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Interferon-α Stimulates the Hypothalamic-Pituitary-Adrenal Axis in vivo and in vitro

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Key Words
Interferon-α
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Abstract
The successful therapeutic use of interferon-α (IFN-α) in myeloproliferative disorders offered the possibility to test its acute and long-term effects on the hypothalamic-pituitary-adrenal (HPA) axis in humans. ACTH and cortisol plasma concentrations were measured in 8 patients hourly starting from 4 p.m. through 12 p.m. on three occasions. The first time all patients were studied before initiation of therapy, when the vehicle was injected alone. The patients were studied again on day 1 of IFN-α therapy (5 million units) and once more after 3 weeks of therapy. On the control day, plasma concentrations of ACTH and cortisol were in the range expected for this time of day. In contrast, after the first administration of IFN-α a significant stimulation of the HPA axis was observed. After 3 weeks of IFN-α therapy, no significant stimulation of the HPA axis occurred after administration of IFN-α. IFN-α-induced adaptive changes in the HPA axis were also indicated by a significantly enhanced ACTH and cortisol response to exogenously administered supramaximal doses of corticotropin-releasing hormone (CRH) when the patients had been on IFN-α treatment for 3 weeks. To determine the exact locus of the IFN-α action, in vitro experiments were performed using rat hypothalamic organ and primary pituitary and adrenal cell culture systems. Thereby a significant stimulation of hypothalamic CRH secretion and rat adrenal corticosterone production was observed after INF-α at concentrations of 5×10^{-8} *M* or 10^{-7} *M* respectively. In contrast, no direct IFN-α effect on pituitary ACTH secretion could be observed in vitro. It is concluded that IFN-α stimulates the HPA axis. The locus of action seems to be the hypothalamus, as well as the adrenal glands.

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Table 1. Characteristics of the patients treated with IFN-α

Pat. No.	Age	Sex	Diagnosis	Therapy
1	56	f	essential thrombocythemia	digoxin 0.1 mg spironolactone 50 mg butizide 5 mg
2	85	m	esential thrombocythemia	digitoxin 0.1 mg
3	66	f	polycythemia vera	digitoxin 0.1 mg
4	90	f	chronic myeloid leukemia	digoxin 0.1 mg dergocrinmesylate 1 mg
5	69	f	chronic myeloid leukemia	
6	46	f	essential thrombocythemia	
7	52	m	essential thrombocythemia	nifedipine 40 mg atenolol 100 mg chlorthalidone 25 mg
8	72	f	polycythemia vera	digoxin 0.1 mg spironolactone 50 mg butizide 5 mg piracetam 2,400 mg

Several years ago, direct interactions between the immune and neuroendocrine systems were first described [1, 2], and the existence of a lymphoid adrenal axis has been postulated [3]. Further studies have attempted to elucidate this relationship between the immune and neuroendocrine systems [4–9]. Concerning the signals directed from the immune to the neuroendocrine system, the soluble mediator substances of the immune system – the cytokines – seem to be of particular interest. Various cytokines – interleukin (IL)-1, IL-2 and IL-6, as well as interferon (IFN)-α and IFN-γ – have been reported to activate the hypothalamic-pituitary-adrenal (HPA) axis [10–21]. The therapeutic use of IFN-α [22] in a variety of diseases offered the opportunity to test its effect in vivo in humans.

This study evaluated the effects of both acute and chronic IFN-α administration on the HPA axis in humans. In addition, the effect of IFN-α was tested in vitro in hypothalamic organ and in primary pituitary and adrenal cell culture systems.

Materials and Methods

In vivo Study

Eight patients with myeloproliferative disorders (table 1), who received IFN-α for therapeutic reasons [22], were studied on three separate occasions after informed consent was obtained. On day 1, patients received placebo; on day 2 the patients were injected recombinant human IFN-α (5 million units subcutaneously) (Berofor; Boehringer Ingelheim, FRG); and finally the patients were studied again after they had been on therapy for 3 weeks (5 × 5 million units/week). To minimize the effect of diurnal variation of ACTH and cortisol concentrations, all tests were started at 3.30 p.m. IFN-α or placebo was injected at 4 p.m. Blood was collected everey 30 min until 5 p.m. and at hourly intervals thereafter until 12 p.m. In addition, a corticotropin-releasing hormone (CRH) test was performed in the same patients before starting the treatment and after 3 weeks of treatment. These tests were done 2–3 days prior to the 8-hour study described above, and the patients did not receive IFN-α on this day. All subjects were given 100 μg of the hypothalamic-releasing hormone for ACTH, i.e. CRH, in an intravenous bolus injection. For the benefit of a quiescent HPA axis, these tests were performed at 6 p.m. Blood was collected in prechilled EDTA tubes, immediately placed on ice and centrifuged within 2 h. Plasma was then separated and stored at –20 °C until assayed.

IFN-α plasma levels were determined using a cytopathic effect inhibition assay [23]. Test plasma were assayed for their ability to protect human A549 lung cancer cells from cytopathic effect induced by mouse encephalomyocarditis virus (EMC). In brief: the test cell line was incubated in microtiter plates, and 2 or 3 days later they were treated with plasma samples or IFN overnight before challenge with EMC. The cytopathic effect after 2 days was scored under a microscope. IFN-α2c (specific activity 320 IU/ng), laboratory standard HS12, calibrated to the international reference preparation G023-901-527 was used as positive control. The investigations were carried out in duplicate, the detection limit was 4 IU/ml. ACTH was measured by a commercially available IRMA (Euro-Diagnostics BV, Apeldoorn, The Netherlands), cortisol by a RIA (Baxter, USA). Body temperature was recorded whenever blood was drawn.

In vitro Studies

Male Sprague-Dawley rats weighing 200–250 g were sacrificed after CO_2 analgesia by decapitation. The hypothalamus, the pituitary gland and the adrenal glands were then removed for the respective organ or cell cultures.

Hypothalamic Organ Cultures. The hypothalamic region was removed with sterile scissors between the posterior border of the optic chiasma, the anterior border of the mammillary bodies and the lateral hypothalamic sulci as described previously [24]. Immediately after dissection, whole hypothalami were placed in 24-well plates (Costar, Cambridge, Mass., USA) at 37 °C in an atmosphere containing 5% CO_2. Each well contained 2 ml of Dulbecco's modified Eagle's medium (DMEM; Gibco, Paisley, UK), supplemented with 2% fetal calf serum (FCS) (Gibco) and antibiotics (Gibco). After 2 h preincubation, the hypothalami were moved from well to well at 20-min intervals. The first three wells consisted of medium alone, the next two wells of medium with varying concentrations of IFN-α or plain medium as a negative control, the next two wells again of medium alone, and the last

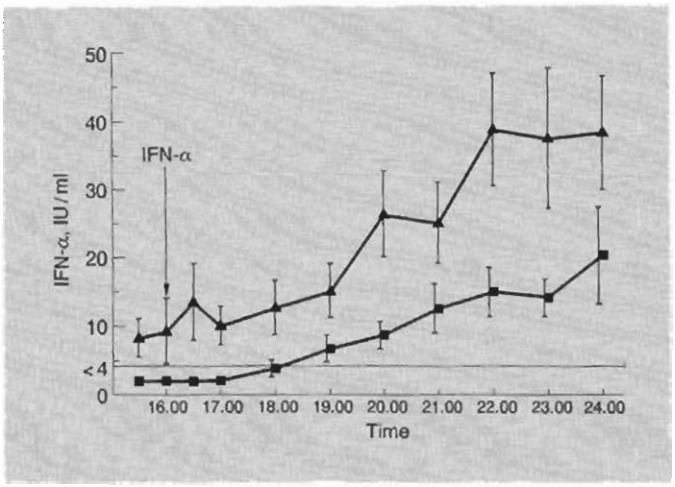

Fig. 1. Mean (± SEM) plasma IFN-α concentrations in patients with myeloproliferative disorders (■ = on day of first subcutaneous application of 5 million units IFN-α, ▲ = after 3 weeks of IFN-α therapy, 5 × 5 million units/week). The line across the bottom at 4 IU/ml IFN-α indicates the limit of detection of the assay used.

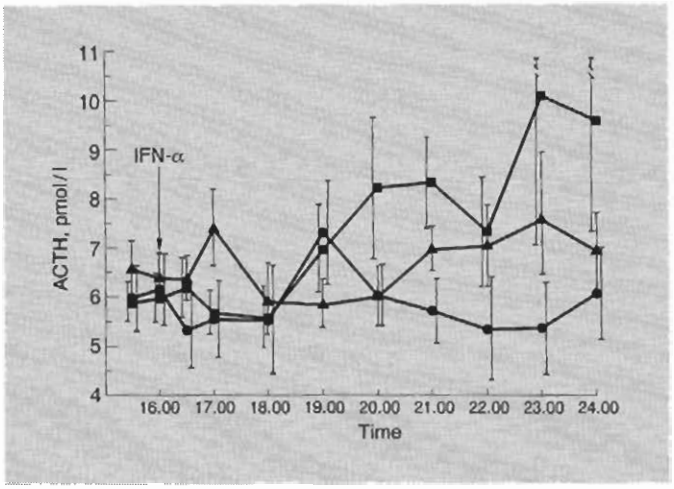

Fig. 2. Mean (± SEM) plasma ACTH concentrations in patients with myeloproliferative disorders (● = before IFN-α therapy, ■ = on day of the first subcutaneous application of 5 million units IFN-α, ▲ = after 3 weeks of IFN-α therapy 5 × 5 million units/week).

well of medium with 60 m*M* KCl for depolarization of the membrane to serve as positive control. Twelve hypothalami were used for each concentration, and hypothalami that failed to respond to KCl with at least a 70% increase of CRH release compared to basal release were excluded from analysis.

Pituitary Cell Culture. After dissection of the posterior and neurointermediate lobes, the anterior lobe of the pituitary gland was mechanically and enzymatically dispersed as described previously [24]. In short, the anterior lobes were first minced in Petri dishes and subsequently dispersed by a 20-min incubation with 0.1% collagenase type 4 (Sigma, St. Louis, Mo., USA) in DMEM. The pellets were washed twice by suspension and centrifugation at 160 *g* and resuspended in DMEM supplemented with 10% FCS. Cell yield was approximately 2×10^6 cells/gland and cell viability determined by trypan blue exclusion was always >95%. The cells were incubated for 4 days in a 24-well plate at a density of 5×10^5 cells/ml in DMEM with 10% FCS, 1% nonessential amino acids (Gibco) and antibiotics at 37 °C under 5% CO_2. The cells were then washed 3 times and incubated for 3 h with DMEM with or without IFN-α at concentrations ranging from 10^{-11} to 10^{-7} *M* in triplicate at 37 °C in an atmosphere of 5% CO_2. rCRH at a concentration of 10^{-7} *M* served as positive control. After incubation, the 24-well plate was centrifuged and the supernatant was collected and stored at –20 °C until determination of ACTH.

Adrenal Cell Culture. Adrenal glands were treated as described above for the pituitary gland. ACTH (Ciba, Basel, Switzerland) at a concentration of 10^{-7} *M* served as positive control for adrenal corticosterone release. Supernatants were stored at –20 °C until corticosterone was determined by RIA (ICN Biomedicals, Costa Mesa, Calif., USA).

Statistical Analysis

All results are expressed as the mean ± SEM. To evaluate a hormone response in vivo, the area under the curve was calculated by integration of the hormone levels in conventional units and time of testing in minutes. Comparing ACTH and cortisol plasma concentrations in vivo as well as body temperatue (fig. 2–4), the statistical evaluation was done using one-way analysis of variance, because some patients did not have complete values for the 3 days of investigation. The statistical analysis of CRH test was done as before comparing the area under the curve using paired t test. To evaluate a hormone response in vitro, mean increases of the respective hormone release over baseline secretion at varying IFN-α concentrations were compared by the Kruskal-Wallis test. Significance was accepted at $p \leq 0.05$.

Results

In vivo Study (fig. 1–4)

IFN-α plasma levels on day 1 of IFN-α treatment, as shown in figure 1, increased after 3 h and reached the maximum after 6 h. The curve of IFN-α plasma concentrations after 3 weeks of IFN-α treatment ran parallel, but shifted to a higher level. Recombinant human IFN-α at a dose of 5 million units induced an increase of cortisol plasma levels (fig. 3) and led to an elevation of the body temperature (fig. 4). Compared to their normal pattern

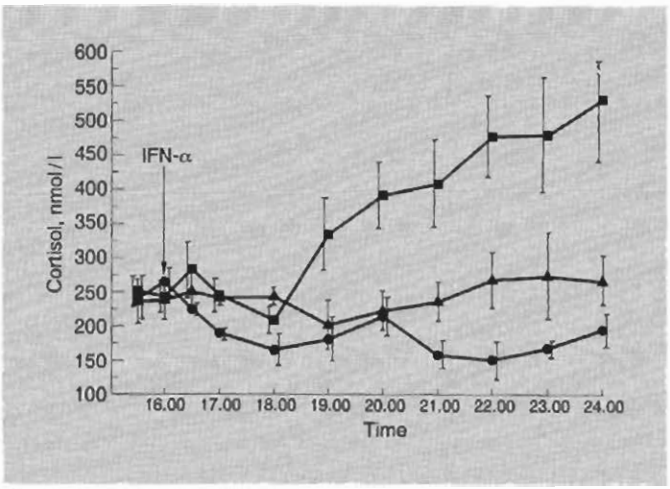

Fig. 3. Mean (± SEM) plasma cortisol concentrations in patients with myeloproliferative disorders (symbols are the same as in figure 2).

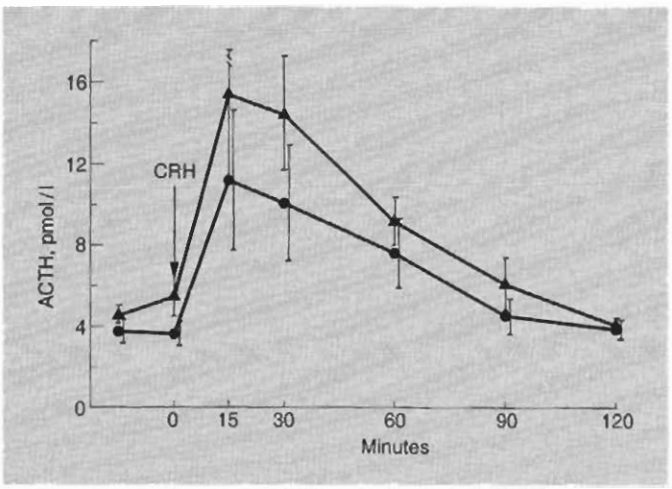

Fig. 5. Mean (± SEM) ACTH concentrations in patients with myeloproliferative disorders, before and after intravenous application of 100 μg CRH (● = before therapy with IFN-α, ▲ = after 3 weeks of subcutaneous therapy with 5 × 5 million units IFN-α/week).

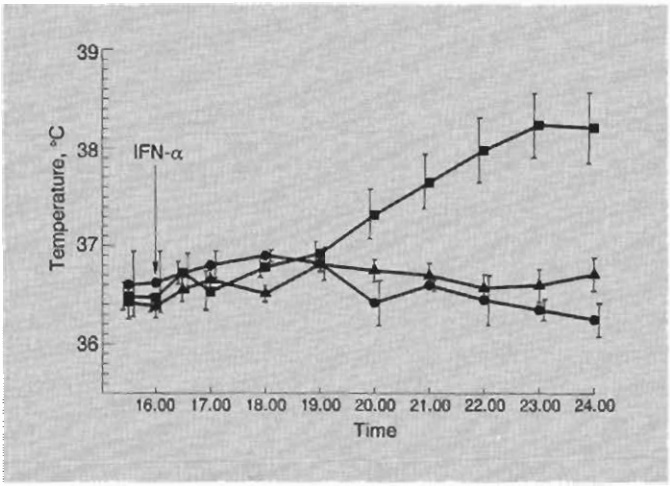

Fig. 4. Mean (± SEM) of axillary body temperature (°C) in patients with myeloproliferative disorders (symbols are the same as in figure 2).

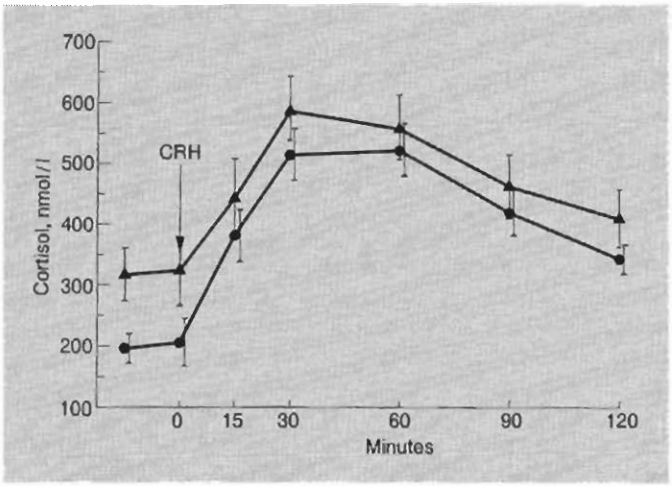

Fig. 6. Mean (± SEM) cortisol concentrations in patients with myeloproliferative disorders before and after intravenous application of 100 μg CRH (symbols are the same as in figure 5).

observed between 4 and 12 p.m. on the baseline day, when the patients received no IFN-α, ACTH plasma concentrations did not increase significantly (fig. 2) whereas cortisol plasma levels increased markedly, as expressed by a significant increase of the area under the curve ($p < 0.01$; fig. 3) after the first injection of IFN-α. The onset of this IFN effect was seen after 3 h and plasma cortisol concentrations remained elevated thereafter until the end of the observation period of 8 h and thus paralleled the plasma concentrations of IFN-α shown in figure 1. When the patients were studied again after they had been on IFN-α for 3 weeks, only slightly but not significantly elevated ACTH and cortisol plasma levels compared to the baseline day could be observed. Body temperature increased after the first injection, which is also expressed by a significant increase of the area under the curve ($p < 0.05$; fig. 4) and returned to the baseline level after 3 weeks of IFN-α treatment.

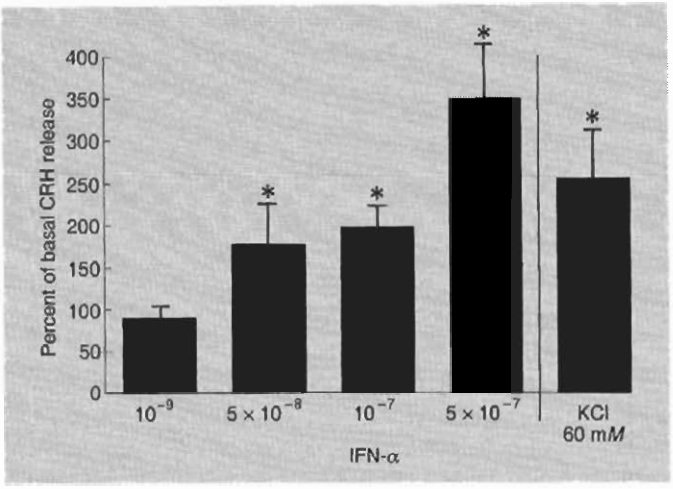

Fig. 7. Effect of graded IFN-α concentrations on the CRH secretion in primary rat hypothalamic organ cultures expressed as percent of control. KCl (60 m*M*) for depolarization of the cell membrane served as a positive control (*p < 0.05 compared to basal CRH release).

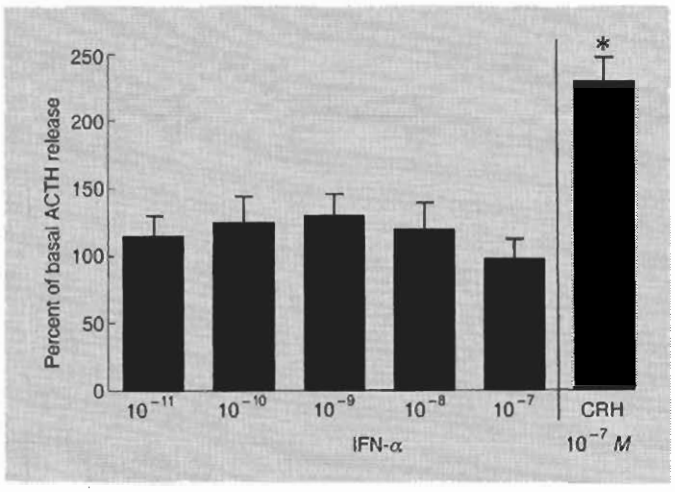

Fig. 8. Effect of graded IFN-α concentrations on the ACTH secretion in primary rat pituitary cell cultures expressed as percent of control. CRH (10^{-7} *M*) served as a positive control (*p < 0.05).

CRH Test (fig. 5, 6)

Despite the return of body temperature to the baseline level after 3 weeks of IFN-α, the area under the curve of ACTH plasma concentrations was significantly greater ($p < 0.01$) than on the baseline day. This significant difference ($p < 0.05$) could also be observed in the area under the curve of the plasma cortisol levels.

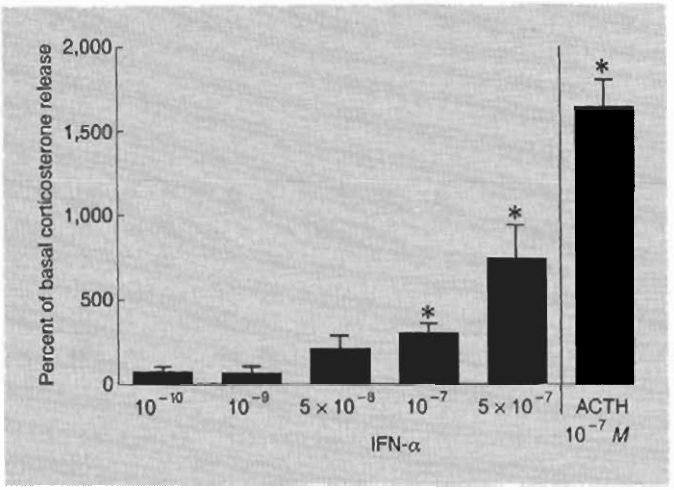

Fig. 9. Effect of graded IFN-α concentrations on the corticosterone secretion in primary rat adrenal cell cultures expressed as percent of control. ACTH (10^{-7}*M*) served as a positive control (*p < 0.05).

In vitro Studies

In the primary rat hypothalamic organ cultures, a significant dose-dependent increase of CRH release could be observed starting at IFN-α concentrations of 5×10^{-8}*M* (fig. 7). In the primary dispersed pituitary cell cultures, no effect of IFN-α on ACTH release could be registered over an IFN-α concentration range from 10^{-11} to 10^{-7} *M* (fig. 8). In primary dispersed adrenal cells, IFN-α at a concentration of 10^{-7} *M* induced a significant increase of corticosterone release, which was still higher at 5×10^{-7} *M* IFN-α (fig. 9).

Discussion

This study demonstrates that IFN-α stimulates the HPA axis in vivo and in vitro. When patients received IFN-α for the first time a significant increase in plasma cortisol concentrations and body temperature was observed. Chronic subcutaneous application of IFN-α led to adaptive changes such that the cortisol response to IFN-α was clearly diminished. Furthermore, in the CRH test, where supramaximal doses of the hypothalamic releasing peptide are administered, increased ACTH and cortisol responses could be registered after 3 weeks of IFN-α therapy. A direct effect of IFN-α on the hypothalamus and the adrenal gland described here for the first time might be responsible for this activation of the HPA axis.

Since the rise of cortisol following administration of IFN-α is not as abrupt as after other known activators of the HPA axis, such as CRH, one might argue that the stimulation is either unspecific, e.g. caused by IFN-α-induced fever or through other mediators. Holsboer et al. [19], for instance, interpreted their observation of IFN-α-induced stimulation of the HPA axis in this way. A favorite candidate for a mediator substance involved in the mediation of the IFN-α effects would be the generation of other ILs by IFN-α, such as IL-1 and IL-6, which have both a pyrogenic and ACTH-releasing activity. Although elevation of body temperature as cause for the activation of the HPA axis cannot be totally ruled out, several points argue against this possible mechanism of action. First, in vitro a direct action of IFN-α on the hypothalamus and adrenal gland has been observed, and second, after chronic IFN-α administration the rise in temperature was abolished whereas the HPA axis was still activated, as expressed by the findings of the CRH test. Furthermore, a specific activation of the HPA axis through stimulation of hypothalamic CRH release unrelated to its pyrogenic effect has also been proposed for another cytokine, namely IL-1 [16]. In addition, the time course of the ACTH and cortisol curves followed the course of IFN-α plasma concentrations after subcutaneous injection.

The chronic activation of the HPA axis by IFN-α is reminiscent of the alterations seen in anorexia nervosa, depression, alcoholism and compulsive running [25], and could also be responsible for the neuropsychiatric disorders previously described in patients receiving treatment with IFN-α [26].

The previously reported inhibitory effect of IFN-α on insulin [27] and sex steroid production [28] in conjunction with the activation of the HPA axis would fit well into Selye's [29] concept of the stress reaction and the general adaptation syndrome. In order to optimally oppose a stressor, e.g. a severe infection, the organism, by elevation of IFN-α plasma concentrations, would activate endocrine systems like the HPA axis that has powerful desirable effects on the cardiovascular system and metabolism, and deactivate systems like the sex steroids, that are not necessary in the acute stress reaction. Thus, the immune system with its mediator substances – the cytokines – would function as a sensory organ as proposed previously by Blalock [5], by stimulating or inhibiting the various endocrine systems. In turn, in situations of prolonged elevation of IFN-α, when the initial stressor persists, glucocorticoids will act upon the immune system to prohibit exaggerated and self-destroying activiy of the immune system.

In summary, IFN-α must be added to the ever-growing list of substances with stimulating effects on the HPA axis. Adding to the results of other studies, this effect was demonstrated in vivo and in vito. It should be noted that only pharmacological doses of IFN-α were effective in vivo, so that any physiological role of IFN-α in the regulation of the HPA axis remains speculative.

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