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Effect of Interferon-γ Treatment on 24-Hour Variations in Plasma ACTH, Growth Hormone, Prolactin, Luteinizing Hormone and Follicle-Stimulating Hormone of Male Rats

Pilar Cano[a] Daniel P. Cardinali[c] Vanessa Jimenez[a] Maria P. Alvarez[b]
Rodolfo A. Cutrera[c] Ana I. Esquifino[a]

[a]Departamento de Bioquímica y Biología Molecular III y [b]Departamento de Biología Celular, Facultad de Medicina, Universidad Complutense, Madrid, España; [c]Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

Key Words
Interferon-γ · ACTH · Growth hormone · Prolactin · Luteinizing hormone · Follicle-stimulating hormone

Abstract
Objective: Interferon-γ (IFN-γ) is a cytokine produced by T helper cells on antigenic challenge that may affect the release of several pituitary hormones. However, in vitro or in vivo studies have yielded disparate results with stimulatory, inhibitory or absent effects of IFN on pituitary hormone release. One of the reasons for these discrepancies could be that hormone changes were commonly assessed at a single time point in the day-night cycle. In this study we measured the circadian pattern of plasma ACTH, growth hormone (GH), prolactin, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) at 6 different time points within a 24-hour cycle in adult male Wistar rats. ***Methods:*** Groups of 6–8 rats kept under light from 08:00 to 20:00 h daily received 5 daily injections intraperitoneally of human IFN-γ (10^5 IU/kg body weight) or saline at 08:30 h. Plasma ACTH, GH, prolactin, LH and FSH levels were measured by a homologous specific double antibody RIA. ***Results:*** A factorial ANOVA for main effects indicated a significant 43% increase of circulating prolactin in IFN-γ-treated rats. Time of day changes were significant for the five hormones examined and these diurnal variations became altered by IFN-γ administration, with a phase advance of ACTH peak, a suppression of the rest phase peak of GH, the appearance of a second peak of prolactin at an early phase of daily photoperiod, and the blunting of the 24-hour variations of plasma FSH. ***Conclusion:*** The data point out an effect of IFN-γ on the mechanisms responsible for the circadian organization of pituitary hormone release.

Interferon-γ (IFN-γ) is a cytokine that participates in the mechanisms of host defense and immune response and is produced by T helper cells on antigenic challenges. In addition, IFN-γ modulates the activity of the endocrine axis, induces fever and sleep, and suppresses food intake and locomotor activity [1].

IFN-γ affects the release of several pituitary hormones. However, in vitro or in vivo studies in a number of species have yielded disparate results with stimulatory, inhibitory or absent effects of IFN on hormone release. For example, luteinizing hormone (LH) or follicle-stimulating hormone (FSH) were reported to be unmodified [2–6]

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Ana I. Esquifino, PhD
Departamento de Bioquímica y Biología Molecular III, Facultad de Medicina
Universidad Complutense, Avda Complutense s/n
ES–28040 Madrid (Spain)
Tel. +34 91 394 16 78, Fax +34 91 394 16 91, E-Mail pelayos@med.ucm.es

or to decrease [7] after IFN administration. Prolactin levels were found unmodified [5, 8, 9], stimulated [9–11] or decreased after IFN treatment [12–16]. A similar picture was obtained for ACTH (stimulation [5, 8, 9, 17–19], inhibition [12], no effect [2, 20–22]) or for growth hormone (GH; stimulation [5, 8, 9, 23, 24], inhibition [12, 14–16], no effect [2, 9]).

One of the reasons for these discrepancies may be that hormone changes were generally assessed at a single time point in the day-night cycle, thus failing to take account of the intricacies of the significant daily variation in pituitary hormone release [25–29]. This may be relevant in the case of IFN-γ since we recently reported that its intracerebroventricular administration during the day to golden hamsters affected both amplitude and phase of the locomotor activity rhythm [30].

In this study we measured the circadian pattern of plasma ACTH, GH, prolactin and gonadotropins at 6 different time points within a 24-hour cycle in adult male rats administered with IFN-γ or vehicle for 5 days in the early morning.

Materials and Methods

Animals and Experimental Design

Adult male Wistar rats bred in our animal facilities were used. Animals were kept under standard conditions of light from 08:00 to 20:00 h daily and temperature (22 ± 2°C) and with access to food and water ad libitum. The studies were conducted in accord with the principles and procedures outlined in the NIH guide for the Care and Use of Laboratory Animals.

Groups of 6–8 animals received 5 daily injections intraperitoneally of human IFN-γ (10^5 IU/kg body weight) or saline at 08:30 h. Supraphysiological doses of IFN-γ like those employed in the present study had been used before in, for example, prevention of procollagen gene expression in rat liver fibrosis [31], rejection of mesencephalic retinal xenografts in rats [32], induction of major histocompatibility complex expression in murine brain [33] or exacerbation of concanavalin A-induced T cell-dependent hepatitis [34]. Rats were killed by decapitation under conditions of minimal stress on the day after the last injection at 6 different times throughout a 24-hour cycle. Blood was collected from the trunk wound in heparinized tubes and was centrifuged at 1,500 *g* for 15 min. The plasma was collected and stored at –20°C until further analysis.

Hormone Determination

Plasma ACTH, GH, prolactin, LH and FSH levels were measured by a homologous specific double antibody RIA, using materials kindly supplied by the NIDDK's National Hormone and Pituitary Program. The intra- and interassay coefficients were 6–8%. Sensitivities of RIAs were 0.2, 0.04, 0.045, 0.097 and 0.048 ng/ml using the NIDDK rat ACTH-RP-1, rat GH-RP-2, rat prolactin RP-3, rat FSH-RP-2 and rat LH-RP-3, respectively. Results were expressed as pg/ml of plasma [25, 27–29].

Statistical Analysis

Statistical analysis of results was performed by a two-way factorial analysis of variance (ANOVA). Generally, the analysis included assessment of the group effect (i.e. the occurrence of differences in mean values between IFN-γ- and vehicle-treated rats), of time-of-day effects (the occurrence of daily changes) and of the interaction between the two factors (treatment and time, from which inference about differences in timing and/or amplitude could be obtained). Post hoc Student-Newman-Keuls multiple comparison tests were then employed to show which time points were significantly different within each experimental group to define the existence of peaks. p values lower than 0.05 were considered indicating statistical significance.

Results

Figure 1 shows the levels of plasma ACTH, GH and prolactin throughout the day in rats receiving IFN-γ or vehicle treatment. A factorial ANOVA for main effects indicated a significant 43% increase of circulating prolactin in IFN-γ-treated rats ($F_{1,75} = 13.4$, $p < 0.0005$) but absence of significant main effects of treatment on plasma ACTH or GH levels. Time of day changes were significant for the three hormones examined ($p < 0.01$) with significant interactions between time of day and treatment in every case ($p < 0.01$), i.e., the ACTH peak seen at 17:00 h in controls was phase-advanced after IFN-γ injection, the maximum of circulating GH found in vehicle-treated controls at 13:00 h was no longer observed and a second maximum of plasma prolactin at 21:00 h was seen in cytokine-treated rats (fig. 1).

Figure 2 depicts the changes in circulating LH and FSH levels after IFN-γ administration. In the case of LH there were no significant effects of treatment, both groups exhibiting maxima at 09:00 h. In the case of FSH, the maximum observed at 09:00 h was no longer found after IFN-γ injection ($p < 0.01$).

Discussion

For the release of most of the hormones examined in the present report, in vitro or in vivo studies indicated stimulatory, inhibitory or absent effects of IFN on their release [2–24]. Previous results, derived from a study at 6 different time points within a 24-hour cycle in rats treated with IFN-γ for 5 days, indicate that the cytokine caused a significant increase of circulating prolactin, with absence of significant effects on ACTH, GH, LH or FSH levels, when treatment was analyzed as a main factor in a factorial ANOVA. IFN-γ administration did alter sig-

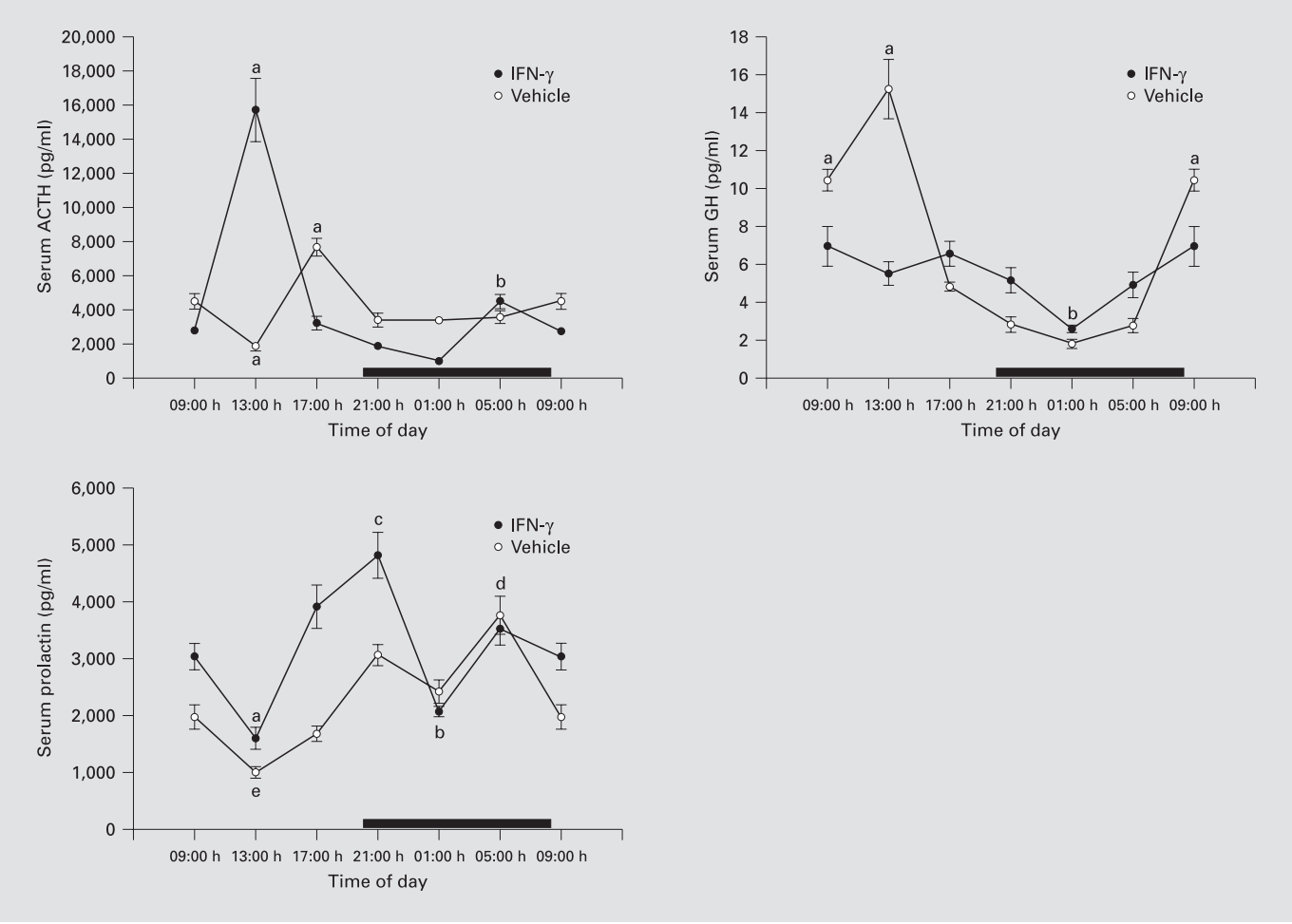

Fig. 1. Effect of IFN-γ on 24-hour changes of plasma ACTH, GH and prolactin concentration. Groups of 6–8 rats were killed by decapitation at 6 different time points throughout a 24-hour cycle. Values at the 09:00 h time point are repeated on the 'second' day. Bar indicates scotophase duration. Shown are the means ± SEM. Letters indicate the existence of significant differences between time points within each group after a one-way ANOVA and Student-Newman-Keuls test, as follows: ACTH: [a] $p < 0.01$ vs. all remaining groups, [b] $p < 0.05$ vs. 01:00 h; GH: [a] $p < 0.01$,[b] $p < 0.05$ vs. all remaining groups; prolactin: [a] $p < 0.01$ vs. 09:00, 17:00,21:00 and 05:00 h, [b] $p < 0.01$ vs. 09:00, 17:00, 21:00 and 05:00 h, [c] $p < 0.01$ vs. 01:00, 05:00, 09:00 and 13:00 h, [d] $p < 0.01$ vs. 01:00, 09:00, 13:00 and 17:00, $p < 0.05$ vs. 21:00 h, [e] $p < 0.01$ vs. 01:00, 05:00, 09:00 and 21:00 h, $p < 0.05$ vs. 17:00 h. For further statistical analysis, see text.

nificantly 24-hour variations of plasma ACTH, GH, prolactin and FSH, by phase advancing the ACTH peak, by suppressing the rest phase peak of GH, by inducing a second peak of prolactin at an early phase of the cycle and by suppressing the daily maximum of plasma FSH. Hence, these data point out an effect of IFN-γ on the mechanisms responsible for the circadian organization of pituitary hormone release.

The suprachiasmatic nucleus (SCN) of the anterior hypothalamus is the dominant pacemaker for most 24-hour rhythms [35]. It acts like a multifunctional timer to adjust the homeostatic system, including hormonal secretion, immune response and various other bodily functions, to the 24-hour cycle. SCN is strongly entrained by light pulses impinging on the retina, when they are given during the night. In contrast, the phase shifts produced by nonphotic stimuli, e.g. physical exercise or social interactions, occur during the day [35].

In a recent study we reported that IFN-γ is another nonphotic stimulus for SCN inasmuch as its intracerebro-

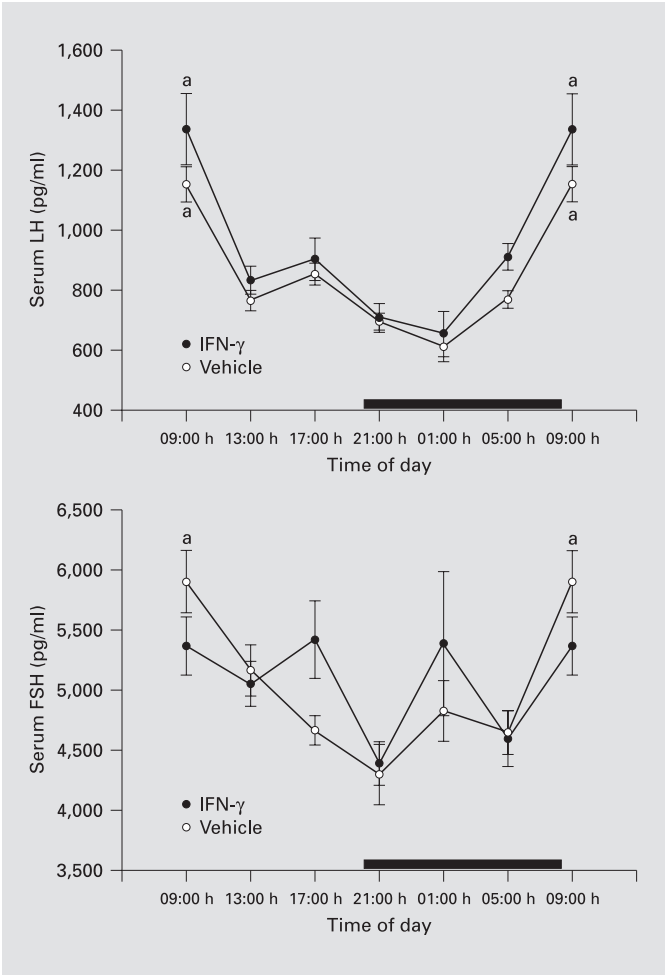

Fig. 2. Effect of IFN-γ on 24-hour changes of plasma LH and FSH concentration. Groups of 6–8 rats were killed by decapitation at 6 different time points throughout a 24-hour cycle. Values at the 09:00 h time point are repeated on the 'second' day. Bar indicates scotophase duration. Shown are the means ± SEM. Letters indicate the existence of significant differences between time points within each group after a one-way ANOVA and a Student-Newman-Keuls test, as follows: [a] $p < 0.01$ vs. all remaining groups. Time of day variations in plasma FSH of IFN-γ-treated rats were not significant in a one-way ANOVA. For further statistical analysis, see text.

ventricular administration during the day but not during the night brought about a phase advance in locomotor activity rhythm in hamsters [30]. In addition a decrease in amplitude of rhythm was observed. Other results indicated a disruptive effect of systemic administration of IFN-α on the circadian rhythm of locomotor activity, body temperature and clock gene mRNA expression in SCN [36].

There are several mechanisms by which a systemic increase of IFN-γ concentration can modify central clock structures [37–39]. As a large, hydrophilic protein, IFN-γ can only cross the blood-brain barrier at leaky points (a circumventricular organ like the organum vasculosum laminae terminalis) or via specific active transport mechanisms [40]. Cytokines act at the level of circumventricular organs by inducing synthesis and release of second messenger systems [41]. It must be noted that a central compartment for cytokines also exists and that there are data indicating that an increase in peripheral cytokines can evoke a mirror increase in brain levels of cytokines [see 37]. Inflammatory cytokines can also induce CNS responses through afferent peripheral neural signaling [42].

IFN-γ receptors have been detected in neuronal elements of ventrolateral SCN [43]. Expression of SCN IFN-γ receptors followed a 24-hour rhythm, coinciding with the expression of Janus kinase 1 and 2 as well as the signal transducer and activator of transcription factor 1, the main intracellular signaling pathway for IFN-γ. Therefore, the effects reported here on changes of the 24-hour variations of pituitary hormone release after administration of IFN-γ at the beginning of the light phase of daily photoperiod may well derive from direct effects of the cytokine on the central pacemaker.

In summary, a significant increase of circulating prolactin and the disruption of 24-hour rhythms of prolactin, ACTH, GH and FSH release, but not of LH release, were found in rats administered intraperitoneally with human IFN-γ. Collectively, the data point to an effect of IFN-γ on the mechanisms responsible for the circadian organization of pituitary hormone release.

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