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Interleukin-6 and ACTH act synergistically to stimulate the release of corticosterone from adrenal gland cells

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SUMMARY

We investigated whether interleukin-6 (IL-6) could cause the release of corticosterone by a direct interaction with the adrenal gland. Primary cultures of rat adrenal glands were obtained by dispersion with collagenase and incubated for 24 h with different doses of IL-6. Levels of corticosterone were measured by competitive protein binding assay. A significant ($P < 0{\cdot}025$) dose-dependent increase in corticosterone levels was seen at all doses used. Time course experiments demonstrated that IL-6 stimulated corticosterone release over a period of 24 h but not after 12 or 3 h. The stimulation of adrenal cells with different doses of $ACTH_{1-24}$ and 40 U/ml of IL-6 showed a synergistic effect when IL-6 was combined with low concentrations of $ACTH_{1-24}$ (2 and 20 pmol/*l*). This effect was not evident at higher doses. Our results suggest that IL-6 may act at different levels of the hypothalmic pituitary adrenal axis. Moreover the finding of a synergistic effect with $ACTH_{1-24}$ indicates that IL-6 could play a role in the long term response to stress.

Keywords interleukin-6 corticosterone adrenocorticotropin (ACTH)

INTRODUCTION

During the course of an immune response hormonal changes occur including an increase in corticosterone levels (Besedovsky *et al.*, 1975). Conversely, the activation of the neuroendocrine system during stress leads to changes in immune function, such as inhibition of the production and action of several cytokines (Snyder & Unanue, 1982; Lee *et al.*, 1988). These observations suggest that a bi-directional communication pathway exists between the immune system and the hypothalamic pituitary adrenal axis. Among the mediators that might play a role in this interaction are the cytokines. Interleukin-1 (IL-1) has been demonstrated to stimulate the release of ACTH, acting on hypothalmic and pituitary cells (Besedovsky *et al.*, 1986; Sapolsky *et al.*, 1987; Bernton *et al.*, 1987; Kehrer *et al.*, 1988; Tsagarakis *et al.*, 1989). It has also been reported that IL-1 is able to induce corticosterone release directly from adrenal gland cells (Roh *et al.*, 1987; Whitcomb *et al.*, 1988), although this observation is contradicted by other reports (Woloski *et al.*, 1985; Sapolsky *et al.*, 1987). Several other cytokines are released during the course of the inflammatory response, and one, interleukin-6 (IL-6), has been shown to play a major role in the regulation of the acute phase response (Ramadori *et al.*, 1988; Castell *et al.*, 1988). Since IL-1 is a potent inducer of IL-6 production in many cell types (Helle *et al.*, 1988; Walther, May & Seghal, 1988), it is possible that the corticosterone-releasing activity which has been attributed to IL-1 is mediated by IL-6. The aim of our study was to investigate whether IL-6 itself could cause the release of corticosterone by acting directly on adrenal gland cells.

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MATERIALS AND METHODS

Animals

Male Wistar rats weighing 180–200 g were maintained in a temperature-controlled environment with 12-h light/dark cycles. Food and water were freely available. All experiments were started between 9 and 12 AM.

Reagents

Collagenase type IA was purchased from Sigma Chemical Company (St Louis, MO). Fetal calf serum (FCS), penicillin and streptomycin were obtained from GIBCO (Paisley, UK). Ham's nutrient F12 medium was purchased from Imperial Laboratories (Andover, UK). Tetracosactrin ($ACTH_{1-24}$, Synacthen) was purchased from Ciba (Sussex, UK). Recombinant human IL-6 (specific activity $5{\cdot}2 \times 10^3$ U/μg) (Hirano *et al.*, 1986) was a gift from Dr T. Hirano.

Experimental procedure

The animals were killed by decapitation and the adrenal glands were immediately removed. Preparation and incubation of adrenal cells were carried out in Ham's nutrient F12 medium

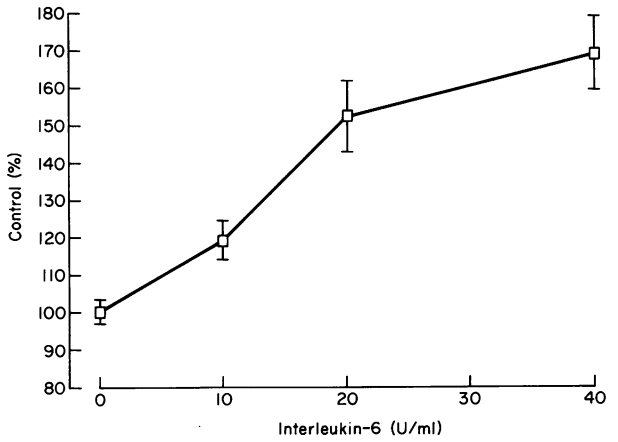

Fig. 1. Effect of interleukin-6 on corticosterone release by adrenal cells. Data are expressed as a percentage of the corticosterone values present in control cultures. Each point is mean ± s.e.m. of three experiments.

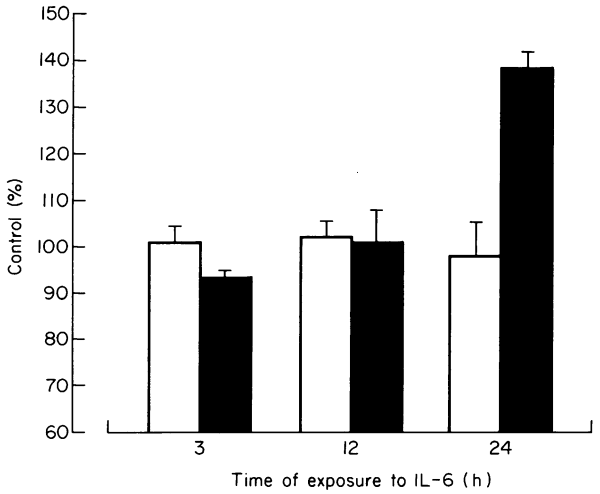

Fig. 2. Time course of interleukin-6 (IL-6) induced corticosterone release. All cells were incubated for 24 h. IL-6 (■) or medium (□) was added at 0, 12 or 21 h. In each experiment, the mean corticosterone production of the controls (at all three times) was taken as 100%. Data from three independent experiments are combined. Bars are s.e.m.

supplemented with antibiotics and 10% charcoal-treated FCS (Whitcomb *et al.*, 1988). Adrenal cells were obtained by digestion with 1 mg/ml collagenase at 37°C for 40 min. The cell suspension was filtered through a nylon gauze (100 μm) washed twice and resuspended in medium. The cells were placed in 24-well plates (Falcon 3047, Becton Dickinson, Lincoln Park, NJ) at a density of $2{\cdot}3 \times 10^5$ cells/well in a volume of 0·9 ml, and then incubated at 37°C under a humidified atmosphere of 5% CO_2 in air. Additions of IL-6, medium or $ACTH_{1-24}$ were made in 0·1 ml. Cell viability was >85% at the beginning of all experiments, as estimated by trypan blue exclusion.

Corticosterone assay

Corticosterone was measured using a competitive protein binding assay in which diluted pooled plasma was the source of corticosteroid binding protein (Murphy, 1967).

Statistical analysis

Due to differences between the activities of different cell preparations, statistical significances of changes were evaluated by analysis of variance using data from all the experiments.

RESULTS

Effect of IL-6 on corticosterone release

To investigate whether IL-6 was able to stimulate the release of corticosterone, primary cultures of adrenal gland cells were stimulated for 24 h with 10, 20 and 40 U/ml of IL-6 (Fig. 1). Analysis of variance of data from three experiments showed significant increases of corticosterone at each dose of IL-6 ($P<0{\cdot}025$ at 10 U/ml; $P<0{\cdot}001$ at other concentrations). In each experiment a significant increase was found, although the maximal stimulation achieved with IL-6 was variable from 23 to 100% above control.

Time dependence of IL-6-induced corticosterone release

Adrenal cells were cultured for a final period of 24 h. IL-6 (40 U/ml) or medium were added at 0, 12 or 21 h, the cells being exposed to the cytokine for 24, 12 or 3 h, respectively (Fig. 2). Analysis of variance of data from three experiments showed a significant increase in corticosterone release ($P<0{\cdot}01$) only in those cultures stimulated for 24 h.

Table 1. Effect of interleukin-6 (IL-6) on corticosterone production in the absence or presence of $ACTH_{1-24}$

$ACTH_{1-24}$ (pmol/*l*)	Stimulation due to IL-6*	Stimulation due to ACTH†
0	1·27 ± 0·04	
2–20	1·66 ± 0·09‡	0·8–3·3
200–2000	0·98 ± 0·05	2·6–36

* Corticosterone production with IL-6/ production without IL-6. Each point is the mean ± s.e.m. of three combined experiments.

† Corticosterone production with ACTH/ production without ACTH. Data show the range of stimulation obtained from three experiments.

‡ $P<0{\cdot}001$ compared with stimulation due to IL-6 alone.

Interaction between ACTH and IL-6 on corticosterone release

Since the major physiological stimulus for corticosterone release is believed to be the ACTH released by the pituitary gland, we investigated a possible interaction between IL-6 and $ACTH_{1-24}$ on corticosterone release. Cell cultures were stimulated for 24 h with concentration of $ACTH_{1-24}$ from 2 pmol/*l* to 20 nmol/*l* alone or in combination with 40 U/ml of IL-6. As shown in Table 1, there was a synergistic effect of $ACTH_{1-24}$ and IL-6 at physiological $ACTH_{1-24}$ concentrations (i.e. 2 pmol/*l* and 20 pmol/*l*). The data are expressed as stimulation due to IL-6 (i.e. corticosterone production with IL-6 and corticosterone production without IL-6) in the absence or presence of

$ACTH_{1-24}$. At higher concentrations of $ACTH_{1-24}$, where stimulation approaches maximal, IL-6 had no further effect.

DISCUSSION

The results presented demonstrate that IL-6 stimulates the release of corticosterone from rat adrenal cells *in vitro*. Moreover, this cytokine is able to act synergistically with low doses of $ACTH_{1-24}$. This finding is particularly interesting in view of possible implications for the stress response. Following severe trauma such as burns or bone fractures, plasma adrenal corticosteroids levels rise within minutes. This coincides with an increase in ACTH concentrations which is presumably its cause. However, in humans, several days after trauma there is a marked lack of correlation between levels of ACTH and the high concentrations of cortisol still present at that time (Vaughan *et al.*, 1982; Barton & Stoner, 1987). These observations suggest that factors other than ACTH contribute to the control of the long-term adrenal response. The stimulatory effect of IL-6 we describe here was not present within the first 12 h but only at 24 h. Since serum levels of IL-6 have also been shown to rise and remain elevated for several days following severe trauma (Nijsten *et al.*, 1987), this cytokine may be one of the factors controlling the long-term adrenal response.

Our findings are in contrast with those reported by Woloski *et al.* (1985), who were unable to induce corticosterone release from the adrenocortical tumour-derived cell line Y1 using purified hepatocyte-stimulating factor (HSF, now recognized to be IL-6; Gauldie *et al.*, 1987). The use of a tumour cell line which might have different characteristics from the normal tissue could explain this discrepancy.

It has been reported that i.v. injection of IL-6 in rats increases the levels of ACTH in plasma and that this effect could be completely abolished by a previous injection of antiserum againt corticotropin-releasing hormone, indicating that one site of action of IL-6 is at hypothalamic level or higher (Naitoh *et al.*, 1988). Our results demonstrate that IL-6 also stimulates adrenal cells directly. Thus, IL-6 acts at more than one level of the hypothalamic pituitary adrenal axis.

Most of the corticosterone from the rat adrenal comes from the zona fasciculata/reticularis. A smaller amount is secreted by the functionally distinct zona glomerulosa. In preliminary experiments, partially purified zona glomerulosa cells showed no response to IL-6, suggesting that the stimulation is confined to the zona fasciculata/reticularis. However, we can not exclude the possibility that other cell types, also present in the cell preparation, are involved in this response. For example, the preparation could contain cells from adrenal medulla, one product of which, adrenaline, stimulates corticosterone release (Walker *et al.*, 1988).

The variable response to IL-6 suggests a sensitivity to some aspect of cell preparation. Microscopic examination of cells showed no obvious differences. It may be that expression of the IL-6 receptor is unstable or, as suggested above, that another cell type present as a variable contaminant has an important role.

The stimulation of purified fasciculata, reticularis and glomerulosa cells and the characterization of IL-6 receptors on those cells will enable us to understand the mechanism of this effect better.

REFERENCES

Barton, R.N. & Stoner, H.B. (1987) Why does a high cortisol concentration persist after fracture of the femoral neck in the elderly? *Circ. Shock*, **21**, 292.

Bernton, E.W., Beach, J.E., Holaday, J.W., Smallridge, R.C. & Fein, H.G. (1987) Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. *Science*, **238**, 519.

Besedovsky, H., Del Rey, A., Sorkin, E. & Dinarello, C.A. (1986) Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science*, **233**, 652.

Besedovsky, H., Sorkin, E., Keller, M. & Muller, J. (1975) Changes in blood hormone levels during the immune response. *Proc. Soc. exp. Biol. Med.* **150**, 466.

Castell, J.V., Gomez-Lechon, M.J., David, M., Hirano, T., Kishimoto, T. & Heinrich, P.C. (1988) Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes. *FEBS Lett.* **232**, 347.

Gauldie, J., Richards, C., Harnish, D., Lansdorp, P. & Baumann, H. (1987) Interferon β_2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc. natl Acad. Sci. USA*, **84**, 7251.

Helle, M., Brakenhoff, J.P.J., De Groot, E.R. & Aarden, L.A. (1988) Interleukin 6 is involved in interleukin 1-induced activities. *Eur. J. Immunol.* **18**, 957.

Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K., Iwamatsu, A., Tsunasawa, S., Sakiyama, F., Matsui, H., Takahara, Y., Taniguchi, T. & Kishimoto, T. (1986) Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature*, **324**, 73.

Kehrer, P., Turnill, D., Dayer, J.M., Muller, A.F. & Gaillard, R.C. (1988) Human recombinant interleukin-1 beta and alpha, but not recombinant tumour necrosis factor alpha stimulate ACTH release from rat anterior pituitary cells in vitro in a prostaglandin E_2 and cAMP independent manner. *Neuroendocrinology*, **48**, 160.

Lee, S.W., Tsou, A.P., Chan, H., Thomas, J., Petrie, K., Eugui, E.M. & Allison, A.C. (1988) Glucocorticoids selectively inhibit the transcription of the interleukin 1β gene and decrease the stability of interleukin 1β mRNA. *Proc. natl Acad. Sci. USA*, **85**, 1204.

Murphy, B.E.P. (1967) Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. *J. clin. Endocrinol. Metab.* **27**, 973.

Naitoh, Y., Fukata, J., Tominaga, T., Nakai, Y., Tamai, S., Mori, K. & Imura, H. (1988) Interleukin-6 stimulates the secretion of adrenocorticotropic hormone in conscious, freely-moving rats. *Biochem. Biophys. Res. Commun.* **155**, 1459.

Nijsten, M.W.N., de Groot, E.R., Ten Duis, H.J., Klasen, H.J., Hack, C.E. & Aarden, L.A. (1987) Serum levels of interleukin-6 and acute phase responses. *Lancet*, **ii**, 921.

Ramadori, G., Van Damme, J., Rieder, H. & Meyer Zum Buschenfelde, K.H. (1988) Interleukin 6, the third mediator of acute-phase reaction, modulates hepatic protein synthesis in human and mouse. Comparison with interleukin 1β and tumour necrosis factor-α. *Eur. J. Immunol.* **18**, 1259.

Roh, M.S., Drazenovich, K.A., Barbose, J.J., Dinarello, C.A. & Cobb, C.F. (1987) Direct stimulation of the adrenal cortex by interleukin-1. *Surgery*, **102**, 140.

Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P. & Vale, W. (1987) Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science*, **238**, 522.

Snyder, D.S. & Unanue, E.R. (1982) Corticosteroids inhibit murine macrophage Ia expression and interleukin 1 production. *J. Immunol.* **129**, 1803.

TSAGARAKIS, S., GILLIES, G., REES, L.H., BESSER, M. & GROSSMAN, A. (1989) Interleukin-1 directly stimulates the release of corticotrophin releasing factor from rat hypothalamus. *Neuroendocrinology*, **49,** 98.

VAUGHAN, G.M., BECKER, R.A., ALLEN, J.P., GOODWIN, C.W., PRUITT, B.A. & MASON, A.D. (1982) Cortisol and corticotrophin in burned patients. *J. Trauma*, **22,** 263.

WALKER, S.W., LIGHTLY, E.R.T., MILNER, S.W. & WILLIAMS, B.C. (1988) Catecholamine stimulation of cortisol secretion by 3-day primary cultures of purified zona fasciculata/reticularis cells isolated from bovine adrenal cortex. *Mol. Cell. Endocrinol.* **57,** 139.

WALTHER, Z., MAY, L.T. & SEGHAL, P.B. (1988) Transcriptional regulation of the interferon- β_2/B cell differentiation factor BSF-2/hepatocyte-stimulating factor gene in human fibroblasts by other cytokines. *J. Immunol.* **140,** 974.

WHITCOMB, R.W., LINEHAN, W.M., WAHL, L.M. & KNAZEK, R.A. (1988) Monocytes stimulate cortisol production by cultured human adrenocortical cells. *J. clin. Endocrinol. Metab.* **66,** 33.

WOLOSKI, B.M.R.N.J., SMITH, E.M., MEYER, W.J. III, FULLER, G.M. & BLALOCK, J.E. (1985) Corticotrophin-releasing activity of monokines. *Science*, **230,** 1035.