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Interferon-α-2a Is a Potent Inhibitor of Hormone Secretion by Cultured Human Pituitary Adenomas

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ABSTRACT

Interferon-α (IFNα) may exert direct inhibitory effects on cell proliferation and on the production of different peptide hormones. We investigated the effect of IFNα on hormone production by 15 GH-secreting pituitary adenomas, 4 clinically nonfunctioning or gonadotroph pituitary adenomas, and 4 prolactinomas *in vitro*. In the GH-secreting pituitary adenoma cultures, a short term (72-h) incubation with IFNα (50–100 U/mL) significantly inhibited GH secretion in 3 of 7 cases and PRL secretion in 6 of 7 cultures. During prolonged incubation (14 days) with IFNα, GH and/or PRL secretion was significantly inhibited in 7 of 8 cultures (GH, 17–78% inhibition; PRL, 39–88% inhibition). In the clinically nonfunctioning or gonadotroph cultures, incubation with IFNα resulted in inhibition of the secretion of gonadotropins and/or α-subunit in all cases (27–62%), whereas in the prolactinoma cultures PRL secretion was inhibited by IFNα in all cases (37–76%). The effect of IFNα was additive to the inhibitory effects of the dopamine agonist bromocriptine (10 nmol/L) or the somatostatin analog octreotide (10 nmol/L). The inhibition of hormone secretion by IFNα was accompanied by inhibition of the intracellular hormone concentrations. The effect of IFNα was dose dependent, with an IC_{50} for inhibition of hormone secretion of 2.3 ± 0.3 U/mL (n = 5), which is relatively low compared with the concentrations that are reached in patients treated with IFNα for various malignancies. In conclusion, the potent antihormonal effect of IFNα on cultured pituitary adenomas suggests that this drug might be of benefit in the treatment of selected patients with secreting pituitary adenomas. As treatment with IFNα is associated with considerable adverse reactions, studies with this drug should only be considered in inoperable, invasive aggressive, and dopamine agonist- and/or somatostatin analog-resistant functioning pituitary macroadenomas. (*J Clin Endocrinol Metab* **84:** 3336–3343, 1999)

INTERFERONS (IFNs) are glycoproteins produced in response to viral and certain nonviral stimuli. Apart from having antiviral activity, IFNs are potent inhibitors of cell proliferation as well. Because of these properties IFNs have been approved for clinical use to treat certain viral diseases, autoimmune diseases, and malignancies (1). During the past 10 yr evidence has emerged that IFNs may play a regulatory role in pituitary hormone secretion as well. Both stimulatory and inhibitory effects of IFNs (IFNα and IFNγ) on the secretion of ACTH, PRL, and GH have been reported (2–6). Apart from cells of the immune system, pituitary endocrine cells may also contain IFNα (7), providing further evidence for a local regulatory role for this group of proteins at the pituitary level. Moreover, Katahira *et al.* (8) recently showed that prolonged incubation with IFNα or IFNγ induces a potent inhibitory effect on POMC gene expression in mouse AtT-20 pituitary tumor cells.

Until now, IFNα has been used with variable success in the treatment of hormone-secreting gastroenteropancreatic tumors (mainly carcinoids). Treatment with IFNα may control tumor growth, but also reduces the secretion of tumor-related products in patients harboring these tumors, thereby improving clinical symptomatology (9–11). The decrease in the production of different peptide hormones is caused by its blocking effect on intracellular messenger ribonucleic acid formation (12) and appears to be signalled via the JAK (Janus kinase)-STAT (signal transducer and activator of transcription) pathway (13).

Medical therapy of human pituitary adenomas comprises the use of dopamine (DA) agonists and somatostatin (SS) analogs. In patients with prolactinomas, treatment with DA agonists effectively reduces circulating PRL levels and tumor size in the majority of them, probably due to an inhibitory effect on PRL synthesis. However, a small subgroup of patients with prolactinomas is either resistant to DA agonist therapy or cannot be treated due to intolerance to therapy with DA agonists (14). In patients with acromegaly, good inhibition of circulating GH levels has been achieved using SS analogs such as octreotide, whereas tumor shrinkage is observed less frequently (15). This is probably due to the absence of an inhibitory effect of SS analogs on GH synthesis (16, 17). Finally, the effectiveness of the use of DA agonists and/or SS analogs in the medical treatment of clinically nonfunctioning pituitary adenomas (NFA) is still under discussion (18).

The presence of IFNα in pituitary endocrine cells, its modulating effect on pituitary hormone secretion (and/or production), and the observations that IFNs act at the transcriptional level suggest that IFNs may also influence hormone secretion by pituitary adenomas. To further explore the possibilities of medical therapy of pituitary adenomas, we investigated in the present study the effect of IFNα-2a on hormone secretion by different types of cultured human pituitary adenoma cells. In addition, the interrelationship

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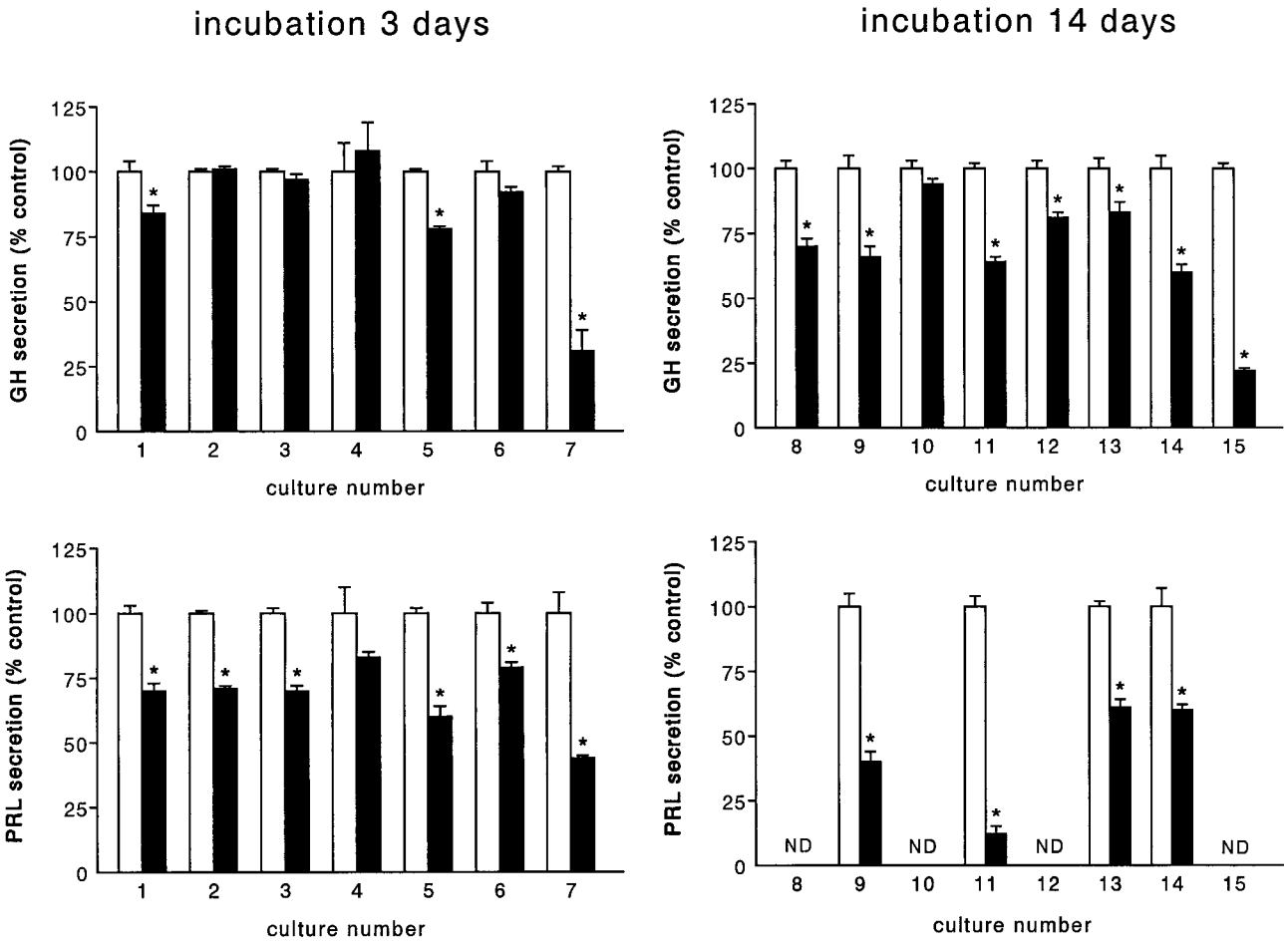

FIG. 1. The effect of IFNα (50–100 U/mL) on GH and/or PRL secretion by 15 cultured human GH-secreting pituitary adenomas. Incubation time was 3 days in multiwell plates (*left panel*) or 14 days in Transwell tissue culture inserts (*right panel*). Values are expressed as the percentage of control hormone release and represent the mean ± SE. *, $P < 0.05$ *vs.* control. ND, Not detectable. □, Control; ■, IFNα.

between the effects of IFNα and octreotide and/or bromocriptine was studied. Moreover, a comparison was made with the effects of IFNα on hormone secretion by primary cultures of three insulinomas and one gastrinoma.

Subjects and Methods

Patients

Pituitary tumor samples were obtained by transsphenoidal operation from 15 patients with GH-secreting pituitary adenomas, from 4 patients with clinically nonfunctioning (n = 2) or gonadotroph (n = 2) adenomas, and from 4 patients with prolactinomas, as described previously (19). Tumor samples of 3 insulinomas and 1 gastrinoma were obtained within 30 min after surgical removal of the tumors. Diagnosis was made on the basis of clinical and biochemical characteristics of the patients in combination with (immuno)histochemistry of the tumor samples. The two patients with gonadotroph adenomas had elevated preoperative levels of FSH. All patients gave their informed consent for the use of tumor material for research purposes.

Cell dispersion and cell culture

Single cell suspensions of the pituitary adenoma tissues were prepared by enzymatic dissociation with dispase as described in detail previously (19). Tumor tissue from the insulinomas and the gastrinoma was dissociated as described (20). For short term incubation of monolayer cultures, the dissociated cells were plated in 48-well plates (Costar, Cambridge, MA) at a density of 10^5 (GH-secreting pituitary adenomas, prolactinomas, insulinomas, and gastrinoma) or 2×10^5 (NFAs) cells/well·1 mL culture medium. After 3–4 days the medium was changed, and 72-h incubations without or with test substances were initiated. At the end of the incubation the medium was removed and centrifuged for 5 min at $600 \times g$. The supernatant was collected and stored at −20 C until analysis. For long term incubation studies in Transwells (21), the isolated tumor cells were plated in Transwell-COL membranes (Costar, Badhoevedorp, The Netherlands) at a density of 10^5 (GH-secreting pituitary adenomas, prolactinomas, insulinomas, and gastrinoma) or 2×10^5 (NFAs) cells/well. The Transwells were then placed into multiwell plates (24-well; Costar) containing 1 mL culture medium. After 72 h the Transwells were transferred to wells containing fresh medium (without or with test substances). Every 3–4 days the cells were placed into fresh medium, and the incubation media were collected and stored at −20 C until determination of hormone concentrations. In some experiments, intracellular hormone concentrations were determined in cell lysates obtained by lysis of the cells in 150 μL ammonia solution (0.2%, vol/vol), followed by the addition of 1 mL assay buffer (100 mmol/L NaCl, 10 mmol/L ethylenediamine tetraacetate, and 10 mmol/L Tris-HCl, pH 7.0), as described previously (22). In the NFA cultures cell lysates were obtained by lysis of the cells in distilled water containing 1 g/L BSA followed by repeated freezing and thawing, as described previously (21).

The cells were cultured at 37 C in a CO_2 incubator. The culture medium consisted of MEM D-valine with Earle's salts supplemented with non essential amino acids, sodium pyruvate (1 mmol/L), 10% FCS, penicillin (1×10^5 U/L), fungizone (0.5 mg/L), L-glutamine (2 mmol/L), and sodium bicarbonate (2.2 g/L), pH 7.6. Unfortunately, generally not

enough tumor material was obtained to test each tumor for its responsiveness to all of the above indicated drugs.

Hormone determinations

Human GH, PRL, LH, and FSH concentrations in the media and cell extracts were determined by immunoradiometric assays (MedGenix Diagnostics, Fleurus, Belgium) as described previously (19, 21). Glycoprotein α-subunit concentrations were determined using an immunoradiometric assay from Immunotech S.A. (Marseille, France). Insulin and gastrin concentrations were determined by double antibody RIAs as previously described (20). Dilution of GH, PRL, LH, FSH, α-subunit, insulin, and gastrin in the media was parallel to that of the respective standards supplied with kits.

Testsubstances

Octreotide (Sandostatin) and bromocriptine were obtained from Novartis Pharma A.G. (Basel, Switzerland). IFNα-2a (Roferon-A) was obtained from Hoffmann-La Roche B.V. (Mijdrecht, The Netherlands).

Measurement of DNA content

The DNA content in the cell lysates (see above) was measured using the bisbenzimide fluorescent dye (Behring, La Jolla, CA) as described in detail previously (22).

Statistical analysis of the data

All data for hormone release are expressed as the mean ± SE (n = 4 wells/treatment group). All data were analyzed by ANOVA to determine overall differences between treatment groups. When significant differences were found by ANOVA, a comparison between treatment groups was made using the Newman-Keuls test.

Results

Pituitary adenomas

First, tumor cell preparations from 15 acromegalics were studied. IFNα (50–100 U/mL) significantly inhibits GH secretion in 3 of 7 cultures (16–69% inhibition) and PRL secretion in 6 of 7 cultures (21–56% inhibition) during a 72-h incubation (Fig. 1, *left panel*). A significantly higher number of cultures responded to IFNα after prolonged exposure. After 14 days of treatment, GH secretion was significantly inhibited in 7 of 8 other tumor cell cultures (17–78% inhibition), and PRL secretion was significantly inhibited in 4 of 4 cultures (39–88% inhibition), as shown in Fig. 1, *right panel*.

In all prolactinoma (n = 4; Fig. 2) and NFA or gonadotroph cultures (n = 4; Fig. 3) IFNα significantly inhibited hormone secretion after 14 days of treatment. The percent inhibition varied between 37–76% in prolactinoma cultures and between 27–62% in NFA and gonadotroph cultures after 14 days of treatment.

In the majority of the cultures the effects of IFNα and octreotide or bromocriptine on hormone secretion were additive. Figure 4 (GH-secreting pituitary adenoma cells) and Fig. 5 (NFA cells) show the increasing, time-dependent, additive effect of these drugs. Tables 1-3 show that significant additive effects on hormone secretion were found in three of five GH-secreting pituitary adenoma cultures, in two of two prolactinoma cultures, and in one NFA culture in which the combination of these drugs was studied. IFNα also significantly inhibited intracellular GH concentrations in two of four GH-secreting adenoma cultures (Table 1), whereas at the same time treatment with octreotide or bromocriptine induced a statistically significant accumulation of intracellular GH concentrations (Table 1), suggesting a different mechanism of action between IFNα, on the one hand, and octreotide and bromocriptine, on the other hand. Both bromocriptine and IFNα significantly inhibited intracellular PRL concentrations in the GH-secreting pituitary adenoma cultures (n = 3; Table 1) and in the prolactinoma cultures (n = 3; Table 2) and intracellular α-subunit and/or FSH concentrations in the NFA cultures (n = 2; Table 3).

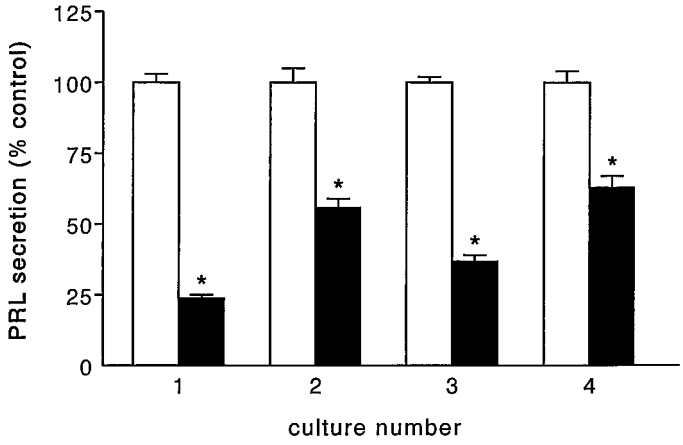

FIG. 2. The effect of IFNα (50 U/mL) on PRL secretion by four cultured human prolactinomas. Incubation time was 14 days in Transwell tissue culture inserts. Values are expressed as the percentage of control hormone release and represent the mean ± SE. *, $P < 0.05$ *vs.* control. □, Control; ■, IFNα.

We also studied the effect of drug withdrawal on day 14 of treatment. In the majority of the cases we found that withdrawal from IFNα treatment resulted in a recovery of hormone secretion, suggesting that the inhibitory effect of IFNα represents inhibition of hormone secretion and/or production and not inhibition of cell proliferation or any cytotoxic effect. Representative examples of the effect of drug withdrawal are shown in Fig. 4 (GH-secreting pituitary adenoma), Fig. 5 (NFA), and Fig. 6 (prolactinoma). The absence of any cytotoxic effect is further illustrated by the absence of an effect of IFNα on the DNA content of the pituitary adenoma cells, which is shown in Fig. 7 for a GH-secreting pituitary adenoma culture. No statistically significant inhibition of IFNα on the DNA content of the cells was demonstrated in six additional pituitary adenoma cultures (data not shown).

Finally, the effect of IFNα on hormone secretion was dose dependent, with IC_{50} values between 1.7–3.3 U/mL (mean, 2.3 ± 0.3; Table 4). Data are shown in Fig. 7 (GH-secreting pituitary adenoma), Table 2 (prolactinoma), and Table 3 (gonadotroph adenomas).

Gastroenteropancreatic tumors

For comparison we also evaluated the effect of IFNα on insulin secretion by three insulinomas and on gastrin secretion by a gastrinoma. In two of three insulinomas insulin secretion was inhibited by 18% and 37% only at a high dose of IFNα (50 U/mL), whereas in the gastrinoma a more significant reduction of gastrin secretion was found (45% inhibition by 50 U IFNα U/mL), as shown in Table 5.

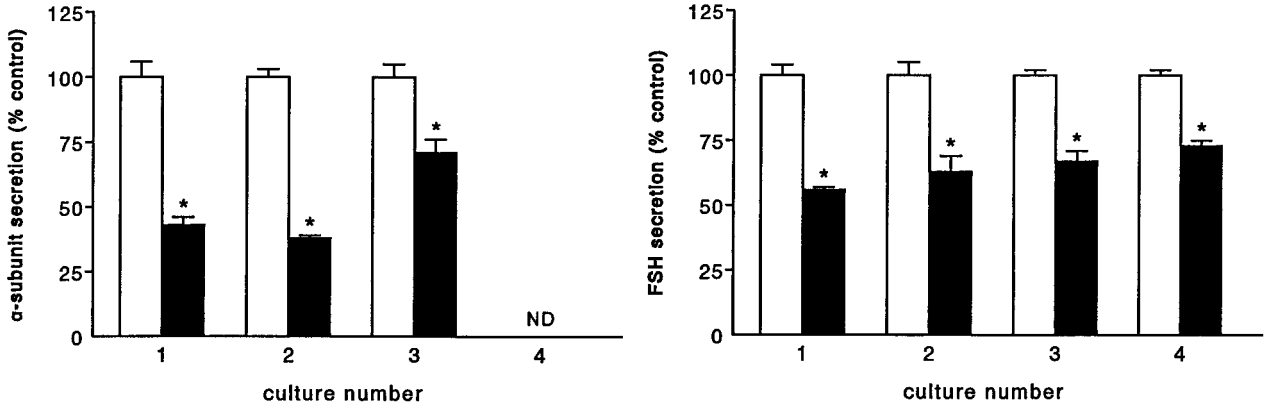

FIG. 3. The effect of IFNα (50–100 U/mL) on α-subunit and/or FSH secretion by cells from four cultured human NFA or gonadotroph adenomas. Incubation time was 14 days in Transwell tissue culture inserts. Values are expressed as the percentage of control hormone release and represent the mean ± SE. *, $P < 0.05$ *vs.* control. ND, Not detectable. □, Control; ■, IFNα.

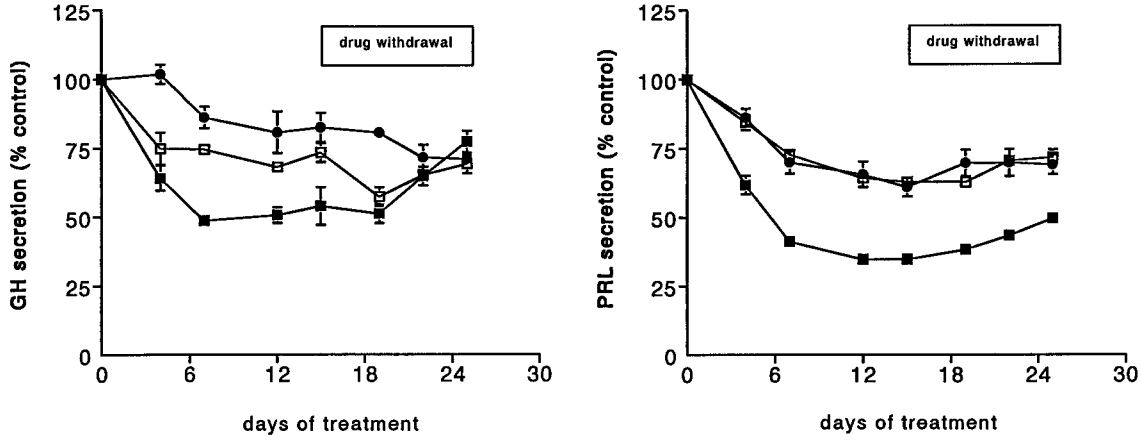

FIG. 4. Additive inhibitory effect of octreotide (10 nmol/L) and IFNα (50 U/mL) on GH (*left panel*) and PRL (*right panel*) secretion by cultured human GH-secreting pituitary adenoma cells (no. 12). The cells were incubated for 25 days without or with drugs in Transwell tissue culture inserts. Values are the mean ± SE and are expressed as the percentage of control hormone release at each time point. □, Octreotide (10 nmol/L); ●, IFNα (50 U/mL); ■, octreotide plus IFNα.

Discussion

In the majority of patients with GH-secreting pituitary adenomas medical treatment with SS analogs and/or DA agonists is, after transsphenoidal surgery, the therapy of second choice (15). First choice medical therapy of this type of pituitary adenomas is not frequently applied because SS analogs and/or DA agonists induce only slight tumor shrinkage in approximately 50% of the patients treated. This is probably related to the fact that these drugs do not inhibit hormonal synthesis in this type of pituitary adenomas (16, 17). In contrast, medical therapy with DA agonists is the first choice therapy in patients with prolactinomas (23). The majority of patients with prolactinomas can be effectively treated with DA agonists, resulting in lowered circulating PRL levels and a reduction of tumor volume due to a potent inhibition of hormonal synthesis by the drug (23). On the other hand, some patients with prolactinomas cannot be treated due to either resistance or adverse reactions to DA agonists (14). Finally, at present no effective medical therapy of patients with NFA is available (18). Taken together, these data indicate that novel drugs that inhibit both hormonal

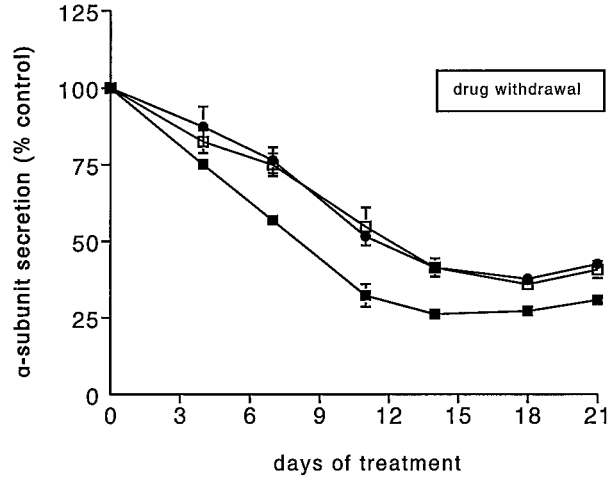

FIG. 5. Additive inhibitory effect of bromocriptine (10 nmol/L) and IFNα (50 U/mL) on α-subunit secretion by cultured human NFA cells (no. 2). The cells were incubated for 21 days without or with drugs in Transwell tissue culture inserts. Values are the mean ± SE and are expressed as the percentage of control hormone release at each time point. □, Bromocriptine (10 nmol/L); ●, IFNα (50 U/mL); ■, bromocriptine plus IFNα.

TABLE 1. The effect of long term treatment with IFNα and/or octreotide or bromocriptine on GH and PRL secretion and intracellular concentrations by cultured human GH-secreting pituitary adenoma cells

Culture no.	Treatment	GH secretion (ng/well ± SE)	Intracellular GH (ng/well ± SE)	PRL secretion (ng/well ± SE)	Intracellular PRL (ng/well ± SE)
8	Control	465 ± 14	98 ± 5		
	Octreotide (10 nmol/L)	283 ± 8[a]	292 ± 11[a]	Not detectable	Not detectable
	Bromo (10 nmol/L)	429 ± 24	184 ± 8[a]		
	IFNα (50 U/mL)	323 ± 17[a]	122 ± 2[a]		
9	Control	385 ± 19	38 ± 2	1337 ± 63	267 ± 36
	Octreotide (10 nmol/L)	63 ± 3[a]	424 ± 21[a]	118 ± 12[a]	90 ± 15[a]
	Bromo (10 nmol/L)	158 ± 7[a]	317 ± 8[a]	74 ± 3[a]	99 ± 17[a]
	IFNα (100 U/mL)	253 ± 15[a]	57 ± 4[a]	536 ± 54[a]	184 ± 6[a]
10	Control	62 ± 2			
	Octreotide (10 nmol/L)	51 ± 2[a]	Not determined	Not detectable	Not detectable
	IFNα (50 U/mL)	58 ± 1			
	Octreotide + IFNα	51 ± 2[a]			
11	Control	107 ± 2		173 ± 7	
	Octreotide (10 nmol/L)	73 ± 1[a]		164 ± 7	
	Bromo (10 nmol/L)	98 ± 1[a]	Not determined	13 ± 2[a]	Not determined
	IFNα (100 U/mL)	68 ± 2[a]		21 ± 5[a]	
	Octreotide + IFNα	58 ± 1[a,b]		29 ± 5[a,b]	
	Bromo + IFNα	54 ± 1[a,b]		9 ± 1[a]	
12	Control	602 ± 22			
	Octreotide (10 nmol/L)	355 ± 19[a]			
	Bromo (10 nmol/L)	572 ± 26	Not determined	Not detectable	Not detectable
	IFNα (50 U/mL)	500 ± 24[a]			
	Octreotide + IFNα	228 ± 13[a,b]			
	Bromo + IFNα	409 ± 14[a,b]			
13	Control	803 ± 61	32 ± 4	7187 ± 229	1100 ± 226
	Bromo (10 nmol/L)	370 ± 32[a]	58 ± 3[a]	678 ± 84[a]	575 ± 51
	IFNα (50 U/mL)	648 ± 63[a]	19 ± 3[a]	4387 ± 282[a]	310 ± 61[a]
	Bromo + IFNα	337 ± 32[a]	44 ± 10	495 ± 68[a,b]	468 ± 117
14	Control	338 ± 19	270 ± 13	862 ± 63	534 ± 19
	Octreotide (10 nmol/L)	194 ± 22[a]	811 ± 42[a]	308 ± 5[a]	329 ± 17[a]
	IFNα (50 U/mL)	202 ± 12[a]	172 ± 10[a]	519 ± 21[a]	331 ± 21[a]
	Octreotide + IFNα	85 ± 4[a,b]	365 ± 11[a,b]	163 ± 5[a,b]	175 ± 11[a,b]

Cells (10^5/well) were incubated for 14 days in Transwells without or with the drugs indicated. The medium was changed every 3–4 days. Values represent hormone concentrations in the medium from days 12–14 of culture (3 days).

[a] $P < 0.05$ vs control.

[b] $P < 0.05$ *vs.* octreotide or bromocriptine (bromo) alone.

hypersecretion and/or synthesis by pituitary adenomas might be beneficial along with current medical therapy.

During the past 10 yr evidence has emerged that IFNs may also play a regulatory role in pituitary hormone secretion (2–8). IFNα-2a is a drug that has been used with variable success in the treatment of several malignancies (1). In patients with neuroendocrine tumors such as carcinoids, treatment with IFNα may control tumor growth, but also reduces the secretion of tumor-related products in patient harboring these tumors, thereby improving clinical symptomatology (9–11). The decrease in the production of different peptide hormones is caused by its blocking effect on intracellular messenger ribonucleic acid formation (12).

In the present study we found that IFNα significantly inhibits both hormone secretion and intracellular hormone concentrations in primary cultures of human GH-secreting pituitary adenomas, prolactinomas, and NFA or gonadotroph adenomas. In the GH-secreting pituitary adenoma cultures in which octreotide and/or bromocriptine significantly inhibited hormone secretion, intracellular GH concentrations were concomitantly increased, as has been reported previously (16). These data suggest that IFNα, on the one hand, and octreotide and bromocriptine, on the other hand, act via different mechanisms of action. Although octreotide and bromocriptine act to inhibit hormone secretion via inhibition of adenylyl cyclase activity and/or inhibition of calcium fluxes (24), IFNα may inhibit hormone synthesis via inhibition of transcription of the GH gene, as has been proposed for the effect of the drug on peptide production in neuroendocrine tumors (12). As we found that hormone secretion recovered after withdrawal of treatment with IFNα *in vitro*, and because the drug did not affect the DNA content of the adenoma cell cultures, it is unlikely that IFNα inhibited cell proliferation or induced cytotoxic effects. Moreover, we found that even very low concentrations significantly inhibited both hormone secretion and intracellular hormone concentrations in a dose-dependent manner.

In primary cultures of human prolactinomas, we found that both bromocriptine and IFNα significantly inhibited hormone secretion and intracellular hormone concentrations. The observation that bromocriptine increased intracellular GH concentrations in human GH-secreting pituitary adenoma cultures, whereas the same drug significantly inhibited intracellular PRL concentrations in prolactinoma cul-

tures suggests a different responsiveness of hormonal synthesis to DA agonists between these two types of pituitary adenomas. Of interest in this respect is that after long term treatment *in vitro* both bromocriptine and IFNα significantly inhibited intracellular PRL concentrations in the GH-secreting pituitary adenomas despite a concomitant rise in intracellular GH levels induced by bromocriptine. The inhibitory effect of IFNα on intracellular PRL concentrations in prolactinomas suggests that this drug might also induce tumor shrinkage in patients with prolactinomas. Again of importance is our observation that very low concentrations induced significant inhibition of intracellular PRL concentrations. However, the four prolactinoma cultures that were studied showed a high sensitivity to bromocriptine *in vitro*. It remains to be established, therefore, whether DA agonist-resistant prolactinomas respond in a comparable manner to IFNα. Of special interest is a case report of a 45-yr old woman suffering from antihepatitis C virus-positive chronic active hepatitis and amenorrhea-galactorrhea syndrome due to a PRL-secreting microadenoma, in whom IFNα treatment resulted in normalization of plasma PRL levels and disappearance of related symptoms (25).

TABLE 2. The effect of long term treatment with IFNα and/or bromocriptine on PRL secretion and intracellular concentrations of cultured human prolactinoma cells

Culture no.	Treatment	PRL secretion (ng/well ± SE)	Intracellular PRL (ng/well ± SE)
1	Control	3199 ± 34	102 ± 13
	Bromo (10 nmol/L)	115 ± 15[a]	13 ± 0[a]
	IFNα (50 U/mL)	495 ± 32[a]	14 ± 0[a]
	Bromo + IFNα	63 ± 6[a,b]	6 ± 0[a,b]
2	Control	5143 ± 290	
	Bromo (10 nmol/L)	815 ± 44[a]	Not determined
	IFNα (50 U/mL)	2897 ± 151[a]	
	Bromo + IFNα	530 ± 50[a,b]	
3	Control	4730 ± 135	109 ± 9
	IFNα (100 U/mL)	1190 ± 32[a]	65 ± 11[a]
	IFNα (50 U/mL)	1757 ± 103[a]	64 ± 2[a]
	IFNα (10 U/mL)	1873 ± 123[a]	78 ± 4[a]
	IFNα (5 U/mL)	2557 ± 67[a]	73 ± 5[a]
	IFNα (1 U/mL)	4400 ± 48	101 ± 5
4	Control	1940 ± 75	379 ± 14
	Bromo (10 nmol/L)	375 ± 26[a]	108 ± 7[a]
	IFNα (50 U/mL)	1218 ± 70[a]	283 ± 31[a]

Cells (10^5/well) were incubated for 14 days in Transwells without or with the drugs indicated. The medium was changed every 3–4 days. Values represent hormone concentrations in the medium from days 12–14 of culture (3 days).

[a] $P < 0.05$ *vs.* control.

[b] $P < 0.05$ *vs.* bromocriptine (bromo) alone.

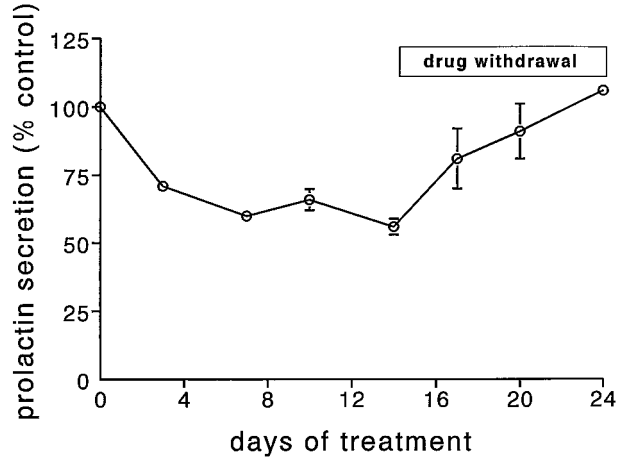

FIG. 6. Recovery of PRL secretion by cultured human prolactinoma cells (no. 2) after withdrawal of IFNα treatment (50 U/mL) on day 14 of treatment. Values are the mean ± SE and are expressed as the percentage of control hormone release at each time point.

TABLE 3. The effect of long term treatment with IFNα and/or bromocriptine on α-subunit and FSH secretion and intracellular concentrations of cultured human clinically nonfunctioning (no. 1 and 2) or gonadotroph (no. 3 and 4) adenoma cells

Culture no.	Treatment	α-Subunit secretion (mU/well ± SE)	Intracellular α-subunit (mU/well ± SE)	FSH secretion (mU/well ± SE)	Intracellular FSH (mU/well ± SE)
1	Control	1.0 ± 0.1	1.8 ± 0.1	16.0 ± 0.6	24.4 ± 1.4
	Bromo (10 nmol/L)	0.3 ± 0.1[a]	1.0 ± 0.1[a]	4.0 ± 0.4[a]	11.5 ± 0.6[a]
	IFNα (100 U/mL)	0.4 ± 0.0[a]	0.7 ± 0.1[a]	8.6 ± 0.2[a]	7.4 ± 0.5[a]
2	Control	2.3 ± 0.1	0.7 ± 0.0	1.0 ± 0.1	
	Bromo (10 nmol/L)	0.8 ± 0.1[a]	0.3 ± 0.1[a]	0.4 ± 0.0[a]	Not detectable
	IFNα (100 U/mL)	0.9 ± 0.1[a]	0.4 ± 0.0[a]	0.6 ± 0.1[a]	
	Bromo + IFNα	0.6 ± 0.0[a,b]	0.3 ± 0.0[a]	0.4 ± 0.1[a]	
3	Control	0.6 ± 0.1		19.3 ± 0.6	
	IFNα (50 U/mL)	0.4 ± 0.0[a]		12.9 ± 0.7[a]	
	IFNα (10 U/mL)	0.3 ± 0.1[a]	Not determined	12.7 ± 0.7[a]	Not determined
	IFNα (5 U/mL)	0.4 ± 0.0[a]		15.0 ± 0.8[a]	
	IFNα (1 U/mL)	0.6 ± 0.1		16.7 ± 0.9[a]	
4	Control			3.6 ± 0.1	
	Bromo (10 nmol/L)			2.3 ± 0.1[a]	
	IFNα (50 U/mL)	Not detectable	Not determined	2.6 ± 0.1[a]	Not determined
	IFNα (10 U/mL)			2.8 ± 0.1[a]	
	IFNα (1 U/mL)			3.2 ± 0.1[a]	
	IFNα (0.1 U/mL)			3.4 ± 0.1	

Cells (10^5/well) were incubated for 14 days in Transwells without or with the drugs indicated. The medium was changed every 3–4 days. Values represent hormone concentrations in the medium from days 12–14 of culture (3 days). LH was not detectable in the culture medium or cell extracts.

[a] $P < 0.05$ *vs.* control.

[b] $P < 0.05$ *vs.* bromocriptine (bromo) alone.

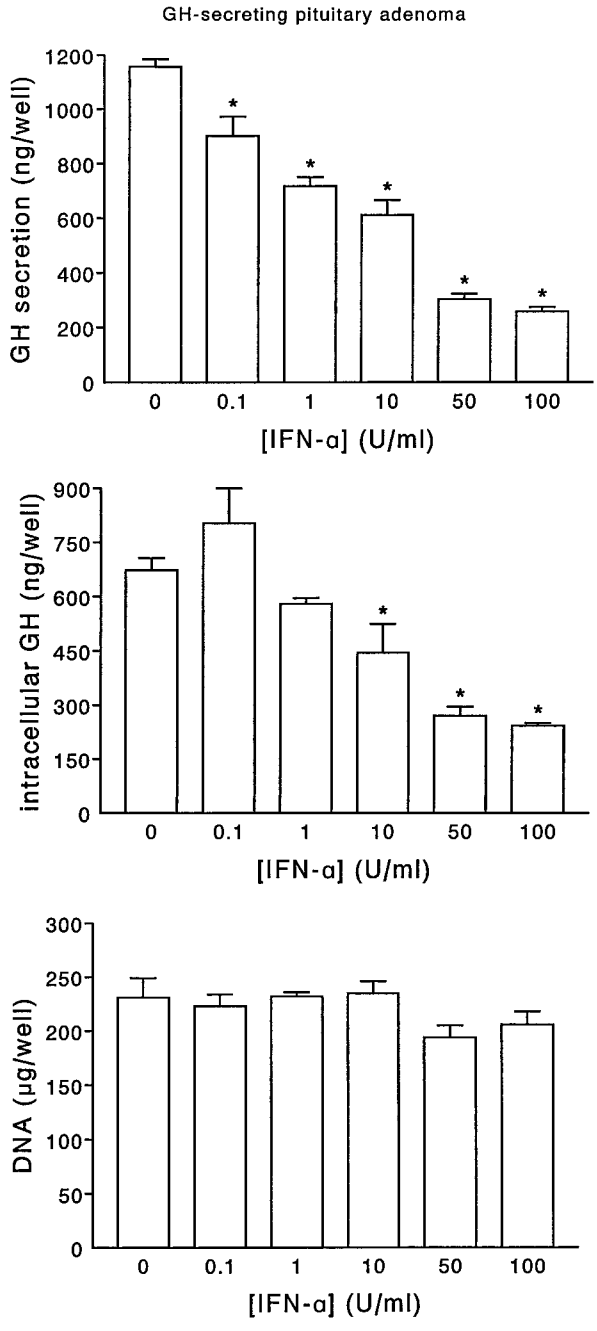

FIG. 7. Dose-dependent inhibition of GH secretion (*upper panel*) and intracellular GH concentrations (*middle panel*) of cultured GH-secreting pituitary adenoma cells (no. 15) by IFNα. Cell number, as measured by the DNA content of the cells, was not inhibited (*lower panel*). Values are the mean ± SE. *, $P < 0.05$ *vs.* control.

Finally, in line with the observations in prolactinoma cultures, we found that both bromocriptine and IFNα significantly inhibited both the secretion and intracellular hormone concentrations of α-subunit and/or FSH in the NFA cultures. The inhibitory effect of prolonged treatment with bromocriptine on hormone production is in agreement with our previous observations (21). However, treatment with DA agonists infrequently induces (minor) tumor shrinkage in patients with NFA despite an inhibitory effect on circulating gonadotropin and/or subunit levels (18). This may be related to the low hormonal activity of this type of pituitary adenoma, and in this respect the effect of treatment with IFNα on tumor volume is more uncertain than that in secreting pituitary adenomas.

TABLE 4. Dose dependency of inhibition by IFNα-2a of hormone secretion by cultured human pituitary adenoma cells

Type of adenoma	Hormone(s)	IC_{50} (U/mL)	Maximal inhibition (%)
GH-secreting (no. 15)	GH	1.7	68
Prolactinoma (no. 3)	PRL	3.3	70
Gonadotroph (no. 3)	FSH/α-subunit	2.0/2.5	36/39
Gonadotroph (no. 4)	FSH	2.0	32

IC_{50}, Concentration of IFNα-2a inducing 50% of the maximal inhibition of hormone secretion. IC_{50} values were calculated from the data presented in Fig. 7, Table 2, and Table 3.

In the present study we showed that in the majority of the cultures the inhibitory effects of IFNα were clearly additive to the inhibitory effects of octreotide and bromocriptine. These observations seem in line with studies in patients with carcinoid tumors, which showed that the combination treatment with octreotide and IFNα appears to be more effective, especially in controlling clinical symptomatology rather than on tumor mass, than treatment with either drug alone (26). Our observations suggest that IFNα treatment does not interfere in a negative manner with the effects of the drugs currently used for medical treatment of prolactinomas and GH-secreting pituitary adenomas and can thus be used in combination. Moreover, in patients with carcinoid tumors IFNα therapy was better tolerated when used in conjunction with SS analogs (26).

Recently, Katahira *et al.* (8), showed that both IFNα and IFNγ have a potent inhibitory effect on POMC gene expression in mouse AtT-20 pituitary tumor cells, thereby providing evidence for an inhibitory action of this group of proteins at the level of transcription. If such an inhibition also occurs in the human corticotropic tumor cell, ACTH production by human corticotroph adenomas may be influenced by IFNα as well. This may be very important, because at present no medical treatment for this type of pituitary adenoma is available.

Although the inhibitory effects of IFNα on hormone production in secreting pituitary adenomas seems promising, several points for the use of IFNα have to be stressed. Treatment with IFNα is associated with considerable adverse reactions, including flu-like syndrome, fatigue, anorexia, and depression (1). This is in sharp contrast with the use of SS analogs and/or DA agonists, which have only mild adverse reactions. On the other hand, we found in the present study that a very low concentration IFNα (<5 U/mL) induced significant inhibitory effects on both hormone secretion and intracellular hormone concentrations. This concentration is much lower than the concentrations reached in patients currently treated with IFNα for various malignancies or viral infections (concentrations around 50–100 U/mL) (27–29). Therefore, lower dose, long term IFNα treatment might be considered. In this respect it is of interest that in treating other malignancies lower dose treatment regimens have been suggested to be more effective than high doses (30, 31). As a result, lower dosages may be related to fewer adverse reac-

TABLE 5. Effect of long term *in vitro* treatment with IFNα-2a on insulin or gastrin secretion by cultured insulinoma and gastrinoma cells

Culture no.	Treatment	Insulin or gastrin secretion (mU/L · well ± SE)	% of control
Insulinoma no. 1	Control	127 ± 5	100
	IFNα (10 U/mL)	123 ± 21	97
	IFNα (50 U/mL)	134 ± 12	106
Insulinoma no. 2	Control	14,749 ± 409	100
	IFNα (10 U/mL)	12,083 ± 329	82
	IFNα (50 U/mL)	9,247 ± 631[a]	63
Insulinoma no. 3	Control	3,304 ± 102	100
	IFNα (50 U/mL)	2,721 ± 55[a]	82
Gastrinoma	Control	26,930 ± 2,893	100
	IFNα (50 U/mL)	14,840 ± 2,281[a]	55

Cells (10^5/well) were incubated for 14 days in Transwells without or with IFNα-2a. The medium was changed every 3–4 days. Values represent hormone concentrations in the medium from days 12–14 of culture (3 days).

[a] $P < 0.05$ *vs.* control cells.

tions. This may be related to the fact that IFNα is part of a physiologically active, complex biological system and should thus not be considered as a conventional drug (30).

In conclusion, the potent inhibitory effect of IFNα on hormone production by cultured pituitary adenomas suggests that the drug might be a novel tool for medical treatment of pituitary adenomas. On the other hand, considering the above-indicated adverse reactions of IFNα treatment, the use of this drug in the medical treatment of pituitary adenomas should only be considered in inoperable, invasive aggressive, DA agonist- and/or SS analog-resistant, secreting pituitary macroadenomas. Of additional interest is that IFNs may have antiangiogenic activity as well (31).

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