Ultrastructural localization of calcium and Ca^{2+}-ATPase activity in gonadotrops and stellate cells of the catfish pituitary

J. Peute[1] *, A.T.M. van Linder[1], M.A. Zandbergen[1], and W.C. de Bruijn[2]

[1] Department of Experimental Zoology, Research Group for Comparative Endocrinology, Padualaan 8, 3584 CH Utrecht, The Netherlands
[2] Clinical Pathological Institute, A.E.M. Unit, Erasmus University, K 902, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

Summary. In the pituitary of the African catfish, *Clarias gariepinus*, calcium precipitates were ultrastructurally visualized with the oxalate-pyroantimonate procedure (OPP). The presence of calcium in these precipiates was validated with several methods, including "Electron Energy Loss Spectrometry" (EELS). In the OPP-treated tissue calcium precipitates were seen in a) non-secretory stellate cells and b) gonadotropic (GTH-) cells. In the latter the amount of precipitate is generally low, but stimulation of the gonadotropin release, either in vivo or in vitro, resulted in a considerable increase. This increase is discussed in relation to the role of calcium as second messenger in the GTH-cells. Ca^{2+}-ATPase was exclusively represented in stellate cells and GTH-cells, its strongest activity associated with the plasma membrane and with the membranes of the endoplasmic reticulum. The localization of this enzyme is discussed in relation to its role in the regulation of the intracellular calcium concentration in the GTH-cells. The stellate cells are considered to be involved in the regulation of extracellular calcium concentrations in the pituitary.

Introduction

In the pituitary of the African catfish, *Clarias gariepinus*, the GTH-cells are exclusively located in the proximal pars distalis (PPD) (Peute et al. 1984). Secretion of gonadotropin (GTH) is stimulated by gonadotropin-releasing hormone (GnRH) (De Leeuw et al. 1987) and inhibited by dopamine (DA) (De Leeuw et al. 1985, 1986). At the ultrastructural level this dual regulation was confirmed with immunocytochemical techniques, showing two types of nerve fibers in contact with the GTH-cells, one type containing GnRH and the other DA (Peute et al. 1987). Recently, the receptors for GnRH (De Leeuw et al. 1988) and DA (Van Asselt et al. 1990) were characterized. For teleost species scarce information is

available about post-receptor systems in GTH-cells. In mammals it was shown that inositol 1,4,5-triphosphate (IP_3) and diacylglycerol are important second messengers (Berridge 1984; Hirota et al. 1985; Sortino et al. 1988; Clayton 1989). Likewise, Ca^{2+} is a necessary factor in the regulation of GTH release, as was illustrated by the rise in intracellular Ca^{2+}-concentration after binding of GnRH to its receptor, whereas absence of Ca^{2+} or blocking the Ca^{2+}-influx attenuates GTH-secretion in response to GnRH (Conn 1982; Bates and Conn 1984; Chang et al. 1988; Drouva et al. 1988). The increase in cytosolic Ca^{2+}, initiated by GnRH, is a result of the release of Ca^{2+} from the intracellular Ca^{2+}-source, the endoplasmic reticulum (ER), and of an influx of extracellular Ca^{2+} via Ca^{2+}-channels (Bates and Conn 1984; Clapper and Conn 1985; Guillemette et al. 1987; Chang et al. 1988; Clayton 1989).

It is assumed that after stimulation of the GTH-cell by GnRH the elevated Ca^{2+}-concentration in the cytoplasm has to be reduced to its basal level. This can be achieved in two ways: 1) Ca^{2+} is transported out of the cell by Ca^{2+}-ATPase and by a Na^+/Ca^{2+} exchanger, 2) Ca^{2+} is pumped back into the ER by means of a similar Ca^{2+}-ATPase mechanism (Schatzman 1985; Carafoli and Longoni 1987).

Investigations of the post-receptor mechanisms in teleost GTH-cells likewise point towards a requirement of Ca^{2+} in the secretion process. In GTH-cells of tilapia Ca^{2+}-ions are involved in GnRH-action (Levavi-Zermonsky and Yaron 1989). Recent in vitro investigations on the African catfish pituitary proved that either the absence of Ca^{2+} in the medium or blocking Ca^{2+}-channels in the plasma membrane diminished GTH-secretion following stimulation with the GnRH analogue Buserelin (Van Asselt et al. 1989).

The purpose of the present study was to obtain additional information about Ca^{2+} in the GTH-cells of the African catfish after stimulated gonadotropin release. The presence and distribution of both Ca^{2+} and Ca^{2+}-ATPase were examined at the ultrastructural level in GTH-cells both under in vivo and in vitro conditions.

The in vivo stimulation was performed by injecting fish with Pimozide/GnRH (De Leeuw et al. 1985); in vitro (in a perifusion system) the basal GTH-secretion is higher than under in vivo conditions in untreated catfish, due to the absence of inhibitory control (De Leeuw et al. 1986).

Material and methods

African catfish, *Clarias gariepinus*, were bred in the laboratory, as described by De Leeuw et al. (1985). For the in vivo stimulation experiment mature female catfish (8 to 10 months old) were injected with the dopamine antagonist Pimozide (10 mg/g) and a gonadotropin-releasing hormone analogue (LHRHa, 0.1 mg/g); 1 ml blood was taken just before the injection. At 30, 45 and 60 min after injection the pituitaries were removed and fixed, and blood was collected for establishing GTH-levels with a homologous radioimmunoassay (RIA) (Goos et al. 1986).

For the in vitro experiment mature female catfish (6–9 months old) were decapitated, and their pituitaries removed and sagitally devided in two halves. The parts were placed in chambers of a perifusion system, as described by De Leeuw et al. (1986). The perifusion-medium was Leibovitz's L15 medium (L15, Serva Heidelberg, FRG) buffered with 15 mM Hepes (pH 7.4) and containing 25% BSA (fraction V; Sigma St. Louis, Mo., USA). The flow rate was 15 ml/h. During the first 2 h no fractions were collected, since the GTH-secretion has to stabilize first. During the next 2-h fractions were collected every 10 min. In these fractions the GTH-content was determined by means of a RIA. After perifusion the pituitary parts were fixed according to one of the following methods.

Calcium (Method according to Borgers et al. 1984)

After dissection (in vivo experiment) or perifusion (in vitro experiment) the pituitaries (parts) were fixed in 0.09 M oxalic acid (pH set at 7.4 with KOH 1 M) with 3% glutaraldehyde and 1.4% sucrose, during 2 h at 4° C. The tissue was rinsed in 0.09 M potassium oxalate (pH 7.4) with 7.5% sucrose for 15 min and postfixed in 1% OsO_4 and 2% potassium-pyroantimonate for 2 h at 4° C. The tissue was then rinsed in aquadest (pH set at 10.0 with KOH 0.1 M) for 15 min, followed by dehydration in a graded series of ethanol and propylene oxide and embedding in Epon. Ultrathin sections were stained with uranylacetate and leadcitrate and observed in a Zeiss (Oberkochen, FRG) EM10 A electron microscope.

Specificity controls:

1. treatment of the sections containing Ca^{2+}-precipitation with 5 mM EGTA solution, 15 min at 60° C.
2. EELS analysis of the precipitate: Electron Energy Loss Spectroscopic (EELS) analyses were performed in a Zeiss EM 902 transmission microscope equipped with an integrated electron energy filter according to Castaign/Henry/Ottensmeyer (Sorber et al. 1990). Untreated ultrathin sections, collected on unfilmed 400 mesh copper grids, were analyzed at 80 kV. Spectra were recorded with the use of the photomultiplyer (PMT) from a probe area of 0.2 µm² of the section with, upon visual inspection, the precipitate-containing parts. Objective-lens apperture = 20 mrad, slit width 1–2 mm, energy resolution: 2–3 eV, with two relative scan speeds. The beam intensity variations over the Δ eV range from 300–400 eV were recorded on a printer-plotter. This range was selected to record the calcium $L_{2,3}$ ionisation edge around 346 eV, which is well-separated from the $M_{4,5}$ ionisation edge of antimony around 580 eV.

Ca^{2+}-ATPase activity (Method according to Ando et al. 1981)

Complete pituitaries (in vivo) or equal parts (in vitro) were fixed in a mixture containing 2% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), for 45 min at 0° C and then washed in cacodylate buffer (pH 7.2) with 8% sucrose for 15 min. The pituitaries (parts) were embedded in 5% agar solution and placed in the cacodylate buffer overnight. With a Vibratome slices of 100 mµ in thickness were cut and subsequently incubated for 30 min at 37° C in Ando's incubation medium, consisting of 250 mM glycine-KOH (pH 8.8), 3 mM ATP (Sigma), 10 mM $CaCl_2$, 2 mM leadcitrate in 100 mM KOH and 8 mM levamisole (Sigma). After rinsing in 0.1 M cacodylate buffer (pH 7.2) with 8% sucrose for 15 min, the tissue was postfixed in 1% OsO_4 in 0.1 M cacodylate buffer (pH 7.2) for 1 h at 0° C. Finally the sections were dehydrated and embedded in Epon. Ultrathin sections were stained with uranylacetate.

Specificity controls:

1. Addition of 5 mM ouabeine (Sigma) to the incubation medium, to exclude the possibility of visualizing reaction products of Na^+-K^+-ATPase.
2. Preheating of the tissue at 80° C before incubation.
3. Incubation in medium lacking enzyme-substrate.
4. Incubation in medium containing 10 mM Vanadate (inhibitor of ATPase).
5. Incubation in medium containing a different substrate (AMP) (Sigma).
6. Incubation in medium containing 3.0 mM PCMB (p-chloromercuribenzoic acid) (Sigma) (phosphatase inhibitor).
7. Incubation in medium containing 3.5 mM n-ethylmaleimide (Sigma) (inhibitor of ATPase)
8. Incubation in medium lacking $CaCl_2$ (with or without preincubation with 50 mM EGTA, 1 h, 0° C).

Results

Oxalate-pyroantimonate procedure

In the proximal pars distalis of the catfish pituitary in which the gonadotropin secretion was not stimulated, electron-dense precipitates were observed in the GTH-cells and in the non-glandular stellate cells. In the GTH-cells fine granular precipitate was present in nucleoplasm, the ER (not all compartments), mitochondria, vacuoles and cytosol (Fig. 1). In the stellate cells precipitates were located mainly in the cytosol and in association with the inner plasma membrane, both in the cell body and in their long extensions (Fig. 2).

After in vivo stimulation of gonadotropin secretion by means of LHRHa/Pimozide injection (Table 1), the amount of precipitation present in the GTH-cells was higher than in the instimulated control animals. This increase was especially noticed in both small and large cisternae of the ER and, on visual expection, most obvious at 45 min after injection (Fig. 3). However, there were marked differences in the amount of precipitate between different GTH-cells within one pituitary.

The gonadotropin secretion by pituitaries under in vitro conditions was also high, as shown in Table 1. The majority of the gonadotropic cells in such pituitary frag-
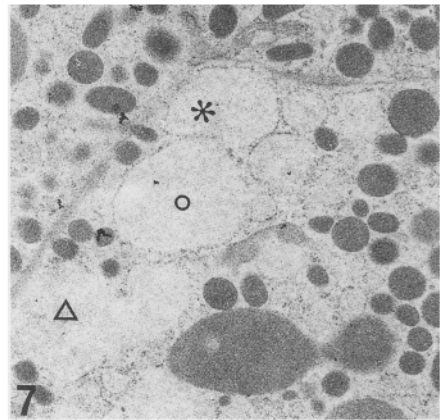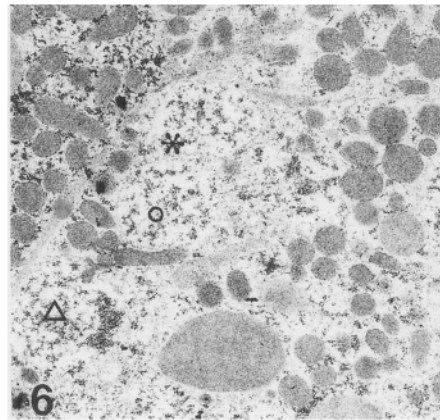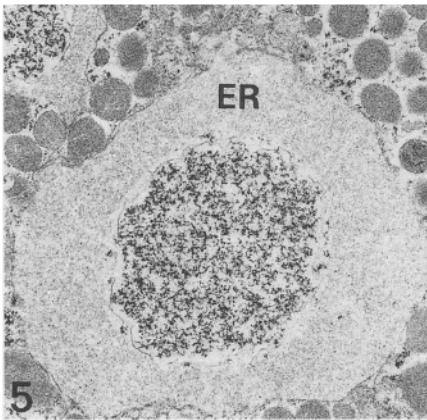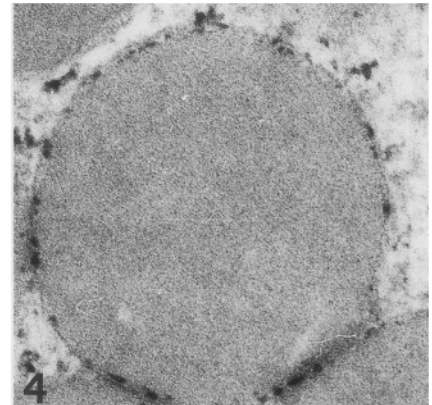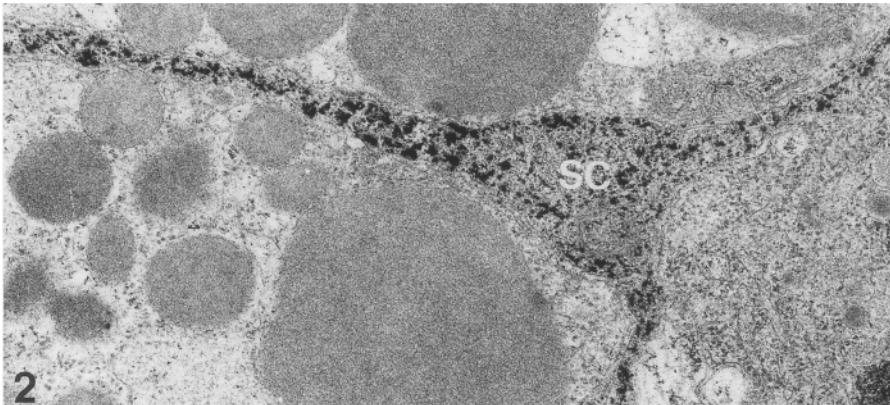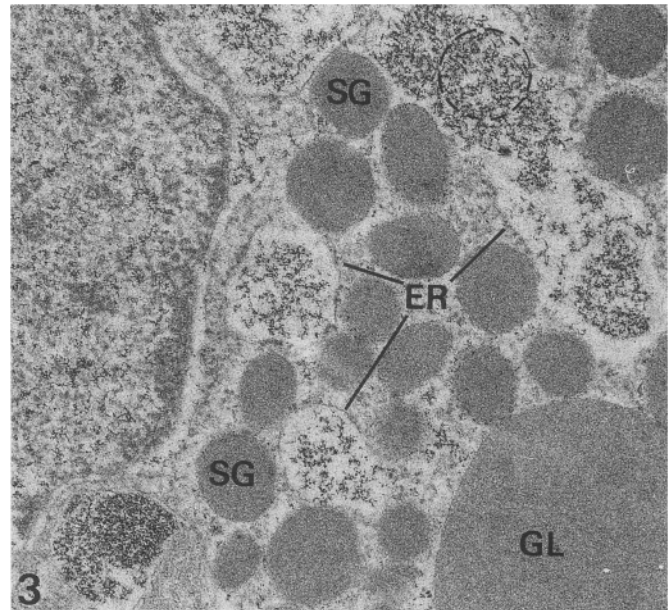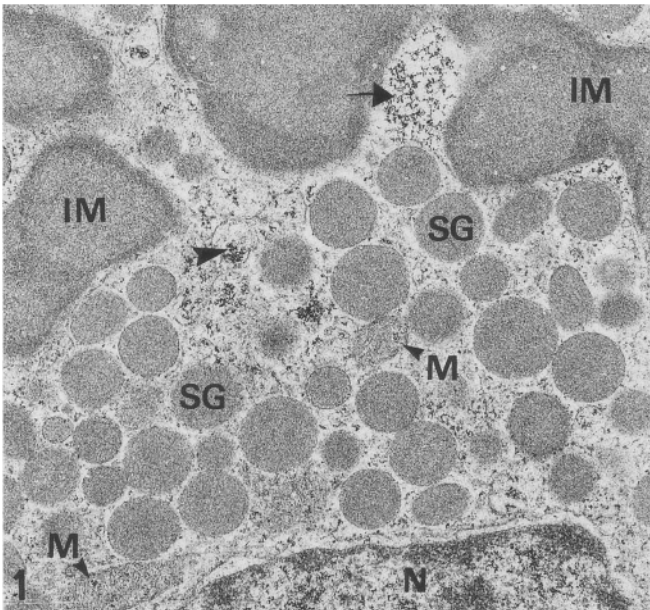
Fig. 1. Gonadotropic cell of uninjected catfish (in vivo); pituitary treated with oxalate-pyroantimonate procedure (*OPP*). Accumulations of fine granular deposits in a number of small endoplasmic reticulum (*ER*) cisternae (➤) and dispersed deposits in large cisternae (→), in mitochondria (*M*) and in the cell nucleus (*N*); *IM* irregular masses, *SG* secretory granules. ×20000

Fig. 2. Part of stellate cell (*SC*) between two gonadotropic cells; OPP-treatment of pituitary of uninjected fish. Heavy deposits in the SC, especially immediately underneath the plasma membrane in the slender extensions. ×62000

Fig. 3. Gonadotropic cell of pituitary after in vivo stimulation of gonadotropin release; OPP-treatment. Large amounts of deposits inside dilated *ER* cisternae. *Dotted circle* indicates probe area

for EELS measurement (0.2 μm²). *SG* secretory granules, *GL* globules. ×20000

Fig. 4. Gonadotropic cell from in vitro pituitary after OPP-treatment. Thin layer of fine granular deposits underneath the limiting membrane of secretory granule. ×121000

Fig. 5. Gonadotropic cell from in vitro pituitary after OPP-treatment. Central core of precipitate in large cisternae of *ER*. ×20000

Figs. 6, 7. Two serial ultrathin sections of (OPP-treated) in vitro pituitaries: Fig. 6 standard section, Fig. 7 section treated with 10 mM EGTA. Note the absence of precipitate in the ER cisternae of the gonadotropic cells after EGTA treatment. *, △ and ○ corresponding cisternae. ×20000
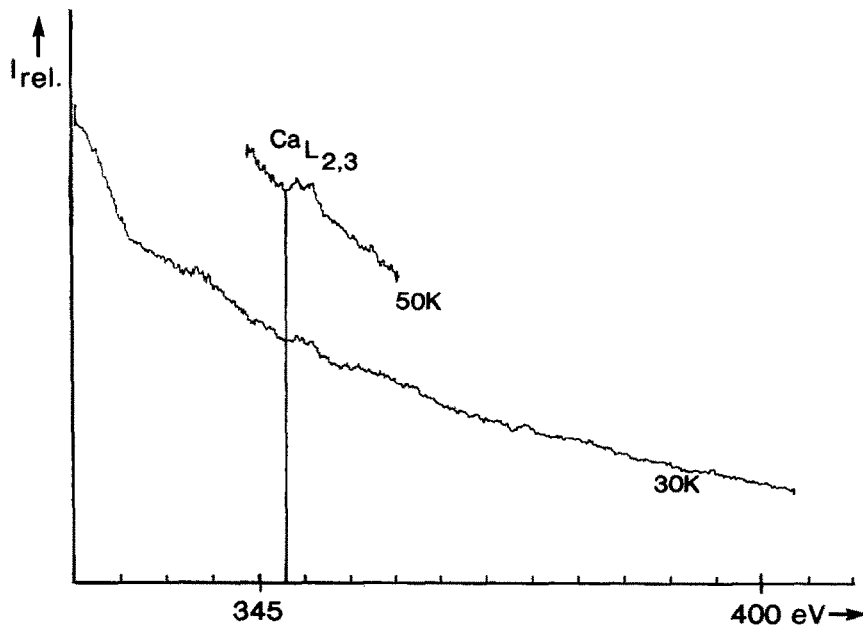
Fig. 8. EELS – analysis of precipitate. Two spectra (taken at two different scan speeds) from the same precipitate show the relative intensity (I_{rel}) versus ΔeV (the energy loss), indicating the calcium $L_{2,3}$ edge at 346 eV. 30 K analyzed area 2200 nm; 50 K analyzed area 500 nm

ments contained a considerable amount of precipitate, but also in this material several GTH-cells were practically devoid of precipitate. In most of the GTH-cells some secretory granules had a small layer of fine deposits just underneath their limiting membrane (Fig. 4). The presence of a central core of precipitate in large cisternae of the ER was a regular phenomenon in the gonadotrops of pituitaries kept under in vitro conditions (Fig. 5).

The amount of precipitate in the stellate cells was visually not influenced neither by the in vitro conditions, nor by the in vivo stimulation of gonadotropin secretion.

Specificity controls:

1. Treatment of sections of in vitro pituitary fragments (characterized by large amounts of precipitate in the GTH-cells) with a 5 mM EGTA-solution, resulted in a complete disappearance of precipitate from the ER cisternae in these cells (Figs. 6 and 7).
2. The spectra (taken at two different scan speeds) clearly show the presence of the calcium ionisation edge (Fig. 8). The analyzed area from which the information is retrieved is, as an example, shown in Fig. 3 to scale.

Ca^{2+}-ATPase activity

Irrespective of the gonadotropin secretory activity of the pituitary gland, Ca^{2+}-ATPase activity was visible in several GTH-cells and non-glandular stellate cells, but not in the other glandular cells of the PPD. The Ca^{2+}-ATPase reaction product was irregularly distributed among the GTH-cells i.e., some cells displaying heavy electron dense deposits, whereas others were practically devoid of reaction product (Fig. 9). In the GTH-cells (Figs. 11–

12) the reaction product was located along the plasma membrane and the membranes of the ER (Fig. 11); occasionally reaction product was associated with the limiting membrane of secretory granules (Fig. 12). The stellate cells generally exhibited a fairly constant enzyme distribution, in the cell body as well as in the long extensions located between the glandular cells (Fig. 10).

Specificity controls:

Addition of ouabeine (1) to the incubation medium did not change the amount of precipitation. Tissue that was preheated (2) or tissue incubated in medium without substrate (3) or in medium containing Vanadate (4) or AMP (5) was free of reaction product. Tissue incubated in medium containing PCMB (6), n-ethylmaleimide (7), or in medium without $CaCl_2$ (8) showed a roughly estimated 50% decrease in reaction product. If the tissue was preincubated with EGTA before incubation in medium without $CaCl_2$, no reaction product was visible.
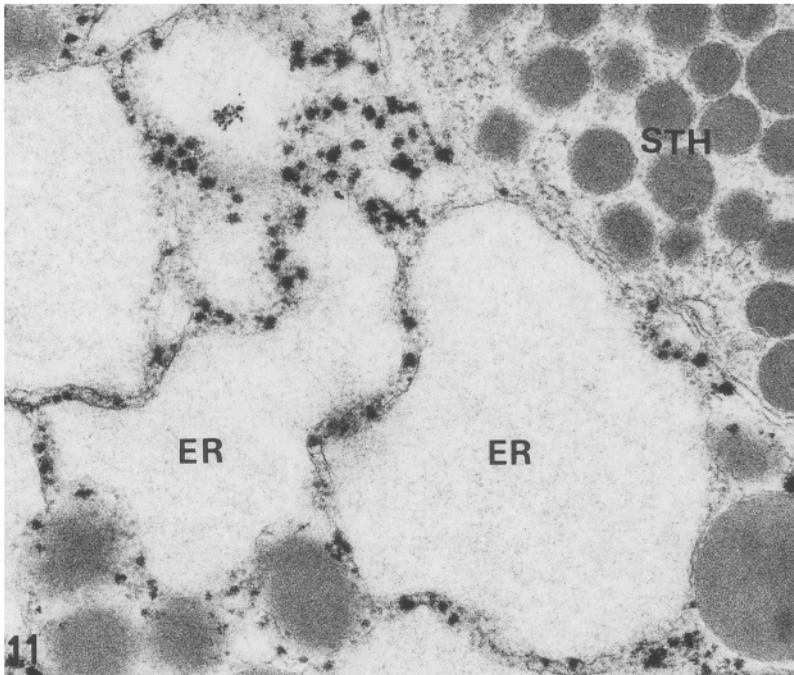
Table 1. GTH-level

In vivo: no injection: 0.6 ng/ml plasma
30 min after injection with Pimozide/LHRHa: 3.6 ng/ml plasma.
45 min after injection with Pimozide/LHRHa: 12.4 ng/ml plasma.
60 min after injection with Pimozide/LHRHa: 14.7 ng/ml plasma.
In vitro: GTH collected from one pituitary during 2 h: 102.5 ng/ml medium.
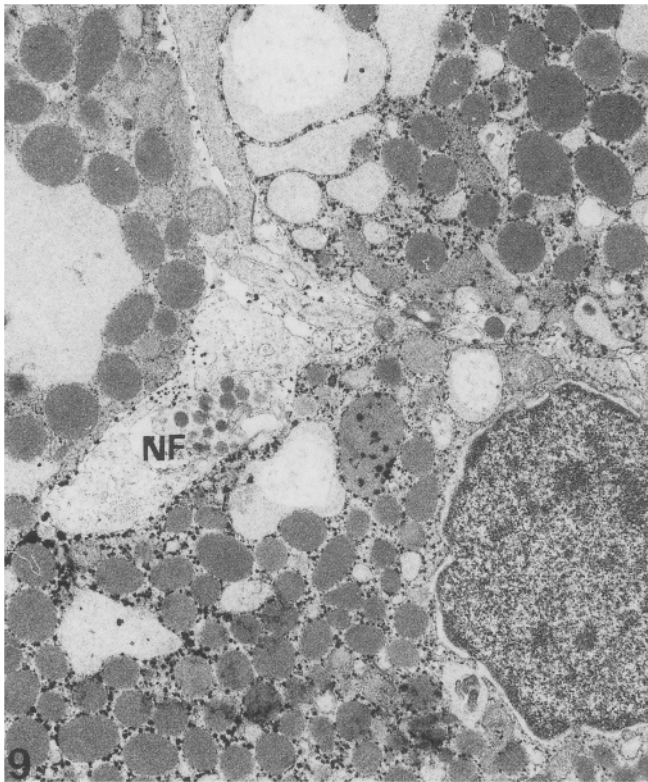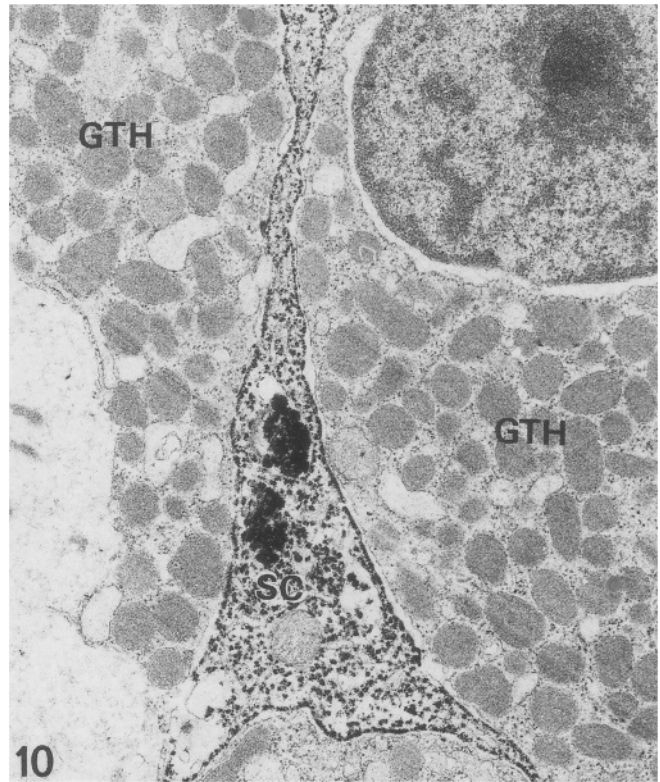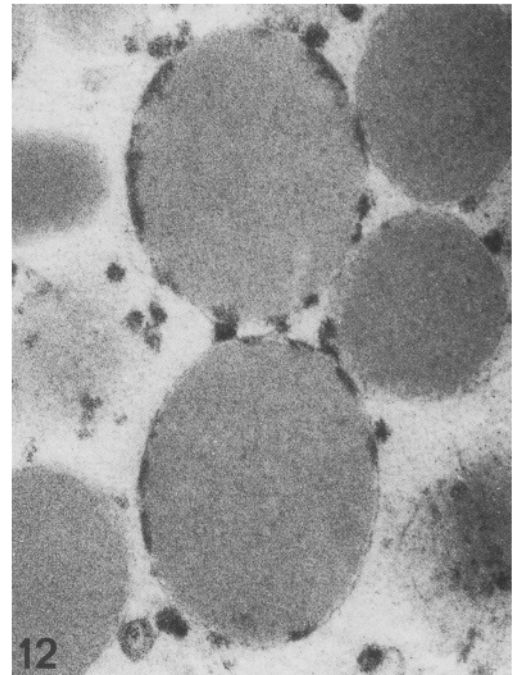
Fig. 9. Gonadotropic cells of uninjected catfish (in vivo): amounts of Ca^{2+}-ATPase reaction product (black deposits) differ from cell to cell. *NF* nerve fiber. ×16000

Fig. 10. Stellate cell (SC) in pituitary of uninjected catfish (in vivo): Ca^{2+}-ATPase reaction product in SC; *GTH* gonadotropic cell. ×16000

Fig. 11. Gonadotropic cell in pituitary of uninjected catfish (in vivo): Ca^{2+}-ATPase reaction product associated with membranes of large *ER* cisternae. Note the absence of reaction product in the neighbouring somatotropic cell (*STH*). ×50000

Fig. 12. Gonadotropin secretory granules: Ca^{2+}-ATPase reaction product associated with limiting membrane. ×80000

Discussion

The oxalate-pyroantimonate method is a common procedure for the ultrastructural visualization of intracellular calcium (Poenie and Epel 1987; Leslie et al. 1988; Susuki and Sugi 1989). In several tissues the presence of calcium in pyroantimonate – induced deposits has been successfully revealed with X-ray microanalysis (Van der Wal et al. 1985; Susuki and Sugi 1989). Likewise, validation of similar precipitates with EELS analysis in other tissues lead to the same conclusion (Heinrich et al. 1990). By performing a double control (EGTA on the section, EELS analysis of the precipitate) on the reliability of the method it has been sufficiently proven that in catfish gonadotrops and stellate cells the precipitate indeed contains Ca^{2+}.

In the GTH-cells Ca^{2+} was localized mainly in compartments of the endoplasmic reticulum; it was more prominently present in GTH-cells of catfish after in vivo stimulation of GTH-release by injection with Pimozide/LHRH than in uninjected control fish. Also in the in vitro perifused pituitaries, which maintain high secretion levels of gonadotropin (De Leeuw et al. 1986), the amount of Ca^{2+}-precipitate in the GTH-cells had increased compared to that in GTH-cells of pituitaries immediately fixed after removal from the brain. A relationship between gonadotropin release and intracellular Ca^{2+}-concentrations was also observed in catfish gonadotrops by Van Asselt et al. (1989). These authors found a stimulated gonadotropin release with the Ca^{2+}-ionophore A23187, but a significant decrease of gonadotropin from catfish pituitary fragments after in vitro treatment with the Ca^{2+}-channel blocker Nifedipine.

The ER, with its Ca^{2+}-binding proteins, is an intracellular Ca^{2+}-store (Macer and Koch 1988). Release of Ca^{2+} from this intracellular source is mediated by IP_3, a second messenger which is formed after binding of GnRH to its receptor (Guillemette et al. 1987; Sortino et al. 1988). It appears that in several investigated cell types only certain compartments of the ER release Ca^{2+} in respons to IP_3, in fact there are IP_3-sensitive and IP_3-insensitive Ca^{2+}-pools (Jean and Klee 1986; Nicchitta et al. 1987; Leslie et al. 1988; Dawson and Comerford 1989; Schulz et al. 1989). Only the IP_3-sensitive compartments are permeable to oxalate (Leslie et al. 1988; Dawson and Comerford 1989). Since in the present study Ca^{2+}-precipitation is based on the binding of Ca^{2+} to oxalate, it is conceivable that Ca^{2+}-precipitate is visible in the IP_3-sensitive compartments of the endoplasmic reticulum only. This could explain the irregular occurrence of Ca^{2+}-precipitates in ER cisternae within one cell, as well as between individual GTH-cells. The ultrastructural visualization of Ca^{2+}-ATPase activity in the catfish pituitary was performed according to the method of Ando et al. (1981). This method has been succesfully applied by other investigators on several organs, resulting in a specific localization of the enzyme (Bambauer et al. 1985; El-Sherif and Bácsy 1989; Mata and Fink 1989; El-Sherif et al. 1990). The results of the various control experiments, as applied in the present study, virtually exclude the formation of reaction products exerted by other enzymes.

In the catfish pituitary Ca^{2+}-ATPase activity was exclusively localized in gonadotropic cells and in stellate cells, but not in other parenchymal cell types of the proximal pars distalis. This is partly in accordance with the results of Bambauer et al. (1985), who observed in the Guinea pig pituitary Ca^{2+}-ATPase activity in stellate cells and in none of the parenchymal cell types. On the other hand, in the rat pituitary Ca^{2+}-ATPase activity was ultrastructurally localized in several parenchymal cell types and in stellate cells as well (El-Sherif and Bácsy 1989). The inconsistent results with regard to Ca^{2+}-ATPase localization in the parenchymal cells and the apparently consistent presence of the enzyme in stellate cells may indicate the importance of the latter in regulating extracellular calcium concentrations in the pituitary (Bambauer et al. 1985). This statement is corroborated by the findings of Ca^{2+}-deposits in the stellate cells, as observed in the present study. This regulation of extracellular Ca^{2+}, however, is only one of several functions attributed to the stellate cells (Perryman 1989).

Elevated Ca^{2+}-concentrations which coincide with increased gonadotropin release have to be reduced to basal levels by pumping Ca^{2+} a) across the plasma membrane towards the extracellular space and/or b) into the endoplasmic reticulum. In both cases a Ca^{2+}-ATPase serves as the Ca^{2+}-pump (Schatzman 1985; Sasaki and Garant 1987; Carafoli and Longoni 1987). The presence of Ca^{2+}-ATPase associated with both the plasma membrane and the membranes of ER cisternae in the GTH-cells is in accordance with this functional role of the enzyme and once more emphasizes the importance of calcium in the process of gonadotropin release in this species, as established by Van Asselt et al. (1989). However, unlike the increase in calcium precipitate in stimulate GTH-cells, no changes in Ca^{2+}-ATPase activity were observed in GTH-cells after stimulation.

Calcium is also known to be directly involved in secretion processes (Garrahan 1986). The presence of both Ca^{2+} and Ca^{2+}-ATPase in association with the limiting membrane of secretory granules might reflect their role in the process of membrane fusion between granules and plasma membrane (for review: Campbell 1983).

References

Ando T, Fujimoto K, Mayahare H, Miyajima H, Ogawa K (1981) A new one-step method for the histochemistry and cytochemistry of Ca-ATPase. Acta Histochem Cytochem 14:705–726

Bambauer HJ, Ueno S, Umar H, Ueck M (1985) Histochemical and cytochemical demonstration of Ca^{2+}-ATPase activity in the stellate cells of the adenohypophysis of the guinea pig. Histochemistry 83:195–200

Bates MB, Conn MP (1984) Calcium mobilisation in the pituitary gonadotrope: relative roles of intra- and extracellular sources. Endocrinology 115:1380–1384

Berridge MJ (1984) Inositol triphosphate and diacylglycerol as second messengers. Biochem J 220:345–360

Borgers M, Thone F, Verheyen A, ten Keurs HEDJ (1984) Localisation of calcium in skeletal and cardiac muscle. Histochem J 16:295–309

Campbell AK (1983) Intracellular calcium: its universal role as regulator. J. Wiley and Sons, Chichester New York Brisbane, pp 1–556

Carafoli E, Longoni S (1987) The plasma membrane in the control

of the signaling function of calcium. In: Mandell LJ, Eaton DC (eds) Cell calcium and the control of membrane transport. The Rockefeller University Press, New York, pp 21–28

Chang JP, Stoijkovic SS, Graeter JS, Catt KJ (1988) Gonadotropin-releasing-hormone stimulates luteinizing hormone secretion by extracellular calcium-dependent and -independent mechanisms. Endocrinology 123:87–97

Clapper DL, Conn MP (1985) Gonadotropin releasing hormone stimulation of pituitary gonadotrope cells produces an increase in intracellular calcium. Biol Reprod 32:269–278

Clayton RN (1989) Gonadotrophin-releasing hormone: its actions and receptors. J Endocrinol 120:11–19

Conn MP (1982) Gonadotropin-releasing hormone stimulation of pituitary gonadotropin release: a model system for receptor mediated, Ca^{2+}-dependent secretion. In: Conn MP (ed) Cellular regulation of secretion and release. Academic Press NY, pp 460–486

Dawson AP, Comerford JG (1989) Effects of GTP on Ca^{2+} movements across endoplasmic reticulum membranes. Cell Calcium 10:343–350

De Leeuw R, Resink JW, Rooyakkers EJM, Goos HJTh (1985) Pimozide modulates the luteinizing hormone-releasing hormone effect on gonadotrophin release in the African catfish, *Clarias lazera*. Gen Comp Endocrinol 58:120–127

De Leeuw R, Goos HJTh, Van Oordt PGWJ (1986) The dopaminergic inhibition of the gonadotropin-releasing hormone-induced gonadotropin release: An in vitro study with fragments and cell suspension from pituitaries of the African catfish, *Clarias gariepinus* (Burchell). Gen Comp Endocrinol 63:303–310

De Leeuw R, Goos HJTh, Van Oordt PGWJ (1987) The regulation of gonadotropin release by neurohormones and gonadal steroids of the African catfish, *Clarias gariepinus*. Aquaculture 63:43–58

De Leeuw R, Conn PM, Van 't Veer C, Goos HJTh, Van Oordt PGWJ (1988) Characterization of the receptor for gonadotropin-releasing hormone in the pituitary of the African catfish, *Clarias gariepinus*. Fish Physiol Biochem 5:99–107

Drouva SV, Rerat E, Bihoreau C, Laplante E, Rasolonjanahary R, Clauser H, Kordon C (1988) Dihydropiridine-sensitive calcium channel activity related to prolactin, growth hormone, and luteinizing hormone release from anterior pituitary cells in culture: interactions with somatostatin, dopamine, estrogens. Endocrinology 123:2762–2773

El-Sherif G, Bácsy E (1989) Ultrastructural study of Ca^{2+}-ATPase activity in the rat adenohypophyseal cells. Histochemistry 91:501–505

El-Sherif G, Van Noorden CJF, Bácsy E (1990) Specificity of the metal-salt method for the localization of Ca^{2+}-ATPase activity studied in rat adenohyphysis tissue in a model system of polyacrylamide gel films. Histochem J 22:51–56

Garrahan PJ (1986) Calcium and cell function. In: Rega AF, Garrahan PJ (eds) The Ca^{2+} pump of plasma membranes. CRC Press, Boca Raton, pp 21–39

Goos HJTh, Burzawa-Gerard B, Terlou M, Richter CJJ (1986) Purification of gonadotropic hormone from the pituitary of the African catfish, *Clarias gariepinus* (Burchell), and the development of a homologous radioimmunoassay. Gen Comp Endocrinol 63:162–170

Guillemette G, Balla T, Baukal AJ, Catt KJ (1987) Inositol 1,4,5 triphosphate binds to a specific receptor and releases microsomal calcium in the anterior pituitary gland. Proc Natl Acad Sci USA 84:8195–8199

Heinrich U-R, Drechsler M, Kreutz W, Mann W (1990) Identification of precipitable Ca^{2+} by electron spectroscopic imaging and electron energy loss spectroscopy in the organ of Corti of the guinea pig. Ultramicroscopy 32:1–6

Hirota K, Hirota T, Aguilera G, Catt KJ (1985) Hormone-induced redistribution of calcium-activated phospholipid-dependent protein kinase in pituitary gonadotrophs. J Biol Chem 260:3243–3246

Jean T, Klee CB (1986) Calcium modulation of inositol 1,4,5 triphosphate-induced calcium from neuroblastoma X gliomahybrid (NG 108-15) microsomes. J Biol Chem 261:16414–16420

Leslie BA, Burgess GM, Putney JW Jr (1988) Persistent inhibition by inositol 1,4,5-triphosphate of oxalate-dependent ^{45}calcium accumulation in permeable guinea-pig hepatocytes. Cell Calcium 9:9–16

Levavi-Zermonsky B, Yaron Z (1989) Calcium ions, protein kinase C, and cyclic AMP mediate GnRH effect on GTH-release in Tilapia. Gen Comp Endocrinol 74:257

Macer DR, Koch GLE (1988) Identification of a set of calcium-binding proteins in reticuloplasm, the luminal content of the endoplasmic reticulum. J Cell Sci 91:61–70

Mata M, Fink DJ (1989) Ca^{++}-ATPase in the central nervous system: an EM cytochemical study. J Histochem Cytochem 37:971–980

Nicchitta CV, Joseph SK, Williamson JR (1987) GTP-mediated Ca^{2+} release in rough endoplasmic reticulum. Correlation with a GTP-sensitive increase in membrane permeability. Biochem J 248:741–747

Perryman EK (1989) Folliculo-stellate cells of the pituitary gland. What role does these star-shaped cells play? Bioscience 39:81–88

Peute J, De Leeuw R, Goos HJTh, Van Oordt PGWJ (1984) Ultrastructure and immunolabeling of gonadotrops and thyrotrops in the pituitary of the African catfish, *Clarias lazera*. Cell Tissue Res 238:95–103

Peute J, Schild RG, Schild VA, Buijs RM, Van Asselt LAC, Van Oordt PGWJ (1987) Immunocytochemical evidence for peptidergic (GnRH) and dopaminergic innervation of the gonadotropic cells in the pituitary of the African catfish, *Clarias gariepinus*. Gen Comp Endocrinol 67:303–310

Poenie M, Epel D (1987) Ultrastructural localisation of intracellular calcium stores by a new cytochemical method. J Histochem Cytochem 35:939–956

Sasaki T, Garant PR (1987) Calmodulin blocker inhibits Ca^{++}-ATPase activity in secretory ameloblast of rat incisor. Cell Tissue Res 248:103–110

Schatzman HJ (1985) Calcium extrusion across the plasmamembrane by the calcium-pump and the Ca^{2+}-Na^{+} exchange system. In: Marmé D (ed) Calcium and cell physiology. Springer, Berlin Heidelberg New York, pp 18–43

Schulz I, Thévenod F, Dehlinger-Kremer M (1989) Modulation of intracellular free Ca^{2+} concentration by IP_3-sensitive and IP_3-insensitive nonmitochondrial Ca^{2+} pool. Cell calcium 10:325–336

Sorber CWJ, De Jong AAW, Den Breejen NJ, De Bruijn WC (1990) Quantitative energy-filtered image analysis in cytochemistry. I. Morphometric analysis of contrast-related images. Ultramicroscopy 32:55–68

Sortino MA, Evans WS, Speciale C, Thorner MO, Scapagnini U, MacLeod RM, Canonico PL (1988) Gonadotropin-releasing-hormone-stimulated phosphoinositide hydrolysis in the anterior pituitary. Neuroendocrinology 48:544–550

Susuki S, Sugi H (1989) Evaluation of the pyroantimonate method for detecting intracellular calcium localization in smooth muscle fibers by the X-ray microanalysis of cryosections. Histochemistry 92:95–101

Van Asselt LAC, Goos HJTh, Van Dijk W, Braas J (1989) Role of calcium ions in action of gonadotropin-releasing hormone on gonadotropin secretion in the African catfish, *Clarias gariepinus*. Gen Comp Endocrinol 76:46–52

Van Asselt LAC, Goos HJTh, De Leeuw R, Peter RE, Hol EM, Wassenberg FP, Van Oordt PGWJ (1990) Characterization of dopamine D_2 receptors in the pituitary of the African catfish, *Clarias gariepinus*. Gen Comp Endocrinol (in press)

Van der Wal P, De Bruijn WC, Westbroek P (1985) Cytochemical and X-ray microanalysis studies of intracellular calcium pools in scale-bearing cells of the coccolithophorid *Emiliana huxleyi*. Protoplasma 124:1–9