Journal of Cellular Biochemistry 65:25–31 (1997)

Retinoic Acid Stimulates Growth Hormone Synthesis in Human Somatotropic Adenoma Cells: Characterization of Its Nuclear Receptors

Jean Guibourdenche,[1] Charlotte Djakouré,[2] Dominique Porquet,[3] Patrick Pagésy,[2] Cécile Rochette-Egly,[4] Françoise Peillon,[2] Jacques Yuan Li,[2] and Danièle Evain-Brion[1]*

[1]Unité INSERM 427, Faculté des Sciences Pharmaceutiques et Biologiques, Université René Descartes Paris V, Paris, France
[2]Unité INSERM 223, Faculté de Médecine Pitié-Salpêtrière, Paris, France
[3]Service de Biochimie-Hormonologie, Hôpital Robert Debré, Paris, France
[4]IGBHC, CNRS/INSERM/Université Louis Pasteur, Ilkirch, France

Abstract In order to gain a better understanding on the possible role of retinoic acid (RA) on human GH secretion, we have characterized the expression of its nuclear receptors in somatotropic adenoma cell extracts. By immunoblotting with rabbit polyclonal antibodies directed against RARα, β, and γ and RXRα and β, we could only detect the presence of RARα and RXRα proteins. The predominant expression of RXRα was confirmed at the mRNA level by Northern and slot-blot analysis. We then investigated the effect of RA on GH synthesis in cell culture of adenomatous somatotrophs. In cultured cells, RA (1 μM) stimulated GH secretion, increased intracellular GH content and GH mRNA levels within 72 h, suggesting a modulation of GH synthesis by RA. J. Cell. Biochem. 65:25–31.

Key words: growth hormone; retinoic acid; retinoic acid nuclear receptors; pituitary adenomas; human pituitary

Vitamin A is involved in a number of essential processes, such as maintenance of normal fetal development and the regulation of growth and differentiation of various tissues, including the pituitary [Bauernfeind, 1983; Agazawa et al., 1989; Hofman and Eichele, 1994]. In addition, vitamin A is required for normal growth and development [Dowling and Wald, 1960]. Beside its specific role in visual process and spermatogenesis, most of the effects of vitamin A are mediated by its physiological acid metabolite, retinoic acid (RA). Biological actions of RA or its derivatives are exerted at the cellular level by two different types of specific nuclear receptors, RARs (RARα, RARβ and RARγ) and RXRs (RXRα, RXRβ, RXRγ), which belong to the superfamily of steroid/thyroid hormone receptors. Those receptors function as ligand-activated transcription factors and regulate gene expression by binding to DNA response elements associated with their target genes [for review, see De Thé and Dejean, 1991; Chambon, 1993; Gudas et al., 1994; Mangelsdorf et al., 1994].

We recently showed that vitamin A deficiency might be involved in abnormal nocturnal growth hormone (GH) secretion observed in some children with short stature [Evain-Brion et al., 1994], suggesting a possible role of vitamin A or its derivatives in human GH secretion. Numerous in vitro studies have shown that RA stimulates growth hormone gene expression in rat pituitary cells [Bedo et al., 1989; Morita et al., 1989, 1990; Garcia-Villalba et al., 1993], while in a recent in vivo study no change in GH pituitary gene expression was observed in vitamin A deficiency [Breen et al., 1995].

Therefore, in order to gain a better understanding on the possible role of RA in human GH secretion, we have characterized the expression of its nuclear receptors in pituitary adenomas from acromegalic patients. We have then investigated effect of RA on GH secretion in pituitary adenoma cell in vitro (i.e., culture).

MATERIALS AND METHODS

Surgical fragments of somatotropic adenomas from six patients (two women and four

Contract grant sponsor: l'Association pour la Recherche contre le Cancer.

*Correspondence to: Dr. Danièle Evain-Brion, Unité INSERM 427, Faculté des Sciences Pharmaceutiques et Biologiques, Université René Descartes Paris V, 4 Avenue de l'Observatoire, 75006 Paris, France.

Received 10 September 1996; accepted 23 October 1996

men aged 26–56 years) were studied. The diagnosis of somatotropic adenoma was established by clinical, radiological and biochemical criteria before transsphenoidal surgery and confirmed by immunocytochemistry. All patients had plasma GH levels of >5 ng/ml; plasma levels of IGF-I were also elevated, within a range of 733–1,850 ng/ml (normal values <463 ng/ml). Owing to the small amount of tissue, three adenomas were used for receptor studies and three for culture studies.

Receptor Studies

Western and Northern blot studies were performed on total cell extracts as previously described [Roulier et al., 1994]. Briefly, for Western blot analysis, proteins (20 µg) were denatured in Laemmli sample buffer containing 5% mercaptoethanol, boiled for 3 min, fractionated on sodium dodecyl sulfate (SDS)-10% polyacrylamide gels, and blotted onto nitrocellulose membranes. The nitrocellulose filters were blocked in phosphate-buffered saline (PBS)/1% nonfat powdered milk for 30 min at room temperature and then incubated overnight at 4°C with rabbit polyclonal antibodies at the required dilution. After extensive washing in PBS containing 1% Tween 20, the filters were incubated with peroxidase-labeled antirabbit immunoglobulins, and specific complexes were revealed by chemiluminescence according to the manufacturer's protocol (Amersham, Les Ulis, France).

Poly(A)+ RNA from pituitary adenomas was isolated with the Micro-Fastrack mRNA isolation kit (Invitrogen, San Diego, CA). RNA samples, denatured at 55°C, were then separated on 1% agarose/formaldehyde gel, transferred to NC membrane in 20× SSC, fixed by 3 min of ultraviolet (UV) radiation and hybridized by using randomly primed ^{32}P-labeled probes for detection of mRNAs specific for RXRα and glyceraldehyde phosphate dehydrogenase (GAPDH). An approximately 840 base pair (bp) *Eco*RI, *Bam*HI restriction fragment, containing the 5′ untranslated region from RXRα, was used as a template for the RXRα probe. The hybridization was carried out in 5× SSC, 2× Denhardt's solution, 0.1% SDS, 50% formamide, 100 mg/ml herring sperm DNA at 42°C. The membranes were then washed twice with 2× SSC, 0.1% SDS at room temperature and once with 0.5× SSC, 0.1% SDS at 55°C. The filters were then placed with X-ray film (Kodak X-Omat, Eastman Kodak, Rochester, NY) for autoradiography at −80°C.

Cell Cultures

After mechanical dispersion, 10^6–$3.5\ 10^6$ cells from pituitary adenoma were plated in culture wells (3.5-cm diameter) in medium 199 Hepes supplemented with fetal calf serum (FCS) (10%), horse serum (10%) and antibiotics in a humidified atmosphere of 95% air–5% CO_2. After 3 days, the medium was replaced and 1 µM RA or the vehicle alone were added and the cells were incubated for 24–72 h. At the end of the experiments, in each individual well, the medium was collected for hormone assay and the cells were harvested for GH cell content or RNA studies.

RNA was extracted from cell cultures with the RNA Quick II (Bioprobe Systems, Paris, France) and was used for dot-blot analysis with an oligonucleotide probe as previously described [Pagesy et al., 1989].

Cell extracts were performed by scraping the cells from the tissue culture wells in 1 ml of 1 M acetic acid and frozen at −20°C. After lyophilization, the cell extracts were resuspended in assay buffer containing 1 mM of 2-aminoethyl-P-benzol-sulfonylfluoride (Gerbu, Gaiberg, Germany) and sonicated.

GH Assay

GH was assayed in the cell extracts, the perifusion and culture media with a commercially available kit (ICN, Paris, France).

Analysis of the Data

Student's *t*-test was used for comparisons. The significance level chosen was $P < 0.05$.

RESULTS

Specific Detection of RARs and RXRs Nuclear Proteins by Immunoblotting in Human Pituitary Adenoma

The distribution of nuclear RA receptors was first analyzed in different anterior pituitary adenomas by immunoblotting, using rabbit polyclonal antibodies directed against either RARα [Gaub et al., 1992], RARβ [Rochette-Egly et al., 1992], RARγ [Rochette-Egly et al., 1991], or RXR (α, β) [Rochette-Egly et al., 1994]. Extracts of COS-1 cells transfected with expression vectors for the corresponding RARs or RXRs were analyzed in parallel. With the polyclonal antibody RPα(F) directed against the F region

of human RARα, a major band with an apparent molecular mass of 51 kDa was detected in all adenomatous cell extracts (Fig. 1A+, lane H). This band migrated at the same position as RARα from transfected COS-1 cells (Fig. 1A+, lane COS-1) and decreased when immunoblotting was performed with depleted antibodies (Fig. 1A−). This band was not detected in case of omission of the primary or the secondary antibody (data not shown). Similarly, with the antibody directed against RXRα [RPRXα(A)], a 54-kDa band with the same electrophoretic mobility as RXRα from transfected COS-1 cells was detected (Fig. 1D+). This band also disappeared when immunoblotting was performed with depleted antibodies (Fig. 1D−). A doublet

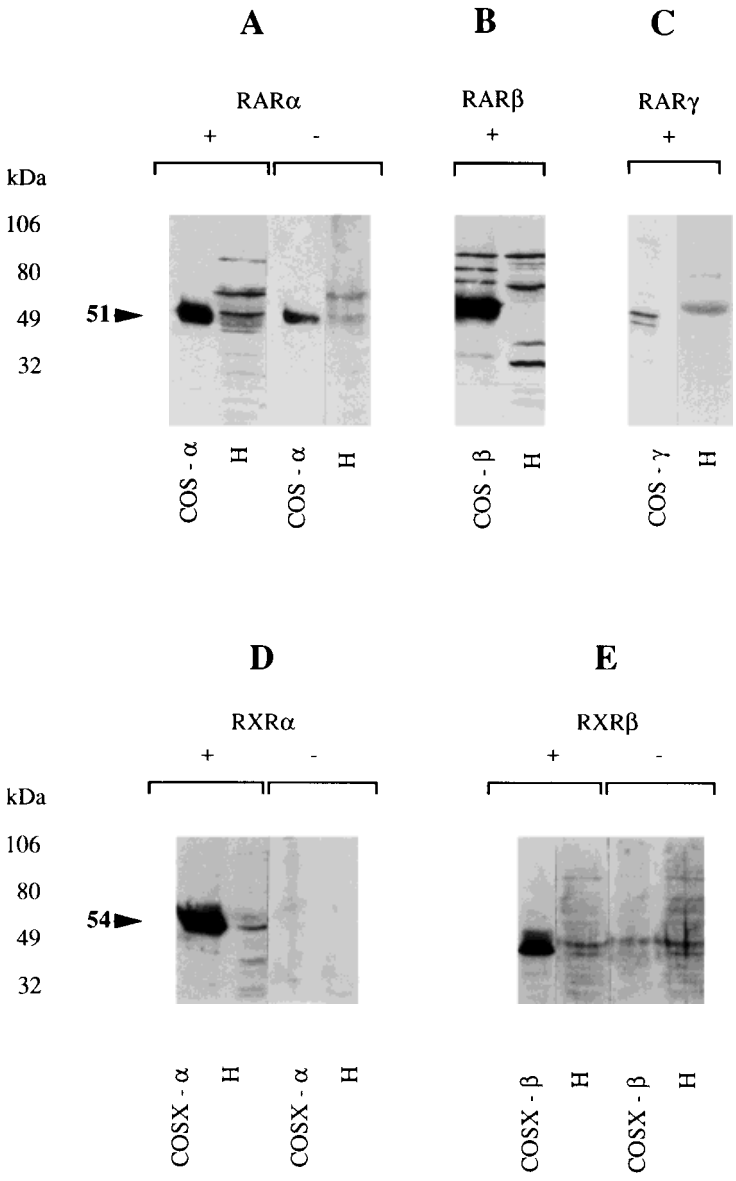

Fig. 1. Characterization of RARs and RXRs in human pituitary adenoma. Western blotting was performed on pituitary adenoma cells extracts (H) with rabbit polyclonal antibodies against RARs (*A,B,C*) and RXRs (*D,E*) without (+) or with (−) previous incubation with the specific peptide targets beforehand in order to test the specificity of the band (*A*) RPα(F) directed against RARα-F region; (*B*) RPβ(F)2 directed against RARβ-F region; (*C*) RPγ(F) directed against RARγ-F region; (*D*) RPRXα(*A*) directed against RXRα-A region; (*E*) RPRXβ(A) directed against RXRβ-A region. As positive control, cell extracts from COS-1 cells transfected with vectors expressing the corresponding RAR or RXR isoform were run in parallel (*C*). *Arrows,* position of the specific band detected in transfected COS-1 cells. *Arrowheads, thin arrows,* position of the specific bands detected in pituitary adenoma cells.

with an apparent molecular mass of 51–47 kDa was detected using the RPRXβ(A) rabbit polyclonal antibody directed against the RXRβ-A region [Rochette-Egly et al., 1991] (Fig. 1E+). However, this signal did not seem to be specific, as it did not disappear in pituitary adenoma cells when using depleted antibodies (Fig. 1E−). With the RPβ(F)2 antibody directed against the RARβ-F region (Fig. 1B) and the RPγ(F) antibody directed against the RARγ-F region (Fig. 1C), no signal could be detected in pituitary adenoma cells at the same position than the band revealed in the corresponding transfected COS-1 cells. According to these results we can conclude that RARα and RXRα, but not RARβ, RARγ, and RXRβ proteins, could be detected in human pituitary adenomas.

Expression of RXRα and RARα mRNA in Human Pituitary Adenoma Cells

Poly(A)+ RNAs were extracted, denatured, blotted, and hybridized with ^{32}P-labeled RXRα cDNA. A band (5, 6 kb) was observed in pituitary adenoma cells (Fig. 2A, lane H) identical to the one that was very largely present in human trophoblastic cells (Fig. 2A, lane P) and therefore used as a positive control [Roulier et al., 1994]. This indicated the presence of RXRα mRNA and was confirmed by slot-blot analysis in every human pituitary adenoma tested (Fig. 2B). Using the same technology, no expression of RARα mRNA could be clearly detected (data not shown).

Effects of RA on GH Synthesis and Secretion

The effect of 1 μM RA was tested for 24–72 h on GH secretion and intracellular GH content in cultured pituitary adenoma cells (Fig. 3). As shown in Figure 3A, basal secretion of GH in the culture medium was significantly increased at 72 h in the presence of RA. Similarly the intracellular content of GH (panel B) was increased by 1.8-fold at 72 h of treatment by RA ($P < 0.05$).

A significant increase in GH mRNA induced by RA was detected in these two cases. Dot-blot

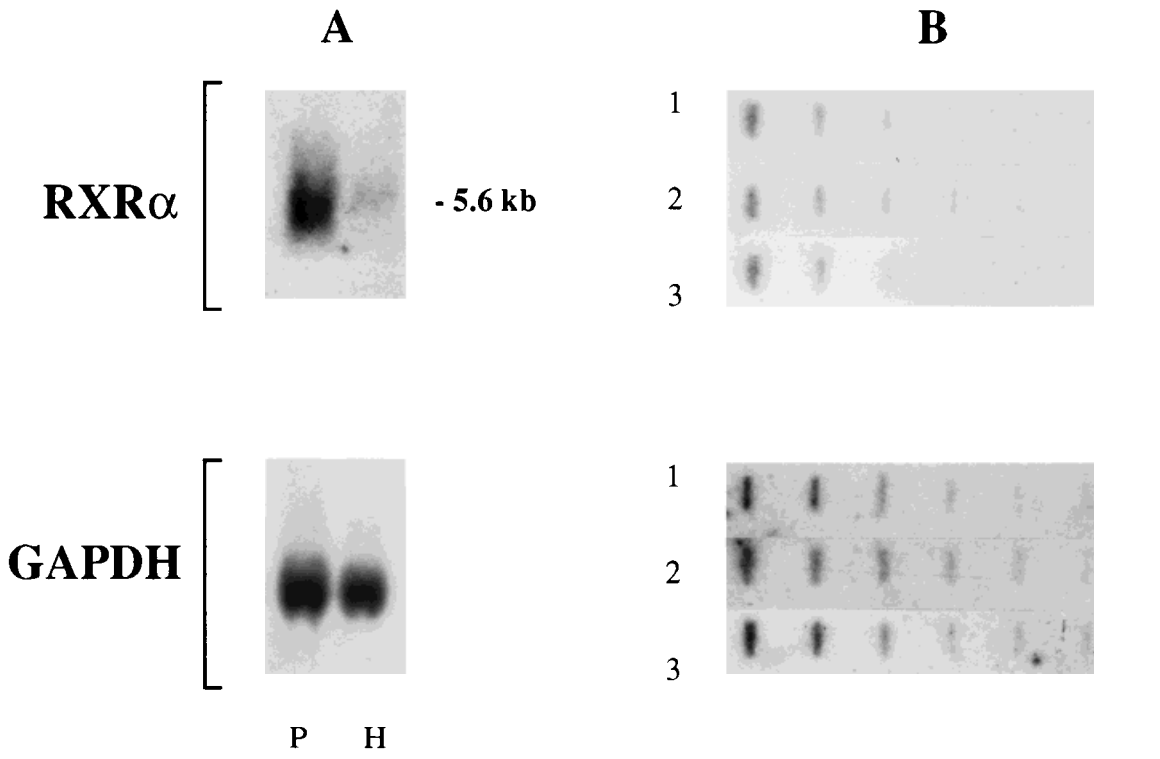

Fig. 2. Northern blot (**A**) and slot-blot analysis (**B**) of poly(A)+ RNA from human pituitary adenoma. Poly(A)+ RNA from human pituitary adenoma (H) and from trophoblastic cells (P) used as controls, was denatured, blotted, and hybridized with ^{32}P-labeled RXRα-cDNA. The size of RXRα mRNA was estimated from the migration of molecular-weight markers (0.24–9.5-kb RNA ladder from Gibco BRL, Cergy Pontoise, France) in the same run (A). RXRα mRNA was detected by slot-blot analysis in all pituitary adenomas tested (*lanes 1–3*) and GAPDH was used to quantify the amount of mRNA blotted. Trophoblastic cells were used as control because they express very high level of RXRα mRNA.

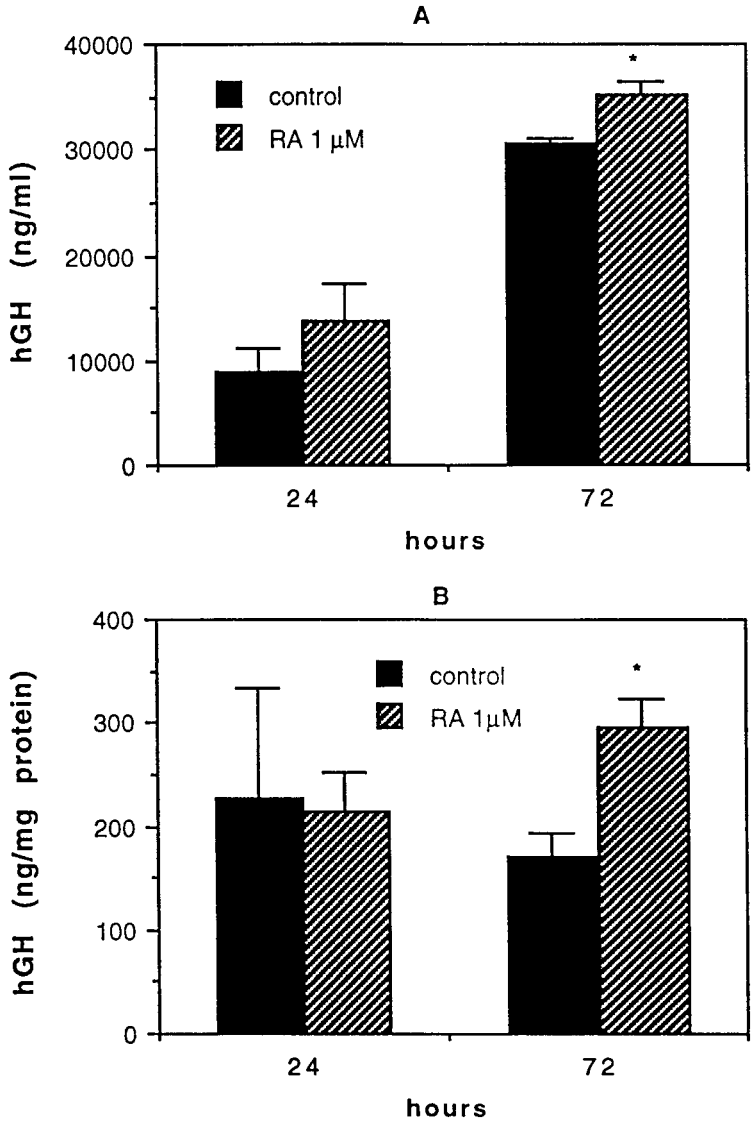

Fig. 3. Effect of retinoic acid (RA) on growth hormone (GH) secretion (**A**) and intracellular GH levels (**B**) in human pituitary adenoma cells in culture. Cells were incubated in the presence of retinoic acid (1 μM) or vehicle only. GH concentrations in the medium or in the cell extracts were measured when indicated. Each bar represents the mean ±SEM of the values obtained from three wells in a representative experiment performed three times. *$P \leq 0.05$ (vs. vehicle only). Three cultures from three different adenomas were studied.

analysis showed that this increase was significant as soon as 24 h after the start of the culture and was still observed at 72 h (Fig. 4). RA stimulated GH mRNA 2.1- and 2.8-fold at 24 and 72 h, respectively.

DISCUSSION

In this study, using immunoblotting experiments and mRNA analyses, we present evidence for the expression of RARα and RXRα proteins in human pituitary adenomas. The pattern of distribution of RARs and RXRs varies during development and differentiation and from one tissue to another, suggesting that each of them may have specific functions. They have been studied at the RNA level by Northern blot analysis and by in situ hybridization in mouse embryos [Mangelsdorf et al., 1994; Dollé et al., 1994]. These studies revealed an ubiquitous distribution of RARα and RXRβ transcripts, whereas the others were more restricted. RARα and RXRα proteins were shown to be present in human trophoblastic cells [Roulier et al., 1994, 1996; Stephanou et al., 1994]. Furthermore, we recently demonstrated that growth factors such as epidermal growth

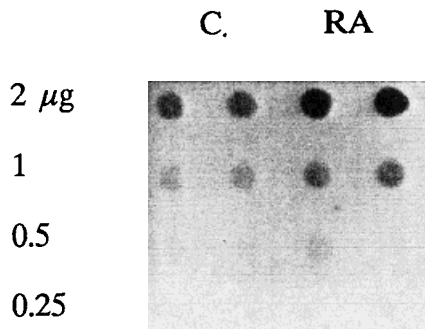

Fig. 4. Dot-blot analysis of growth hormone (GH) mRNA expression in control (C) and retinoic acid (RA)-treated cultured adenomatous cells. Cells were cultured for 72 h. Amounts on total RNA blotted are indicated on the left. The autoradiograph was exposed for 48 h.

factor increase RXRα expression in these cells [Roulier et al., 1996]. The presence of RARα protein in human somatotropic adenoma cell extracts detected by immunoblot analysis is in agreement with immunohistochemical studies showing a low expression of RARα in human pituitary adenomas [Chorny et al., 1995].

Bioactivity of retinoic acid nuclear receptors is also modulated by post-transcriptional modification, such as phosphorylation. We previously showed [Gaub et al., 1992; Rochette-Egly et al., 1991, 1992, 1994, 1996] that RARα and RXRα are nuclear proteins that can be phosphorylated. Therefore they appear very often as a smear of bands in immunoblots. The same observation was made in pituitary adenoma cell extracts (Fig. 1A,D).

We observed that the expression of RXRα was predominant at the protein as well as at the mRNA levels suggesting a role of this isoform of retinoic acid receptor in the regulation of GH secretion. Following this hypothesis, a direct role of RXRs in regulating GH secretion was described in rat pituitary cells [Sugawara et al., 1994, 1995; Davis et al., 1994].

In adenomatous somatotrophs in culture, RA induced an increase in GH mRNA and in GH cell content as well as stimulation of GH secretion. These effects likely reflect an increase in GH synthesis. Similar effects have been reported in the rat in vivo and in vitro, in normal rat pituitary cells or in tumor cell lines [Bedo et al., 1989; Morita et al., 1989, 1990; Garcia-Villalba et al., 1993]. The time course of these effects and the increase in GH gene expression are compatible with a transcriptional effect of RA through RAR and RXR nuclear receptors. However, this effect might be indirect, through a cascade of gene activation, because, in contrast to murine species, no retinoid-responsive element has been reported on the human GH gene [Seeburg, 1982].

We previously showed that RA stimulates, within minutes, cAMP release and GH secretion in human adenoma cells in perifusion [Djakouré et al., 1996]. This paper shows that RA also has a direct effect on GH synthesis in human pituitary cells in culture. These results are of interest regarding our clinical findings of a potential role of a vitamin A deficiency in some children with neurosecretory GH dysfunction and growth retardation [Evain-Brion et al., 1994].

ACKNOWLEDGMENTS

We thank the neurosurgeons of Hôpital Foch (Suresnes, France) for providing the pituitary fragments, l'Institut Electricité Santé and Diepal NSA for their financial supports. This work was supported by a grant from l'Association pour la Recherche contre le Cancer. The authors appreciate the skillful technical assistance of A.M. Brandi.

REFERENCES

Agazawa N, Taniguchi K, Mikami S (1989): Effects of vitamin A deficiency on the function of pituitary–gonadal system in male rats. Nippon Juigaku Sasshi 51:1209–1217.

Bauernfeind JC (1983): Vitamin A: Technology and application. World Rev Nutr Diet 41:110–199.

Bedo G, Santisteban P, Aranda A (1989): Retinoic acid regulates growth hormone gene expression. Nature 339: 231–234.

Breen JJ, Matsuura T, Ross AC, Gurr JA (1995): Regulation of thyroid-stimulating hormone b-subunit and growth hormone messenger ribonucleic acid levels in the rat: Effect of vitamin A status. Endocrinology 136:543–549.

Bression D, Michard M, Le Dafniet M, Pagesy P, Peillon F (1986): Evidence for a specific estradiol binding site on the rat pituitary membrane. Endocrinology 119:1048–1051.

Chambon P (1993): The molecular and genetic dissection of the retinoid signalling pathway. Gene 135:223–228.

Chertow BS, Buschmann RJ, Kaplan RL (1979): Cellular mechanisms of insulin release. Effects of retinol on insulin release and islet ultrastructure. Diabetes 28:754–761.

Chorny JA, Kruse CA, Kleinschmidt-DeMasters BK, Garza-Williams S, Conway DR, Gaub MP, Rochette-Egly C, Franklin WA (1995): Immunohistochemical study of the retinoic acid receptor a in normal versus neoplastic human tissues. Appl Immunohistochem 3:64–71.

Davis KD, Berrodin TJ, Stelmach JE, Winkler JD, Lazar MA (1994): Endogenous retinoid X receptors can function as hormone receptors in pituitary cells. Mol Cell Biol 14:7105–7110.

De Luca LM (1991): Retinoids and their receptors in differentiation, embryogenesis and neoplasia. FASEB J 5:2924–2933.

De Thé H, Dejean A (1991): The retinoic acid receptors. In Saurat JH (ed): "Retinoids: 10 years on." Basel: S. Kargers, pp 2–9.

Djakouré C, Guibourdenche J, Porquet D, Pagesy D, Peillon F, Li JY, Evain-Brion D (1996): Vitamin A and retinoic acid stimulate within minutes cAMP release and growth hormone secretion in human pituitary cells. J Clin Endocrinol Metab 81:3123–3126.

Dollé P, Fraulob V, Kastner P, Chambon P (1994): Developmental expression of murine retinoid X receptor (RXR) genes. Mech Dev 45:91–104.

Dowling JE, Wald G (1960): The biological function of vitamin A acid. Proc Natl Acad Sci USA 46:587–608.

Evain-Brion D, Porquet D, Thérond P, Paulsen A, Czernichow P (1994): Vitamin A deficiency and nocturnal GH secretion in short children. Lancet 343:87–88.

Garcia-Villalba P, Au-Fliegner M, Samuels HH, Aranda A (1993): Interaction of thyroid hormone and retinoic acid receptors in the regulation of the rat growth hormone gene promoter. Biochem Biophys Res Commun 191:580–586.

Gaub MP, Rochette-Egly C, Lutz Y, Ali S, Matthes H, Scheuer I, Chambon P (1992): Immunodetection of multiple species of retinoic acid receptor a: Evidence for phosphorylation. Exp Cell Res 201:335–346.

Gudas LJ, Sporn MB, Roberts AB (1994): Cellular biology and biochemistry of the retinoids. In Sporn MB, Roberts AB, Goodman DS (eds): "The Retinoids: Biology, Chemistry and Medicine." 2nd Ed. New York: Raven Press, pp 443–520.

Hofman C, Eichele G (1994): Renoids in development. In Sporn MB, Roberts AB, Goodman DS (eds): "The Retinoids: Biology, Chemistry and Medicine." 2nd Ed. New York: Raven Press, pp 387–441.

Horvath JE, Groot K, Schally AV (1995): Growth hormone-releasing hormone stimulates cAMP release in superfused rat pituitary cells. Proc Natl Acad Sci USA 92:1856–1860.

Kurie JM, Younes A, Miller WH Jr, Burchert M, Chiu CF, Kolesnick R, Dmitrovsky E (1993): Retinoic acid stimulates the protein kinase C pathway before activation of its b-nuclear receptor during human teratocarcinoma differentiation. Biochim Biophys Acta 1179:203–207.

Mangelsdorf DJ, Umesono K, Evans RM (1994): The retinoid receptors. In Sporn MB, Roberts AB, Goodman DS (eds): "The Retinoids: Biology, Chemistry and Medicine." 2nd Ed. New York: Raven Press, pp 319–349.

Morita S, Fernandez-Mejia C, Melmed S (1989): Retinoic acid selectively stimulates growth hormone secretion and messenger ribonucleic acid levels in rat pituitary cells. Endocrinology 124:2952–2956.

Morita S, Matsuo K, Tsuruta M, Leng S, Yamashita S, Izumi M, Nagataki S (1990): Stimulatory effect of retinoic acid on GH gene expression: the interaction of retinoic acid and tri-iodothyronine in rat pituitary cells. J Endocrinol 125:251–256.

Nemere I, Zhou LX, Norman AW (1993): Nontranscriptional effects of steroid hormones. Receptor 3:277–291.

Nemere I, Dormanon MC, Hammond MW, Okamura WH, Norman AW (1994): Identification of a specific binding protein for 1a25-dihydrovitamin D in basal-lateral membranes of chick intestinal epithelium and relationship to transcaltachia. J Biol Chem 269:23750–23796.

Pagesy P, Li JY, Rentier Delrue F, Lebouc Y, Martial JA, Peillon F (1989): Evidence of the prosomatostatin (prepro-SRIF) messenger ribonucleic acid (mRNA) in human normal and tumoral anterior pituitary gland. Mol Endocrinol 3:1289–1294.

Rochette-Egly C, Lutz Y, Saunders M, Scheuer I, Gaub MP, Chambon P (1991): Retinoic acid receptor g: Specific immunodetection and phosphorylation. J Cell Biol 115: 535–545.

Rochette-Egly C, Gaub M, Lutz Y, Ali S, Scheuer I, Chambon P (1992): Retinoic acid receptor-b: Immunodetection and phosphorylation on tyrosine residues. Mol Endocrinol 6:2197–2209.

Rochette-Egly C, Lutz Y, Pfister V, Heiberger S, Scheuer I, Chambon P, Gaub MP (1994): Detection of retinoid X receptors using specific monoclonal and polyclonal antibodies. Biochem Biophys Res Commun 204:525–536.

Rochette-Egly C, Oulad-Abdelghami M, Staud B, Pfister V, Scheuer I, Chambon P, Gaub MP (1995): Phosphorylation of the retinoic acid receptor α by protein kinase A. Mol Endocrinol 9:860–871.

Roulier S, Rochette-Egly C, Rebut-Bonneton C, Porquet D, Evain-Brion D (1994): Nuclear retinoic acid receptor characterization in cultured human trophoblast cells: Effect of retinoic acid on epidermal growth factor receptor expression. Mol Cell Endocrinol 105:165–173.

Roulier S, Rochette-Egly C, Alsat E, Dufour S, Porquet D, Evain-Brion D (1996): EGF increases retinoid X receptor-a expression in human trophoblastic cells in culture: Relationship with retinoic acid induced human chorionic gonadotropin secretion. Mol Cell Endocrinol 118:125–135.

Seeburg RH (1982): The human growth hormone gene family: nucleotide sequence show recent divergence and predict a new polypeptide hormone. DNA 1:238–249.

Stephanou A, Sarlis NJ, Richards R, Handwerger S (1994): Expression of retinoid acid receptor subtypes and cellular retinoic acid biding protein-II mRNAs during differentiation of human trophoblast cells. Biochem Biophys Res Commun 202:772–780.

Sugawara A, Yen PM, Chin WW (1994): 9-cis retinoic acid regulation of rat growth hormone gene expression: Potential roles of multiple nuclear hormone receptors. Endocrinology 136:1956–1962.

Sugawara A, Yen PM, Qi Y, Lechan RM, Chin WW (1995): Isofom-specific retinoid-X receptor (RXR) antibodies detect differential expression of RXR proteins in the pituitary gland. Endocrinology 136:1766–1774.

Umesono K, Giguere V, Glass CK, Rosenfeld MG, Evans RM (1988): Retinoic acid and thyroid hormone induce gene expression through a common responsive element. Nature 336:262–265.