Pergamon

Biochemical Pharmacology, Vol. 47, No. 1, pp. 63–76, 1994.

0006-2952/94 $6.00 + 0.00

INTERNALIZATION AND NUCLEAR LOCALIZATION OF PEPTIDE HORMONES

GÉRARD MOREL*
CNRS URA 1454, Neuroendocrinology, Lyon-Sud School of Medicine, Oullins, France

Abstract—The presence of neuropeptide receptors on the plasma membrane is well accepted, as is its internalization and down-regulation. The analysis of the fate of these peptides within their target-cells is difficult. Endogenous peptides or administered native peptides are visualized in these cells using immunocytology after cryoultramicrotomy. Labelled peptides can be injected and their internalization kinetics studied using ultrastructural autoradiography. The pituitary gland is a suitable model for the study of the neuropeptide mechanism, with the lactotroph function being taken as an example in the present case. Prolactin (PRL) release depends on two main neuropeptides: thyrotropin-releasing hormone (TRH) and somatostatin (SS). The TRH immunoreactivity obtained from endogenous as well as injected material was restricted to the plasma membrane, secretory granules, cytoplasmic matrix and nucleus. The internalization kinetics of exogenous native TRH showed an increase of immunoreactive material in all compartments including the nucleus. The endogenous SSs (SS14 and SS28) were detected in the same subcellular lactotroph compartments. Injection of ^{125}I-SS showed a rapid binding of SS at the plasma membrane level before internalization. For 60 min of *in vivo* uptake, ^{125}I-SS28, the large SS molecule, was detected in the cytoplasm only, while ^{125}I-SS14 was found in the nuclear matrix. *In vitro* ^{125}I-SS28 was restricted to the nuclear membrane. Under physiological conditions the endogenous neuropeptides were visualized in the nucleus, but after injection of labelled peptides only small molecules were found in the nucleus. The significance of the presence of these neuropeptides is discussed.

The first description of a neuropeptide in the nucleus of its target cell, which was produced in 1973 by Tixier-Vidal's group [1], showed that [^{3}H]TRH† could be internalized into the nuclei of the thyrotropic cells of the pituitary in primary culture. However, the difficulty of preparing a purified nuclear fraction, without plasma membrane contamination, has meant that the characterization of the nuclear binding site remains obscure. The presence of plasma membrane peptide receptors is well accepted, as is the internalization and down-regulation of peptides, but the fate of these peptides within the cell is difficult to analyse. The presence of exogenous molecules in lysosomes is associated with degradation, in the Golgi apparatus with synthesis or recycling, and in nucleus with gene expression. The difficulty resides in the fact that neuropeptides show a high affinity for endogenous ligands, but their concentration in tissues is low [2].

The pituitary gland is a suitable model for the study of neuropeptide mechanisms, given that all the neuropeptides characterized in the brain have been found to have a modulatory effect in the pituitary. The number of these neuropeptides increases each year, and now includes SS [3, 4], TRH [5], GH-releasing hormone [6, 7], gonadotropin-releasing hormone [8, 9], corticotropin-releasing hormone [10, 11], vasointestinal peptide [12], substance P [13], neuropeptide Y [14], oxytocin [15], calcitonin [16], arginine vasopressin [17], angiotensin II [18] and atrial natriuretic peptide [19]. Each of these endogenous peptides has been detected by immunocytology in one or more pituitary target cells, but none has been detected in all the pituitary cell types. All these peptides were internalized into the nucleus from the plasma membrane. Administration of labelled or unlabelled peptide makes it possible to follow the internalization process [5, 19–31].

In order to illustrate this phenomenon, the lactotrophic function was used as a model. PRL release depends on two main neuropeptides: TRH and SS. The isolation of the pure form of TRH as a tripeptide was first described in 1966 by Schally *et al.* [32]. SS was described by Brazeau *et al.* [33] as the hypothalamic GH release-inhibiting factor of 14 amino acids (SS14), following which an NH_2-terminally extended form of SS containing 28 amino acids (SS28) was isolated and characterized [34–36]. Native and synthetic peptides stimulate or inhibit the secretion of PRL both *in vivo* and *in vitro*. These peptides bind to a receptor which has been biochemically characterized in pituitary membrane fractions [37, 38]. Other peptides and hormones act to regulate this lactotrophic function.

MATERIALS AND METHODS

Products

For immunocytological studies. Anti-TRH serum [5] was raised in rabbit injected with TRH coupled to a sunflower globulin by the bis-diazotized benzidine method. The characteristics of this highly specific anti-TRH antibody have been described

* Correspondence: CNRS URA 1454, Faculté de Médecine Lyon Sud, BP 12-F69921 Oullins, France. Tel. (33) 78 86 31 20; FAX (33) 78 86 31 16.

† Abbreviations: GH, growth hormone; PRL, prolactin; SS, somatostatin; SS14, somatostatin (1–14); SS28, somatostatin (1–28); TRH, thyrotropin-releasing hormone.

previously [5]; anti-SS sera (ref. S309 and S310) were kindly supplied by Dr R. Benoit (Montréal, Canada); these highly specific anti-SS sera have been described previously [39]; anti-rPRL-R sera (ref. U5, U6, T1 and T6) were supplied by Dr P. A. Kelly (Paris, France); anti-rGH and anti-rPRL sera were supplied by Dako (Trappes, France).

For autoradiographical studies. SS14 agonist ([D-Tyr^0,D-Trp^8]-SS14) was purchased from Peninsula (CA, U.S.A.) and the SS28 agonist ([Leu^8, D-Trp^{22}, Tyr^{25}]-SS28) was kindly supplied by Dr J. Rivier (CA, U.S.A.).

Animals

Adult male Wistar rats (about 250 g) were purchased from Iffa Credo (St Germain s/l'Arbresle, France) and were housed at room temperature in a 12 hr light–dark cycle. The animals were fed a diet of rat chow and water *ad libitum*.

For immunocytological studies. Anterior pituitaries were removed and fixed by immersion in 4% paraformaldehyde (2 hr), or in 2.5% glutaraldehyde (1 hr) and post-fixed in 1% buffered osmium tetroxide (1 hr). Washing (1 hr) and fixation steps were performed in 100 mM mono-di-phosphate buffer at pH 7.4. The tissues were cut into small pieces (1 mm^3) at the beginning of the fixation. After the last washing, the tissues were incubated in 0.4 M sucrose as a cryoprotectant. Freezing was performed in a cold gradient of fuming nitrogen (Biogel, CFPO, Lyon, France) to −4° before total immersion in liquid nitrogen as described previously [40].

For autoradiographical studies. Shortly before stimulation with the ^{125}I-peptide, the animals were deeply anaesthetized with pentobarbital (65 mg/kg body weight) and a cannula was inserted into the right jugular vein, extending to the atrium. The animals were then immediately stimulated with labelled peptide. At several points, a number of animals were decapitated and the pituitary glands quickly removed, minced and fixed by immersion in glutaraldehyde 2.5% (1 hr) in 0.1 M phosphate buffer (pH 7.4). After washing, the tissues were post-fixed in 1% OsO_4 for 1 hr, dehydrated in ethanol and embedded in Araldite. If signs of awakening occurred during the time between injection of labelled hormone and decapitation, more pentobarbital was injected in order to maintain deep anesthesia.

Immunocytological studies

Ultrathin frozen sections. Ultrathin frozen sections (100 nm) were cut at −120° on an Ultracut (Reichert-LEICA, Vienna, Austria) fitted with an FC_4D cryosectioning system [40].

Immunocytological reaction using gold-conjugated anti-IgG [41]. These sections were incubated with primary antiserum (dilution 10^{-5}–10^{-7}) in a mono-di-phosphate buffer: 100 mM, NaCl; 665 mM, Tween 20; 0.05%, ovalbumin; 1% (pH 7.4) for 60 min and then washed, after which the antigen–antibody complexes were revealed by incubation with goat anti-rabbit IgG conjugated to 15 nm gold particles (BioCell, Cardiff, U.K.) for 30 min. After washing in the buffer, the sections were washed with distilled water and contrasted using 2% neutral uranyl acetate (5 min) and then embedded in methyl cellulose [40].

Gold particles can be counted in each pituitary cell type and expressed as density of gold particles per 100 μm^2.

Immunocytological reaction using the peroxidase anti-peroxidase (PAP) method [8]. Ultrathin frozen sections of the pituitary gland were cut from three or four blocks per animal. For each block, 24–36 ultrathin sections were incubated with (a) normal sheep serum (dilution 1:100), and (b) various dilutions of the anti-peptide serum for 10 min. The antigen–antibody complexes were revealed as follows: (c) sheep antiserum to rabbit-γ-globulins (dilution 1:10,000) for 10 min; (d) peroxidase–anti-peroxidase complex (dilution 1:500) for 10 min; (e) 4-chloro-1-naphthol (ICN Pharmaceuticals, Plainview, NY, U.S.A.) solution in 50 mM Tris–HCl buffer saline (pH 7.6) as previously described [5]) for 3 min; (f) 2% osmium tetroxide for 10 min; (g) uranyl acetate aqueous solution (2%) for 10 min. After steps a, b, c and d, the grids were washed with Tris–HCl buffer. After steps e, f and g, the grids were washed with distilled water. All washing steps were performed for 10 min.

Semi-quantitative evaluation of TRH immunoreactivity. The intensity or magnitude of intracellular immunoreactivity was established semi-quantitatively by using as an index the "Maximum Dilution factor of the Antiserum" (MDA), as described previously [5, 41, 42]. Consecutive ultrathin pituitary slices were analysed by progressive dilutions of the primary serum (10-fold dilution cascade), the MDA representing the last dilution of the antiserum that allowed a significant intracellular localization. For the sake of convenience, the transformed pMDA value was used for the expression of the result, with pMDA representing the absolute value of the logarithm of the MDA (pMDA = −log MDA), by analogy with the pH expression used for indicating the concentration of the hydrogen ion. For example, a pMDA of 7 means that the antiserum dilution 10^7 was the last one that gave rise to a visible immunocytochemical reaction. An increase of 3 in the pMDA value corresponds to achieving the same last positive localization using an antiserum 1000 times more dilute.

Checking the immunocytological reaction. The specificity of the immunocytological reaction was checked by (a) using normal rabbit IgG rather than primary antiserum, (b) omitting the primary antibody, (c) using primary antiserum after incubation with the homologous antigen (Peninsula) at a concentration of 1 or 10 μg/mL of undiluted antibody and (d) using primary antiserum after incubation with a heterologous antigen at a concentration 40 times higher than that of the homologous antigen.

Autoradiographical studies

Radioiodination. SS14 and SS28 agonists were radioiodinated using the standard chloramine T method and purified by Sephadex G-100 gel preparation [43]. Radiolabelled peptides were used the next day. Specific radioactivity measured by self-displacement was approx. 400 μCi/μg.

In vivo *autoradiography of* ^{125}I*-peptides.* Uptake

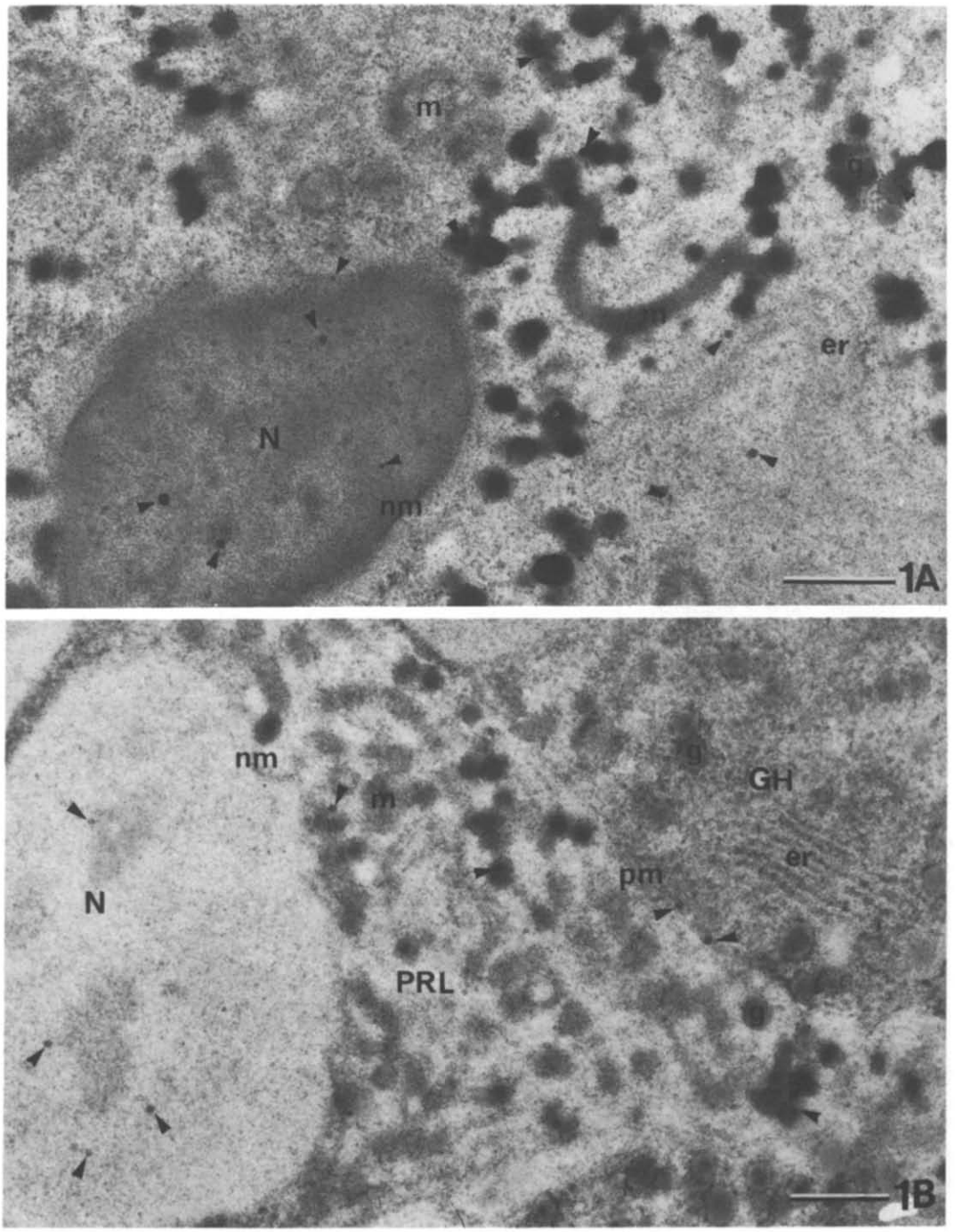

Fig. 1. Ultrastructural evidence for endogenous SS14-like (A) and SS28-like (B) immunoreactivities in lactotrophs. Immunoreactivity, using the PAP method (arrowhead) on ultrathin frozen sections, is observed at the plasma membrane level (pm) in the cytoplasmic matrix, secretory granules (g) and nucleus (N). GH, somatotroph; er, endoplasmic reticulum; m, mitochondrion; nm, nuclear membrane. Bar = 1 μm [4].

of ^{125}I-peptide was studied in pentobarbital anaesthetized rats. The animals received 3×10^8 dpm of ^{125}I-peptide (0.5 μg) via indwelling intravenous catheters. At time intervals of 2, 5, 15, 30 and 60 min after injection, two rats were decapitated and their pituitary glands dissected out. At each step in the histological processing, the dpm of each solution was determined so as to measure loss of radioactivity. This loss represented approximately 20% of the initial values [20, 21, 23–30].

In vitro *autoradiography of* ^{125}I*-peptides*. Female rats (Wistar, 200–220 g) were decapitated and the pituitaries were dissected rapidly. The cells were then dispersed according to the procedure described by Hopkins and Farquhar [44], maintained in DMEM and supplemented with calf serum from which the steroids had been removed by overnight adsorption on Norit A charcoal (1%) and dextran (0.1%) for 5–6 days. A steroid [oestradiol (E_2)] was added to the medium within the first 24 hr of culture. Mouse AtT20 cells were cultured in the same medium [25].

Primary cultures of pituitary cells were incubated with mono-iodinated SS28 agonist (1.4×10^6 dpm/bottle) for 30 min and cultures of AtT20 for 1, 4 and 18 hr, respectively.

Checking the autoradiographic technique. To test the specificity of ^{125}I-peptide uptake, some animals were also injected with ^{125}I-peptide, as above, together with 50 μg of unlabelled peptide and then killed 5 min after injection. The specificity of uptake by cells in the culture was checked by concomitant incubation with an excess of SS28 or SS14 (5×10^{-7} M).

Preparation of autoradiograms. For light microscopic autoradiography, the resin was removed using 50% ethanolic sodium methoxide solution. The semithin sections (1 μm) were then dipped in Kodak NTB_2 (New Haven, CT, U.S.A.). The slides were developed in Dektol (Kodak) for 2 min and stained with toluidine blue after 2 weeks of exposure.

For electron microscopic autoradiography, ultrathin sections of anterior pituitary cells were coated with Ilford L4 emulsion (St Priest, France) using the loop technique [43] to obtain a monolayer film of densely-packed silver bromide crystals. The sections were developed in D19 developer (Kodak) (2 min) and stained with uranyl acetate (10 min) and lead citrate (5 min) as previously described [43].

Analysis of autoradiograms. Analysis of silver grain distribution was done only at the ultrastructural level. A random selection of cells sectioned through the nucleus was photographed in a given grid square. The percentage of grains associated with organelles was determined on micrographs (magnification ×20,000) by the probability circle method [45]. A circular area of 460 nm in diameter around the silver grain was taken to represent the radioactive source, with a probability of 95% [45]. The grain was classified as exclusive if only one organelle was localized within the circle, and as shared if it was observed over more than one organelle. Each shared grain was assigned, on the basis of probability, to only one structure, according to the method of Nadler [45]. The corrected number of grains for each organelle was the sum of the exclusive and adjusted-shared grains and was expressed as a percentage of the total number of corrected grains.

The relative concentration of label in each organelle was calculated by dividing the percentage of corrected silver grain count by the percentage of area occupied by the organelles. A grain density greater than 1 was taken to represent "significant" labelling, following Salpeter and McHenry [46].

RESULTS

Only results concerning PRL and GH functions will be illustrated, as a model of visualization of neuropeptides in target cells, which in this case are the lactotrophs and somatotrophs. No controls are given here, but to obtain them see [3–5, 23, 25–27, 40, 41, 43, 47].

Localization of endogenous SS14 and SS28 by immunocytology

Endogenous SS was visualized as described pre-

Table 1. Percentage of pituitary labelled cells according to intensity of labelling

	Number of silver grains per cell					
Pituitary cell types	NSB*	2	3	4	5	≥6
Control						
GH	85%	3%	9%	3%		1%
PRL	84%	10%	4%	2%		
TSH	67%	33%				
LH-FSH	100%					
ACTH	100%					
Oestradiol treatment						
GH	76%	10%	4%			
PRL	76%	12%		6%	3%	3%
TSH	83%	17%				
LH-FSH	100%					
ACTH	100%					

Pituitary cells were cultured for 6 days, and exposed to oestradiol for 5 days. Autoradiography was carried out after incubation of cells for 30 min with radiolabelled SS28 analogue in control and oestradiol-treated cell populations. More than 100 cells were analysed for each cell type. Specific binding is represented here by quantities of silver grain higher than 1 per cell.

PRL, lactotrophs; GH, somatotrophs; TSH, thyrotrophs; LH-FSH, gonadrotrophs; ACTH, corticotrophs.

* NSB, non specific binding.

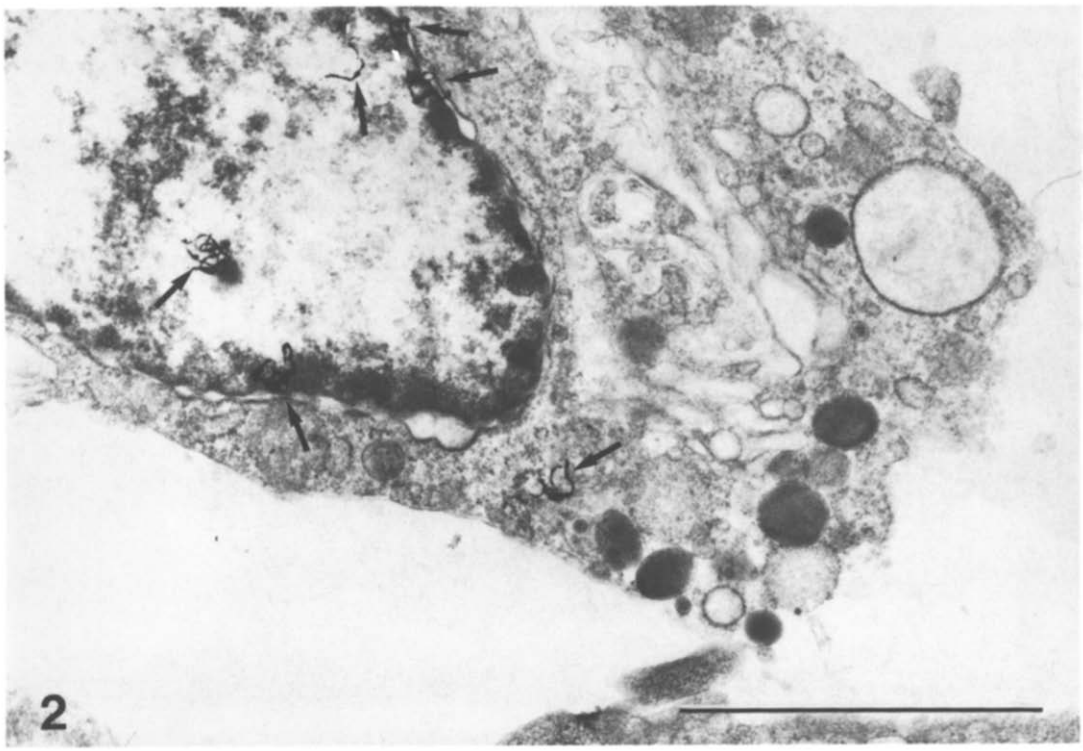

Fig. 2. Ultrastructural autoradiography of ^{125}I-[Leu^{8}-D-Trp^{22}-Tyr^{25}]-SS28 in a lactotroph cultured for 6 days, with exposure to oestradiol for 5 days. Autoradiography was carried out after incubation of cells for 30 min with radiolabelled analogue. Silver grains (arrow) are located over the nucleus, nuclear membrane and cytoplasmic matrix. Bar = 1 μm.

viously [3, 4], in three pituitary cell types: somatotrophs, lactotrophs and thyrotrophs. The density of immunoreactive material detected depended on each pituitary cell type [4]. In these three cell types the same localization was observed. Low SS-like immunoreactivity (LI) was always detected at the plasma membrane level. However, a high level of endogenous SS-LI was visualized in the cytoplasmic matrix, secretory granules and nucleus. Figure 1 illustrates the presence of SS-LI in lactotrophs.

Localization of monoiodated SS agonists by autoradiography

In primary cultures of pituitary cells, the uptake was restricted to these same three cell types (Table 1, Fig. 2). The kinetics of internalization in AtT20 cells showed that monoiodated SS28 agonist first binds to the plasma membrane, before internalization in the lysosomes, Golgi apparatus and secretory granules. At the nuclear level, SS28 was not detected in the matrix, but at the nuclear membrane level it was detected throughout.

SS14 and SS28 were internalized as previously described [23, 26] in three pituitary cell types: somatotrophs, lactotrophs and thyrotrophs. Administration of ^{125}I-SS14 agonist in intact animals, with the use of an ultrastructural autoradiographic method, showed that this labelled peptide, after binding to the plasma membrane, was internalized into the cytoplasmic matrix, secretory granules and, finally, the nucleus (Fig. 3), while ^{125}I-SS28 agonist did not give a significant signal at the nuclear level [23].

Localization of endogenous and exogenous TRH by immunocytology

Endogenous TRH-LI was found in two particular pituitary cell types: the thyrotrophs and the lactotrophs. This immunoreactivity was restricted to the plasma membrane, secretory granules, cytoplasmic matrix and nucleus (Fig. 4).

Exogenous TRH (100 ng) was injected into rats, as previously described [5]. TRH-LI was predominantly found in lactotrophs and thyrotrophs (Fig. 4).

The internalization process studied in lactotrophs, via MDA parameters (Fig. 5), was similar to those described in thyrotrophs [5]. After binding to the plasma membrane, TRH-LI was internalized into the secretory granules and then into the nuclei of somatotrophs and also those of the lactotrophs and thyrotrophs.

Localization of receptor molecules

PRL receptors-LI have been found in all anterior pituitary cells, including somatotrophs and lactotrophs. In these cells PRL receptor-LI was

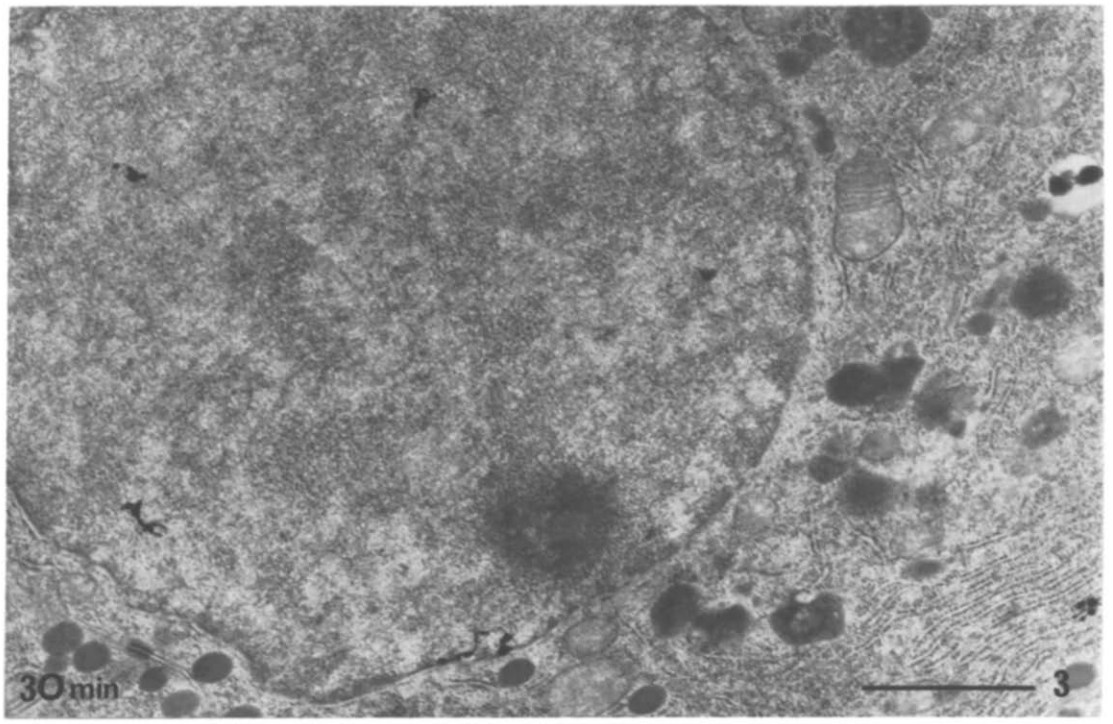

Fig. 3. Ultrastructural *in vivo* autoradiography of ^{125}I-[Tyr^0-D-Trp^8]-SS14 in a lactotroph. Silver grains (arrow) are detected over the cytoplasm and nucleus (nuclear membrane and nuclear matrix) 30 min after injection. Bar = 0.5 μm.

detected not only at the plasma membrane level, but also in the nucleus (Fig. 6).

DISCUSSION

We have shown the presence, in the pituitary gland, of 10 neuropeptides, using immunocytology to demonstrate the presence of endogenous molecules (taken up or synthesized *in situ*) and used autoradiography to study their internalization kinetics. In the same way, using immunocytology, we used agonist molecules or native peptides to demonstrate the internalization process and to amplify the immunocytological reaction, respectively. We chose the combination of immunocytology and ultrathin frozen sections to obtain the detection of antigens present at very low levels. The extreme sensitivity of this technique has been demonstrated by Hemming *et al.* [47], who reported that a signal could be detected in frozen sections using specific antibodies diluted 1000-fold compared to the same antigen in epoxy resin-embedded sections. The localization of these endogenous peptides is summarized in Fig. 7. They were found in the nucleus, cytoplasmic matrix, secretory granules and, sometimes, at the plasma membrane level of their target cells. No neuropeptides were detected in any anterior pituitary cells, indicating a cellular specificity of this detection.

The presence of peptide at the plasma membrane level is always difficult to show, given that this step, as binding studies demonstrate, is carried out in physiologial conditions and occurs very rapidly. However, when the administration of exogenous native neuropeptides was carried out before immunocytological detection, the signal detected at this level was intense, as was shown with TRH administration versus endogenous detection of this peptide [5]. This suggests that in physiological conditions the concentration of endogenous peptide in contact with the plasma membrane of its target cells is extremely low and that the internalization process occurs rapidly. A similar process seems to take place at the nuclear membrane level. Distinct significant labelling can be observed after injection of iodated GH-releasing hormone agonist into this compartment [30], or after injection of the same unlabelled agonist [7, 22]. Without treatment, however, the signal observed for native endogenous neuropeptides is of extremely low intensity.

The presence of binding sites at the plasma membrane level is well documented, but the presence of neuropeptides or protein hormones at the nuclear membrane level is disputable. This presence may suggest that the barrier between transcription and translation is a potential point of regulation [48]. Its effectiveness is attested to by the fact that most mRNA never leaves the nucleus and is eventually degraded. Dexamethasone, a synthetic glucocorticoid, induces α_{2u}-globulin production in rat liver [49]. After adrenalectomy, the mRNA for this protein accumulates in the nucleus, whereas the

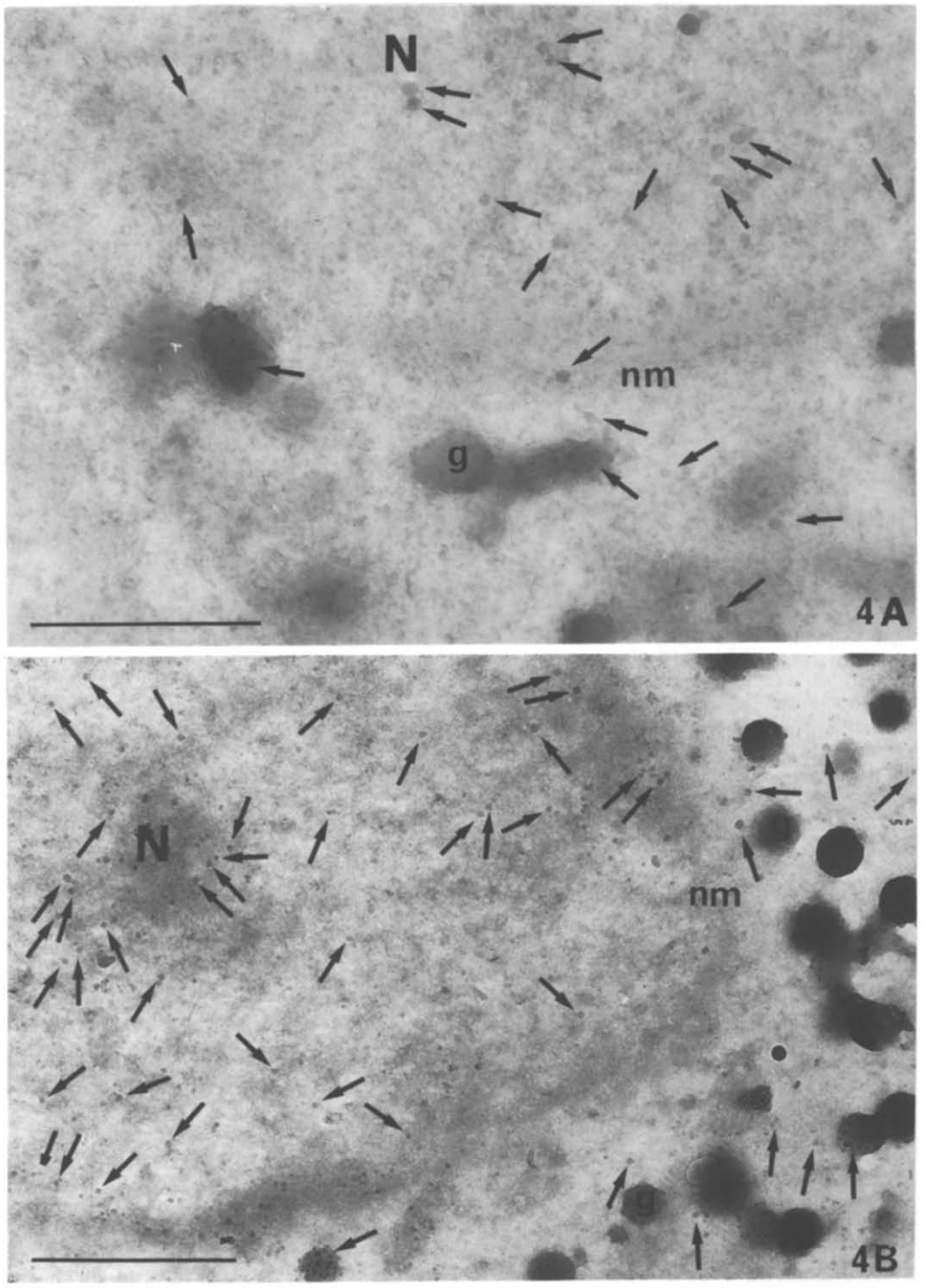

Fig. 4. TRH internalization in lactotrophs. TRH-like immunoreactivity (arrow) is revealed using the PAP method on ultrathin frozen sections. (A) Endogenous TRH is observed in the cytoplasmic matrix, secretory granules (g) and nucleus (N). (B) Exogenous TRH is detected 15 min after i.v. injection of 100 ng native TRH. TRH-like immunoreactivity is particularly localized in the nucleus. nm, Nuclear membrane. Bar = 1 μm [5].

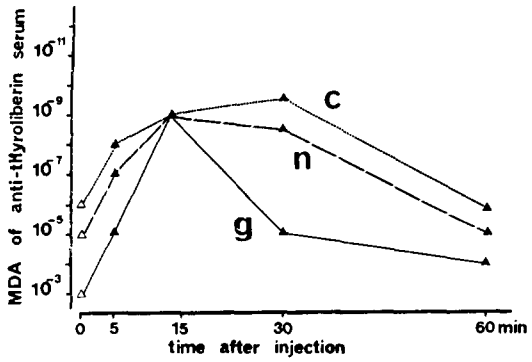

Fig. 5. Influence of time on MDA values (see Materials and Methods for details) in lactotrophs after native TRH injection. c, Cytoplasmic matrix compartment; g, secretory granule compartment; n, nuclear compartment [5].

mRNA content in the polysome falls. For protein hormones, insulin and epidermal growth factor, the nuclear efflux of endogenous mRNA and the nuclear influx of dextran were stimulated by a supraphysiological concentration of hormones [50, 51]. The latter studies also described the hormone which regulates the poly(A) mRNA efflux from intact nuclei by influencing the nucleoside triphosphatase activity in the nuclear envelope [52, 53]. However, binding sites have been described at the nuclear membrane level for insulin [54] and gonadotropin [55]. The regulation of this mechanism could imply the presence of a nucleotide triphosphate phosphatase in the nucleus. Human chorionic gonadotropin stimulates this enzyme in the isolated nuclei of lutheal cells [56], but not in the nuclei of non-target tissue. Insulin and cAMP have more modest effects in their target tissues [57], but such effects have been shown by influencing nucleoside triphosphatase activity in the nuclear envelope [52, 53]. In the same way, 1 hr after the *in vivo* injection of GH-releasing hormone or SS in intact rats, mRNA encoding for GH, detected by ultrastructural *in situ* hybridization, showed an accumulation of this mRNA in the nuclear matrix and the nuclear membrane [58].

In the nuclear matrix, on the other hand, the signal detected was strong and constant. In target cells neuropeptides were always detected in this compartment. Signal density depends on the peptide and the method used. The detection of an endogenous peptide by the use of immunocytology means that this peptide is present in physiological conditions, and can be revealed, while injection of iodated peptide does not produce a significant signal in the nuclear matrix [23, 28, 30], except for short peptides, such as monoiodated SS14 [26] or tritiated TRH [1] which can be revealed in the nucleus. The kinetics of internalization for these modified peptides, which conserve a certain biological activity, are too short to show nuclear translocation. Moreover, the percentage of labelled molecule uptaken by the nucleus is too low to be visualized. However, the presence in the nucleus of similar (but unlabelled) injected peptides, such as TRH [5] or GH-releasing hormone [7, 22], can be visualized within a short time. However, the accumulation of polypeptide hormones in the nuclei of target cells has been observed for insulin [59, 60], GH [61, 62], PRL [60, 63], opioid [64], nerve growth factor [65, 66], epidermal growth factor [67], platelet-derived growth factor [68], fibroblast growth factor [69] and β- and δ-interferon [70].

Recent data have provided both direct and indirect evidence indicating that in the nucleus, neuropeptides behave, like steroid hormones, in the way they alter gene expression; this has been demonstrated for nerve growth factor [71–73], TRH [74] and GH-releasing hormone [58, 75, 76] and is possible in the case of SS [77]. The signal generated by the binding of TRH to its plasma membrane receptor rapidly reaches the cell nucleus, where it increases the transcription of the PRL gene [72, 73]. In addition, post-transcriptional action of TRH seems to amplify and extend the regulation which occurs at the transcriptional level [72]. Interestingly, TRH, which is only detected in somatotrophs, can also inhibit GH synthesis by modulating the level of specific mRNAs [72, 78], injection of TRH.

Neuropeptides such as substance P and substance K [79], detected in lactotrophs and gonadotrophs [13], vasopressin [80], detected in lactotrophs, corticotrophs and gonadotrophs [16] and bombesin [81], a non-mammalian analogue of gastrin-releasing peptide, detected in lactotrophs, somatotrophs and gonadotrophs, have been reported to stimulate DNA synthesis. The mechanism of these effects remains to be elucidated. On the other hand, nuclear binding sites have been described for gonadotropin-releasing homone [82], gonadotropin [55], GH [83, 84], angiotensin II [69, 85], opioid [64] and growth factors (such as epidermal growth factor, nerve growth factor, platelet-derived growth factor [68] and fibroblast growth factor [86]), using binding studies and for GH [83, 87] and PRL [88] using specific antibodies.

Numerous neuropeptides and polypeptide hormones, notably insulin and epidermal growth factor such as poly(A) mRNA efflux regulator [52, 53] have been reported to show specific nuclear effects. Moreover, insulin may convert GH-secreting cells into PRL-secreting cells [89] and, when applied directly to isolated frog oocyte nuclei, may markedly stimulate RNA synthesis [90]. Gonadotropin-releasing homone [8, 9, 20] and angiotensin II [18] peptides have been described in anterior pituitary cells, where their effects are known [91, 92]. Moreover, angiotensin II enhances the susceptibility of chromatin to nuclease digestion [69], which would be consistent with the induction of transcriptional activity. By contrast, epidermal growth factor, through these nuclear receptors in chromatin [68], increases DNA resistance to nuclease digestion. The presence of fibroblast growth factor within the nucleus and more precisely the nucleolus, is correlated with stimulation of transcription of the ribosomal gene [86]. This growth factor also has a stimulatory effect on RNA polymerase I in isolated nuclei. Activin inhibits the synthesis of GH [93] and proopiomelanocortin [94]. In the same way, GH and

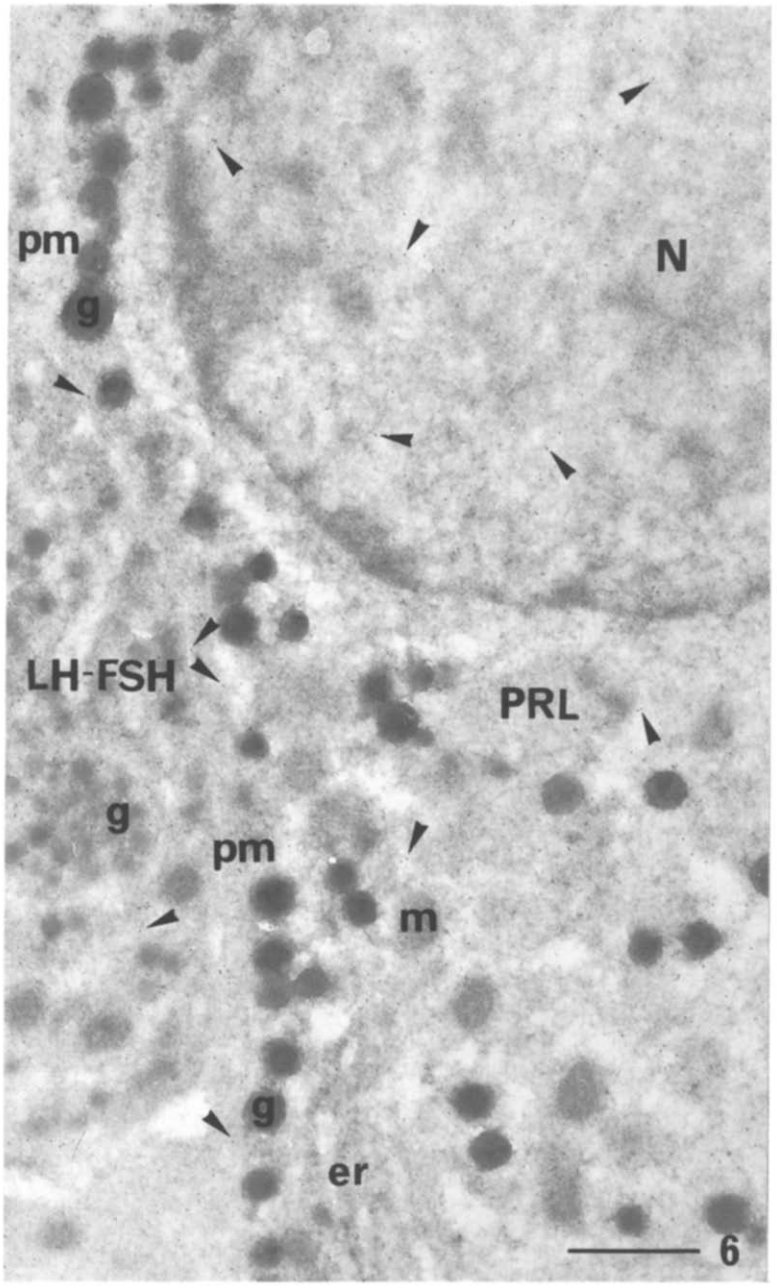

Fig. 6. Localization of PRL receptor-like immunoreactivity in lactotroph (PRL) and gonadotroph (LH-FSH). Immunoreactivity, using the colloidal gold particle method (arrowhead) on ultrathin frozen sections, is observed at the plasma membrane level (pm), in the cytoplasmic matrix and in the nucleus (N) and is occasionally associated with the nuclear membrane (nm). er, endoplasmic reticulum; m, mitochondrion. Bar = 1 μm.

PRL are known to regulate their own receptors [95–97]. The presence of PRL receptor in the nucleus [88] could explain this phenomenon.

The nature of the signalling mechanism in question indicates that protein kinase C is a promising candidate. Protein kinase C, which is also a nuclear enzyme [98], has been demonstrated to have implications for the rapidity of the cell response and for the down-regulation [99–103] of the lymphokine receptor family [104]. It is possible that GH acts on protein kinase C at the nuclear level to regulate transcription through a specific transacting element [83, 105]. Corticotropin-releasing hormone has been found to modulate protein kinase C in corticotrophs [106]. It has been shown, however, that only cAMP concentrations are correlated with the modulation of GH and proopiomelanocortine mRNA, in rat anterior pituitary cells in culture [107]. In addition,

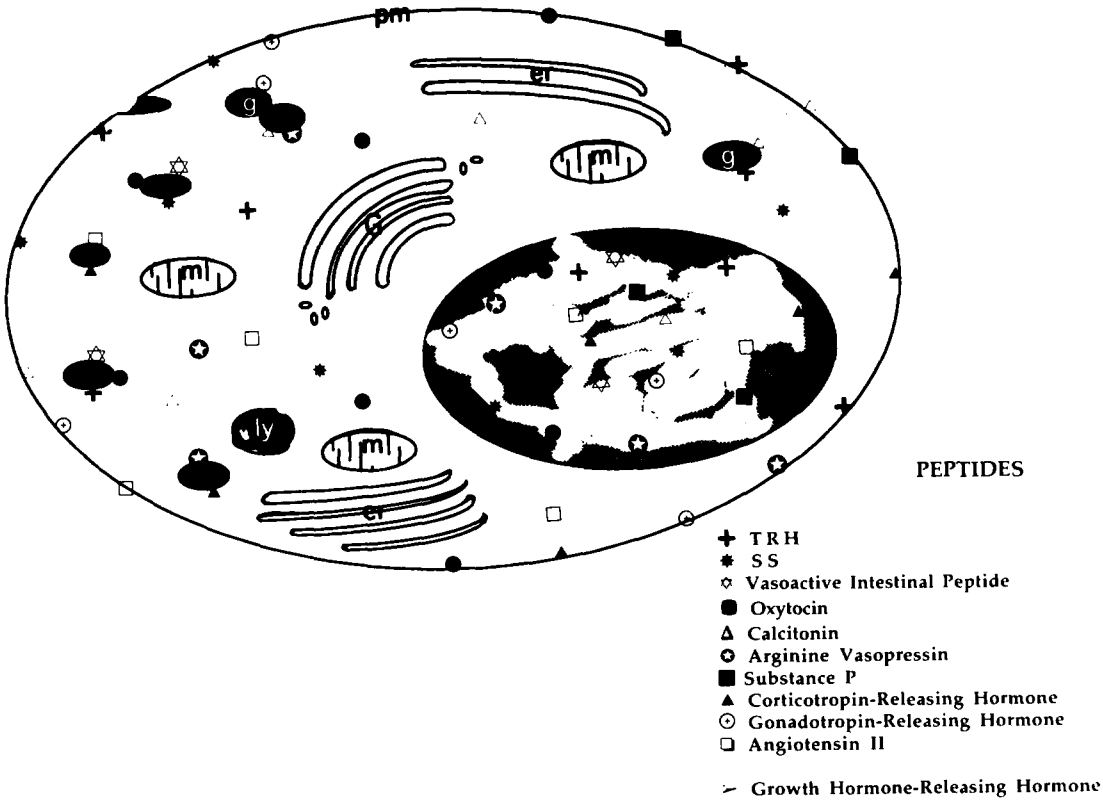

Fig. 7. Schematic localization of neuropeptide-like immunoreactivity in lactotroph. All these peptides were localised in lactotrophs, except for the GH-releasing hormone detected in somatotrophs only. pm, Plasma membrane level; g, secretory granule; ly, lysosome; er, endoplasmic reticulum; G, Golgi apparatus; m, mitochondrion; N, nucleus.

the same group has previously suggested that the possible role of cAMP in the transcriptional activity of the anterior pituitary is through phosphorylation of histone and non-histone acidic nuclear proteins [108].

Another form of nuclear action of neuropeptides can be hypothesized, through the oncogenes. Recently, Lin *et al.* [109] have demonstrated that Pit-1 expression depends on the expression of the receptor for GH-releasing hormone which mediates pituitary cell growth. GH-releasing hormone also induces c-*fos* expression in cultured primary pituitary cells [110]. In the same way, an apparent (human) hGH-induced protein kinase C activation of c-*fos* activation, but not of insulin-like growth factor gene expression has been reported in Ob1771 cells [99]. TRH also increases the levels of c-*fos* mRNA in GH3B6 pituitary tumour cells [111].

Internalization process through the plasma membrane is well documented for certain target cells, but in pituitary cells endosome structures have never been found in the visualizing of endogenous neuropetides. However, the mode of uptake of neuropeptides into the nucleus, i.e. the way they arrive from the plasma membrane through the cytoplasm, is as yet unknown. Jiang and Schindler [112] have reported that, for the epidermal growth factor, plasma membrane-bound EGF receptor–EGF complexes find their way to the nucleus and stimulate both nucleocytoplasmic transport and DNA synthesis.

The physiological function of these neuropeptides, which are internalized from the extracellular compartment of the pituitary cell into the nucleus, may play an intracellular communication role within the pituitary, by influencing not only pituitary hormone release but also the release (for review see Refs. 97, 113) or synthesis [16, 29, 114–121] of the peptides which are known to be present in particular pituitary cell types. However, further studies will need to be carried out in order to understand the functions of these neuropeptides in pituitary or other target tissues.

REFERENCES

1. Gourdji D, Tixier-Vidal A, Morin A, Pradelles P, Morgat JL, Fromageot P and Kerdelhué B, Binding of a tritiated thyrotropin-releasing factor to a prolactin secreting clonal cell line (GH3). *Exp Cell Res* **82**: 39–46, 1973.
2. Hökfelt T, Johansson O and Goldstein M, Chemical anatomy of the brain. *Science* **225**: 1326–1334, 1984.
3. Morel G, Mesguich P, Dubois MP and Dubois PM, Ultrastructural evidence for endogenous somatostatin-like immunoreactivity in the pituitary gland. *Neuroendocrinology* **36**: 291–299, 1983.

4. Mesguich P, Benoit R, Dubois PM and Morel G, Somatostatin-28- and somatostatin-14-like immunoreactivities in the rat pituitary gland. *Cell Tissue Res* **252**: 419–427, 1988.
5. Morel G, Gourdji D, Grouselle D, Brunet N, Tixier-Vidal A and Dubois PM, Immunocytochemical evidence for *in vivo* internalization of thyroliberin into rat pituitary target cells. *Neuroendocrinology* **41**: 312–320, 1985.
6. Morel G, Mesguich P, Dubois MP and Dubois PM, Ultrastructural evidence for endogenous growth hormone-releasing factor (GRF)-like immunoreactivity in the monkey pituitary gland. *Neuroendocrinology* **38**: 123–133, 1984.
7. Dubois PM, Mesguich P, Hemming F and Morel G, Effects and distribution of hpGRF in somatotropes of rat pituitary gland. In: *Monoclonal Antibodies and New Trends in Immunoassays* (Ed. Bizollon ChA), pp. 229–237. Elsevier Science Publishers, Amsterdam, 1984.
8. Sternberger LA and Petrali JP, Quantitative immunocytochemistry of pituitary receptors for luteinizing hormone-releasing hormone. *Cell Tissue Res* **162**: 141–176, 1975.
9. Morel G and Dubois PM, Immunocytochemical evidence for gonadoliberin in rat anterior pituitary gland. *Neuroendocrinology* **34**: 197–206, 1982.
10. Morel G, Hemming F, Tonon M-C, Vaudry H, Dubois MP, Coy D and Dubois PM, Ultrastructural evidence for corticotropin-releasing factor (CRF)-like immunoreactivity in the rat pituitary gland. *Biol Cell* **44**: 89–92, 1982.
11. Morel G, Enjalbert A, Proulx L, Pelletier G, Barden N, Gossard F and Dubois PM, Effect of corticotropin-releasing factor on the release and synthesis of prolactin. *Neuroendocrinology* **49**: 669–675, 1989.
12. Morel G, Besson J, Rosselin G and Dubois PM, Ultrastructural evidence for vasoactive intestinal peptide (VIP) in rat pituitary gland. *Neuroendocrinology* **34**: 85–89, 1982.
13. Morel G, Chayvialle JA, Kerdelhue B and Dubois PM, Ultrastructural evidence for endogenous substance P-like immunoreactivity in the rat pituitary gland. *Neuroendocrinology* **35**: 86–92, 1982.
14. Chabot J-G, Enjalbert A, Pelletier G, Dubois PM and Morel G, Evidence for direct action of neuropeptide Y (NPY) in rat pituitary gland. *Neuroendocrinology* **47**: 511–517, 1988.
15. Morel G, Chabot J-G and Dubois PM, Ultrastructural evidence for oxytoxin in the rat anterior pituitary gland. *Acta Endocrinol (Copenh)* **117**: 307–314, 1988.
16. Morel G, Chabot J-G, Enjalbert A, Priam M and Dubois PM, Evidence for direct action of calcitonin in the rat pituitary gland. *Acta Endocrinol (Copenh)* **120**: 682–688, 1989.
17. Terrier C, Chabot J-G, Pautrat G, Jeandel L, Gray D, Lutz-Bucher B, Zingg HH and Morel G, Arginine-vasopressin in anterior pituitary cells: *in situ* hybridization of mRNA and ultrastructural localization of immunoreactivity. *Neuroendocrinology* **54**: 303–311, 1991.
18. Chabot J-G, Gray DA, Dubois PM and Morel G, Presence of angiotensin II in the adult male rat anterior pituitary gland: immunocytochemical study after cryoultramicrotomy. *Exp Cell Res* **180**: 189–191, 1989.
19. Morel G, Chabot J-G, Belles-Isles M and Heisler S, Synthesis and internalization of atrial natriuretic factor in anterior pituitary cells. *Mol Cell Endocrinol* **55**: 219–231, 1988.
20. Pelletier G, Dube D, Guy J, Seguin C and Lefebvre FA, Binding and internalization of a luteinizing hormone-releasing hormone agonist by rat gonadotrophic cells. A radioautographic study. *Endocrinology* **111**: 1068–1076, 1982.
21. Duello TM, Nett TH and Farquhar MG, Fate of gonadotropin-releasing hormone agonist internalized by rat pituitary gonadotrophs. *Endocrinology* **112**: 1–10, 1983.
22. Mesguich P, Morel G and Dubois PM, Ultrastructural evidence for *in vivo* internalization of exogenous growth hormone-releasing factor (GRF) in rat somatotrophs. In: *Regulatory Peptides in Digestive, Nervous and Endocrine Systems*, INSERM Symposium (Eds. Lewin MJM and Bonfils S), pp. 47–50. Elsevier Science Publishers, Amsterdam, 1985.
23. Morel G, Leroux P and Pelletier G, Ultrastructural autoradiographic localization of somatostatin-28 in the rat pituitary gland. *Endocrinology* **116**: 1615–1620, 1985.
24. Leroux P and Pelletier G, Radioautographic study of binding and internalization of corticotropin-releasing factor by rat anterior pituitary corticotrophs. *Endocrinology* **114**: 14–21, 1984.
25. Morel G, Pelletier G and Heisler S, Internalization and subcellular distribution of radiolabeled somatostatin-28 in mouse anterior pituitary tumor cells. *Endocrinology* **119**: 1972–1979, 1986.
26. Morel G and Pelletier G, Ultrastructural autoradiographic localization of somatostatin-14 (SS-14) in the rat pituitary gland. *Life Sci Adv* **5**: 46–53, 1986.
27. Morel G, Dihl F, Aubert ML and Dubois PM, Binding and internalization of native gonadoliberin (GnRH) by anterior pituitary gonadotrophs of the rat. A quantitative autoradiographic study after cryo-ultramicrotomy. *Cell Tissue Res* **248**: 541–550, 1987.
28. Morel G and Heisler S, Internalization of endogenous and exogenous atrial natriuretic peptide by target tissues. *Electron Microsc Rev* **1**: 221–259, 1988.
29. Morel G, Chabot J-G, Gossard F and Heisler S, Is atrial natriuretic peptide synthesized and internalized by gonadotrophs? *Endocrinology* **124**: 1703–1710, 1989.
30. Morel G, Uptake and ultrastructural localization of a [^{125}I] growth hormone releasing factor agonist in male rat pituitary gland: evidence for internalization. *Endocrinology* **129**: 1497–1504, 1991.
31. Child GV, Hazum E, Amsterdam A, Limor R and Naor Z, Cytochemical evidence for different routes of gonadotropin-releasing hormone processing by large gonadotropes and granulosa cells. *Endocrinology* **119**: 1329–1338, 1986.
32. Schally AV, Bowers CY, Redding TW and Barrett JF, Isolation of thyotropin-releasing factor (TRF) from porcine hypothalamus. *Biochem Biophys Res Commun* **25**: 165, 1966.
33. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J and Guillemin R, Hypothalamic polypeptide that inhibits the secretion of pituitary growth hormone. *Science* **179**: 77–79, 1973.
34. Schally AV, Huang WY, Chang RC, Arimura A, Redding TW, Millar RP, Hunkapillar MW and Ood RC, Isolation and stucture of prosomatostatin. A putative somatostatin precursor from pig hypothalamus. *Proc Natl Acad Sci USA* **77**: 4488–4493, 1980.
35. Pradayrol L, Jornvall H, Mutt V and Ribet A, N-terminally extended somatostatin: the primary structure of somatostatin-28. *FEBS Lett* **109**: 55–58, 1980.
36. Esch F, Böhlen P, Ling N, Benoit R, Brazeau P and Guillemin R, Primary structure of ovine hypothalamic somatostatin-28 and somatostatin-25. *Proc Natl Acad Sci USA* **77**: 6827–6831, 1980.
37. Schonbrunn A and Tashjian Jr AH, Characterization of functional receptors for somatostatin in rat pituitary cells in culture. *J Biol Chem* **253**: 6473–6483, 1978.

38. Labrie F, Barden N, Poirier G and De Lean A, Binding of thyrotropin-releasing hormone to plasma membrane of bovine anterior pituitary gland. *Proc Natl Acad Sci USA* **69**: 283–287, 1972.
39. Morrisson JH, Benoit R, Magistretti PJ and Bloom FE, Immunohistochemical distribution of prosomatostatin-related peptides in cerebral cortex. *Brain Res* **262:** 344–351, 1983.
40. Morel G, Coupes semi-fines et ultrafines de tissus congelés: congélation, cryo-ultramicrotomie, contraste. In: *Techniques en Microscopie Électronique: Cryométhodes, Immunocytologie, Autoradiographie, Hybridation in situ* (Ed. Morel G), pp. 31–54. INSERM, Paris, 1991.
41. Morel G, Immunocytologie après cryo-ultramicrotomie. In: *Techniques en Microscopie Électronique: Cryométhodes, Immunocytologie, Autoradiographie, Hybridation in situ* (Ed. Morel G), pp. 195–210. INSERM, Paris, 1991.
42. Aubert ML, Begeot M, Winiger BP, Morel G, Sisonenko PC and Dubois PM, Ontogeny of hypothalamic luteinizing hormone-releasing hormone (GnRH) and pituitary GnRH receptors in fetal and neonatal rats. *Endocrinology* **116**: 1565–1576, 1985.
43. Morel G, Electron microscopic autoradiographic techniques. In: *Electron Microscopy of Tissues, Cells and Organelles* (Ed. Harris JR), pp. 83–123. IRL Press, Oxford, 1991.
44. Hopkins CR and Farquhar MG, Hormone secretion by cells dissociated from rat anterior pituitaries. *J Cell Biol* **59**: 276–303, 1973.
45. Nadler NJ, The interpretation of grain counts in electron microscope radioautography. *J Cell Biol* **49:** 877–882, 1971.
46. Salpeter MM and McHenry FA, Electron microscope autoradiography. Analysis of autoradiograms. In: *Advanced Techniques in Biological Electron Microscopy* (Ed. Koehler JK), pp. 113–131. Springer, New York, 1973.
47. Hemming FJ, Mesguich P, Morel G and Dubois PM, Cryoultramicrotomy versus plastic embedding: comparative immunocytochemistry of rat anterior pituitary cells. *J Microsc* **131**: 26–34, 1983.
48. Blobel G, Gene gating: a hypothesis. *Proc Natl Acad Sci USA* **82:** 8527–8529, 1985.
49. Fulton R, Birnie GD and Knowler JT, Post-transcriptional regulation of rat liver gene expression by glucocorticoids. *Nucleic Acid Res* **13**: 6467–6482, 1985.
50. Schumm DE and Webb TE, Insulin-modulated transport of RNA from isolated liver nuclei. *Arch Biochem Biophys* **210**: 275–279, 1981.
51. Schindler M and Jiang L-W, Epidermal growth factor and insulin stimulate nuclear pore-mediated macromolecular transport in isolated rat liver nuclei. *J Cell Biol* **104:** 849–853, 1987.
52. Purello F, Burnham DB and Goldfine ID, Insulin regulation of protein phosphorylation in isolated rat liver nuclear envelopes: potential relationship to mRNA metabolism. *Proc Natl Acad Sci USA* **80**: 1189–1193, 1983.
53. Schröder HC, Wenger R, Urgakovic D, Friese K, Bachmann M and Muller WEG, Differential effect of insulin and epidermal growth factor on the mRNA translocation system and transport of specific Poly(A+) mRNA and Poly(A−) mRNA in isolated nuclei. *Biochemistry* **29**: 2368–2378, 1990.
54. Vigneri R, Goldfine ID, Wong KY, Smith GJ and Pezzino V, The nuclear envelope. The major site of insulin binding in rat liver nuclei. *J Biol Chem* **253:** 2098–2103, 1978.
55. Rajendran KG and Menon KMJ, Evidence for existence of gonadotropin receptors in the nuclei isolated from rat ovary. *Biochem Biophys Res Commun* **111**: 127–134, 1983.
56. Ramani N and Rao CV, Direct stimulation of nucleoside triphosphatase activity in bovine luteal nuclear membranes by human chorionic gonadotropin. *Endocrinology* **120**: 2468–2473, 1987.
57. Goldfine ID, Clawson GA, Smuckler EA, Purrello F and Vigneri R, Action of insulin at the nuclear envelope. *Mol Cell Biochem* **48**: 3–14, 1982.
58. Morel G, Dihl F and Gossard F, Ultrastructural distribution of growth hormone (GH) mRNA and GH intron I sequences in rat pituitary gland: effect of GH-releasing hormone and somatostatin. *Mol Cell Endocrinol* **65**: 81–89, 1989.
59. Oldfine ID, Jones AL, Hradek GT, Wong KY and Mooney JS, Entry of insulin into human cultured lymphocytes: electron microscope autoradiographic analysis. *Science* **202:** 760–763, 1978.
60. Giss BJ and Walker AM, Mammotroph autoregulation: intracellular fate of internalized prolactin. *Mol Cell Endocrinol* **42**: 259–267, 1985.
61. Bonifacino JS, Rogin LP and Paladini AC, Formation of complex between ^{125}I-labelled human or bovine somatotropins and binding proteins *in vivo* in rat liver and kidney. *Biochem J* **214:** 121–134, 1983.
62. Rezvani I, Maddaiah VT, Collipp PJ, Thomas J and Chen SY, Kinetics of uptake and subcellular distribution of human growth hormone in liver slices. *Biochem Med* **7**: 432–440, 1973.
63. Cleviger CV, Sillman AL and Prystowsky MB, Interleukin-2 driven nuclear translocation of prolactin in cloned T-lymphocytes. *Endocrinology* **127:** 3151–3159, 1990.
64. Belcheva M, Barg J, Rowinski J, Clark WG, Gloeckner CA, Ho A, Gao XM, Chuang DM and Coscia C, Novel opioid binding sites associated with nuclei of NG108-15 neurohybrid cells. *J Neurosci* **13**: 104–114, 1993.
65. Yanker BA and Shooter EM, Nerve growth factor in nucleus: interaction with receptor on the nuclear membrane. *Proc Natl Acad Sci USA* **76**: 1269–1273, 1979.
66. Marchisio PC, Nadin L and Calissano P, Intracellular distribution of nerve growth factor in rat pheochromocytoma PC12 cells: evidence for a perinuclear and intranuclear location. *Proc Natl Acad Sci USA* **77:** 1656–1660, 1980.
67. Savion N, Vlodavsky I and Gospodarowicz D, Nuclear accumulation of epidermal growth factor in cultured bovine corneal endothelial and granulosa cells. *J Biol Chem* **256:** 1149–1154, 1981.
68. Rakwicz-Szulczynska EM, Rodeck U, Herlyn M and Koprowski H, Chromatin binding of epidermal growth factor, nerve growth factor and platelet derived growth factor in cells bearing the appropriate surface receptors. *Proc Natl Acad Sci USA* **83**: 3728–3732, 1986.
69. Re RN, Vizard DL, Brown J and Bryan SE, Angiotensin II receptors in chromatin fragments generated by micrococcal nuclease. *Biochem Biophys Res Commun* **119**: 220–227, 1984.
70. Macdonald HS, Kushnaryov VM, Sedmak JJ and Grossberg SE, Transport of χ-interferon into the nucleus may be mediated by nuclear membrane receptors. *Biochem Biophys Res Commun* **138**: 254–260, 1986.
71. Evans GA and Rosenfeld MG, Regulation of prolactin in RNA analysed using aspecific cDNA probe. *J Biol Chem* **254**: 8023–8030, 1979.
72. Laveriere JN, Morin A, Tixier-Vidal A, Truong AT, Gourdji D and Martial JA, Inverse control of prolactin and growth hormone gene expression: effect of

thyroliberin on transcription and RNA stabilization. *EMBO J* **2:** 1493–1499, 1983.
73. Murdoch GH, Franco R, Evans GA and Rosenfeld MG, Polypeptide hormone regulation of gene expression. Thyrotropin-releasing hormone rapidly stimulates both transcription of the prolactin gene and the phosphorylation of a specific nuclear protein. *J Biol Chem* **258**: 15329–15335, 1983.
74. Evans GA and Rosenfeld MG, Regulation of prolactin in RNA analysed using a specific cDNA probe. *J Biol Chem* **254**: 8023–8030, 1979.
75. Barinaga M, Yamamoto G, Rivier C, Vale W, Evans R and Rosenfeld MG, Transcriptional regulation of growth hormone gene expression by growth hormone-releasing factor. *Nature (London)* **306:** 84–85, 1983.
76. Barinaga M, Bilezikjan LM, Vale W, Rosenfeld MG and Evans RM, Independent effects of growth hormone-releasing factor on growth hormone release and gene transcription. *Nature (London)* **314**: 279–281, 1985.
77. Sara VR, Rutherford R and Smythe GA, The influence of maternal somatostatin administration on foetal brain cell proliferation and its relationship to serum growth hormone and brain trophin activity. *Horm Metab Res* **11:** 147–149, 1979.
78. Evans GA, David DN and Rosenfeld MG, Regulation of prolactin and somatotropin mRNAs by thyroliberin. *Proc Natl Acad Sci USA* **75**: 1294–1298, 1978.
79. Nilsson J, von Euler AM and Dalsgaard CJ, Stimulation of connective tissue cell growth by substance P and substance K. *Nature (London)* **315**: 61–63, 1985.
80. Rozengurt E, Legg A and Pettican P, Vasopressin stimulation of mouse 3T3 cell growth. *Proc Natl Acad Sci USA* **76**: 1284–1287, 1979.
81. Rozengurt E and Sinnet-Smith J, Bombesin stimulation of DNA synthesis and cell division in cultures of Swiss 3T3 cells. *Proc Natl Acad Sci USA* **80**: 2936–2940, 1983.
82. Millar R, Rosen H, Badminton M, Pasqualini C and Kerdelhué B, Luteinizing hormone-releasing hormone (LH-RH) binding to purified rat pituitary nuclei. *FEBS Lett* **153**: 382–386, 1983.
83. Lobie PE, Barnard R and Waters MJ, The nuclear growth hormone receptor binding protein. *J Biol Chem* **266**: 22645–22652, 1991.
84. Hoquette JF, Postel-Vinay MC, Kayser C, de Hemptine B and Amar-Costesec A, The human liver growth hormone receptor. *Endocrinology* **125**: 2167–2174, 1989.
85. Re RN, The cellular biology of angiotensin: paracrine, autocrine and intracrine actions in cardiovascular tissues. *J Mol Cell Cardiol* V (Suppl): 63–69, 1989.
86. Bouche G, Gas N, Prats H, Badin V, Tauber JP, Teissie J and Almaric F, Basic fibroblast growth factor enters the nucleolus and stimulates the transcription of ribosomal genes in ABAE cells undergoing $G_0 \rightarrow G_1$ transition. *Proc Natl Acad Sci USA* **84:** 6770–6774, 1987.
87. Baumbach WR, Horner DR and Logan JS, The growth hormone-binding protein in rat serum is an alternatively spliced form of the rat growth hormone receptor. *Gene Dev* **3**: 1199–1206, 1989.
88. Morel G, Ouhtit A and Kelly PA, Prolactin receptor immunoreactivity in rat anterior pituitary. *Neuroendocrinology*, in press.
89. Inoue K and Sakai T, Conversion of growth hormone-secreting cells into prolactin-secreting cells and its promotion by insulin and insulin-like growth factor-1 *in vitro*. *Exp Cell Res* **195**: 53–58, 1991.
90. Miller DS, Stimulation of RNA and protein synthesis by intracellular insulin. *Science* **240:** 506–508, 1988.
91. Chieffi G, Pierantoni R and Fasano S, Immunoreactive GnRH in hypothalamic and extrahypothalamic areas. *Int Rev Cytol* **127**: 1–55, 1991.
92. Saavedra JM, Brain and pituitary angiotensin. *Endocr Rev* **13**: 329–380, 1992.
93. Billestrup N, Gonzalez-Manchon C, Potter E and Vale W, Inhibition of somatotroph growth and growth hormone biosynthesis by activin *in vitro*. *Mol Endocrinol* **4**: 356–362, 1990.
94. Bilezikjian LM, Blount AL, Campen CA, Gonzalez-Manchon C and Vale W, Activin A inhibits proopiomelanocortin messenger RNA accumulation and adrenocorticotropin secretion of AtT20 cells. *Mol Endocrinol* **5**: 1389–1395, 1991.
95. Topper YJ and Freeman CS, Multiple hormone interactions in the developmental biology of the mammary gland. *Physiol Rev* **60:** 1049–1106, 1980.
96. Rosenthal SM, Silverman BL and Wehrenberg WB, Exogenous growth hormone inhibits bovine but not murine pituitary growth hormone secretion *in vitro*: evidence for a direct feedback of growth hormone on pituitary. *Neuroendocrinology* **53**: 597–600, 1991.
97. Schwartz J and Cherny R, Intercellular communication within the anterior pituitary influencing the secretion of hypophysial hormones. *Endocr Rev* **13**: 453–475, 1992.
98. Masmoudi A, Labourdette G, Mersel M, Huang FL, Huang K-P, Vincendon G and Malviya AN, Protein kinase C located in rat liver nuclei. *J Biol Chem* **264**: 1172–1179, 1989.
99. Doglio A, Dani C, Grimadi P and Ailhaud G, Growth hormone stimulates c-*fos* gene expression by means of protein kinase C without increasing inositol lipid turnover. *Proc Natl Acad Sci USA* **86**: 1148–1152, 1989.
100. Smal J and Meyts P, Role of kinase C in the insulin-like effects of human growth hormone in rat adipocytes. *Biochem Biophys Res Commun* **147**: 1232–1240, 1987.
101. Smal J, Kathuria S and Meyts P, Acridine orange, an inhibitor of protein kinase C, abolishes insulin and growth hormone stimulation of lipogenesis in rat adipocytes. *FEBS Lett* **244**: 465–468, 1989.
102. Russell DH, New aspects of prolactin and immunity: a lymphocyte-derived prolactin-like product and nuclear protein kinase C activation. *Trends Pharmacol Sci* **10**: 40–44, 1989.
103. Mason Garcia M, Weill CL and Beckman BS, Rapid activation by erythropoietin of protein kinase C in nuclei of erythroid progenitor cells. *Biochem Biophys Res Commun* **168**: 490–497, 1990.
104. Bazan JF, Haemopoietic receptors and helical cytokines. *Immunol Today* **11**: 350–354, 1990.
105. Elsholtz HP, Mangalam HJ, Potter E, Albert VR, Supowit S, Evans RM and Rosenfeld MG, Two different cis-active elements transfer the transcriptional effects of both EGF and phorbol esters. *Science* **234**: 1552–1557, 1986.
106. Childs GV and Unabia G, Activation of protein kinase C and L calcium channel enhances binding of biotinylated corticotropin-releasing hormone by anterior pituitary corticotropes. *Mol Cell Endocrinol* **2**: 117–126, 1989.
107. Simard J, Labrie F and Gossard F, Regulation of growth hormone mRNA and pro-opiomelanocortin mRNA levels by cyclic AMP in rat anterior pituitary cells in culture. *DNA* **5**: 263–271, 1986.
108. Jolicoeur P and Labrie F, Phosphorylation of nuclear proteins from bovine anterior pituitary gland induced by adenosine 3′:5′-monophosphate. *Eur J Biochem* **48:** 1–9, 1974.
109. Lin C, Lin S-C, Chang C-P and Rosenfeld MG, Pit-1-dependent expression of the receptor for growth hormone-releasing factor mediates pituitary cell growth. *Nature (London)* **360**: 765–768, 1992.

110. Billestrup N, Mitchell RL, Vale W and Verma IM, Growth hormone-releasing factor induces c-*fos* expression in cultured primary pituitary cells. *Mol Endocrinol* **1**: 300–305, 1987.
111. Weisman AS, Tixier-Vidal A and Gourdji D, Thyrotropin-releasing hormone increases the level of c-*fos* and b-actin mRNA in GH3B6 pituitary tumor cells. *In Vitro* **23:** 585–590, 1987.
112. Jiang L-W and Schindler M, Nucleocytoplasmic transport is enhanced concomitant with nuclear accumulation of epidermal growth factor (EGF) binding activity in both 3T3-1 and EGF receptor reconstituted NR-6 fibroblasts. *J Cell Biol* **110**: 559–568, 1990.
113. Denef C, Paracrine interaction in the anterior pituitary. *Clin Endocrinol Metab* **15**: 1–32, 1986.
114. O'Halloran DJ, Jones PM and Bloom SR, Neuropeptides synthesized in the anterior pituitary: possible paracrine role. *Mol Cell Endocrinol* **75**: C7–C12, 1991.
115. Deschesper CF, Mellon SH, Cumin F, Baxter JD and Ganong WF, Analysis by immunocytohemistry and *in situ* hybridization of renin and its mRNA in kidney, testis, adrenal gland and pituitary of the rat. *Proc Natl Acad Sci USA* **83:** 7552–7556, 1986.
116. Deschesper CF, Crumrine DW and Ganong WF, Evidence that the gonadotrophs are the likely site of production of angiotensin II in the anterior pituitary of the rat. *Endocrinology* **119**: 36–43, 1986.
117. May V, Wilber JF, U'Pritchard DC and Child GV, Persistence of immunoreactive TRH and GnRH in long-term primary anterior pituitary cultures. *Peptides* **8**: 543–558, 1987.
118. Jones PM, Ghatei MA, Steele J, O'Halloran D, Gon G, Legon S, Burrin JM, Leonhardt U, Polak JM and Bloom SR, Evidence for neuropeptide Y synthesis in the rat anterior pituitary and the influence of thyroid hormone status: comparison with vasoactive intestinal peptide, substance P, and neurotensin. *Endocrinology* **125:** 334–341, 1989.
119. Bruhn TO, Bolduc TG, Maclean DB and Jackson IMD, ProTRH peptides are synthesized and secreted by anterior pituitary cells in long-term culture. *Endocrinology* **129**: 556–558, 1991.
120. Arnaout MA, Garthwaite TL, Martison DR and Hagen TC, Vasoactive intestinal polypeptide is synthesized in anterior pituitary tissue. *Endocrinology* **119**: 2052–2057, 1986.
121. O'Halloran D, Jones PM, Ghatei MA, Domin J and Bloom SR, The regulation of neuropeptide expression in rat anterior pituitary following chronic manipulation of estrogen status: a comparison between substance P, neuropeptide Y, neurotensin and vasoactive intestinal peptide. *Endocrinology* **127**: 1463–1469, 1990.