ELSEVIER

European Journal of Pharmacology 339 (1997) 215–221

ejp

# Effect of the phosphodiesterase III inhibitor amrinone on cytokine and nitric oxide production in immunostimulated J774.1 macrophages

Zoltán H. Németh [a], Csaba Szabó [a,b], György Haskó [a], Andrew L. Salzman [b], E. Sylvester Vizi [a,*]

[a] *Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, P.O.B. 67, H-1450 Budapest, Hungary*
[b] *Division of Critical Care Medicine, Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA*

Received 7 May 1997; revised 10 September 1997; accepted 3 October 1997

**Abstract**

The level of intracellular cyclic nucleotides is a regulatory factor in a variety of immune processes. Increases in intracellular cyclic AMP (cAMP) and/or cyclic GMP (cGMP) concentration by the inhibition of phosphodiesterase have been shown to modulate the inflammatory response. Amrinone is a clinically used positive inotropic agent which elevates intracellular cAMP and cGMP levels by selective inhibition of the phosphodiesterase III isoenzyme. In the current study, we investigated the effect of various concentrations (1–300 $\mu$M) of amrinone on lipopolysaccharide-induced production of pro- and anti-inflammatory cytokines and of nitric oxide (NO) in vitro. In cultured murine J774.1 macrophages, 1 ng/ml–10 $\mu$g/ml of lipopolysaccharide from *Escherichia coli* O55:B5 induced production of tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-10, and nitrite (breakdown product of NO). Pretreatment of cells with amrinone caused a dose-dependent suppression of TNF-$\alpha$ production in the concentration range of 1–100 $\mu$M. Furthermore, this drug suppressed NO production in the range of 30–300 $\mu$M. Similarly to the results in the J774.1 cells, amrinone also inhibited TNF-$\alpha$ and NO production in the range of 10–100 $\mu$M in primary rat peritoneal macrophages. At 300 $\mu$M, but not at lower concentrations, amrinone inhibited interleukin-10 production in lipopolysaccharide-treated J774.1 macrophages. Pretreatment of the macrophages with 100 and 300 $\mu$M amrinone increased the lipopolysaccharide-elicited translocation of nuclear factor-$\kappa$B. Taken together, our results indicate that the phosphodiesterase III inhibitor amrinone modulates the activation/production of many pro- and anti-inflammatory factors in endotoxin-stimulated cells. It remains to be further investigated how such immunomodulatory effects contribute to the clinical profile of the agent.


*Keywords:* Lipopolysaccharide; cAMP; cGMP; Phosphodiesterase III inhibitor; Monocyte; Macrophage; Interleukin-10; TNF-$\alpha$ (tumor necrosis factor-$\alpha$); Nitric oxide (NO)

## 1. Introduction

A number of stimuli such as microbial infection, tissue injury, and tumor cells can activate macrophages or mononuclear phagocytes. These immune cells have complex and multifactorial regulatory activities exerted through the production of various pro- or anti-inflammatory cytokines, reactive oxygen metabolites, and nitric oxide (NO). The intensive work of the past decade has demonstrated that the systemic inflammatory response caused by bacterial lipopolysaccharide is a consequence of enhanced release of these macrophage-derived mediators. Many studies have described cytokines such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$) (Beutler, 1995), and interleukin-1 (Dinarello, 1991) as key intermediates of an overresponsive host-defense reaction. These two cytokines are essential in the induction of NO synthesis in response to immune stimuli (Szabó et al., 1993a,b; Szabó, 1996b). The excessive formation of NO by the macrophage-type inducible nitric oxide synthase contributes to the peripheral vasodilatation and vascular hyporeactivity to vasoconstrictor agents, and eventually leads to circulatory shock in endotoxemia (Szabó, 1996a,b). In addition, the host also provides counterbalancing signals to terminate the hyperreactive phase of the immune response. A prominent example for this is interleukin-10, which has been shown to be a natural suppressor of proinflammatory mediators (Gerard et al., 1993). Interleukin-10 decreases the production of TNF-$\alpha$ in lipopolysaccharide-treated macrophages (Fiorentino et

* Corresponding author. Tel.: +36-1-2100810; fax: +36-1-2100813.

al., 1991), and down-regulates lipopolysaccharide-evoked TNF-$\alpha$ and NO production in endotoxemia in vivo (Mossmann, 1994).

One of the ways by which the inflammatory response could be down-regulated is to reduce the activity of macrophages. It is widely accepted that intracellular cyclic adenosine monophosphate (cAMP) exerts a broad suppressant effect on most inflammatory cells (Torphy and Undem, 1991). Agents that are known to elevate intracellular cAMP levels, such as prostaglandin $E_2$ (Strassmann et al., 1994) or ligands of $\alpha_2$-adrenoceptors (Haskó et al., 1995), $\beta$-adrenoceptors (Elenkov et al., 1995), dopamine (Haskó et al., 1996a), and adenosine receptors (Haskó et al., 1996b) can influence the production of pro- and anti-inflammatory cytokines and of NO in response to lipopolysaccharide. In addition, drugs able to elevate intracellular cAMP and/or cyclic guanosine monophosphate (cGMP) concentrations via inhibition of phosphodiesterases, the enzymes that catalyze the breakdown of these cyclic nucleotides, also regulate the cytokine response (Pastores et al., 1996). Although seven different isotypes of phosphodiesterases have been characterized, recent studies have demonstrated that inflammatory cells contain predominantly type III and type IV phosphodiesterases (Murray et al., 1990; Verghese et al., 1995; Souness et al., 1996).

Recently, Giroir and Beutler (1992) and our group (Németh et al., 1997) showed that amrinone, a selective inhibitor of the phosphodiesterase III isotype, suppressed the lipopolysaccharide-evoked production of TNF-$\alpha$ and nitrite/nitrate in endotoxemic mice. We also demonstrated that this drug caused a biphasic time-course response of interleukin-10 production in endotoxemic mice; intraperitoneal pretreatment of animals with amrinone decreased the plasma interleukin-10 concentration in the first phase of the response, but enhanced it in the second part (Németh et al., 1997). These results prompted us to investigate whether the phosphodiesterase III inhibitor amrinone can also influence lipopolysaccharide-induced TNF-$\alpha$, interleukin-10, and NO production in vitro in cultured macrophages. Since the translocation of nuclear factor-$\kappa$B (NF-$\kappa$B) is an essential event in the activation of macrophages in response to lipopolysaccharide (Müller et al., 1993), we also aimed to determine the effect of amrinone on lipopolysaccharide-stimulated activation of NF-$\kappa$B in vitro.

## 2. Materials and methods

### 2.1. Materials

Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Gibco Life Technologies (Grand Island, NY). Amrinone, lipopolysaccharide from *Escherichia coli* O55:B5, and all other drugs were obtained from Sigma Chemical (St. Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits for murine interleukin-10 and TNF-$\alpha$ were obtained from Genzyme (Genzyme, Boston, MA). These kits are specific for mouse cytokines, but the TNF-$\alpha$ kit also cross-reacts with the rat TNF-$\alpha$. Amrinone did not interfere with the detection of cytokines using this kit.

### 2.2. Cell culture

The murine monocyte/macrophage cell line J774.1 was grown in DMEM supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 $\mu$g/ml streptomycin at 37°C in 5% $CO_2$/95% air. Cells were treated with various concentrations (1–300 $\mu$M) of amrinone or drug vehicle (DMEM supplemented as mentioned above) 30 min prior to lipopolysaccharide challenge and thereafter with lipopolysaccharide (1 ng/ml–10 $\mu$g/ml) for 24 h. At 24 h, TNF-$\alpha$, interleukin-10 and nitrite were measured in the supernatant and viability was measured with the MTT method as described below.

### 2.3. Preparation of peritoneal macrophages

Peritoneal macrophages from male Wistar rats (Charles River, Wilmington, MA) were harvested by peritoneal lavage with RPMI medium containing L-glutamine (3.5 mM), penicillin (50 U/ml), streptomycin (50 $\mu$g/ml) and heparin sodium (10 U/ml) as described (Zingarelli et al., 1996). The cells were plated on 12-well plastic plates at 1 million cells/ml and incubated for 2 h at 37°C in a humidified 5% $CO_2$ incubator. Non-adherent cells were removed by rinsing the plates three times with 5% dextrose in phosphate-buffered saline. Cells were stimulated with lipopolysaccharide (10 $\mu$g/ml) for 24 h. At 24 h, TNF-$\alpha$ and nitrite were measured in the supernatant and viability was measured with the MTT method as described below.

### 2.4. Measurement of interleukin-10 and TNF-$\alpha$ concentration

Production of interleukin-10 and TNF-$\alpha$ 24 h after lipopolysaccharide stimulation was measured in samples of cell culture supernatant using solid-phase ELISA, employing the multiple antibody sandwich principle that specifically detects TNF-$\alpha$ and interleukin-10 (Genzyme, Boston, MA). Assays were performed according to the manufacturer's instructions. Plates were read at 450 nm by a microplate reader (Model 450, BIO-RAD, Richmond, CA). Absorbancy was recalculated as concentration (ng/ml) using a standard curve by the Microplate Manager/PC Data Analysis Software (BIO-RAD, Richmond, CA).

### 2.5. Measurement of nitrite (breakdown product of NO) concentration

Nitrite in culture supernatants at 24 h was measured by adding 100 $\mu$l of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamide in 5% phosphoric acid) to

100 μl samples of medium (Haskó et al., 1996b). The optical density at 550 nm ($OD_{550}$) was measured by using the BIO-RAD 450 microplate reader. Nitrite concentrations were calculated by comparison with $OD_{550}$ of standard solutions of sodium nitrite and sodium nitrate. The measurements of nitrite were performed using reagents free of nitrite and nitrate: no basal or background nitrite or nitrate levels were detected. Amrinone did not interfere with the detection of nitrite by this assay.

### 2.6. Measurement of mitochondrial respiration

Mitochondrial respiration, an indicator of cell viability, was assessed by the mitochondria-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan (Szabó et al., 1993c). Cells were incubated in 96-well plates with MTT (0.2 mg/ml) for 1 h at 37°C. Cell medium was removed by aspiration, and cells were solubilized in dimethyl sulfoxide (100 μl/well). The extent of reduction of MTT to formazan within cells was quantitated by measurement of $OD_{550}$ using the BIO-RAD 450 microplate reader. Amrinone did not interfere with this assay.

### 2.7. Preparation of nuclear extracts and NF-κB Western blotting

Preparation of nuclear extracts and NF-κB Western blotting was performed as previously described (Haskó et al., 1996b). Cells were treated with 100 and 300 μM amrinone or drug vehicle 30 min prior to lipopolysaccharide challenge and thereafter with lipopolysaccharide (10 μg/ml) for 90 min. Cells were scraped, and pellets were resuspended in 400 μl of cold buffer A (HEPES, pH 7.9) (10 mM); KCl (10 mM); EDTA (0.1 mM); EGTA (0.1 mM); phenylmethylsulfonyl fluoride (0.5 mM); pepstatin A (1 μg/ml); leupeptin (10 μg/ml); and aprotinin (10 μg/ml) on ice for 15 min in the presence of 25 μl of 1% Nonidet P-40. Then, samples were vortexed and centrifuged for 1 min at $10{,}000 \times g$, and the pellet was resuspended in 100 μl of Buffer B (HEPES, pH 7.9) (20 mM); NaCl (400 mM); EDTA (1 mM); EGTA (1 mM); dithiothreitol (1 mM); phenylmethylsulfonyl fluoride (0.5 mM); pepstatin A (1 μg/ml); leupeptin (10 μg/ml); and aprotinin (10 μg/ml). After shaking on a rocker platform for 15 min at 4°C, samples were centrifuged for 15 min at $10{,}000 \times g$ at 4°C. Seventy-microliter aliquots were then treated with 150 μl of sodium dodecyl sulfate polyacrylamide gel electrophoresis sample buffer. Western blotting was performed (as described above) with rabbit anti-mouse NF-κB as the primary Ab (Santa Cruz Biotechnology, Santa Cruz, CA; 1/750 in Tween/Tris-buffered saline (0.02%)).

### 2.8. Statistical evaluation

All values in the figures and text are expressed as mean ± standard error of the mean (S.E.M.) of $n$ observations, where $n$ represents the number of samples studied. Statistical analysis of the data was performed by one-way analysis of variance followed by Bonferroni multiple comparison test, as appropriate. A $P$-value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Amrinone causes a biphasic response of TNF-α production in lipopolysaccharide-stimulated macrophages

Pretreatment of J774.1 macrophages with 1–100 μM amrinone 30 min prior to lipopolysaccharide (10 μg/ml) challenge caused a significant, concentration-dependent in-

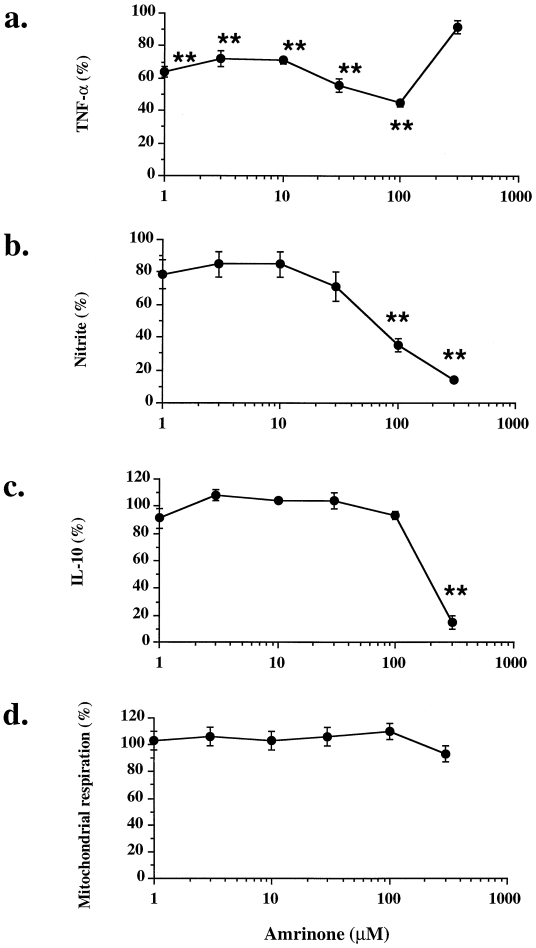


Fig. 1. Effect of pretreatment with amrinone (1–300 μM) on lipopolysaccharide-induced TNF-α (a), nitrite (b), and interleukin-10 (c) production and mitochondrial respiration (d) in J774.1 macrophages. Macrophages were stimulated with lipopolysaccharide (10 μg/ml) for 24 h in the presence or absence of various concentrations (1–300 μM) of amrinone. TNF-α, nitrite, and interleukin-10 concentrations were determined in the cell supernatants. Data (% of lipopolysaccharide-stimulated values) are expressed as the mean ± S.E.M. of 8 wells. Lipopolysaccharide-stimulated concentrations of TNF-α, nitrite, and interleukin-10 in the absence of amrinone were 8.7 ± 0.8 ng/ml, 54 ± 4 μM and 1.13 ± 0.03 ng/ml, respectively. ** Indicates $P < 0.01$.

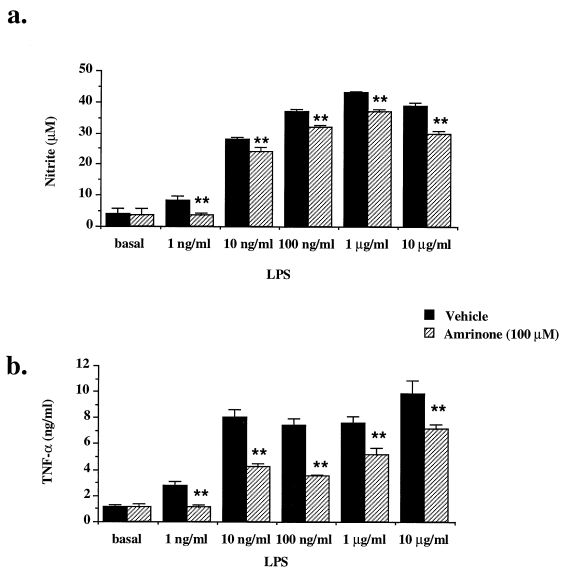


Fig. 2. Effect of pretreatment with amrinone (100 $\mu$M) on lipopolysaccharide-induced TNF-$\alpha$ (a) and nitrite (b) production by J774.1 macrophages stimulated for 24 h with increasing concentrations of lipopolysaccharide (1 ng/ml–10 $\mu$g/ml). Data are expressed as the mean ± S.E.M. of 6 wells. ** Indicates $P < 0.01$.

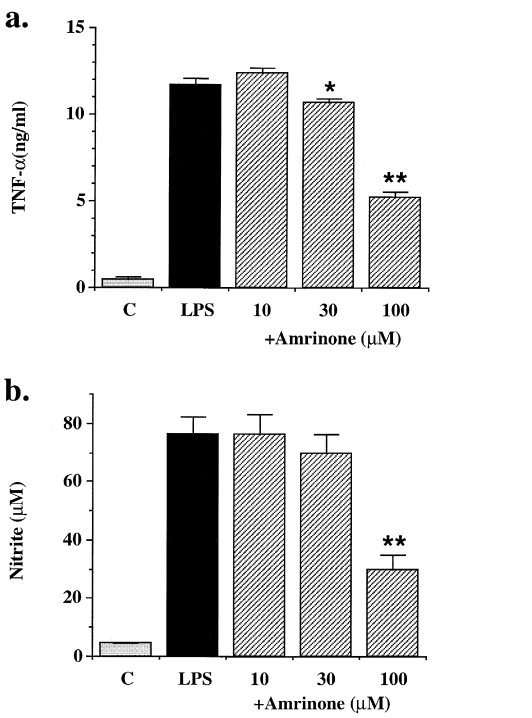


Fig. 3. Effect of pretreatment with amrinone (10–100 $\mu$M) on lipopolysaccharide-induced TNF-$\alpha$ (a) and nitrite (b) production in rat peritoneal macrophages stimulated for 24 h with lipopolysaccharide (10 $\mu$g/ml). Data are expressed as the mean ± S.E.M. of 6 wells. * Indicates $P < 0.05$; ** indicates $P < 0.01$.

hibition of TNF-$\alpha$ production. Interestingly, the TNF-$\alpha$ level returned to the control level at 300 $\mu$M amrinone (Fig. 1a).

### 3.2. Amrinone suppresses formation of nitrite in immunostimulated macrophages

Pretreatment of the J774.1 macrophages with various concentrations (30–300 $\mu$M) of amrinone caused a concentration-dependent suppression of nitrite production in response to 10 $\mu$g/ml of lipopolysaccharide for 24 h (Fig. 1b). Cell viability was not affected by amrinone in this concentration range, as indicated by the MTT test (Fig. 1d).

### 3.3. Effect of amrinone in macrophages stimulated with various concentrations of bacterial lipopolysaccharide

Treatment of the J774.1 macrophages with various concentrations (1 ng/ml–10 $\mu$g/ml) of lipopolysaccharide caused a concentration-dependent increase in nitrite and TNF-$\alpha$ production. Amrinone (as tested at the concentration of 100 $\mu$M), significantly inhibited the production of nitrite and TNF-$\alpha$ at all lipopolysaccharide concentrations (Fig. 2).

### 3.4. Effect of amrinone on lipopolysaccharide-induced interleukin-10 levels

In contrast to the concentration-dependent suppression of TNF-$\alpha$ and nitrite production, amrinone decreased the interleukin-10 release only at 300 $\mu$M (Fig. 1c).

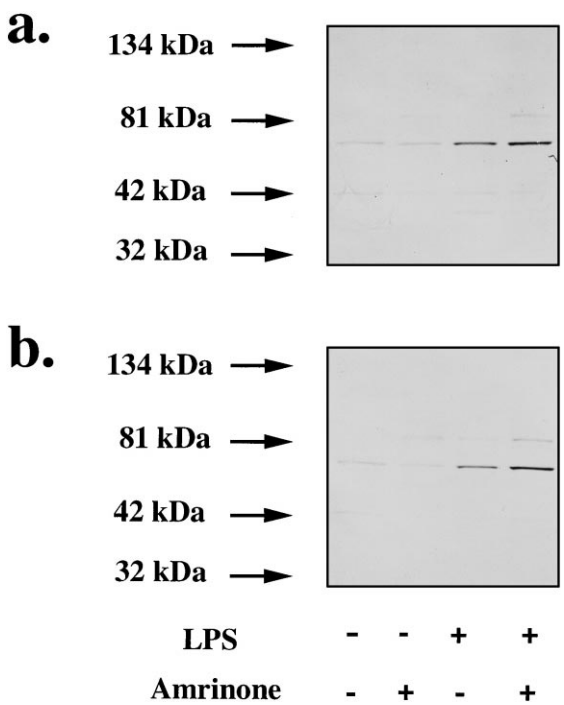


Fig. 4. Pretreatment with 100 $\mu$M (a) and 300 $\mu$M (b) of amrinone increases the nuclear translocation of NF-$\kappa$B by lipopolysaccharide stimulation in J774.1 macrophages. This figure shows the representative NF-$\kappa$B Western blot in nuclear extracts of control J774.1 cells and in cells 90 min after lipopolysaccharide challenge in the presence or absence of 100 and 300 $\mu$M amrinone.

### 3.5. Effect of amrinone on lipopolysaccharide-induced TNF-α and nitrite production in rat peritoneal macrophages

We have also tested the effect of amrinone on TNF-α and nitrite production in primary cells. Peritoneal macrophages from rats produced large amounts of TNF-α and nitrite at 24 h after 10 μg/ml lipopolysaccharide stimulation (Fig. 3). This was inhibited by amrinone at 30–100 μM (Fig. 3). There was no suppression in the cellular viability in response to amrinone. For instance, at the highest concentration of amrinone tested (100 μM), mitochondrial respiration amounted to 98 ± 35% of the control values ($n = 6$).

### 3.6. Effect of amrinone on lipopolysaccharide-evoked nuclear translocation of NF-κB

Challenge of J774.1 cells with lipopolysaccharide (10 μg/ml) for 90 min induced the translocation of the transcription factor NF-κB to the nucleus (Fig. 4). Pretreatment of the macrophages with 100 (Fig. 4a) and 300 μM (Fig. 4b) amrinone potentiated the lipopolysaccharide-elicited nuclear translocation of this transcription factor.

## 4. Discussion

The results reported here clearly show that pretreatment with amrinone caused a concentration-dependent suppression of TNF-α production in a concentration-range of 1–100 μM. Considering that amrinone elevates intracellular cAMP level by inhibition of phosphodiesterase III (Giroir and Beutler, 1992; Endres et al., 1994; Sinha et al., 1995), and that increase in the level of this cyclic nucleotide downregulates TNF-α production (Katakami et al., 1988; Renz et al., 1988), the inhibitory effect of amrinone on TNF-α synthesis may have been caused by a rise of cAMP concentration (Pastores et al., 1996).

Unexpectedly, pretreatment of cells with a higher concentration (300 μM) of amrinone resulted in restoration of TNF-α production. Regarding the mechanism of this relative upregulation, several possibilities may be considered. It can be hypothesized that this high concentration of amrinone can preferentially increase the intracellular level of cGMP rather than that of cAMP. In contrast to the suppressive effect of a rise in cAMP concentration on the production of TNF-α (Katakami et al., 1988), the elevation of cGMP level can potently upregulate TNF-α synthesis in macrophages (Renz et al., 1988; Harbrecht et al., 1995).

Another possibility is that the high concentration of amrinone may have exerted its effect by elevation of the intracellular calcium concentration, since earlier studies have reported that amrinone increases calcium influx from the extracellular space into the cell by influencing a sodium-dependent calcium channel (Mancini et al., 1985). Since augmentation of intracellular calcium concentration is reported to enhance TNF-α production (Lo et al., 1996), it can be assumed that at this high concentration of amrinone the enhancing effect of increase in calcium level may have overridden the suppressive effect of cAMP.

An additional explanation for the restoration of TNF-α production at 300 μM amrinone is that it inhibits the activity of cAMP-dependent protein kinase A by competitively preventing the binding of adenosine triphosphate to the catalytic subunit (Earl et al., 1986). Thus, the elevated intracellular cAMP concentration is unable to exert its effect, because the protein kinase A which mediates this action is directly inhibited by amrinone.

Similarly to our earlier in vivo data (Németh et al., 1997), the lipopolysaccharide-evoked production of NO was concentration-dependently decreased by amrinone at a range of 30–300 μM in J774.1 macrophages as well as in rat primary cells (peritoneal macrophages). The parallel downregulation of TNF-α and NO formation by 30 and 100 μM amrinone can be attributed to the fact that TNF-α is a key intermediate in the activation of inducible nitric oxide synthase in response to lipopolysaccharide in vitro and in vivo (Thiemermann et al., 1993; Szabó, 1995). On the other hand, the difference in the regulation of TNF-α and NO production by 300 μM amrinone may be due to a mechanism involving other cytokines and intracellular signals that play a role in the induction of inducible NO synthase gene expression.

Interestingly, we also demonstrated that pretreatment with 300 μM amrinone resulted in a marked decrease in interleukin-10 production. This was an unexpected finding because earlier studies have reported that increase in cAMP concentration brings about an augmentation of interleukin-10 synthesis (Kambayashi et al., 1995; Jilg et al., 1996). It is conceivable in this case that 300 μM amrinone may more effectively elevate the intracellular level of cGMP rather than that of cAMP. The enhancement of cGMP concentration can inhibit the interleukin-10 production as opposed to the upregulating effect of a rise in cAMP level on the synthesis of this cytokine.

Taken together, our findings provide functional evidence that this clinically used positive inotropic agent modulates the production of pro- and anti-inflammatory cytokines. It is noteworthy in this respect that the concentrations of amrinone which elicited biological effects in the current study are in the same concentration range as the peak plasma levels of this agent (Allen-Webb et al., 1994; Sato et al., 1995). The inhibition of TNF-α and NO production can be regarded as a beneficial action of amrinone. On the other hand, the decrease in interleukin-10 release and the increase in NF-κB nuclear translocation by amrinone is more difficult to evaluate in clinical application. Although interleukin-10 downregulates the inflammatory cascade via inhibition of proinflammatory cytokine production (Gerard et al., 1993; Marchant et al., 1994), the

high level of interleukin-10 may enhance susceptibility to invasion of microorganisms in repeated infection or continuous septic processes (Standiford et al., 1995; Ertel et al., 1996). Moreover, activation of NF-$\kappa$B is expected to upregulate, rather than suppress, pro-inflammatory pathways. Thus, in our in vitro system, amrinone has neither clear pro-inflammatory, nor anti-inflammatory effects. It is conceivable that the actions of this phosphodiesterase inhibitor in vivo might be cell-type specific, and may be different in the early versus the late phase of the inflammatory response. Clearly, further studies are needed in order to determine the relevance of the various immunomodulatory effects of this drug in vivo.

## Acknowledgements

The expert technical assistance of Mr. M. O'Connor is gratefully acknowledged. This work was supported by grants from OTKA (No. T 017567 and T 016756 Hungarian Research Fund) and ETT 200/1996.

## References

Allen-Webb, E.M., Ross, M.P., Pappas, J.B., McGough, E.C., Banner, W. Jr., 1994. Age-related amrinone pharmacokinetics in a pediatric population. Crit. Care Med. 22, 1016–1024.

Beutler, B., 1995. TNF, immunity and inflammatory disease: Lessons of the past decade. J. Invest. Med. 43, 227–235.

Dinarello, C.A., 1991. The proinflammatory cytokines interleukin-1 and tumor necrosis factor and the treatment of the septic shock syndrome. J. Infect. Dis. 163, 1177–1184.

Earl, C.Q., Linden, J., Weglicki, W.B., 1986. Inhibition of cyclic AMP-dependent protein kinase activity by the cardiotonic drugs amrinone and milrinone. Life Sci. 39, 1901–1908.

Elenkov, I.J., Haskó, G., Kovács, K.J., Vizi, E.S., 1995. Modulation of lipopolysaccharide-induced tumor necrosis factor-$\alpha$ production by selective $\alpha$- and $\beta$-adrenergic drugs in mice. J. Neuroimmunol. 61, 123–131.

Endres, S., Sinha, B., Fülle, H.J., 1994. Amrinone suppresses the synthesis of tumor necrosis factor-$\alpha$ in human mononuclear cells. Shock 5, 377–380.

Ertel, W., Keel, M., Steckholzer, U., Ungethüm, U., Trentz, O., 1996. Interleukin-10 attenuates the release of proinflammatory cytokines but depresses splenocyte functions in murine endotoxemia. Arch. Surg. 131, 51–56.

Fiorentino, D.F., Zlotnik, A., Mosmann, T.R., Howard, M., O'Garra, A., 1991. IL-10 inhibits cytokine production by activated macrophages. J. Immunol. 147, 3815–3822.

Gerard, C., Bruyns, C., Marchant, A., Abramowicz, D., Vandenabeele, P., Delvaux, A., Fiers, W., Goldman, M., Velu, T., 1993. Interleukin 10 reduces the release of tumor necrosis factor-$\alpha$ and prevents lethality in experimental endotoxemia. J. Exp. Med. 177, 547–550.

Giroir, B.P., Beutler, B., 1992. Effect of amrinone on tumor necrosis factor production in endotoxic shock. Circ. Shock 36, 200–207.

Harbrecht, B.G., Wang, S.C., Simmons, R.L., Billiar, T.R., 1995. Cyclic GMP and guanylate cyclase mediate lipopolysaccharide-induced Kupffer cell tumor necrosis factor-$\alpha$ synthesis. J. Leukocyte Biol. 57, 297–302.

Haskó, G., Elenkov, I.J., Kvetan, V., Vizi, E.S., 1995. Differential effect of selective block of $\alpha_2$ adrenoceptors on plasma levels of tumor necrosis factor-$\alpha$, interleukin-6 and corticosterone induced by bacterial lipopolysaccharide in mice. J. Endocrinol. 144, 457–462.

Haskó, G., Szabó, C., Merkel, K., Bencsics, A., Zingarelli, B., Kvetan, V., Vizi, E.S., 1996a. Modulation of lipopolysaccharide-induced tumor necrosis factor-$\alpha$ and nitric oxide production by dopamine receptor agonist and antagonists in mice. Immunol. Lett. 49, 143–147.

Haskó, G., Szabó, C., Németh, Z.H., Kvetan, V., Pastores, S.M., Vizi, E.S., 1996b. Adenosine receptor agonists differentially regulate IL-10, TNF-$\alpha$, and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. J. Immunol. 157, 4634–4640.

Jilg, S., Barsig, J., Leist, M., Küsters, S., Volk, H.-D., Wendel, A., 1996. Enhanced release of interleukin-10 and soluble tumor necrosis factor receptors as novel principles of methylxanthine action in murine models of endotoxic shock. J. Pharmacol. Exp. Ther. 278, 421–431.

Kambayashi, T., Jacob, C.O., Zhou, D., Mazurek, N., Fong, M., Strassmann, G., 1995. Cyclic nucleotide phosphodiesterase type IV participates in regulation of IL-10 and the subsequent inhibition of TNF-$\alpha$ and IL-6 release by endotoxin-stimulated macrophages. J. Immunol. 155, 4909–4916.

Katakami, Y., Nakao, Y., Koizumi, T., Katakami, N., Ogawa, R., Fujita, T., 1988. Regulation of tumor necrosis factor production by mouse peritoneal macrophages: The role of cellular cyclic AMP. Immunology 64, 719–724.

Lo, C.-J., Garcia, I., Cryer, H.G., Maier, R.V., 1996. Calcium and calmodulin regulate lipopolysaccharide-induced alveolar macrophage production of tumor necrosis factor and procoagulant activity. Arch. Surg. 131, 44–50.

Mancini, D., LeJemtel, T., Sonnenblick, E., 1985. Intravenous use of amrinone for the treatment of the failing heart. Am. J. Cardiol. 56, 8B–15B.

Marchant, A., Bruyns, C., Vandenabeele, P., Ducarme, M., Gerard, C., Delvaux, A., de Groote, D., Abramowicz, C., Velu, T., Goldman, M., 1994. Interleukin-10 controls interferon-$\gamma$ and tumor necrosis factor production during experimental endotoxemia. Eur. J. Immunol. 24, 1167–1171.

Mossmann, T.R., 1994. Properties and functions of interleukin-10. Adv. Immunol. 56, 1–26.

Müller, J.M., Ziegler-Heitbrock, H.W.L., Baeuerle, P.A., 1993. Nuclear factor kappa B, a mediator of lipopolysaccharide effects. Immunobiology 187, 211–256.

Murray, K.J., England, P.J., Hallam, T.J., Maguire, J., Moores, K., Reeves, M.L., Simpson, A.W.M., Rink, T.J., 1990. The effects of siguazodan, a selective phosphodiesterase inhibitor, on human platelet function. Br. J. Pharmacol. 99, 612–616.

Németh, Z.H., Haskó, G., Szabó, C., Vizi, E.S., 1997. Amrinone and theophylline differentially regulate cytokine and nitric oxide production in endotoxemic mice. Shock 7, 371–375.

Pastores, S.M., Haskó, G., Vizi, E.S., Kvetan, V., 1996. Cytokine production and its manipulation by vasoactive drugs. New Horiz. 4, 252–264.

Renz, H., Gong, J.H., Schmidt, A., Nain, M., Gemsa, D., 1988. Release of tumor necrosis factor-$\alpha$ from macrophages. Enhancement and suppression are dose-dependently regulated by prostaglandin $E_2$ and cyclic nucleotides. J. Immunol. 141, 2388–2393.

Sato, Y., Matsumori, A., Sasayama, S., 1995. Inotropic agent vesnarinone inhibits cytokine production and E-selectin expression in human umbilical vein endothelial cells. J. Mol. Cell. Cardiol. 27, 2265–2273.

Sinha, B., Semmler, J., Eisenhut, T., Eigler, A., Endres, S., 1995. Enhanced tumor necrosis factor suppression and cyclic adenosine monophosphate accumulation by combination of phosphodiesterase inhibitors and prostanoids. Eur. J. Immunol. 25, 147–153.

Souness, J.E., Griffin, M., Maslen, C., Ebsworth, K., Scott, L.C., Pollock, K., Palfreyman, M.N., Karlsson, J.A., 1996. Evidence that cyclic AMP phosphodiesterase inhibitors suppress TNF-$\alpha$ generation from human monocytes by interacting with a 'low-affinity' phosphodiesterase 4 conformer. Br. J. Pharmacol. 118, 649–658.

Standiford, T.J., Strieter, R.M., Lukacs, N.W., Kunkel, S.L., 1995. Neutralization of IL-10 increases lethality in endotoxemia: Coopera-

tive effects of macrophage inflammatory protein-2 and tumor necrosis factor. J. Immunol. 155, 2222–2229.

Strassmann, G., Patil-Koota, V., Finkelmann, F., Fong, M., Kambayashi, T., 1994. Evidence for involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin $E_2$. J. Exp. Med. 180, 2365–2370.

Szabó, C., 1995. Alteration in the production of nitric oxide in various forms of circulatory shock. New Horiz. 3, 2–32.

Szabó, C., 1996a. The pathophysiological role of peroxynitrite in shock, inflammation, and ischaemia-reperfusion injury. Shock 6, 79–88.

Szabó, C., 1996b. Physiological and pathophysiological roles of nitric oxide in the central nervous system. Brain Res. Bull. 41, 131–141.

Szabó, C., Wu, C.C., Gross, S.S., Thiemermann, C., Perretti, M., Vane, J.R., 1993a. Interleukin-1 contributes to the induction of nitric oxide synthase by endotoxin in vivo. Eur. J. Pharmacol. 250, 157–160.

Szabó, C., Wu, C.C., Mitchell, J.A., Gross, S.S., Thiemermann, C., Vane, J.R., 1993b. Platelet-activating factor contributes to the induction of nitric oxide synthase by bacterial lipopolysaccharide. Circ. Res. 73, 991–999.

Szabó, C., Thiemermann, C., Vane, J.R., 1993c. Dihydropyridine antagonists and agonists of calcium channels inhibit the induction of nitric oxide synthase by endotoxin in cultured macrophages. Biochem. Biophys. Res. Commun. 196, 825–830.

Thiemermann, C., Wu, C.C., Szabó, C., Peretti, M., Vane, J.R., 1993. Tumor necrosis factor is an endogenous mediator of the induction of nitric oxide synthase in endotoxin shock in the rat. Br. J. Pharmacol. 110, 177–182.

Torphy, T.J., Undem, B.J., 1991. Phosphodiesterase inhibitors: New opportunities for the treatment of asthma. Thorax 46, 512–523.

Verghese, M.W., McConnell, R.T., Strickland, A.B., Gooding, R.C., Stimpson, S.A., Yarnall, D.P., Taylo, J.D., Furdon, P.J., 1995. Differential regulation of human monocyte-derived TNF-$\alpha$ and IL-1$\beta$ by type IV cAMP-phosphodiesterase (cAMP-PDE) inhibitors. J. Pharmacol. Exp. Ther. 272, 1313–1320.

Zingarelli, B., Salzman, A.L., Szabó, C., 1996. Protective effects of nicotinamide against nitric oxide-mediated vascular failure in endotoxic shock: Potential involvement of polyADP ribosyl synthetase. Shock 5, 258–264.