Pergamon

Biochemical Pharmacology, Vol. 48, No. 11, pp. 2065–2071, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00414-5

MODULATION OF ENDOTHELIN-1 PRODUCTION BY A PULMONARY EPITHELIAL CELL LINE

I. REGULATION BY GLUCOCORTICOIDS

EDUARDO CALDERÓN,* CELSO E. GÓMEZ-SÁNCHEZ, EDUARDO N. COZZA, MINGYI ZHOU, RONALD G. COFFEY, RICHARD F. LOCKEY, LEON D. PROCKOP and ANDOR SZENTIVANYI

Departments of Internal Medicine, Pharmacology and Therapeutics and Neurology, University of South Florida, College of Medicine; and James A. Haley Veterans Administration Hospital, Tampa, FL, U.S.A.

(*Received* 23 *June* 1994; *accepted* 4 *August* 1994)

Abstract—Endothelin-1 (ET-1) is one of the most potent bronchoconstrictor agents yet described. Bronchial epithelial cells of asthmatic patients *in vivo* express preproET-1 and *in vitro* release high amounts of ET-1. Healthy and chronic bronchitic controls do not express preproET-1 or release ET-1. Interleukin-2 (IL-2) and other cytokines up-regulate the *in vitro* ET-1 release in guinea pig airway epithelial cells. We explored whether two glucocorticoids, dexamethasone (Dex) and triamcinolone acetonide (TA), inhibit the synthesis and release of ET-1 by A549 cells, a transformed human pulmonary epithelial cell line, since ET-1 may have a basic role in the pathogenesis of asthma. Cells were grown to confluence in RPMI 1640 plus 10% fetal bovine serum (FBS). Cells were then cultured for 3 days without serum to obtain ET-1 basal levels. The effects of 10% FBS, IL-2 (10 U/mL), Dex, TA or mifepristone, a steroid antagonist (1, 10 or 100 nM), were evaluated on ET-1 as measured by radioimmunoassay (RIA). ET-1 production increased from 57.6 ± 5 pg/mg cell protein at 6 hr to 170 ± 9 pg/mg cell protein at 72 hr in control cultures. Ten percent FBS increased ET-1 production from 58.7 ± 9.6 to 399 ± 14.5 pg/mg cell protein. IL-2 significantly increased ET-1 from 100.7 ± 6.1 to 144 ± 6.7 at 24 hr and from 170 ± 9 to 207.7 ± 24 at 72 hr. Dex and TA (10 and 100 nM) at 24–72 hr decreased ET-1 under basal conditions. Both drugs (only at 100 nM) decreased ET-1 production in 10% FBS- and IL-2-stimulated cells. Mifepristone (10 and 100 nM) reversed the decreased production of ET-1 induced by Dex (100 nM) at 24–72 hr. Northern blot analysis showed that Dex (100 nM) decreased the expression of ET-1 mRNA at 6 and 24 hr, but that mifepristone (100 nM) reversed this effect in cells cultured with Dex. In conclusion, Dex and TA down-regulate the synthesis and production of ET-1 by this human pulmonary epithelial cell line under basal or stimulated conditions, and these effects are reversed by mifepristone. These findings suggest a novel mechanism of glucocorticoid effect during the treatment of asthma.

Key words: endothelin; glucocorticoids; mifepristone; A549 cells; asthma; interleukin-2

ET-1†, one of the most potent smooth muscle constrictor agents currently known, is one of the four peptides of the family of endothelins and was first isolated from porcine vascular endothelial cells. In human lungs, the main nonvascular source of ET-1 synthesis appears to be bronchial epithelial cells. High-affinity binding sites for ET-1 are found on alveolar septa, nerve trunks and bronchial smooth muscle cells [1]. The latter, when stimulated, lead to bronchoconstriction [2]. ET-1 is a potent activator of human alveolar macrophages increasing intracellular [Ca^{2+}], phosphorylation of several proteins, and superoxide production to an extent comparable with the response induced by the chemotactic peptide FMLP and PAF [3]. ET-1 is produced by airway epithelial cells and is present in higher levels in the bronchoalveolar lavage of asthmatic patients when compared with chronic bronchitic or healthy controls [4].

A549 pulmonary type II-like epithelial cells (American Type Culture Collection, Rockville, MD) are a cell line derived from a patient with lung carcinoma [5]. It is reasonable to assume that they behave in some ways like normal pneumocytes since they retain many features of type-II alveolar epithelial cells.

This is the first of a series of papers to explore the regulation of ET-1 production in human pulmonary epithelial cells. In this study using A549 cells, we examined the regulation of ET-1 production and its mRNA expression by Dex and TA since glucocorticoids are preeminent anti-inflammatory agents and there is no single activity that explains their pharmacologic effects in the therapy of asthma.

* Corresponding author: Dr. Eduardo Calderón, Division of Allergy and Clinical Immunology, University of South Florida, c/o VA Medical Center, 13000 Bruce B. Downs Blvd. (VAR 111D), Tampa, FL 33612–4745. Tel. (813)972–7631; FAX (813)972–7623.

† Abbreviations: ET-1, endothelin-1; IL, interleukin; Dex, dexamethasone; TA, triamcinolone acetonide; FMLP, formylmethionylleucylphenylalanine; PAF, platelet-activating factor; RIA, radioimmunoassay; PEG, polyethylene glycol; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; HETE, hydroxyeicosatetraenoic acid; TNF-α, tumor necrosis factor-α; GM-CSF, granulocyte macrophage-colony stimulating factor; hsp90, 90 kDa heat shock protein; and GRE, glucocorticoid response element.

MATERIALS AND METHODS

Reagents. ET-1 was purchased from Peninsula Laboratories (Belmont, CA). Human recombinant IL-2 was obtained from the Genzyme Corp. (Cambridge, MA). Dex, TA, RPMI 1640 cell culture medium and FBS were purchased from the Sigma Chemical Co. (St. Louis, MO). Mifepristone, or RU38486, was a gift from Roussell UCLAF Laboratories, France. Rabbit IgG anti-ET-1 and goat anti-rabbit IgG antiserum were developed in our laboratories [6].

Human cell culture. A549 cells were seeded in 24-well cell culture dishes obtained from the Costar Corp. (Cambridge, MA) to measure the production of ET-1, at three different times, and in 6-well cell culture dishes to study the expression of mRNA of ET-1. Cells were incubated at 37°, 5% CO_2/95% air, grown to confluence in RPMI 1640 plus 10% FBS (3–4 days), and were then cultured for 3 days in serum-free RPMI 1640. Cells were incubated with Dex, TA or mifepristone at concentrations of 1, 10 or 100 nM in serum-free RPMI 1640 or RPMI 1640 with 10% FBS. Cell viability was more than 98% as determined by the trypan blue exclusion test.

Total protein. Total cell protein was measured by the method of Lowry *et al.* [7] after dissolving samples in 1N NaOH.

Radioimmunoassay of ET-1. The concentration of ET-1 was assayed by RIA at room temperature in culture supernatants withdrawn at 24, 48 and 72 hr. The concentrations of ET-1 were measured in the extracted supernatant by comparison with an ET-1 standard. The assay buffer, rabbit IgG anti-ET-1 (final dilution 1:2000), goat anti-rabbit IgG antiserum (final dilution 1:60) and the ^{125}I tracer (8000 cpm/tube) were added to standard and experimental samples. After overnight incubation, the labeled antibody complex was precipitated with 10% PEG in PBS and centrifuged at 4000 *g* for 30 min at 4° [6]. Supernatants were discarded and bound radioactivity in the precipitate was measured in a gamma counter.

Northern blot analysis. Total cellular RNA from A549 cells was obtained using the RNAzol™ B method (Cinna/Biotecx Laboratories International, Inc., Friendswood, TX). After electrophoresis through agarose/formaldehyde gel, the mRNA was transferred to Zeta-Probe®GT (Bio-Rad, Richmond, CA) membrane and hybridized with denatured ^{32}P-labeled cDNA probes of 1.2 kb for ET-1 (supplied by Dr. Kenneth D. Bloch) [8–10]. Autoradiograms were prepared.

Statistical analysis. The amount of ET-1 of each supernatant was expressed in picograms per milligram of total cell protein. Samples were prepared in triplicates, and data are reported as means ± SD. Differences of ET-1 production were analyzed by the use of Student's *t*-test for unpaired data. All analyses were two-tailed [11]. Alpha was set at 0.05.

RESULTS

Time course of ET-1 production by unstimulated and 10% FBS-stimulated A549 cells. ET-1 cell culture supernatants were withdrawn at 6, 12, 24, 48 and 72 hr (Table 1). Extracellular ET-1 production increased from 57.6 ± 5 to 170 ± 9 pg/mg of cell protein in control cultures and from 58.7 ± 9.6 to 399 ± 14.5 in FBS-stimulated cultured cells. After 12 hr of incubation, ET-1 production in the group stimulated with FBS was significantly higher than that of unstimulated cells.

Table 1. Extracellular ET-1 produced by A549 epithelial cells cultured in RPMI 1640 without (control) or with 10% fetal bovine serum (FBS)

Time (hr)	Extracellular ET-1 produced (pg/mg cell protein)		N	P
	Control	10% FBS		
6	57.6 ± 5	58.70 ± 9.6	3	NS*
12	61.6 ± 6	101.0 ± 5	3	<0.005
24	87.5 ± 12	143.8 ± 5.7	3	<0.0001
48	129 ± 17.3	272.0 ± 30	3	<0.0001
72	170 ± 9	399.0 ± 14.5	3	<0.0001

Values are means ± SD.
* Not significant.

Time course of ET-1 production by cells incubated with serum-free RPMI 1640 in the presence of Dex or TA. Supernatants were withdrawn at 24, 48 and 72 hr. Both Dex (Fig. 1) and TA (Fig. 2) at 10 and 100 nM concentrations (but not at 1 nM, data not shown) decreased the extracellular ET-1 production in each time period. Cells incubated with Dex or TA at 100 nM concentrations caused the greatest effect, showing that this inhibition is also concentration dependent. The effects of both glucocorticoids on ET-1 production were basically similar at the same molar concentrations.

Time course of ET-1 production by cells incubated with 10% FBS RPMI 1640 in the presence of Dex or TA. Supernatants were withdrawn at 24, 48 and 72 hr. Extracellular ET-1 production was reduced significantly by either Dex or TA (Table 2) at 10 nM at 24 hr and at 100 nM concentrations from 24 to 72 hr. Both glucocorticoids, at 1 nM, did not reduce ET-1 production (data not shown). This shows that down-regulation induced by Dex and TA is both concentration and time dependent.

Effects of Dex or TA on the time course of ET-1 production by cells incubated with RPMI 1640 and IL-2. Supernatants were withdrawn at 24, 48 and 72 hr. IL-2 significantly increased ET-1 from 100.7 ± 6.1 to 144 ± 6.7 at 24 hr, from 153.2 ± 16 to 183 ± 16 at 48 hr and from 170 ± 9 to 207.7 ± 24 at 72 hr. Extracellular ET-1 production from IL-2-stimulated cells was clearly decreased by both Dex and TA (Table 3) at 100 nM concentrations at 24–72 hr. Cells cultured with Dex or TA (100 nM) decreased the time-dependent ET-1 production compared with the control groups.

Time course of ET-1 production by cells incubated with Dex (100 nM) in the presence of mifepristone. Supernatants were withdrawn at 24, 48 and 72 hr. Mifepristone at 100 nM reversed the down-regulated ET-1 production induced by Dex at 24–72 hr, whereas (10 nM) mifepristone did not affect the

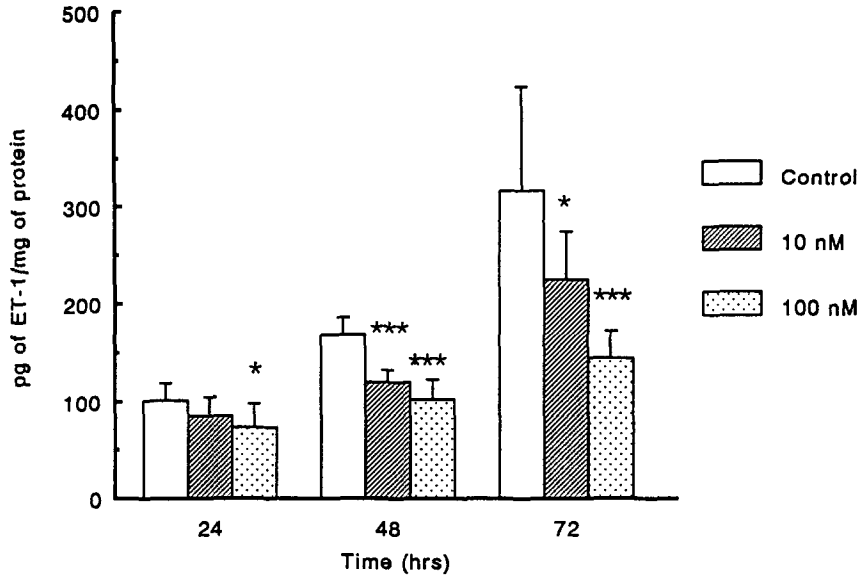

Fig. 1. Time course of ET-1 production by A549 cells incubated with serum-free RPMI 1640 in the presence of dexamethasone (Dex). In each time period, the first bar represents the control and the other two, Dex. The second bar is for Dex at 10 nM and the third for Dex at 100 nM. Three experiments were performed in triplicate. Values are means ± SD. Key: (*) $P < 0.05$, and (***) $P < 0.0001$.

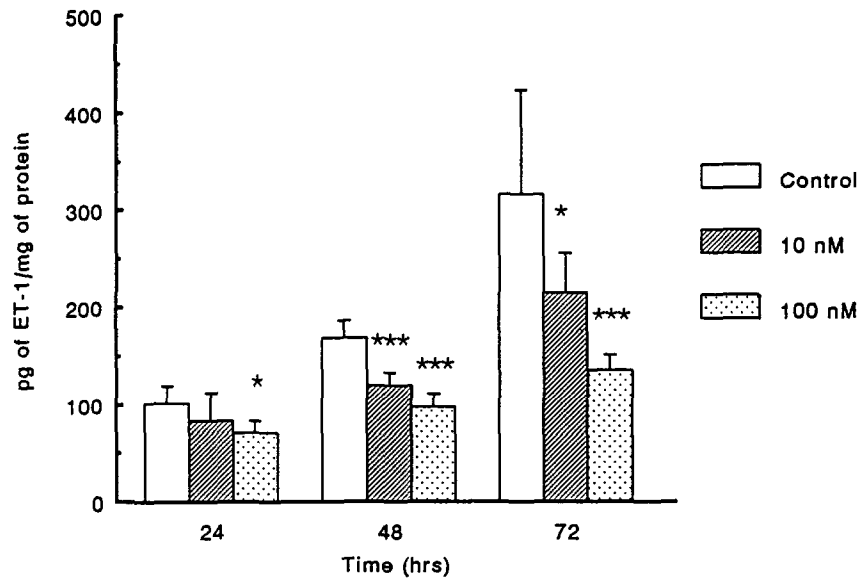

Fig. 2. Time course of ET-1 production by A549 cells incubated with serum-free RPMI 1640 in the presence of triamcinolone acetonide (TA). In each time period, the first bar represents the control and the other two, TA. The second bar is for TA at 10 nM and the third for TA at 100 nM. Three experiments were performed in triplicate. Values are means ± SD. Key: (*) $P < 0.05$, and (***) $P < 0.0001$.

down-regulation induced by Dex at 72 hr (Fig. 3). Mifepristone (1, 10, 100 nM) did not modify ET-1 production in unstimulated A549 cells (data not shown).

Effects of dexamethasone and mifepristone on accumulation of ET-1 mRNA. To further define the mechanism by which glucocorticoids inhibit ET-1 production, we estimated the effect of Dex (100 nM) on the level of ET-1 mRNA in A549 cells (Fig. 4, top). Dex markedly decreased the levels of mRNA

Table 2. Extracellular ET-1 produced by A549 cells cultured in RPMI 1640 with 10% fetal bovine serum (control) or in the presence of dexamethasone or triamcinolone acetonide

Time (hr)	Extracellular ET-1 produced (pg/mg cell protein)					
		Dexamethasone		Triamcinolone acetonide		
	Control	100 nM	10 nM	100 nM	10 nM	N
24	183 ± 30	141 ± 31*	159 ± 31†	121 ± 54*	152 ± 45†	3
48	310 ± 57	198 ± 58‡	269 ± 94	273 ± 14*	313 ± 36	3
72	476 ± 80	252 ± 61‡	426 ± 39	288 ± 17‡	426 ± 16	3

Values are means ± SD.
* $P < 0.01$.
† $P < 0.05$.
‡ $P < 0.001$.

Table 3. Extracellular ET-1 produced by A549 cells cultured in RPMI 1640 and IL-2 (control) or in the presence of dexamethasone or triamcinolone acetonide

Time (hr)	Extracellular ET-1 produced (pg/mg cell protein)			
	Control	Dexamethasone (100 nM)	Triamcinolone acetonide (100 nM)	N
24	144.0 ± 6.7	102.0 ± 17*	119.0 ± 12*	3
48	183.0 ± 16	119.0 ± 38*	150.0 ± 24†	3
72	207.7 ± 24	129.0 ± 61*	130.0 ± 17‡	3

Values are means ± SD.
* $P < 0.01$.
† $P < 0.05$.
‡ $P < 0.001$.

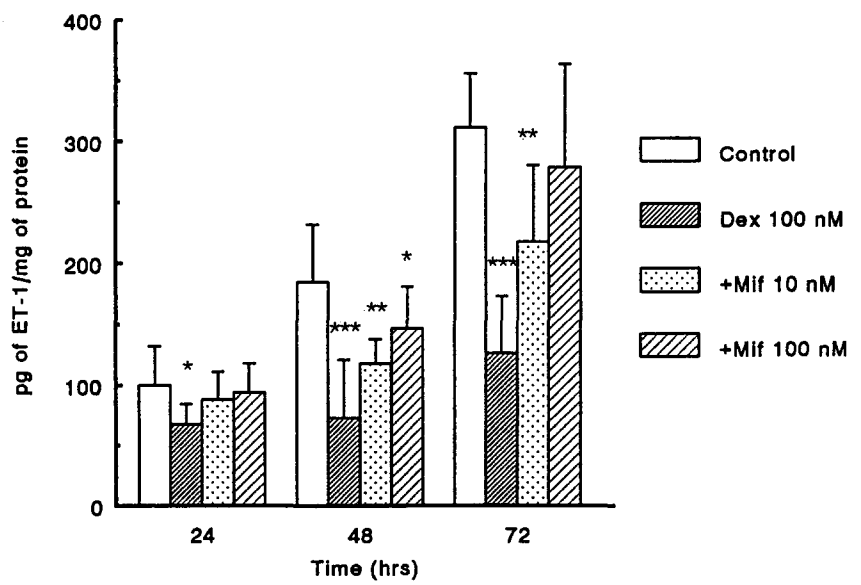

Fig. 3. Time course of ET-1 production by A549 cells incubated with 100 nM Dex, in the presence of mifepristone. In each time period, the first bar represents the control and the other three 100 nM Dex. The second bar is for Dex alone, the third Dex and mifepristone at 10 nM and the fourth Dex and mifepristone at 100 nM. Three experiments were performed in triplicate. Values are means ± SD. Key: (*) $P < 0.05$, (**) $P < 0.001$, and (***) $P < 0.0001$.

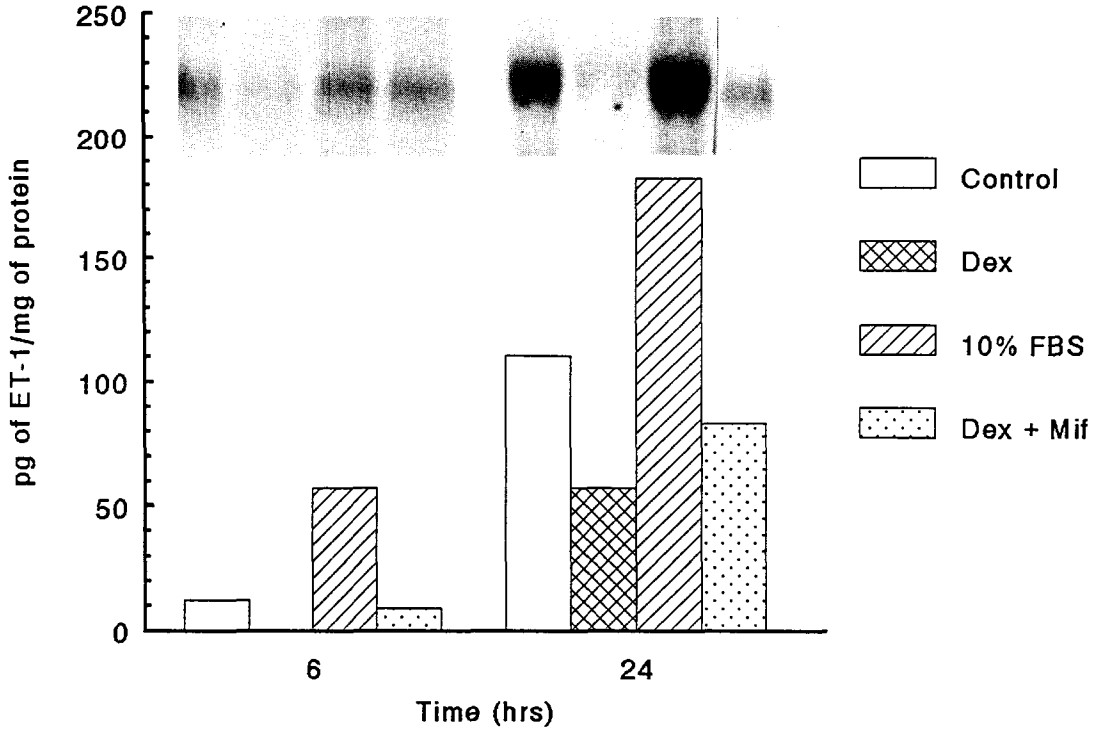

Fig. 4. Kinetic expression of ET-1 mRNA from A549 cells at 6 and 24 hr of incubation with (a) serum-free RPMI 1640, (b) 100 nM Dex, (c) 10% FBS–RPMI 1640, or (d) 100 nM Dex and 100 nM mifepristone. Top: Northern blot analysis of ET-1 mRNA expression. Bottom: ET-1 produced by respective cells.

ET-1 at 6 and 24 hr. Treatment with 10% FBS stimulated an increase in detectable mRNA for ET-1. In contrast, in cells incubated with Dex and mifepristone (both at 100 nM), ET-1 mRNA was preserved. This observation suggests that glucocorticoids act at the transcriptional level, affecting the stability of mRNA for ET-1. In the supernatants of these groups of cells (Fig. 4, bottom), ET-1 production correlated well with the expression of mRNA ET-1.

DISCUSSION

The study of pharmacologic agents that modulate ET-1 production may provide valuable information about specific treatments of allergic diseases, since ET-1 appears to be a paracrine hormone that modulates airway inflammatory responses. Human bronchial, tracheal and nasal epithelial cells produce and express receptors for ET-1 [2, 4, 12]. In nasal mucosa, ET receptors induce serous and mucous cell secretion. ET-1 binds to receptors on primary cultures of feline tracheal epithelial cells and induces the production of arachidonic acid eicosanoid metabolites such as PGE_2, $PGF_{2\alpha}$, 5-HETE and 12-HETE [13]. ET-1 in the guinea pig isolated perfused lung was shown to increase edema formation and stimulate proinflammatory eicosanoid production [14, 15], whereas in rat lung it increased 15-lipoxygenase activity and oxygen radical formation [16]. *In vitro*, ET-1 production is up-regulated by a variety of cytokines with inflammatory properties in allergic diseases, such as IL-2, IL-1, IL-6, IL-8, TNF-α, GM-CSF and others [17].

The present study indicates that two glucocorticoids, Dex and TA, down-regulate ET-1 production in a transformed human pulmonary epithelial cell line. Decreased production of ET-1 occurred at pharmacologic glucocorticoid concentrations (10 and 100 nM Dex or TA). The delay of several hours seen in these experiments prior to the onset of the down-regulation of ET-1 production by Dex and TA is consistent with an effect dependent on new mRNA and/or protein synthesis. Reduction of ET-1 production by glucocorticoids is most probably the result of decreased levels of mRNA transcripts rather than inhibition of translation.

Glucocorticoids enter the cell by passive diffusion and bind to the cytoplasmic receptor, leading to dissociation of the 90-kDa heat shock protein (hsp90) and subsequent translocation to the nucleus of the cell. The receptor binds to specific sites in the genome termed GREs, upstream of the promoter regions of glucocorticoid-responsive genes. Depending on the physiological context, binding of glucocorticoid–receptor complex to the GRE enhances or suppresses transcription of the relevant

gene [18]. The existence of a second class of GREs, the "composite" GREs, which require interaction with other DNA binding proteins such as c-jun and c-fos, components of the transcription factor AP-1, has also been described [19]. Glucocorticoids may also regulate gene expression at post-transcriptional levels by modifying the stability and steady-state levels of mRNAs, such as growth hormone [18].

Glucocorticoids diminished the FBS- and IL-2-increased ET-1 production in A549 cells and also diminished transcription and expression of mRNA ET-1. Since pulmonary epithelial cells have a key role in allergic inflammation, our observations bear directly on the mechanism of the anti-inflammatory effects of glucocorticoids [20].

Mifepristone stabilizes the association of steroid receptor with hsp90 in the presence of ligand, which prevents translocation of glucocorticoid receptors to the nucleus and thereby blocks transcription of genes containing GRE [21–23]. Our demonstration that mifepristone reverses the effects of dexamethasone on ET-1 production and ET-1 mRNA is, therefore, consistent with the hypothesis that the effects of steroids on the epithelium are mediated through glucocorticoid receptors.

Glucocorticoid therapy inhibits the bronchial infiltration of leukocytes during the allergic late phase reaction. This results in inhibition of local production of ET-1, thus preventing bronchial epithelium activation. It is possible, therefore, that among other factors that initiate the accumulation of leukocytes during the late phase response (which may include ET-1), some may be derived from bronchial epithelial cells, which are inhibited from releasing their agents by exposure to steroids [24].

In summary, these results demonstrated that A549 cells synthesize and release ET-1. IL-2 or FBS stimulation increased the synthesis and release of ET-1 in a time-dependent manner. Glucocorticosteroids down-regulated ET-1 production under basal cell conditions or after stimulation in a concentration- and time-dependent manner, in part by diminishing the expression of ET-1 mRNA. Mifepristone, a competitive glucocorticoid receptor antagonist, reversed the decrease of ET-1 production without itself affecting the ET-1 production by allowing the synthesis of ET-1 mRNA. Our findings suggest that (1) ET-1 may be a proinflammatory mediator with a role in the pathogenesis of asthma, where IL-2 and other cytokines are continuously stimulating airway epithelial cells, and (2) during the therapy of asthma, glucocorticoids may have the capability to down-regulate the production of ET-1 by pulmonary epithelial cells, thereby preventing ET-1 actions on its cellular targets, and could help to restore the pulmonary homeostasis by decreasing ET-1 production.

Acknowledgements—The research in this paper was supported, in part, by Grant HL 27737 from the National Institutes of Health (C.E.G.-S.), Medical Research Funds from the Department of Veterans Affairs (C.E.G.-S.) and a grant from The Eleanor Naylor Dana Charitable Trust (A.S.).

REFERENCES

1. Power RF, Wharton J, Zhao Y, Bloom SR and Polak JM, Autoradiographic localization of endothelin-1 binding sites in the cardiovascular and respiratory systems. *J Cardiovasc Pharmacol* **13**: S50–S56, 1989.
2. Mattoli S, Mezzetti M, Riva G, Allegra L and Fasoli A, Specific binding of endothelin on human bronchial smooth muscle cells in culture and secretion of endothelin-like material from bronchial epithelial cells. *Am J Respir Cell Mol Biol* **3**: 145–151, 1990.
3. Haller H, Schaberg T, Lindschau C, Lode H and Distler A, Endothelin increases $[Ca^{2+}]_i$, protein phosphorylation, and O_2^- production in human alveolar macrophages. *Am J Physiol* **261**: L478–L484, 1991.
4. Vittori E, Marini M, Fasoli A, de Franchis R and Mattoli S, Increased expression of endothelin in bronchial epithelial cells of asthmatic patients and effect of corticosteroids. *Am Rev Respir Dis* **146**: 1320–1325, 1992.
5. American Type Culture Collection, *Catalogue of Cell Lines and Hybridomas*, 7th Edn, p. 100. ATCC, Rockville, MD, 1992.
6. Gómez-Sánchez CE, Foecking MF, Ferris MW, Hieda HS and Gómez-Sánchez EP, Rat mesenteric artery endothelial cells in culture secrete ET-1. *Life Sci* **46**: 881–884, 1990.
7. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
8. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K (Eds.), *Current Protocols in Molecular Biology, Volume 1. Preparation and Analysis of RNA*. Greene Publishing Association and Wiley Interscience, New York, 1990.
9. Block KD, Friedrich SP, Lee MP, Eddy RL, Shows TB and Quertermous T, Structural organization and chromosomal assignment of the gene encoding endothelin. *J Biol Chem* **264**: 10851–10857, 1989.
10. Bio-Rad Chemical Division, *Zeta-Probe® GT (Genomic Tested) Blotting Membranes Instruction Manual*. Bio-Rad, Richmond, CA, 1990.
11. Motulsky HJ, *T-EASE™*. Institute for Scientific Information®, Philadelphia, PA, 1987.
12. Mullol J, Chowdhury BA, White MV, Ohkubo K, Rieves D, Baraniuk J, Hausfeld NN, Shelhamer JH and Kaliner MA, Endothelin in human nasal mucosa. *Am J Respir Cell Mol Biol* **8**: 393–402, 1993.
13. Wu T, Rieves RD, Larivee P, Logun C, Lawrence MG and Shelhamer JH, Production of eicosanoids in response to endothelin-1 and identification of specific endothelin-1 binding sites in airway epithelial cells. *Am J Respir Cell Mol Biol* **8**: 282–290, 1993.
14. Tschirhart EJ, Drijfhout JW, Pelton JT, Miller RC and Jones CR, Endothelins: Functional and autoradiographic studies in guinea pig trachea. *J Pharmacol Exp Ther* **258**: 381–387, 1991.
15. De Nucci G, Thomas R, D'Orleans NA, Juste P, Antunes E, Walder C, Warner TD and Vane JR, Pressor affects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostaglandin and endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* **85**: 9797–9800, 1988.
16. Nagase T, Fukuchi Y, Jo C, Teramoto S, Uejima Y, Ishida K, Shimizu T and Orimo H, Endothelin-1 stimulates arachidonate 15-lipoxygenase activity and oxygen radical formation in the rat distal lung. *Biochem Biophys Res Commun* **168**: 485–489, 1990.
17. Endo T, Uchida Y, Matsumoto H, Susuki N, Nomura A, Hirata F and Hasegawa S, Regulation of endothelin-1 synthesis in cultured guinea pig airway epithelial cells by various cytokines. *Biochem Biophys Res Commun* **186**: 1594–1599, 1992.
18. Vankatesh VC and Ballard PL, Glucocorticoids and

gene expression. *Am J Respir Cell Mol Biol* **4**: 301–303, 1991.

19. Diamond MI, Miner JN, Yoshinaga SK and Yamamoto KR, Transcription factor interactions: Selectors of positive and negative regulation from a single DNA element. *Science* **249**: 1266–1272, 1990.
20. Laitinen LA, Laitinen A and Persson CGA, Role of epithelium. In: *Bronchial Asthma, Mechanisms and Therapeutics* (Eds. Weiss EB and Stein M), 3rd Edn, pp. 296–308. Little, Brown & Co., Boston, MA, 1993.
21. Groyer A, Schweizer-Groyer G, Cadepond F, Mariller M and Baulieu E-E, Antiglucocorticosteroid effects suggest why steroid hormone is required for receptors to bind DNA *in vivo* but not *in vitro*. *Nature* **328**: 624–626, 1987.
22. Dalman FC, Scherrer LC, Taylor LP, Akil H and Pratt WB, Localization of the 90-kDa heat shock protein-binding site within the hormone-binding domain of the glucocorticoid receptor by peptide competition. *J Biol Chem* **266**: 3482–3490, 1991.
23. Evans RM, The steroid and thyroid hormone receptor superfamily. *Science* **240**: 889–895, 1988.
24. Szentivanyi A, Immune-neuroendocrine circuitry and its relation to asthma. In: *Bronchial Asthma, Mechanisms and Therapeutics* (Eds. Weiss EB and Stein M), 3rd Edn, pp. 421–438. Little, Brown & Co., Boston, MA, 1993.