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Inflammation Research

The β-adrenoceptor agonist clenbuterol is a potent inhibitor of the LPS-induced production of TNF-α and IL-6 in vitro and in vivo

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Abstract. *Objective and Design:* To investigate the suppressive effects of the β-agonist clenbuterol on the release of TNF-α and IL-6 in a lipopolysaccharide (LPS)-model of inflammation, both in vitro and in vivo.
Material and Subjects: Human U-937 cell line (monocyte-derived macrophages), and male Wistar rats (200–250 g).
Treatment: U-937 macrophages were incubated with LPS at 1 µg/ml, with or without 1.0 mM–0.1 nM test drugs (clenbuterol and other cAMP elevating agents) for 1–24 h. Rats were administered either 1 or 10 µg/kg clenbuterol (or saline) orally, 1 h before intraperitoneal administration of 2 mg/kg LPS.
Methods and Results: TNF-α and IL-6 time-concentration profiles were determined both in culture media and plasma, using ELISA' s and bioassays. LPS-mediated release of both cytokines was significantly suppressed by clenbuterol.
Conclusions: The β-agonist clenbuterol very potently suppresses the LPS-induced release of the pro-inflammatory cytokines TNF-α and IL-6 both in vitro and in vivo.

Key words: Clenbuterol – β-Agonists – Cytokines – Macrophages – Lipopolysaccharide

Introduction

The important regulatory role of the cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) during various acute and chronic inflammatory processes is well known [1–4].

Several potential strategies are currently under investigation aiming to modulate the synthesis and release of these cytokines or to block (part of-) their effects before or after receptor interaction. Regulatory mechanisms leading to diminished pro-inflammatory cytokine production are of immediate clinical interest in the treatment of many chronic and acute inflammatory diseases including rheumatoid arthritis, inflammatory bowel disease, and sepsis.

Results from different studies, mostly in vitro, indicate that the adrenergic system is also involved in the regulation of an inflammatory response [5–8]. It has been shown that elevated intracellular levels of the second messenger cAMP are associated with a reduced release of pro-inflammatory mediators, or an induction of anti-inflammatory mediators [9, 10]. One way to induce elevated intracellular cAMP levels is by inhibiting phosphodiesterase (PDE) IV enzyme which breaks down cAMP [11, 12]. Some of the PDE-inhibitors, including xanthine derivatives such as pentoxifylline, have already found their way into the clinic.

Induction of adenylate cyclase, directly or via stimulation of G-protein coupled receptors (e.g. β-adrenoceptors), is an alternative way to increase cAMP levels and hence inhibit cytokine production. Several agents known for their cAMP elevating capability via the adenylate cyclase pathway have been tested for their anti-inflammatory activity. Among these are forskolin [11], β-agonists [6, 13], adenosine agonists [14], and catecholamines [5, 7, 15].

Although β-agonists are better known for their use as bronchodilators in asthma, they also show some anti-inflammatory characteristics. These anti-inflammatory effects have been studied mainly in vitro [8, 11, 16, 17]. In these studies different cell lines have been used, producing sometimes contrasting results [13, 17–20]. Data about the effect of β-agonists on the inflammatory response in vivo are very rare [13, 21].

Macrophages are a major source of (pro-) inflammatory cytokines [19, 22, 23]. We were interested in therapeutic suppression of pro-inflammatory cytokines during inflammation. The first goal was to compare the effect of some cAMP-elevating agents on the LPS stimulated release of the cytokines TNF-α and IL-6. Therefore several β-agonists and other cAMP-elevating agents were evaluated in vitro, using the human monocyte derived macrophage U-937 cell line. The second goal of this study was to correlate the in vitro

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findings with in vivo data obtained from an animal model of acute inflammation: i.e. LPS treated (conscious) rats, monitoring TNF-α and IL-6 in plasma.

Materials and methods

Chemicals

Adenosine, clenbuterol, terbutaline, (+/–)-isoproterenol, pentoxifylline, rolipram, phorbol-12-myristate-13-acetate (PMA), lipopolysaccharide (*Escherichia coli* O111:B4), 3-(4, 5-dimethyl-thiazol-2-y)2, 5-diphenyltetrazolium bromid (MTT) were obtained from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands).

Culturing U-937 cells

U-937 cells (a human monocyte like, histocytic lymphoma cell line, CRL-1593.2) were purchased from the American Type Culture Collection (Rockville, MD, USA). The cells were grown in plastic flasks (75 cm^2, Corning Costar, Badhoevedorp, The Netherlands) in RPMI-1640 (Sigma Chemical Co., St. Louis, MO, USA), supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS, Life Technologies, Breda, The Netherlands), and 2 mM of L-glutamine (Life Technologies) at 37 °C in a humidified atmosphere of 95% air-5% CO_2. The cells were seeded at densities of $0.1 * 10^6$ cells/ml and subcultured when the cell concentration reached $1.0 * 10^6$ cells/ml.

Differentiation of U-937 cells and incubations

U-937 cells are monocytes which can be easily differentiated into macrophages, and are very often used as model for human macrophages. Differentiating the U-937 monocytes into macrophages was based on the method of Sajjadi et al. [14].

When the cells were prepared for experiments they were seeded at a concentration of $1 * 10^6$ cells/well in 12-wells cell culture plates and incubated overnight with 10 ng/ml PMA, added to the culture medium. U-937 cells were allowed to recover from PMA-treatment for 48 h, by changing the culture medium every day. After this recovery period, U-937 macrophages were incubated with 1 µg/ml LPS alone, or in combination with the following test substances: adenosine; β-agonists: clenbuterol, terbutaline, (+/–)-isoproterenol; PDE-IV inhibitors: pentoxifylline and rolipram.

All stock solutions were prepared on the day of the experiment in phosphate buffered saline (PBS, Life Technologies). Controls were treated similarly and incubated with either the test substance alone or vehicle (PBS). In the comparative study of several cAMP elevating agents culture medium was collected at 3 h after incubation with LPS. In the experiments with only LPS and clenbuterol (4-amino-α-[t-butyl-aminomethyl]-3, 5-dichlorobenzyl-alcohol hydrochloride, at 1 µM, 10 nM, or 0.1 nM) culture medium was collected at regular time intervals, for 24 h, and tested in an ELISA for TNF-α, and IL-6 concentrations. ELISA kits were purchased from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB, Amsterdam, The Netherlands). Kits were used according to protocol provided by the manufacturer. After removal of culture medium the cells were lysed in 1 ml NaOH (0.1 M) and used for protein determination by the modified method of Bradford (Bio-Rad, München, Germany). The amount of cytokines in the culture medium was expressed per total amount of cell protein.

Cell viability determination/MTT-assay

In order to test for possible cytotoxic effects of clenbuterol, alone or in combination with LPS, the cell viability was determined by MTT-assay based on Denizat and Lang [24]. In short, the differentiated U-937 cells were incubated for 3, 6, and 24 h with 10 and 1 µM clenbuterol, and with the combination of 1 µM clenbuterol and 1 µg/ml LPS. At the end of the incubation, 250 µl of a 3 mg/ml MTT-solution (in PBS) was added to each well. The test was terminated after 3 h by the aspiration of culture medium and addition of 500 µl of isopropanol (Mallinckrodt Baker, Deventer, The Netherlands), containing 0.5% (w/v) SDS (Sigma), and 36 mM HCl (Merck, Darmstadt, Germany). Plates were shaken thoroughly, and optical densities were determined spectrophotometrically at 590 nm using a spectrophotometer (Victor^2-multilabel-counter, Wallac Oy, Turku, Finland).

Animals

Male Wistar rats (200–250 g U:WU (CPB)) were purchased from the Utrecht University central animal facilities (GDL, Utrecht, The Netherlands). Animals were housed in macrolon cages, and received food and water ad libitum. Room temperature was kept constant at 22 °C, and light was maintained at a 12-h cycle. The animal experimentation guidelines of our institute were followed.

Experimental setup for plasma TNF-α and IL-6 measurements in rats

Twenty rats, divided in five groups of four each, were fasted the night before the experiment (only water available). The first group received only LPS, the second and third group received LPS and different doses of clenbuterol. The fourth and fifth group were controls to study the effect of saline or clenbuterol only. Just before the experiment a control blood sample was taken (t = 0). At the start of the experiment the rats received orally either drug vehicle (saline) or clenbuterol (1 or 10 µg/kg bodyweight), by gavage. One hour later they were injected intraperitoneally with either vehicle or LPS (2 mg/kg bodyweight). Blood samples were withdrawn from the tail at 1, 2, 3, and 4 h after LPS challenge. Blood was collected in heparinised microvials (Sarstedt, Nümbrecht, Germany), and centrifuged for 10 min at 4 °C. Plasma was stored at –80 °C until assayed.

Determination of rat plasma TNF-α concentration by a PK(15) cell-based bioassay

TNF-α was measured using the PK(15) cell line according to the method as described by Bertoni et al. [25]. The assay is based on the concentration-dependent cytotoxic effect of TNF-α on PK(15) cells. PK(15) cells were purchased from the American Type Culture Collection (CCL-33). Cytotoxicity/inhibited proliferation was measured indirectly using the MTT-assay as described above. Samples were titrated in 3-fold dilutions and for each plate a positive control (3 fold dilutions of recombinant rat TNF-α (Sanvertech, Heerhugowaard, The Netherlands)) and negative control (incubations with medium deprived of TNF-α) were measured.

Determination of rat plasma IL-6 concentration by a B9-cell based bioassay

IL-6 was measured with a murine hybridoma B9 cell line according to the method as described by Helle et al. [26]. B9 cells were a kind gift from the CLB (Amsterdam, The Netherlands). The assay is based on the IL-6 dependent proliferation of B9 cells. Proliferation was measured indirectly using the MTT-assay. Samples were titrated in 3-fold dilutions and for each plate a positive control (3 fold dilutions of recombinant rat IL-6 (Sanvertech) and a negative control (wells incubated with medium deprived of IL-6) were measured.

Statistical analysis

Data are expressed as means ± standard error or standard deviation. Statistical analysis was performed using the Student's *t*-test. The mean values of two groups (LPS-treated vs. LPS/clenbuterol treated) were considered to be significantly different if $p < 0.05$.

Results

Modulation of LPS-induced cytokine release in U-937 cells

Undifferentiated U-937 cells, growing in suspension, were succesfully stimulated to differentiate to a macrophage-like cell type by overnight incubation with PMA. After a recovery period of 48 h, these cells were found to respond strongly to the addition of LPS (1 µg/ml) by releasing TNF-α and IL-6 into the medium.

When U-937 macrophages were incubated with LPS together with several cAMP-elevating agents a strong suppression of the LPS-induced TNF-α release by β-agonists (clenbuterol, terbutaline, and isoproterenol, at 1 µM) was found 3 h after incubation (Fig. 1). The other compounds (adenosine, pentoxifylline, and rolipram) were not able to suppress TNF-α release at this concentration. These compounds needed to be added at much higher concentrations (1 mM, 0.1 mM) to achieve the same inhibition of TNF-α-release (data not shown). β-Agonists were also tested at 10 nM concentration, at this concentration clenbuterol was shown to be the most potent suppressor of LPS-induced TNF-α release.

As a result of these findings the effect of clenbuterol on the LPS-induced TNF-α and IL-6 release was studied in more detail by measuring concentration-time profiles of these cytokines. Concentration-time profiles for TNF-α and IL-6 were found to be different (Fig. 2 and 3). TNF-α levels reached a rather sharp maximum at approximately 3 h after adding LPS and had returned to basal levels within 24 h of exposure to LPS (Fig. 2). The increase in IL-6 concentration started later than that of TNF-α and showed a biphasic response (Fig. 3). A first plateau was seen at approximately 6 h after adding LPS, and a second, higher, plateau at 12 h after adding LPS. Clenbuterol was found to reduce the release of both TNF-α and IL-6 in a concentration-dependent way. This effect lasted for at least 24 h. Neither clenbuterol itself nor saline were found to have any effects on cytokine release.

Cytotoxicity studies

In order to exclude possible non-specific cytotoxic effects as explanation for the effect of clenbuterol on the LPS stimulated cytokine release, cell viability was tested at 3, 6, and 24 h. Clenbuterol (1 and 10 µM) and a combination of clenbuterol and LPS were incubated with differentiated U-937 cells. Using the MTT cytotoxicity assay, it was found that none of the concentrations tested was significantly cytotoxic (Fig. 4).

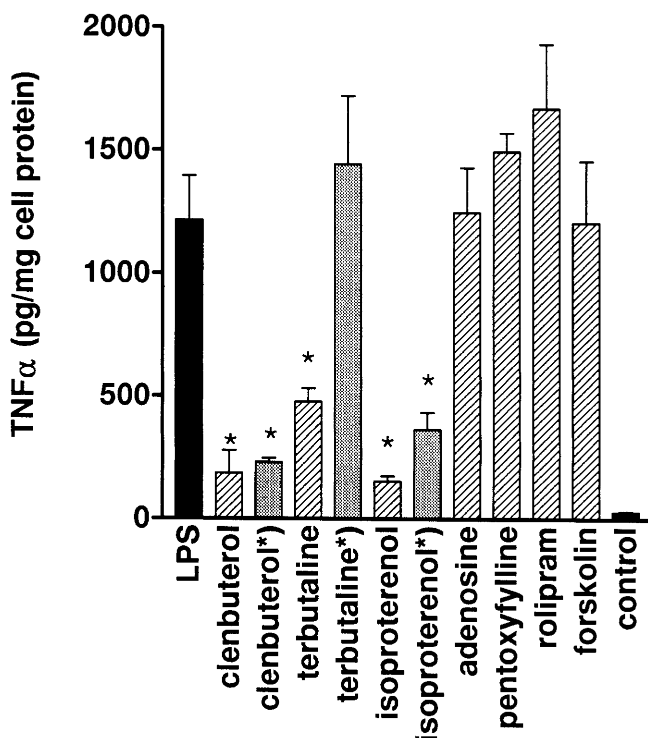

Fig. 1. Inhibitory effect of different compounds on TNF-α release by differentiated U-937 cells at 3 h after LPS incubation. U-937 cells were incubated with only LPS (1 µg/ml), or LPS simultaneously with different concentrations (1 µM or * 10 nM) of the inhibitors. Controls received only vehicle (PBS). Data are means ± SEM for triplicate determinations from the second of a series of three experiments.

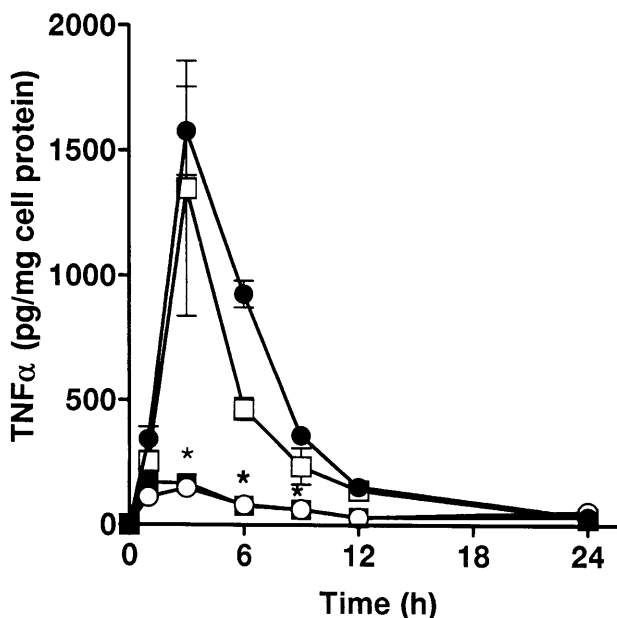

Fig. 2. Time-course of LPS-induced TNF-α release by differentiated U-937 cells. Effect of simultaneous incubation with different concentrations of clenbuterol. U-937 cells were incubated with only LPS (1 µg/ml ●), or LPS together with clenbuterol (1 µM ○; 10 nM ■; 0.1 nM □). Data are means ± SEM for triplicate determinations from the third of a series of three experiments.

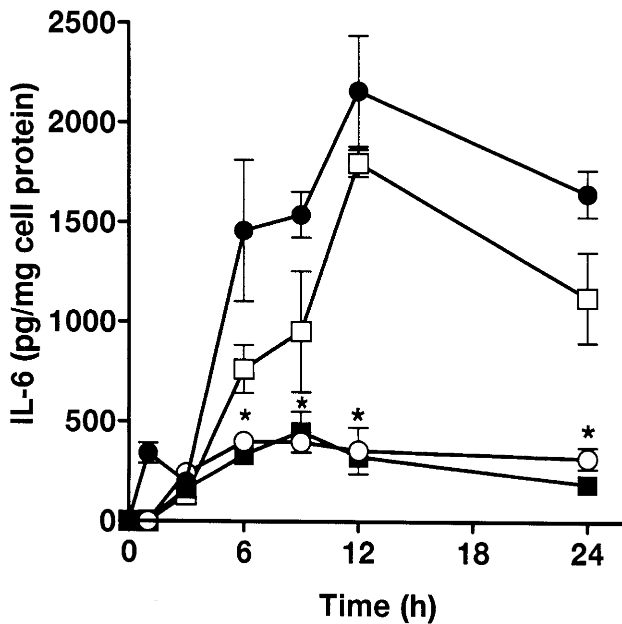

Fig. 3. Time-course of LPS-induced IL-6 release by differentiated U-937 cells. Effect of simultaneous incubation with different concentrations of clenbuterol. U-937 cells were incubated with only LPS (1 µg/ml ●), or LPS together with clenbuterol (1 µM ○; 10 nM ■; 0.1 nM □). Data are means ± SEM for triplicate determinations from the third of a series of three experiments.

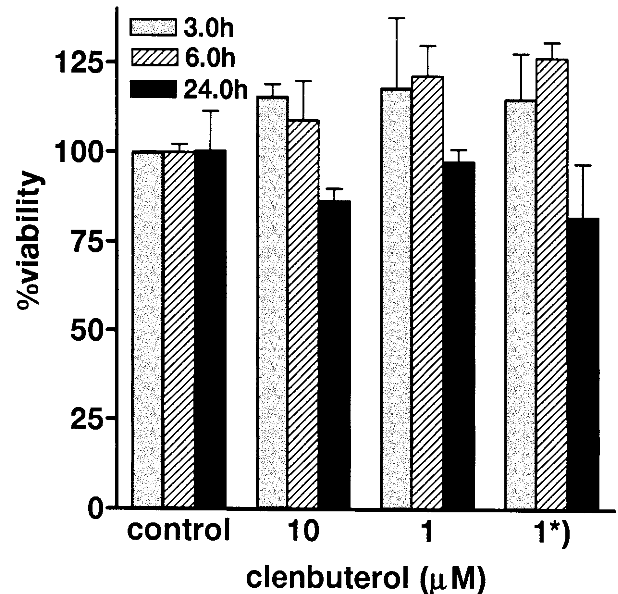

Fig. 4. Effect of different concentrations of clenbuterol and LPS on the viability of differentiated U937-cells in time. U937-cells were incubated with different concentrations of clenbuterol (10, and 1 µM, and * 1 µM + 1 µg/ml LPS). At 3, 6 and 24 h after start of the incubations the cell viability was determined using the MTT-assay. Viability was expressed as percentage of control. Data are means ± SD for triplicate determinations from a representative experiment.

In vivo effects of clenbuterol on LPS-induced cytokine release

Following LPS (2 mg/kg i.p.) administration to rats, high plasma levels of TNF-α and IL-6 were detected. Plasma profiles of both cytokines were slightly different. For TNF-α, peak plasma concentrations were found between 1 and 2 h after LPS injection (Fig. 5). TNF-α had been cleared from plasma at 3 h after LPS administration. Compared to TNF-α, IL-6 concentration in plasma reached its peak somewhat later (between 2 and 3 h). IL-6 levels were back to control values at 4 h after LPS injection (Fig. 6).

The administration of clenbuterol 1 h before injecting LPS resulted in an almost complete block of the LPS-induced release of TNF-α, IL-6. Neither saline nor clenbuterol itself were found to have any effect on cytokine release in vivo.

Discussion

The results of the present study demonstrate that clenbuterol is a very potent inhibitor of LPS-induced release of TNF-α and IL-6 both in vivo (rats) and in vitro (U-937 cells). When the suppressive effect of some eminent cAMP-elevating agents on the LPS-induced pro-inflammatory cytokine release was compared, it was found that the β-adrenoceptor agonists were more potent than adenosine, and phosphodiesterase- inhibitors (pentoxifylline, rolipram). The concentration of clenbuterol, necessary to cause this effect, was remarkably low compared to other β-agonists and other compounds of the classes tested.

Our in vivo results show that clenbuterol, given 1 h before LPS, is able to block the release of the pro-inflammatory cytokines TNF-α and IL-6 almost completely. The clenbuterol dose needed to produce such an effect (1 µg/kg) is in the same order of magnitude as that has been used previously in human therapeutics (asthma), this even without taken into account a correction for species-extrapolation from man to rat.

Clenbuterol is a β-adrenoceptor agonist with relatively high affinity for β2-adrenoceptors, developed as a long acting, orally active compound. Like most other β2-agonists the main indication for use is in asthma therapy where the compound produces relaxation of bronchial smooth muscle. In addition, it has been shown that some β2-agonists possess anti-inflammatory properties [6, 8, 27]

There have been some other studies suggesting an effect of β-adrenoceptor agonists on the release of cytokines. Most papers describe in vitro effects using different cell types such as RAW 264.7 (murine macrophage cell line) [17], HL-60 (human leukemic cell line) [16], or PBMCs (freshly isolated human monocytes) [8]. In these studies, β-agonists such as isoproterenol and salbutamol were found to inhibit the release of TNF-α. In addition, the stimulation of IL-10 and inhibition of IL-1β by β-agonists were reported by Haskó *et al.* [17], and Yoshimura et al. [8], respectively. However, in contrast to our study most authors use a much higher β-agonist concentration.

As far as we know there are only two papers describing an effect of β-agonists on LPS-induced IL-6 release [13, 20]. Remarkably, von Patay et al. [20] report a synergistic effect of isoproterenol on the LPS induced IL-6 release by rat

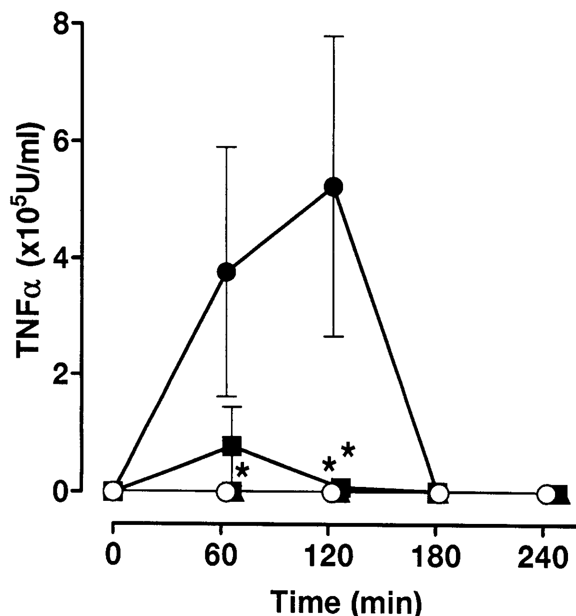

Fig. 5. Effect of clenbuterol on the LPS induced plasma levels of TNF-α. Rats were administered orally either saline or clenbuterol 1 h before intra-peritoneal injection of LPS or saline. Control (saline orally and intraperitoneally; ○), LPS (saline orally and LPS intraperitoneally; ●), LPS and clenbuterol (clenbuterol orally 1 µg/kg □, or 10 µg/kg ■, and LPS intraperitoneally), clenbuterol (orally 10 µg/kg , saline intraperitoneally; ▲). Data are means of 4 animals ± SEM.

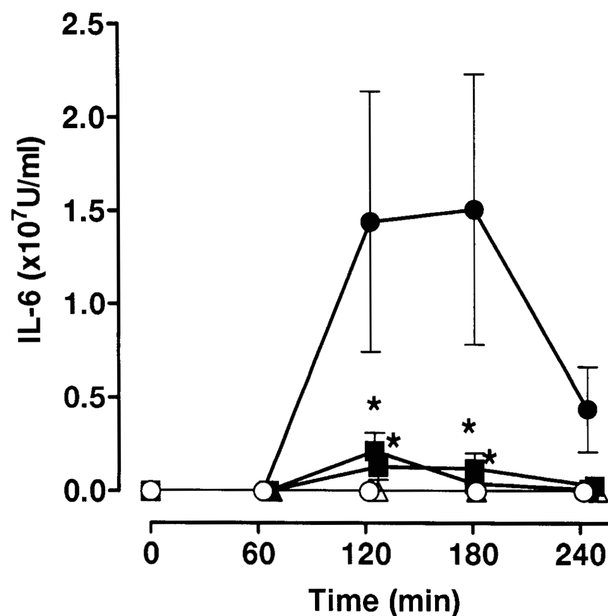

Fig. 6. Effect of clenbuterol on the LPS induced plasma levels of IL-6. Rats were administered orally either saline or clenbuterol 1 h before intra-peritoneal injection of LPS or saline. Control (saline orally and intraperitoneally; ○), LPS (saline orally and LPS intraperionealy; ●), LPS and clenbuterol (clenbuterol orally 1 µg/kg □, or 10 µg/kg ■, and LPS intraperitoneally), clenbuterol (oral 10 µg/kg, saline intraperitoneally; ▲). Data are means of 4 animals ± SEM.

thymic epithelial cells in vitro. Yoshimura et al. [8] studied the effect of four different β-agonists, including clenbuterol, on LPS stimulated human peripheral blood mononuclear cells (PBMCs). They also found an inhibition of the release of TNF-α by LPS treated PBMCs, at concentrations comparable to these used in the present study. As far as we know there are no such data in this respect on the human U-937 cell line.

In contrast, in vivo data seem to be very rare in the literature and apparently only studies in mice are described. Szabó et al. [13] studied the effect of isoproterenol on inflammatory mediator production after LPS stimulation. Using this β-agonist, at a 1000-fold higher dose (10 mg/kg) compared to our study, they found that LPS induced TNF-α release was inhibited. However, in contrast to our findings, the authors describe an increase in LPS induced IL-6 production after isoproterenol pretreatment. Sekut et al. [21] also used mice, and studied the effect of orally administered salmeterol (β-agonist) on TNF-α release (not IL-6) after intraperitoneal injection of LPS. They also showed an inhibitory effect of this β-agonist on TNF-α-release, but a 100-fold higher dose of salmeterol (0.1 mg/kg) was used to achieve this effect. They determined that the best effect (highest inhibition of TNF-α-release) was reached when salmeterol was given at 1 h before LPS injection.

A possible explanation for the difference in magnitude of effect of different β-agonists on cytokine release and a different effect on separate cytokines was already suggested by Sekut et al. [21], who argued that more specific β2-adrenoceptor agonists had different effects on cytokine release than non-selective β-agonists. Furthermore, the difference in response of different cell types on β-agonist stimulation might also be explained by the varying β-adrenoceptor distribution on different cells of the immune system [28, 29].

Additionally, it is likely that pharmacokinetic properties of the compounds tested in vivo are of importance. Altogether this may explain why clenbuterol (high affinity, and more specific for the β2-adrenoceptor) is having such a potent in vivo inhibitory effect on cytokine release.

Our in vitro data are well in accordance with the in vivo findings, although the kinetics of cytokine release appeared to be slower in vitro than in vivo. Macrophage cells are a major source of LPS-induced TNF-α and IL-6 release. Studies that inactivated macrophages in vivo [19, 22] observed a dramatic decrease in plasma levels of LPS induced cytokine release, compared to animals with active macrophages.The results of these studies and the present study suggest that the potent systemic effects of clenbuterol are for a large part achieved by action on macrophages.

In conclusion, we demonstrate that the β-agonist clenbuterol is a very potent inhibitor in vitro and in vivo of the LPS-induced release of the pro-inflammatory cytokines TNF-α and IL-6. Although it seems logical to conclude that the compound exerts its effect via cAMP, additional mechanisms may also play a role. In the present study clenbuterol was given 1 h before LPS challenge. Whether our findings may be of therapeutic value needs to be further investigated. A negative aspect might be that low doses of β-agonists could suppress systemic immune reactions (possibly even when administered locally). This could result in a higher susceptibility of patients for infections.

References

[1] Strieter RM, Kunkel SL, Bone RC. Role of tumor necrosis factor-alpha in disease states and inflammation. Crit Care Med 1993; 21: S447–63.

[2] Henderson B, Poole S, Wilson M. Bacterial modulins: a novel class of virulence factors which cause host tissue pathology by inducing cytokine synthesis. Microbiol Rev 1996; 60: 316–41.

[3] Ohshima S, Saeki Y, Mima T, Sasai M, Nishioka K, Nomura S, et al. Interleukin 6 plays a key role in the development of antigen-induced arthritis. Proc Natl Acad Sci USA 1998; 95: 8222–6.

[4] Yao YM, Redl H, Bahrami S, Schlag G. The inflammatory basis of trauma/shock-associated multiple organ failure. Inflamm Res 1998; 47: 201–10.

[5] Madden S, Sanders VM, Felten DL. Catecholamine influences and sympathetic neural modulation of immune responsiveness. Annu Rev Pharmacol Toxicol 1995; 35: 417–48.

[6] Hetier E, Ayala J, Bousseau A, Prochiantz A. Modulation of interleukin-1 and tumor necrosis factor expression by β-adrenergic agonists in mouse ameboid microglial cells. Exp Brain Res 1991; 86: 407–13.

[7] Guirao X, Kumar A, Katz J, Smith M, Lin E, Keogh C, et al. Catecholamines increase monocyte TNF receptors and inhibit TNF through β_2-adrenoreceptor activation. Am J Physiol 1997; 273: E1203–8.

[8] Yoshimura T, Kurita C, Nagao T, Usami E, Nakao T, Watanabe S, et al. Inhibition of tumor necrosis factor-alpha and interleukin-1-beta production by beta-adrenoreceptor agonists from lipopolysaccharide stimulated human peripheral blood mononuclear cells. Pharmacology 1997; 54: 144–52.

[9] Eigler A, Siegmund B, Emmerich U, Baumann KH, Hartmann G, Endres S. Anti-inflammatory activities of cAMP-elevating agents: enhancement of IL-10 synthesis and concurrent suppression of TNF production. J Leukoc Biol 1998; 63: 101–7.

[10] Leist M, Auer Barth S, Wendel A. Tumor necrosis factor production in the perfused mouse liver and its pharmacological modulation by methylxanthines. J Pharmacol Exp Ther 1996; 276: 968–76.

[11] Seldon PM, Barnes PJ, Meja K, Giembycz MA. Suppression of lipopolysaccharide-induced tumor necrosis factor-α generation from human peripheral blood monocytes by inhibitors of phosphodiesterase 4: interaction with stimulants of adenylyl cyclase. J Pharmacol Exp Ther 1995; 48: 747–57.

[12] Cheng JB, Watson JW, Pazoles CJ, Eskra JD, Griffiths RJ, Cohan VL, et al. The phosphodiesterase type 4 (PDE4) inhibitor CP-80, 633 elevates plasma cyclic AMP levels and decreases tumor necrosis factor-α (TNFα) production in mice: effect of adrenalectomy. J Pharmacol Exp Ther 1996; 280: 621–6.

[13] Szabó C, Haskó G, Zingarelli B, Németh ZH, Salzman AL, Kvetan V, et al. Isoproterenol regulates tumour necrosis factor, interleukin-10, interleukin-6 and nitric oxide production and protects against the development of vascular hyporeactvity in endotoxaemia. Immunology 1997; 90: 95–100.

[14] Sajjadi FG, Takabayashi K, Foster AC, Domingo R, Firestein G. Inhibition of TNF-α expression by adenosine. Role of A3 adenosine receptors. J Immunol 1996; 156: 3435–42.

[15] van der Poll T, Lowry SF. Epinephrine inhibits endotoxin-induced IL-1β production: roles of tumor necrosis factor-α and IL-10. Am J Physiol 1997; 273: r1885–90.

[16] Gu Y, Seidel A. Influence of salbutamol and isoproterenol on the production of TNF and reactive oxygen species by bovine alveolar macrophages and calcitrol differentiated HL-60 cells. Immunopharmacol Immunotoxicol 1996; 18: 115–28.

[17] Haskó G, Németh ZH, Szabó C, Zsilla G, Salzman AL, Vizi ES. Isoproterenol inhibits IL-10, TNF-α, and nitric oxide production in RAW 264.7 macrophages. Brain Res Bull 1998; 45: 183–7.

[18] Straub RH, Hermann M, Frauenholz T, Berkmiller G, Lang B, Schölmerich J, et al. Neuroimmune control of interleukin-6 secretion in the murine spleen. Differential beta-adrenergic effects of electrically released endogenous norepinephrine under various endotoxin conditions. J Neuroimmunol 1996; 71: 37–43.

[19] Straub RH, Dorner M, Riedel J, Kubitza M, van Rooijen N, Lang B, et al. Tonic neurogenic inhibition of interleukin-6 secretion from murine spleen caused by opioidergic transmission. Am J Physiol 1998; 274: 997–1003.

[20] von Patay B, Loppnow H, Feindt J, Kurz B, Mentlein R. Catecholamines and lipopolysaccharide synergistically induce the release of interleukin-6 from thymic epithelial cells. J Neuroimmunol 1998; 86: 182–9.

[21] Sekut L, Champion BR, Page K, Menius Jr JA, Conolly KM. Anti-inflammatory activity of salmeterol: down-regulation of cytokine production. Clin Exp Immunol 1995; 99: 461–6.

[22] Salkowski CA, Neta R, Wynn TA, Strassmann G, van Rooijen N, Vogel SN. Effect of liposome-mediated macrophage depletion on LPS-induced cytokine gene expression and radioprotection. J Immunol 1995; 155: 3168–79.

[23] Decker K. The response of liver macrophages to inflammatory stimulation. Keio J Med 1998; 47: 1–9.

[24] Denizat F, Lang R. Rapid colorimetric assay for cell growth and survival. J Immunol Methods 1986; 89: 271–7.

[25] Bertoni G, Kuhnert P, Peterhans E, Pauli U. Improved bioassay for the detection of porcine tumour necrosis factor using a homologous cell line: PK(15). J Immunol Methods 1993; 160: 267–71.

[26] Helle M, Boeije L, Aarden LA. Functional discrimination between interleukin 6 and interleukin 1. Eur J Immunol 1988; 18: 1535–40.

[27] Butchers PR, Vardey CJ, Johnson M. Salmeterol, a potent and long-acting inhibitor of inflammatory mediator release from human lung. Br J Pharmacol 1991; 104: 627–76.

[28] Radojcic T, Baird S, Darko D, Smith D, Bulloch K. Changes in β-adrenergic receptor distribution on immunocytes during differentiation: an analysis of T-cells and macrophages. J Neurosci Res 1991; 30: 328–35.

[29] Sanders VM, Baker RA, Ramer Quinn DS, Kasprowicz DJ, Fuchs BA, Street NE. Differential expression of the beta2-adrenergic receptor by Th1 and Th2 clones: implications for cytokine production and B cell help. J Immunol 1997; 158: 4200–10.