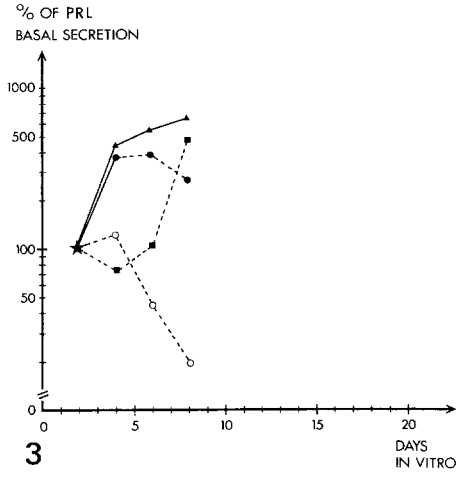
2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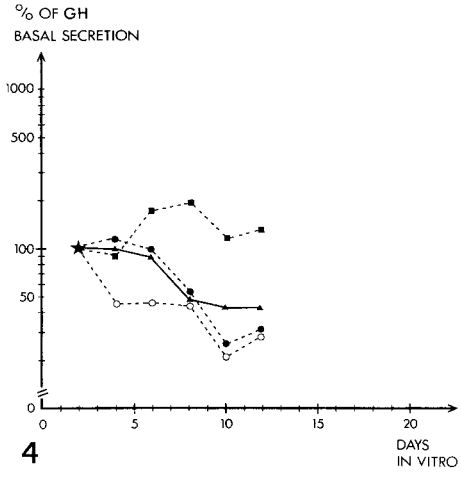
% OF GH
BASAL SECRETION
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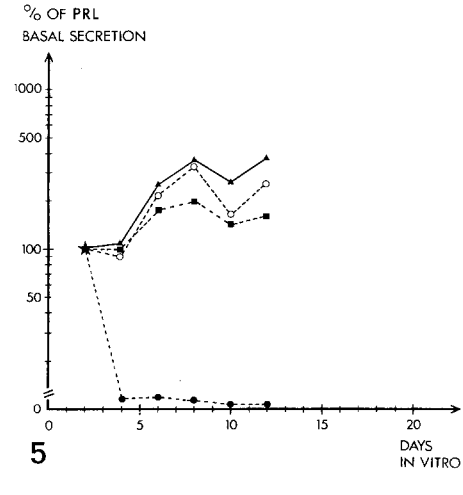
4


% OF PRL
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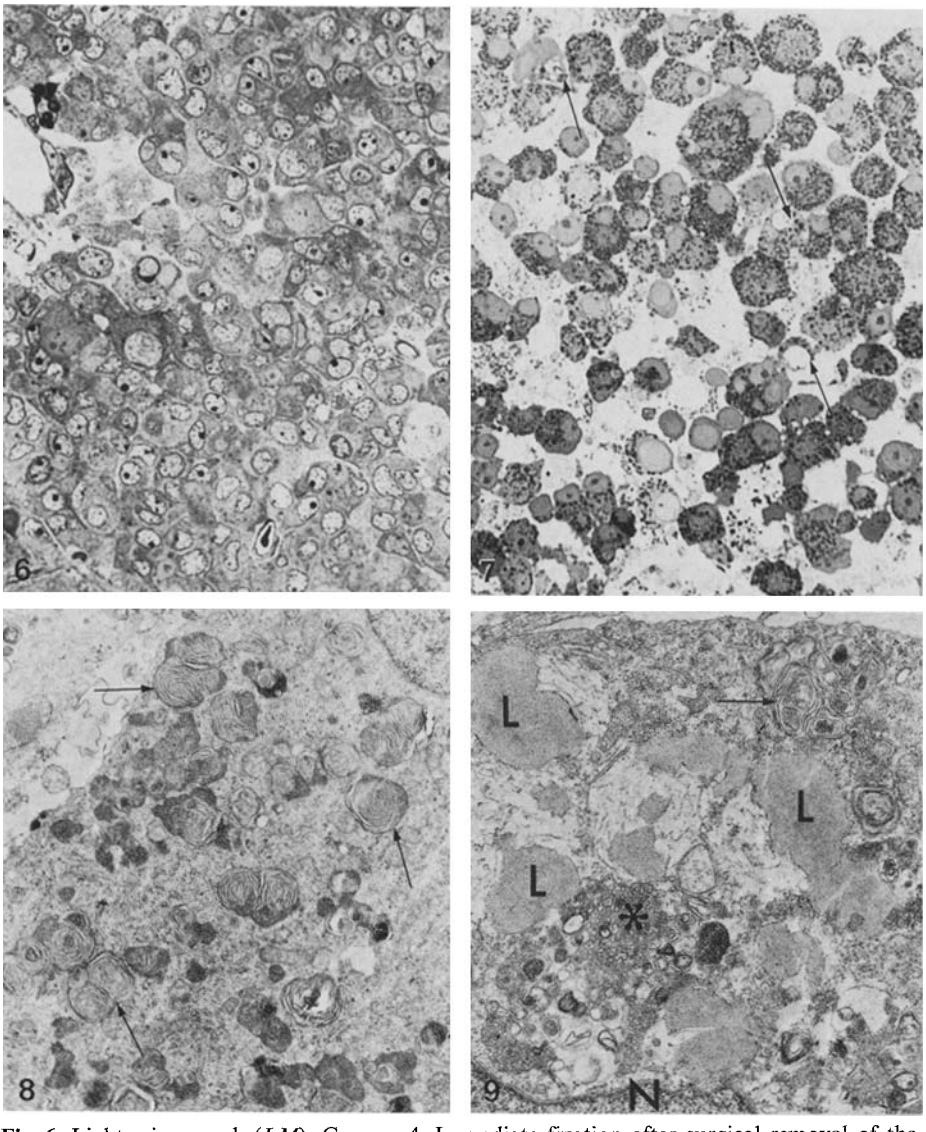


Fig. 6. Light micrograph (*LM*). Case no 4. Immediate fixation after surgical removal of the tumour. A certain pleomorphism of cells occur. Very few non-tumourous cells are identified. Hormone granules are not identified. × 320

Fig. 7. LM. Specimen from tumour no 4. Organ culture during 6 days. Incubation with 1 μg/ml of Estradurin. The cells contain a large number of dense inclusions in the cell cytoplasm. The dense packing of cells has been replaced by loosely dispersed cells. Some cells show vesiculation of cytoplasm (*arrows*). × 320

Fig. 8. Electron micrograph (EM). Organ culture specimen from tumour no 2. Exposure to 0.1 μg/ml of Estradurin during 8 days in vitro. C.f. Fig. 4. Many cells are filled with myelin-figure-like inclusions (*arrows*) in the cytoplasm. Otherwise the ultrastructure appears well preserved. × 8,800

Fig. 9. EM. Organ culture specimen from tumour no 1. Exposure to 1 μg/ml of Estraduring during 8 days in vitro. C.F. Fig. 3. Several cells show obvious signs of degeneration: formation of myelin figures (*arrows*), lipoid-like inclusions (*L*) and aggregations of amorphous material (*asterisk*). The ground substance of the cell is rather heterogeneous with several translucient areas. *N*, nucleus. × 14,900

material (possibly degenerating mitochondria or degenerating hormone granules), but very few hormone granules (Figs. 8 and 9). Such morphological changes were absent both in tissue taken for immediate fixation and in controls. The cell nucleus was normal. Many mitochondria revealed cristal separation/disintegration without swelling or loss of ground substance.

Specimens exposed to 0.001 μg/ml showed a normal morphology.

17-β-oestradiol

a) Hormone secretion. Incubation with 0.1–0.001 μg/ml did not influence the synthesis/secretion of GH or PRL except in case no 7, which was largely composed of normal tissue (post pregnancy hyperplasia). A stimulatory effect was suspected using the concentrations 0.1–0.01 μg/ml but not after incubation with 0.001 μg/ml.

b) Morphology. Specimens incubated with 17-β-oestradiol showed a well preserved general ultrastructure. Cells contained hormone granules of very varying sizes. Accumulations of electron dense inclusion bodies frequently occurred in many cells (Figs. 10 and 11). Most inclusions had an amorphous substructure but some were found with a substructure comprising several small aggregations. When compared with controls and tissue taken for immediate fixation, such findings appeared to be specific for incubation with 17-β-oestradiol. These ultrastructural changes occurred during incubation with even the lowest concentration at 0.001 μg/ml, but to a low extent.

2. Incubation with testosterone (Sustanon®)

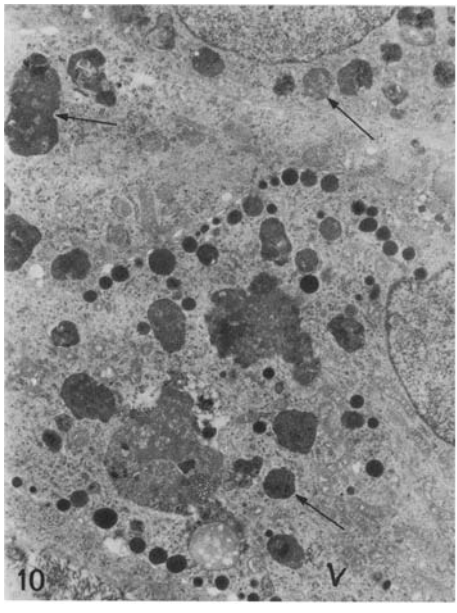
A. Hormone secretion

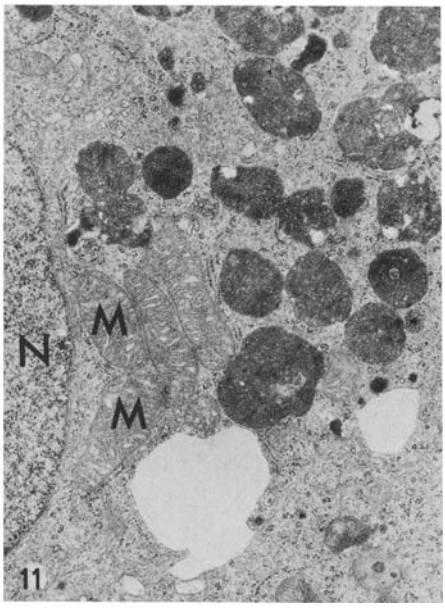
Prolactinomas. An inhibitory effect on PRL secretion was achieved with 1 and 0.1 μg/ml in two tumours (nos 2 & 4) (Fig. 12) whereas incubations of tissue from tumour no 1 did not affect the synthesis/secretion of PRL. In a few specimens from tumour no 2 the concentration of 0.001 μg/ml also inhibited the normal increase of PRL, as encountered in controls (Fig. 12).

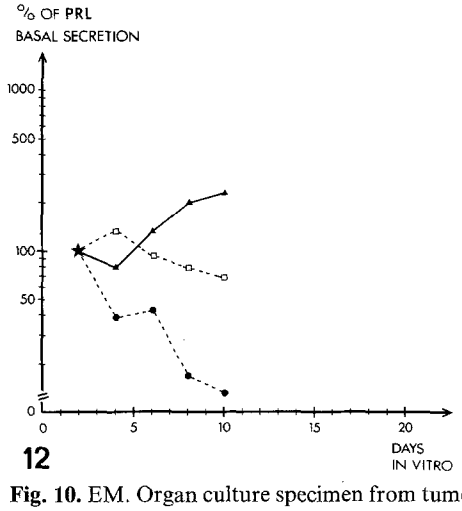
Tumour tissue causing acromegaly. Incubation with testosterone in the concentrations 0.001–1 μg/ml did not affect PRL or GH secretion (Fig. 13).

B. Morphology

In general, a large number of surviving cells occurred. In specimens with a decrease of the secretion of hormone (mainly prolactinomas) morphological changes were evident (Figs. 14 and 15). The number of hormone granules was low. The cell cytoplasm contained myelin figures and accumulations of amorphous electron dense inclusion bodies (Fig. 16). The ultrastructure







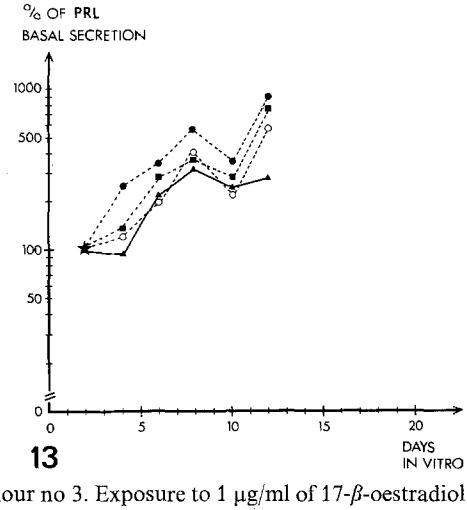


Fig. 10. EM. Organ culture specimen from tumour no 3. Exposure to 1 μg/ml of 17-β-oestradiol during 6 days in vitro. The cells contain a large number of electron optically dense inclusions in the cell cytoplasm (*arrows*). In some cells hormone granules line the cell membrane while other cells are devoid of secretory granules in the same secretion. The cell organelles appear ultrastructurally normal. × 3,100

Fig. 11. EM. Organ culture specimen from tumour no 3. Incubation with 0.1 μg/ml of 17-β-oestradiol during 6 days in vitro. Electron optically dense inclusion bodies occur in the cell cytoplasm. The ground substance, mitochondria (*M*) and the nucleus (*N*) are normal. Some inclusions are suspected to be composed to aggreates from dense material (degenerating hormone granules?). × 9,100

Fig. 12. Diagram showing the secretion of PRL. Case no 2. Incubation with testosterone during 8 days in vitro (*dotted line*). ●, 1 μg/ml. □, 0.001 μg/ml. ▲, control

Fig. 13. Diagram showing the secretion of PRL after exposure to testosteron. Case no 6. ▲, control. ●, 1 μg/ml. ○, 0.1 μg/ml. ■, 0.01 μg/ml. The incubation period is indicated with the *dotted line*

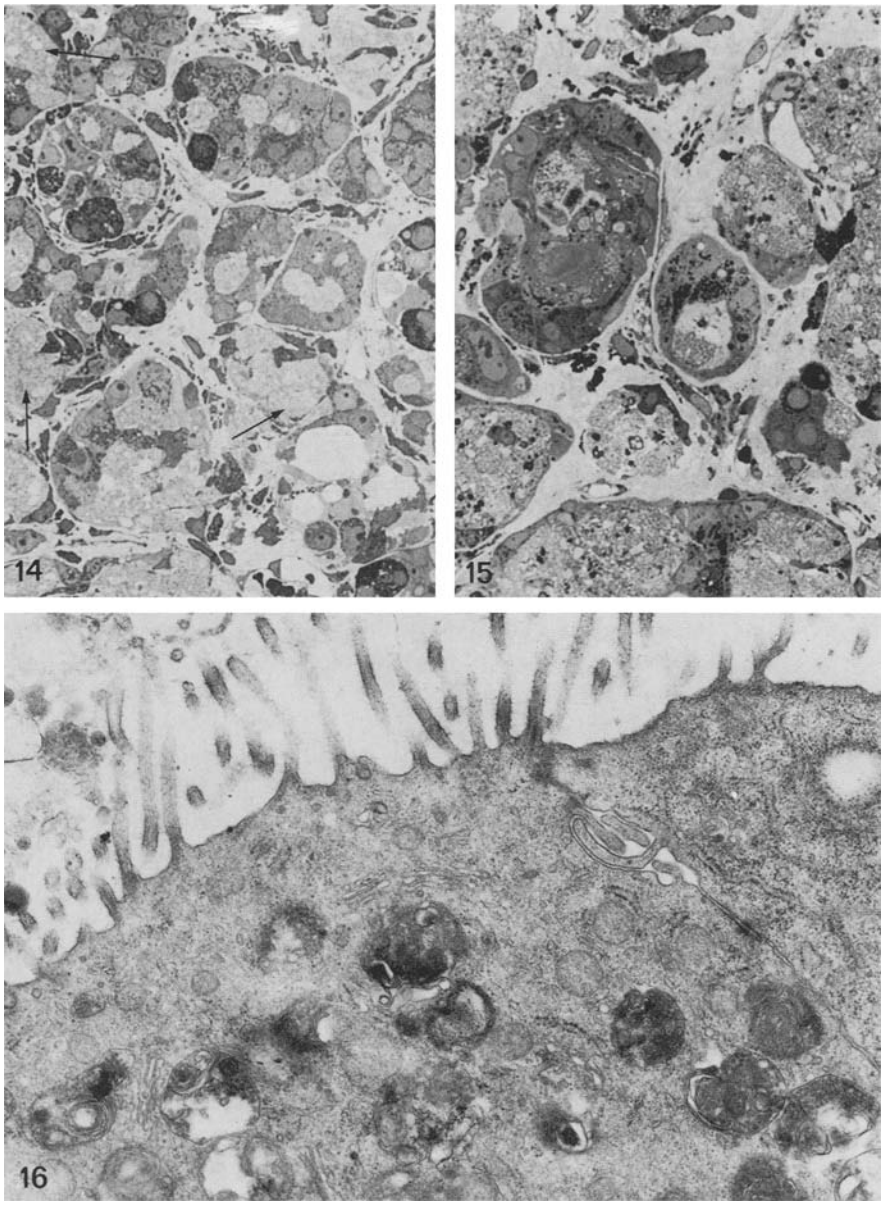


Fig. 14. LM. Organ culture during 8 days. Control specimen, case no 1. A large number of preserved cells occur in the explant. A few cells appear necrotic in the centre aggregates of cells (*arrows*). Some cells stain more intensely and contain a large number of secretory granules. × 270

Fig. 15. LM. Organ culture explant from case no 1. After 4 days in vitro in ordinary culture medium the specimen was exposed to 1 µg/ml of testosterone during 4 days. Considerably more cells appear necrotic than in Fig. 14. Surviving cells are filled with inclusions in the cell cytoplasm. × 240

Fig. 16. EM. A cell from the specimen in Fig. 15. The cytoplasm contains a large number of electron optically dense inclusions with a myelin-figure-like substructure. The cell organelles are ultrastructurally preserved. Frequent microvilli extend from the cell surface. × 12,900

of the cells was otherwise normal. Some cells had microvilli on the cell surface (Fig. 16). Many cells were entirely devoid of any degenerative changes. However, the number of hormone granules was often low in these cells and only occasionally cells were found with a large number of hormone granules.

Discussion

The mechanisms by which steroid hormones take part in the regulation of pituitary hormone secretion have been the subject of a large number of studies. So far most experimental investigations are based on animal models (review: Allen et al. 1977). Species differences have made it difficult to extrapolate animal data to humans. During recent years methods have been developed for in vitro studies of the human pituitary gland, normal and neoplastic (Guyda et al. 1973; Kohler et al. 1969; Peillon et al. 1975 and 1978; Anniko et al. 1979).

There is a general agreement that both in experimental animals and man physiological and pharmacological doses of oestradiol exert an inhibitory effect on gonadotropin secretion (negative feedback) whereas intermediate doses provide the stimulus for the surge of gonadotropins (positive feedback) responsible for ovulation (Libertun et al. 1974; Mahes and McPherson 1977). Muldoon (1977) reported that the pituitary oestrogen receptor levels did not degenerate in the absence of endogeneous oestrogen but could be augmented by acute stimulation with oestrogen. The receptors were maintained at a constant level following chronic exposure to a hormonal stimulus (rat). Greeley et al. (1974 and 1975) reported that gonadotrophs remain functionally competent even when the pituitary is separated from the influence of the hypothalamus (rat).

Whether or not pituitary tumours still possess the normal receptors for oestrogen remains unknown. Pichon et al. (1980) showed that 14/23 human pituitary tumours contained oestrogen receptors. Receptors were more often present and their concentration higher in PRL-secreting tumours than in GH-secreting adenomas and non-secreting tumours. According to earlier reports on normal pituitary function such low levels of oestradiol as those used in the present study should thus cause a negative feedback effect on the secretion of PRL. However, the incubation with 17-β-ooestradiol did not affect the synthesis/secretion of PRL into the culture medium during 4 days in vitro. The ultrastructural analysis of incubated organ cultures revealed that a large number of cells contained many lysosomes and especially inclusion bodies in the cytoplasm. In spite of the initial nonconformity between hormone secretion and ultrastructure, a negative (inhibitory) feedback mechanism still can be indicated. In control specimens, specific ultrastructural changes did not occur. The morphological changes were apparently not due to the in vitro conditions. It seems likely that the alterations in fine structure are induced by 17-β-oestradiol in the culture medium. Initial changes in the hormone regulatory processes can be reflected by changes

in ultrastructure, e.g. specific inclusion bodies in the cell cytoplasm, before changes of hormone secretion. If the culture period had been increased beyond 4 days in vitro, it is most likely that specific morphological changes could have been correlated with a decreased secretion/synthesis of hormone. Haug & Gautvik (1976) showed that in a clonal strain of rat pituitary tumour cells (GH_3) in vitro, sex steroids altered the production of PRL. Physiological concentrations of 17-β-oestradiol increased the production of PRL but this effect did not occur until after 4 days in vitro. The stimulatory effect was reversible. In humans, an increase in pituitary tumours can occur when the patient becomes pregnant or is given oestrogens (Katjar and Tomkin 1971). The fact that not all pituitary tumours are stimulated by oestrogens can be related to the presence or absence of oestrogen receptors in these tumours (Pichon et al. 1980).

The pituitary tumours subject to in vitro culture had been exposed to bromocriptine prior to surgery in some cases. Specimens taken for immediate fixation at surgery showed groups of degenerating cells and/or large numbers of lysosomes within the cells. These morphological features were not present in the control organ cultures and similar observations were made by Anniko and Wersäll (1981). Thus, it was concluded that the bodies in cells exposed to oestrogen had been induced by the incubation with oestrogen (17-β-oestradiol) because intracellular structures were not found in controls. Electron microscopic observations on oestrogen induced pituitary tumours in the rat show cells with hypertrophy with a well developed endoplasmic reticulum, enlarged Golgi apparatus, fat droplets and few secretory granules initially. Later, the cells become exhausted and contain autophagic vacuoles, round myelinated bodies, degranulated endoplasmic reticulum and an atrophied Golgi apparatus (Watari and Tsukagoshi 1969).

The secretion of GH was not affected by the incubation with 17-β-oestradiol. Neither was the cell morphology. The number of cells with possibly specific inclusion bodies was low in the explants from the pituitary tumour with a concomitant secretion of GH and PRL. These cells could possibly be of PRL secreting type and thus react in a similar way as tumour cells in prolactinomas.

A slight stimulatory effect of 0.01 µg/ml of 17-β-oestradiol was suspected in the case with post-pregnancy pituitary tissue. This observation is in agreement with animal experiments (McPherson et al. 1974 and 1975).

Estradurin® is a biologically inactive polymer of 17-β-oestradiol slowly hydrolysing/releasing biologically active units of 17-β-oestradiol. The inactive drug acts as a very strong inhibitor of phosphatases which is the reason for its very long duration for effect (3 weeks) (LEO Corporation, personal communication). The effects of Estradurin® on hormone secretion in organ cultures were interpreted as being caused by the phosphatase inhibiting properties of the drug. Concentrations of 0.001–1 µg/ml caused a strong inhibition of PRL synthesis/secretion both in prolactinomas and in pituitary tumours causing acromegaly. In contrast the secretion of GH was largely unaffected by Estradurin® in both endocrinological types of tumours. The use of this drug in vitro would thus permit to separate the function of

cells responsible for production of GH and PRL. The ultrastructural changes caused by Estradurin® differ from those induced by pure 17-β-oestradiol.

Androgens may excert antioestrogenic effects by nuclear translocation of oestrogen receptors in oestrogen-sensitive tissues in animals (Rochefort et al. 1972; Ruh et al. 1974). However, Schmidt et al. (1976) reported that this effect was not likely to occur under physiological conditions. Androgen receptors would excert their effects independently of the androgen interference with oestrogen receptors. Incubation with testosterone caused inhibition of PRL synthesis/secretion in tumour tissue from two prolactinomas whereas no effect occurred on tumours causing acromegaly. In vitro incubation with testosterone was performed during 4–11 days, i.e. about twice the period of time as used for 17-β-oestradiol.

In prolactinomas the ultrastructural changes caused by testosterone and 17-β-oestradiol were similar. In some testosterone-incubated organ cultures cells were found with microvilli on the surface. The functional significance of these observations is unknown but may indicate de-differentiation of the cell. Otherwise cell structure, including the degenerative changes, was similar as in adjacent cells without microvilli.

In tumours from acromegalic patients degenerative changes in fine structure were not observed when they were exposed to testosterone in vitro. The difference in cell morphology and hormone secretion between GH and PRL secreting tumours following incubation with testosterone may indicate that the PRL producing cells in prolactinomas and tumours causing acromegaly are different. Whether or not the former are tumour cells and the latter normal PRL producing cells, has to be analyzed further.

Conclusions

1. The organ culture model is a suitable tool for studies on feed-back mechanisms of steroid hormones on pituitary tumour tissue. A direct effect is excerted on tumour cells uncoupling the hypothalamic-pituitary regulatory axis.

2. Oestrogen receptors are present on human pituitary tumour cells from patients with prolactinomas and tumours causing acromegaly.

3. In vitro exposure of tumour tissue using pharmacological doses of 17-β-oestradiol (0.001–0.1 μg/ml) caused specific ultrastructural changes in a dose-response pattern. The morphological alterations are likely to cause a subsequent decrease in hormone secretion with increasing time in organ culture.

4. Incubation of prolactinomas with 0.1–1 μg/ml of testosterone caused in 2/3 cases an inhibition of PRL secretion. This likely reflects the presence of androgen receptors.

5. Incubation with testosterone of tumour tissue causing acromegaly did not affect secretion of PRL or GH (3 cases). In these tumours, however, ultrastructural changes occurred in the cells even following incubation with 0.001 μg/ml of testosterone. Androgen receptors are likewisely not lacking but can be rather few. Thus it would require a long time in organ culture to affect the synthesis of hormone.

References

Adams EF, Brajkovich IE, Mashiter K (1979) Hormone secretion by dispersed cell cultures of human pituitary adenomas: effects of theophylline, thyreotropin releasing hormone, somatostatin and 2-bromo-alpha-ergocryptine. J Clin Endocr Metab 49:120–129

Allen MB, Greeley GH, Costoff A, Mahesh VB, Phil D, Little KY (1977) Experimental models for the study of pituitary function. In: Allen MB, Mahesh VB (eds) The pituitary. Academic Press, New York, pp 235–260

Anniko M, Wersäll J (1981) Clinical and morphological findings in two cases of bromocriptine treated prolactinomas. Acta Pathol Microbiol Scand, Sect A 89:41–47

Anniko M, Eneroth P, Werner S, Wersäll J (1980) In vitro preservation of human pituitary tumours in organotypic differentiation. Acta Otolaryngol (Stockh) 88:424–431

Batzdorf U, Gold V, Matthews N, Brown J (1971) Human growth hormones in cultures of human pituitary tumours. J Neurosurg 34:741–748

Goodyer CG, Hall CS, Guyda H, Robert F, Giroud GJP (1977) Human fetal pituitary in culture: hormone secretion and response to somatostatin, luteinising hormone releasing factor, thyrotropin releasing factor and dibuturyl cyclic AMP. J Clin Endocrinol Metab 45:73–85

Greeley GH, Allen MB, Mahesh VB (1974) FSH and LH response in the rat after intravenous, intracarotid or subcutaneous administration of LH-RH. Proc Soc Exp Biol Med 147:859–862

Greeley GH, Allen MB, Mahesh VB (1975) Potentiation of luteninizing hormone release by estradiol at the level of the pituitary. Neuroendocrinology 18:233–241

Guyda H, Robert F, Colle E, Hardy J (1973) Histologic, ultrastructural and hormonal characterization of a pituitary tumour secreting both GH and prolactin. J Clin Endocrinol Metab 36:531–547

Haug E, Gautvik KM (1976) Effects of sex steroids on prolactin secreting rat pituitary cells in culture. Endocrinology 99:1482–1489

Katjar T, Tomkin G (1971) Emergency hypophysectomy in pregnancy after induction of ovulation. Br Med Bull 4:88

Kohler PO, Bridson E, Rayford PL, Kohler E (1969) Adenomas in culture. Metab Clin Exp 18:782–788

Libertun C, Orias R, McCann SM (1974) Biphasic effect of estrogen on the sensitivity of the pituitary to luteinizing hormone-releasing factor (LRF). Endocrinology 94:1094–1100

Mashiter K, Adams E, Beard M, Holley A (1977) Bromocriptine inhibits prolactin and growth hormone release by human pituitary tumours in culture. Lancet 2:197–198

Mahesh VB, McPherson JC (1977) Steroid hormone feedback systems for the control of pituitary function. In: Allen MB, Mahesh VB (eds) The pituitary. Academic Press, New York, pp 261–293

Matsukura S, Kakita T, Hirata Y, Yoshimi H, Fukase M, Iwasaki Y, Kato Y, Imura H (1977) Adenylate cyclase of GH and ACTH producing tumour of human: activation by non-specific hormones and other bioactive substances. J Clin Endocrinol Metab 44:392–397

McPherson JC, Costoff A, Mahesh VB (1975) Influence of estrogen-progesterone combinations on gonadotropin secretion in castrate female rats. Endocrinology 91:771–779

McPherson JC, Costoff A, Eldridge CJ, Mahesh VB (1974) Effects of various progestational preparations on gonadotropin secretion in ovariectomized immature female rats. Fertil Steril 25:1063–1070

Muldoon TG (1977) Pituitary estrogen receptors. In: Allen MB, Mahesh VB (eds) The pituitary. Academic Press, New York, pp 295–329

Peillon F, Gourmelen M, Donnadieu M, Brandi A, Sevaux D, Pham Huu Trung MT (1975) Organ culture of human somatotrophic pituitary adenomas: ultrastructure and growth hormone production. Acta Endocrinol (Kobenh) 79:217–229

Pichon MF, Bression D, Peillon F, Milgrom E (1980) Estrogen receptors in human pituitary adenomas. J Clin Endocrinol Metab 51:897–902

Peillon G, Cesselin F, Garnier PE, Brandi AM, Donnadieu M, Hermite ML, Dubois MP (1978) Prolactin secretion and synthesis in short-term organ culture of pituitary tumours from acromegalic patients. Acta Endocrinol (Kopenh) 87:701–715

Rochefort H, Lignon F, Capony F (1972) Formation of estrogen nuclear receptor in uterus: effects of androgens, estrone and nafoxidine. Biochem Biophys Res Commun 47:662–670

Ruh TS, Wassilik SG, Ruh MF (1974) Androgen-induced nuclear accumulation of the estrogen receptor. Steroids 24:209–224

Schmidt WN, Sadler MA, Katzenellenbogen BS (1976) Androgen-uterine interaction: nuclear translocation of the estrogen receptor and induction of the synthesis of the uterine-induced protein (IP) by high concentrations of androgens in vitro but not in vivo. Endocrinology 98:702–716

Skyler JA, Rogol AD, Lovenberg W, Knazek RA (1977) Characterization of growth hormone and prolactin produced by human pituitary in culture. Endocrinology 100:283–291

Strumpf WE, Sar M, Keefer DA (1975) Localization of hormones in the pituitary: receptor sites for hormones from hypophysial target glands and the brain. In:Tixier-Vidal A, Farquhar MG (eds) The anterior pituitary. Academic Press, New York, pp 63–82

Tixier-Vidal A, Farquhar MG (1975) The anterior pituitary. Academic Press, New York

Watari N, Tsukagoshi N (1969) Electron microscopic observations on the estrogen-induced pituitary tumour. Gunma Symp Endocrinol 6:297–314

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