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Effects of Interferons on Cortisol Production in Bovine Adrenal Fasciculata Cells Stimulated by Adrenocorticotropin

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Abstract

The effects of interferons (IFNs) IFN-α, IFN-β and IFN-γ on the production of cortisol in bovine adrenal fasciculata cells have been investigated.

Pretreatment of the fasciculata cells with recombinant interferon-α-2b from man (over 300 units mL^{-1}), but not with fibroblast IFN-β or recombinant IFN-γ from man, reduced the production of cortisol in cells stimulated with adrenocorticotropin (ACTH) (1 nM). IFN-α-2b inhibited ACTH-induced cortisol production in a concentration- (300–15 000 units mL^{-1}) and time- (2–24 h) dependent manner. The inhibitory effect of IFN-α-2b on the production was abolished when the cells were simultaneously treated with anti-IFN-α antibody, and it was reversible. IFN-α-2b also inhibited dibutyryl cyclic AMP-induced production of cortisol but not pregnenolone-induced production. The effect of IFN-α-2b was not influenced by increases in external ACTH and Ca^{2+} concentrations and IFN-α-2b did not affect the ACTH-induced increase in cyclic AMP level in the cells.

These results strongly suggest that IFN-α-2b reduces ACTH-induced production of cortisol in bovine adrenal fasciculata cells by affecting the early process of cortisol synthesis. The results also indicate that IFNs might not directly affect steroidogenesis in the adrenal cortex in-vivo, because of the requirement of high concentrations of IFN-α-2b for inhibition, and because of the ineffectiveness of IFN-β and IFN-γ.

The interferons (IFNs), a family of bioactive cytokines, are glycoproteins which interfere with virus replication (Isaacs & Lindenmann 1957) and have a variety of other biological effects including modulation of immune systems and regulation of cell proliferation and differentiation (Pestka & Langer 1987; Douglas 1990; Sen 1992). IFNs are classified into three types, IFN-α, IFN-β and IFN-γ, produced mainly by leucocytes, fibroblasts, and T lymphocytes, respectively.

IFNs are widely used as drugs for the treatment of patients with viral infection or carcinoma (Douglas 1990). However, during therapy with IFNs, especially IFN-α, severe psychiatric side-effects such as depression, confusion and behavioural changes have been reported (McDonald et al 1987; Renault et al 1987; Hasford et al 1993) in addition to influenza-like symptoms including fever, chill, headache, and vomiting (Douglas 1990). We have investigated the effects of IFNs on the secretion of catecholamines from bovine adrenal chromaffin cells, widely used as a model of nervous systems (Tachikawa et al 1997). The results showed that recombinant IFN-α-2b (30–500 units mL^{-1}) from man, but not fibroblast IFN-β and recombinant IFN-γ from man, reduces secretions from cells stimulated by acetylcholine and it was suggested that the inhibitory effects of IFN-α-2b might be associated with the side-effects of IFN-α, and that immune systems might regulate the functions of nervous systems or adrenal medulla, or both, via IFN-α in-vivo.

Recent studies have shown that immune systems regulate the function of endocrine and neuroendocrine systems via cytokines in-vivo and in-vitro. The effects of cytokines on the hypothalamo–pituitary–adrenocortical axis have been demonstrated. For example, interleukin (IL)-1 increased

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the release of corticotropic-releasing hormone (CRH) from rat hypothalamus in-vivo and in-vitro (Berkenbosch et al 1987; Sapolsky et al 1987; Saphier & Ovadia 1990), and directly stimulated the secretion of adrenocorticotropin (ACTH) from the anterior pituitary glands (Berkenbosch et al 1987; Bernton et al 1987; Cambronero et al 1992). IL-6 enhanced ACTH secretion from rat hemipituitary glands in-vitro, and the injection of IL-6 into rats also increased both ACTH and corticosterone secretion (Lyson & McCann 1991). The release both of CRH from medial basal hypothalamus (Spinedi et al 1992) and of cortisol from adrenocortical cells (Darling et al 1989) were also increased by tumour necrosis factor-α. Thus, several cytokines regulate the functions of the hypothalamo–pituitary–adrenocortical axis.

The effects of IFNs on their systems have also been observed. The intracerebroventricular administration to rats of recombinant IFN-α from man reduced basal plasma corticosterone levels (Kindron et al 1989; Saphier et al 1993), whereas intravenous administration to rats of rat IFN-α or IFN-β or recombinant IFN-α from man increased concentrations of corticosterone and ACTH in peripheral plasma (Menzies et al 1996). Nolten et al (1993) have reported that the intravenous administration of IFN-β to cancer patients increases both ACTH and cortisol in the plasma. IFN-γ stimulated ACTH secretion from cultured pituitary cells from man (Malarkey & Zvara 1989) or inhibited it from rat cells (Vankelecom et al 1992). Although these reports show that IFNs modulate the functions of the hypothalamo–pituitary–adrenocortical axis, the results conflict.

To examine the direct influence of IFNs as immunotransmitters and drugs upon the adrenal cortex, therefore, we investigated the effect of IFNs on the production of cortisol in bovine adrenal fasciculata cells stimulated by ACTH.

Materials and Methods

Materials

Ca^{2+}-free Krebs–Ringer phosphate (KRP) buffer used for cell isolation had the composition: 154 mM NaCl, 5·6 mM KCl, 2·2 mM $CaCl_2$, 1·1 mM $MgCl_2$, 0·85 mM NaH_2PO_4, 2·15 mM Na_2HPO_4, 10 mM glucose, and 0·5% bovine serum albumin (pH 7·4). Oxygenated Krebs–Ringer–4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) buffer (KRH buffer) (pH 7·4), used as an incubation medium, was of composition: 125 mM NaCl, 4·8 mM KCl, 2·6 mM $CaCl_2$, 1·2 mM $MgSO_4$, 25 mM HEPES, 5·6 mM glucose, and 0·5% bovine serum albumin. Recombinant IFN-α-2b, IFN-γ and ACTH [1–24] from man were purchased from Seikagaku Kogyo (Tokyo, Japan). Sheep anti-human IFN-α polyclonal antibody was from Funakoshi (Tokyo, Japan). Fibroblast IFN-β from man and pregnenolone were from Sigma (St Louis, MO). The cyclic AMP kit was purchased from Yamasa (Chyoshi, Japan). Tissue culture instruments were obtained from Falcon Plastics (Cockeysville, MD). Dulbecco's Modified Eagle medium (DMEM) and F-12 Nutrient Mixture (Ham) were from Life Technologies (Grand Island, NY). Calf serum was obtained from Nacarai Tesque (Kyoto, Japan). Other chemicals were of the highest grade available from commercial sources.

Isolation and culture of bovine adrenal fasciculata cells

Bovine adrenal glands were kindly provided by the Centre of Iwate Livestock Industry. Adrenal fasciculata cells were prepared by the method of collagenase digestion as described elsewhere (Yanagibashi et al 1990; Yamazaki et al 1996). Briefly, adrenal glands were perfused via the adrenal vein with Ca^{2+}-free KRP buffer for 15 min at 37°C to flush the glands. The adrenal glands were then dissected to remove the zona glomerulosa, the zona reticularis and the medulla, and the zona fasciculata was sliced using a Stadie-Rigg slicer. The slices were digested with collagenase for 30 min at 37°C. The isolated cells were filtered through a nylon mesh, washed three times with Ca^{2+}-free KRP buffer, and centrifuged at 600 *g* for 2 min. The cells were immediately suspended in DMEM–Ham's F-12 (1:1) containing 10% foetal bovine serum, cytosine arabinoside (3 μM), penicillin (100 units mL^{-1}), streptomycin (100 μM) and amphotericin B (0·3 $\mu g\,mL^{-1}$), and plated on 15-mm diameter wells at a density of 5×10^5 cells. The cells were maintained in a CO_2 incubator (95% air–5% CO_2) at 37°C.

Measurement of cortisol in the fasciculata cells

After three days of culturing, the fasciculata cells were further cultured for one day at 37°C with IFN-containing or plain culture medium, except as otherwise described below. The cells were washed twice with KRH buffer and then pre-incubated with KRH buffer for 10 min at 37°C. The cells were washed once more with prewarmed KRH buffer and incubated with or without ACTH or other test

agents for 1 h. The reaction was terminated by transferring the incubation medium to tubes in an ice-cold bath. The cortisol produced in the medium was extracted with dichloromethane and quantified by the sulphonic acid condensation method (Silber et al 1958), using a fluorescence spectrophotometer (650-10S; Hitachi, Tokyo, Japan) at an excitation wavelength of 470 nm and an emission wavelength of 520 nm. The amount of cortisol produced from the cells was expressed as ng per well h^{-1}.

Measurement of cyclic AMP concentration in the cells

After incubation of the cultured cells with or without 1 nM ACTH for 1 h, the medium was removed and the cells were immediately frozen on dry ice. The cells were scraped from the well into 6% perchloric acid. The samples were centrifuged at 20 000 *g* for 10 min, and the supernatant was neutralized with KOH. The samples were centrifuged at 20 000 *g* for 10 min, and cyclic AMP in the supernatant was succinylated with triethylamine and succinic anhydride (Cailla et al 1976). The cyclic AMP levels were measured by radioimmunoassay (Steiner et al 1972) and were expressed as pmol per well h^{-1}.

Statistics

Statistical evaluation of data was performed by analysis of variance. When a significant F value was found by analysis of variance, Scheffe's test for multiple comparisons was performed to identify differences among the groups. $P < 0{\cdot}05$ was considered to be indicative of significance.

Results

Effects of IFN-α-2b, IFN-β and IFN-γ on cortisol production in adrenal fasciculata cells

We first examined the effects of three kinds of IFN, IFN-α, IFN-β and IFN-γ, on the production of cortisol in bovine adrenal fasciculata cells. After pretreatment of the fasciculata cells with recombinant IFN-α-2b from man (1500 and 15 000 units mL^{-1}) for 24 h, when the IFN-α-2b-treated cells were stimulated by ACTH (1 nM), cortisol production in the cells was greatly inhibited (Table 1). The inhibitory effect of IFN-α-2b was not observed in the basal (spontaneous) production in non-stimulated cells. The amount of cortisol in the culture medium of IFN-α-2b (1500 and 15 000 units mL^{-1})-treated cells did not significantly change during 24 h in comparison with that of the non-treated cells (data not shown). Pretreatment of the cells with fibroblast IFN-β or recombinant IFN-γ from man (2000 and 15 000 units mL^{-1}) had no effect on ACTH-induced production of cortisol (Table 1).

Table 1. Effects of interferons on cortisol production in bovine fasciculata cells.

Interferon	Amount (units mL^{-1})	Cortisol production (ng per well h^{-1})	
		Control	Adreno-corticotropin
None	–	21 ± 5	1448 ± 72
Interferon-α-2b	1500	28 ± 10	967 ± 56*
	15 000	29 ± 7	448 ± 16*
Interferon-β	2000	19 ± 6	1356 ± 26
	15 000	24 ± 5	1421 ± 25
Interferon-γ	2000	26 ± 9	1447 ± 77
	15 000	25 ± 8	1490 ± 10

The isolated bovine adrenal fasciculata cells were cultured for 72 h and then further cultured with or without recombinant interferon (IFN)-α-2b from man (1500 or 15 000 units mL^{-1}), IFN-β (2000 or 15 000 units mL^{-1}) or recombinant IFN-γ from man (2000 or 15 000 units mL^{-1}) for 24 h. The cells were washed with prewarmed Krebs–Ringer–HEPES buffer and then incubated with or without 1 nM adrenocorticotropin (ACTH) for 1 h at 37°C. Data are means ± s.d. of results from four experiments. *$P < 0{\cdot}01$ compared with ACTH-induced cortisol production.

As shown in Figure 1A, IFN-α-2b inhibited ACTH-induced cortisol production (1499 ± 53 ng per well h^{-1}) in a concentration-dependent manner (IC50 (concentration resulting in 50% inhibition)) = 2500 units mL^{-1}); inhibition by IFN-α-2b was significant at 300 units mL^{-1} (1350 ± 40 ng $well^{-1}$ h^{-1}). At 1500 units mL^{-1}, it was 35% and was maximum (approx. 70%) at 15 000 units mL^{-1}. To examine the properties of IFN-α-2b inhibition of cortisol production, therefore, we used IFN-α-2b at 6000 units mL^{-1} which has a noticeable effect, although the concentration is high.

Time course of IFN-α-2b inhibition of cortisol production

IFN-α inhibition of ACTH (1 nM)-induced production of cortisol was detected after 2 h of cell pretreatment with the cytokine (6000 units mL^{-1}). It was 38% after the 8-h pretreatment and reached a plateau after 24 h (approx. 60% inhibition). IFN-α-2b did not alter spontaneous production (data not shown). Thus, IFN-α-2b inhibited cortisol production in cells stimulated by ACTH in a time-dependent manner (Figure 1B).

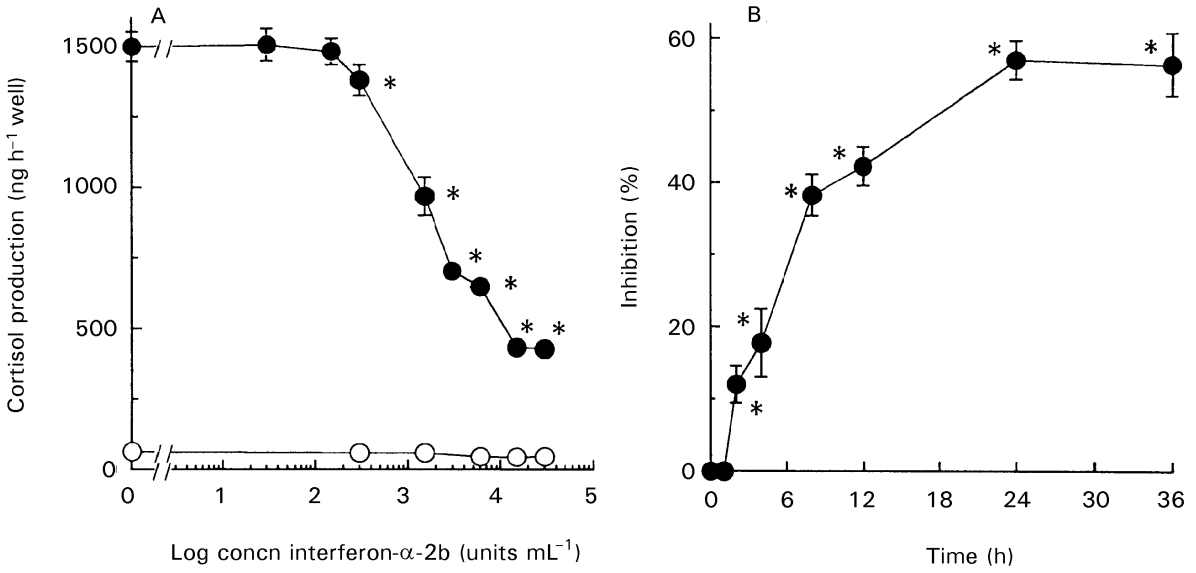

Figure 1. A. Effects of different concentrations of interferon (IFN)-α-2b on cortisol production in bovine adrenal fasciculata cells. After 72 h of culturing the isolated cells, the cells were pretreated for 24 h with different concentrations of IFN-α-2b. The cultured cells were washed with prewarmed Krebs–Ringer–HEPES (KRH) buffer and incubated with (●) or without (○) 1 nM adrenocorticotropin (ACTH) for 1 h at 37°C. Data are means ± standard deviations of results from four experiments. *$P < 0.005$ compared with ACTH-induced cortisol production. B. Effect of IFN-α-2b pretreatment time on cortisol production in the cells. After 72 h of culturing the isolated cells were pretreated with or without IFN-α-2b (6000 units mL^{-1}) for the time indicated. The cultured cells were washed with prewarmed KRH buffer and then incubated with or without 1 nM ACTH for 1 h at 37°C. Basal values were subtracted from the data, and ACTH-induced responses were expressed as percentage inhibition. Basal cortisol production was 51–65 ng per well h^{-1} and ACTH-induced production (in the absence of IFN-α-2b) was 1390–1500 ng per well h^{-1}. Data are means ± s.d. of results from four experiments. *$P < 0.005$ compared with ACTH-induced cortisol production.

Specificity of IFN-α-2b inhibition

By use of anti-human IFN-α antibody we tested whether the inhibitory action of IFN-α-2b on cortisol production is specific. The exposure of anti-IFN-α antibody with IFN-α-2b (6000 units mL^{-1}) to the cells for 24 h antagonized the effect of IFN-α-2b on ACTH (1 nM)-induced cortisol production. Anti-IFN-α at 6000 neutral-izing units mL^{-1} (one neutralizing unit of anti-IFN-α antibody abolishes the activity of one unit of IFN-α) completely overcame inhibition by IFN-α-2b (Figure 2), indicating that the inhibition of the cortisol production is specific for IFN-α-2b.

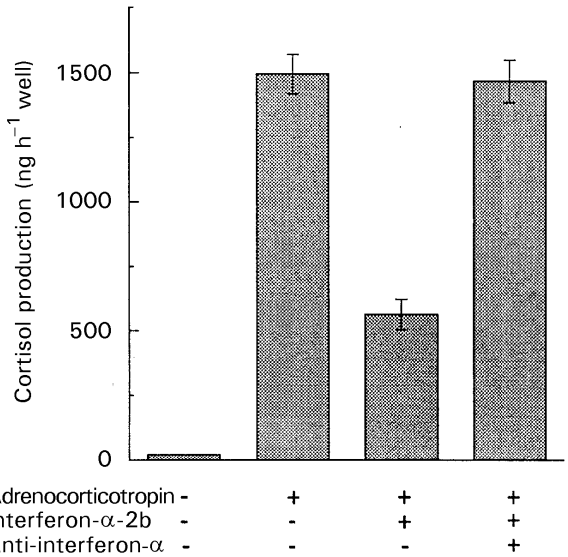

Figure 2. Effect of anti-interferon (IFN)-α antibody on IFN-α-2b inhibition of adrenocorticotropin (ACTH)-induced cortisol production. After 72 h of culturing the isolated cells, the cells were pretreated with or without IFN-α-2b (6000 units mL^{-1}) in the presence or absence of anti-human IFN-α polyclonal antibody (6000 neutralizing units mL^{-1}) for 24 h. The cultured cells were washed with prewarmed Krebs–Ringer–HEPES buffer and then incubated without or with 1 nM ACTH for 1 h at 37°C. The titre of the antibody is expressed as neutralizing units which determines the ability of the antiserum to neutralize IFN-α from man. One neutralizing unit of anti-IFN-α antibody abolishes the activity of one unit IFN-α. Data are means ± s.d. of results from four experiments.

Recovery of IFN-α-2b inhibition of ACTH-induced cortisol production

To examine the reversibility of IFN-α-2b inhibition of cortisol production, the cultured cells were pretreated with or without 6000 units mL^{-1} IFN-α-2b in the culture medium for 24 h, replacing each medium with either normal (plain) or 6000 units mL^{-1} IFN-α-2b-containing culture medium, and were then cultured for an additional 24–48 h. As shown in Figure 3, substantial restoration of ACTH-induced cortisol production was observed for cells pretreated with IFN-α-2b for 24 h and then incubated in the plain medium. The recovery of

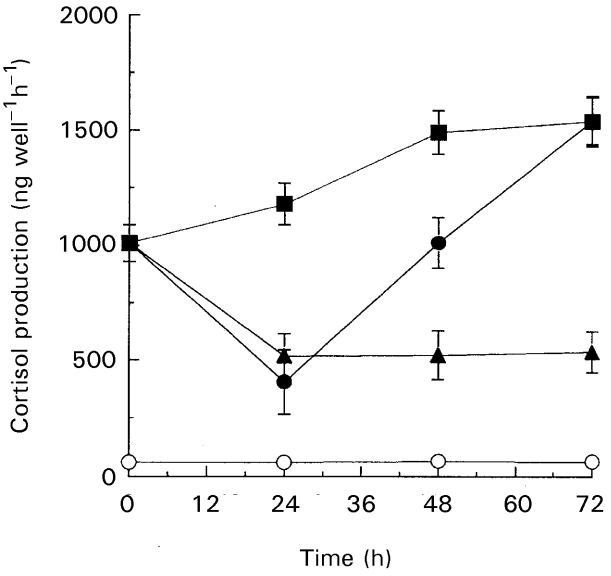

Figure 3. Recovery of interferon (IFN)-α-2b inhibition of adrenocorticotropin (ACTH)-induced cortisol production. After 24 h of culturing the isolated cells, the cells were pretreated with (▲, ●) or without (○, ■) IFN-α-2b (6000 units mL^{-1}) for 24 h and then treated with (▲) or without (○, ●, ■) IFN-α-2b (6000 units mL^{-1}) for the additional times indicated in the figure. The cells were washed with prewarmed Krebs–Ringer–HEPES buffer and then incubated for 1 h with (▲, ●, ■) or without (○) 1 nM ACTH at 37°C. Data are means ± s.d. of results from four experiments.

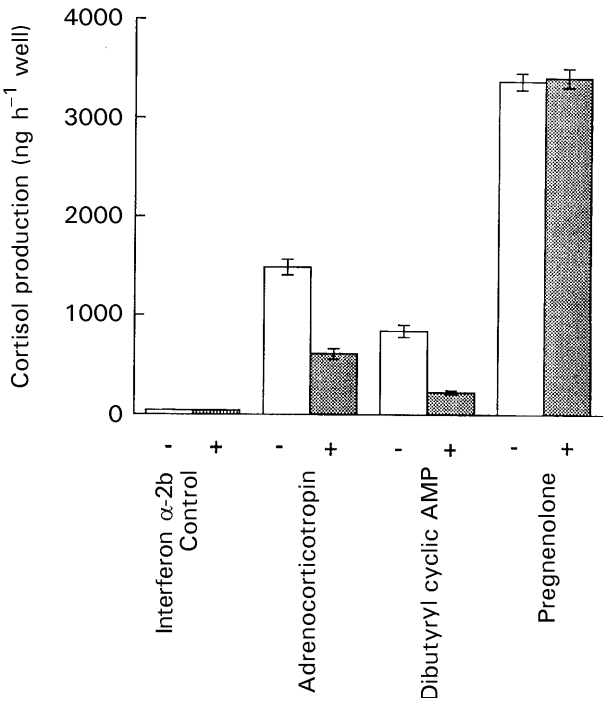

Figure 4. Effects of interferon (IFN)-α-2b on dibutyryl cyclic AMP- and pregnenolone-induced cortisol production in the cells. After 72 h of culturing the isolated cells were pretreated with or without IFN-α-2b (6000 units mL^{-1}) for 24 h. The cells were washed with prewarmed Krebs–Ringer–HEPES buffer and then incubated with or without 1 nM adrenocorticotropin, 2 mM dibutyryl cyclic AMP or 10 μM pregnenolone for 1 h at 37°C. Data are means ± s.d. of results from four experiments.

IFN-α-2b inhibition was observed after incubation with plain medium for 24 h and was almost complete after 48 h.

Effects of IFN-α-2b on dibutyryl cyclic AMP- and precursor-induced cortisol production

To examine where IFN-α-2b influences the process of cortisol synthesis in the cells, we investigated the effect of IFN-α-2b on cortisol production by dibutyryl cyclic AMP, an analogue of cyclic AMP, and from pregnenolone, a precursor of cortisol synthesis. Dibutyryl cyclic AMP (2 mM) and pregnenolone (10 μM) stimulated cortisol production (Figure 4). IFN-α-2b (6000 units mL^{-1}) inhibited not only the ACTH-induced production (61% inhibition) but also dibutyryl cyclic AMP-induced production (73% inhibition), whereas it did not inhibit pregnenolone-induced cortisol production.

Effects of ACTH and Ca^{2+} concentrations on IFN-α-2b inhibition

When ACTH concentrations (10 pM–1 μM) in the incubation medium were increased, cortisol production was dose-dependently augmented as shown in Figure 5A. However, the inhibitory effect of IFN-α-2b (6000 units mL^{-1}) on cortisol production was barely altered by the increases in ACTH concentration (100 pM–1 μM), indicating that IFN-α-2b might not directly compete with ACTH for the receptor. Inhibition by IFN-α-2b was not affected by increasing the external Ca^{2+} concentration (2·6–10·4 mM) (Figure 5B).

Effect of IFN-α-2b on the cyclic AMP content of the cells

ACTH increases the cellular content of cyclic AMP, which plays a crucial role in ACTH-induced cortisol production (Ganguly & Davis 1994; Schimmer & Parker 1996). ACTH (1 nM) increased cyclic AMP content (12·5 pmol h^{-1} well). Pretreatment of the cells with IFN-α-2b (300–15 000 units mL^{-1}) for 24 h did not affect the ACTH-induced increase in cyclic AMP content (Figure 6).

Discussion

This study has demonstrated that relatively long-term exposure (2–24 h) of bovine adrenal fasciculata cells to recombinant IFN-α-2b from man inhibits ACTH-stimulated cortisol production in the cells (Table 1 and Figure 1). The inhibitory

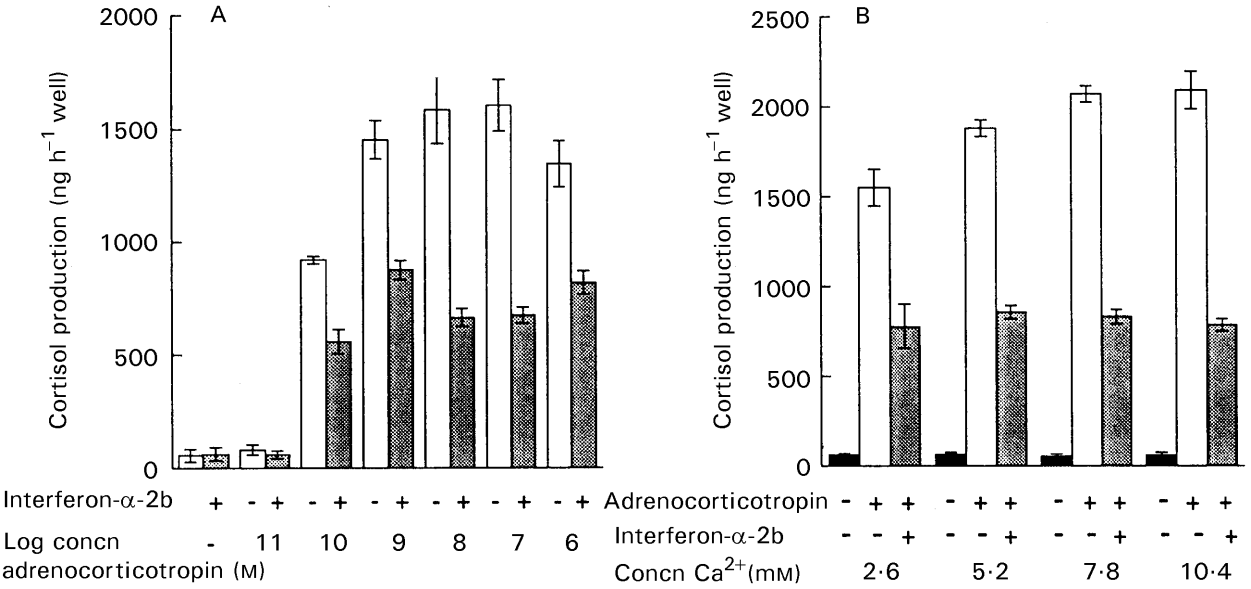

Figure 5. Effects of different concentrations of adrenocorticotropin (ACTH) and external Ca^{2+} on interferon (IFN)-α-2b inhibition of cortisol production. After 72 h of culturing the isolated cells, the cells were pretreated with or without IFN-α-2b (6000 units mL^{-1}) for 24 h. The cells were washed with prewarmed Krebs-Ringer-HEPES buffer and then incubated with different concentrations of ACTH (A) or incubated with different concentrations of the external Ca^{+} in the presence or absence of 1 nM ACTH (B) for 1 h at 37°C. Data are means ± s.d. of results from four experiments.

effect of IFN-α-2b on cortisol production is probably not attributable to the suppression of the cell growth in the culture, although IFNs are known to suppress cell proliferation and differentiation (Sen 1992), because the effect of IFN-α-2b was reversible (Figure 3), and IFN-α-2b inhibited neither cortisol production from pregnenolone, a precursor of the cortisol synthesis, nor the elevation of cyclic AMP by ACTH. This suggests that IFN-α-2b acts on a site, or on sites, in the process of cortisol production without affecting cell proliferation and differentiation.

Cardoso et al (1990) and Gisslinger et al (1993), respectively, have recently shown that IFN-α at low concentrations increases basal cortisol production in adrenal slices from man and in rat adrenal cells. Although the reason for the discrepancies between their observations and ours is not clear, there are several differences between the experimental conditions used in the different laboratories. In the laboratories of Cardoso and Gisslinger adrenal slices from man or rat adrenal cells were used and the adrenal medulla was not removed from the cortex (catecholamines are known to stimulate cortisol production in adrenal cortical cells (O'Connell et al 1994; Mazzocchi et al 1997)). Also in the Cardoso and Gisslinger laboratories the effect of IFN-α on cortisol production was examined in the absence of ACTH. These differences in the prepared materials and in the methodology might account for these discrepancies. We have occasionally observed that ACTH-induced cortisol production in bovine fasciculata cells is potentiated by IFN-α-2b at lower concentrations (130–150 units mL^{-1}), although this stimulatory effect was not reproducible.

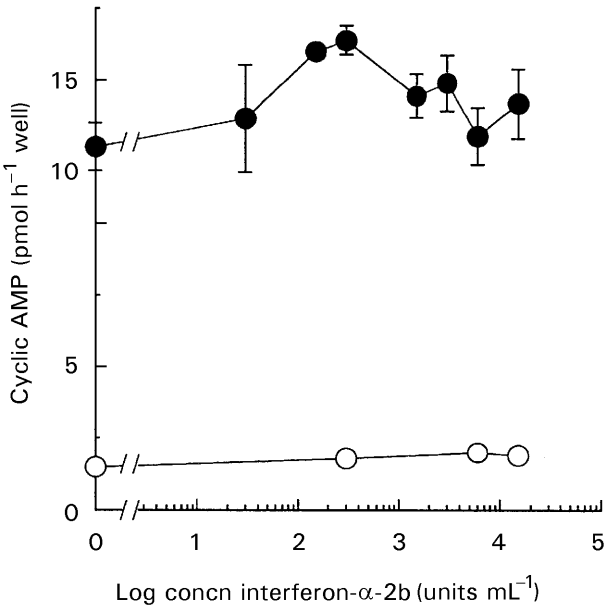

Figure 6. Effect of interferon (IFN)-α-2b on cyclic AMP levels in the cells. After 72 h of culturing the isolated cells, the cells were pretreated for 24 h with different concentrations of IFN-α-2b indicated. The cultured cells were washed with prewarmed Krebs–Ringer–HEPES buffer and incubated with (●) or without (○) 1 nM adrenocorticotropin for 1 h at 37°C. Data are means ± s.d. of results from four experiments.

Neither fibroblast IFN-β nor recombinant IFN-γ from man (2000 and 15 000 units mL^{-1}) altered ACTH-induced cortisol production (Table 1) and the inhibitory effect of IFN-α-2b was overcome by anti-IFN-α antibody (Figure 2), indicating that the inhibitory effect on cortisol production is specific to IFN-α-2b. However, many studies have reported that IFN-α and IFN-β share a common receptor and have similar biological activity (Pestka & Langer 1987). IFN-β and IFN-α-2b, therefore, should inhibit ACTH-induced cortisol production in this study and so the different effects of IFN-α-2b and IFN-β seem to be contradictory. Several explanations should, however, be considered.

IFN-β from man might not affect cells derived from a different species, because some biological activities mediated by IFNs are characterized by species-specificity (Stewart 1979); the pathway of signal transduction in the cells after the IFN-β binding to the receptor might be different from that after IFN-α-2b binding; or the fasciculata cells might have specific receptors for IFN-α-2b. ACTH is considered to produce cortisol in fasciculata cells mainly by the following physiological process (Boyd & Gorban 1980; Nishikawa et al 1996; Schimmer & Parker 1996). ACTH binds its membrane receptors, and cellular concentrations of cyclic AMP are increased by adenylate cyclase activated via receptor-coupled GTP binding protein; cyclic AMP-dependent protein kinase is activated; each step of the hydrolysis of cholesterol ester to cholesterol and of cholesterol transfer into the mitochondria is stimulated by the protein kinase, and cortisol production through pregnenolone produced from cholesterol is increased.

IFN-α-2b affected neither the ACTH-induced increase in the cyclic AMP level (Figure 6) nor the pregnenolone-induced production of cortisol, but it reduced dibutyryl cyclic AMP-induced cortisol production (Figure 4). These results strongly suggest that IFN-α-2b inhibits cortisol production by affecting an early step of the synthetic process. A relatively long period (2–24 h) was required for the appearance of the inhibitory effect of IFN-α-2b (Figure 2B). Hence, it is thought that the IFN-α-2b inhibition arises by a mechanism involving gene expression and protein synthesis. Thus, IFN-α-2b might modulate the syntheses of some enzymes or proteins participating in the process of cortisol synthesis, such as cholesterol ester hydrolase, which hydrolyses cholesterol ester to cholesterol (Boyd & Gorban 1980) or a steroidogenic acute regulatory protein, the import of which into the mitochondria is thought to be a crucial event promoting cholesterol transfer to the membrane (Clark et al 1994). IFNs inhibit the expression of several genes and can reduce the level of the correspondent proteins (Douglas 1990; Sen 1992; Awatsuji et al 1995). It has recently been found that IFN-α stimulates a Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway via its receptor and the activated STATs form the IFN-stimulated gene factor, which activates gene transcription (Pellegrini & Dusanter-Fourt 1997). A very recent report has shown that STAT2 binds tightly to cyclic AMP response element-binding protein (CREB)-binding protein (CBP) and contributes to IFN-α nuclear signaling (Bhattacharya et al 1996; Yoneyama et al 1998). It is known that in adrenal cortex ACTH-induced cyclic AMP activates CREB which requires CBP as a co-activator and increases the gene transcriptions of the steroidogenesis enzymes (Waterman & Bischof 1997). Therefore, it is very interesting to speculate that the signal transduction pathway stimulated by IFN-α might interact with that stimulated by ACTH through CBP as a common messenger. This might be one reason why IFN-α-2b suppresses ACTH-induced cortisol synthesis. Studies on the intracellular mechanism of the IFN-α-2b-induced inhibitory effect are now under investigation.

Recent studies have shown that in adrenal fasciculata ACTH increases the intracellular Ca^{2+} concentration and cortisol production and that both increases are blocked or abolished by an L-type Ca^{2+}-channel blocker or by removal of external Ca^{2+} (Kimoto et al 1996). These findings indicate that Ca^{2+} influx into the cells is critical, and that both Ca^{2+} and cyclic AMP are essential second messengers for stimulation of ACTH-induced cortisol synthesis (Birmingham et al 1953; Cheitlin et al 1985), although the action site of Ca^{2+} is not well understood. The IFN-α-2b effect was not altered by increases in the external Ca^{2+} concentration (Figure 5B). Therefore, IFN-α-2b might influence the stimulation of cortisol synthesis mediated by Ca^{2+} rather than the Ca^{2+} influx induced by ACTH.

As mentioned above, IFNs have been reported to regulate the functions of the hypothalamus and pituitary in the hypothalamo-pituitary-adrenocortical axis. IFN-α has been shown to increase blood levels of ACTH and cortisol in man (Scott et al 1983; Roosth et al 1986; Muller et al 1991) and Menzies et al (1996) have demonstrated that natural rat IFN-α and β and recombinant IFN-α from man augment concentrations of ACTH and corticosterone in rat peripheral plasma. Saphier et al (1993) have observed that administration to rats of recombinant IFN-α from man inhibits corticosterone secretion. In this study, however, rather high concentrations of IFN-α-2b (300–15 000 units mL^{-1})

were required to inhibit ACTH-induced cortisol production (Figure 1A). Neither IFN-β nor IFN-γ affected cortisol production, although IFN-β and IFN-γ can modulate pituitary function (Malarkey & Zvara 1989; Vankelecom et al 1992; Nolten et al 1993). Thus, it is unlikely that IFNs as immunotransmitters or drugs directly affect the steroidogenesis of adrenal fasciculata in-vivo. Therefore, it is highly probable that IFN-α regulates CRH and ACTH release from the hypothalamus and pituitary glands, respectively, and consequently (indirectly) controls glucocorticoid production in the adrenal cortex. However, specific receptors for IFNs have been found in many mammalian cells (Zoon & Arnheiter 1984), including those of the adrenal glands (Chambers et al 1990). Further, the lower concentrations (300–1500 units mL^{-1}) of IFN-α-2b inhibiting cortisol production in this study are very similar to those having biological effects on other tissues (Meldolesi et al 1977; Yap et al 1986; Pfeffer et al 1990; Reich & Pfeffer 1990; Yamazaki et al 1993). Taken together, these results suggest that IFN-α-2b might bind to the receptors and have inhibitory effect in the fasciculata cells. Therefore, the possibility that IFN-α has direct action on the adrenal cortex in-vivo cannot be completely discounted, although it is not clear why such high concentrations (>10 000 units mL^{-1}) of IFN-α-2b are required for the marked effect on cortisol production. Further study is needed.

In conclusion, recombinant IFN-α-2b from man, but not fibroblast IFN-β and recombinant IFN-γ from man, reduced ACTH-induced production of cortisol in bovine adrenal fasciculata cells by affecting an early step in the process of cortisol synthesis. However, IFN-α might not directly affect glucocorticoid production in the adrenal cortex in-vivo, because of the high IFN-α-2b concentrations required for inhibition.

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