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Exogenous and endogenous catecholamines inhibit the production of macrophage inflammatory protein (MIP) 1α *via* a β adrenoceptor mediated mechanism

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1 Noradrenaline (NA) and adrenaline (Ad) are modulators of cytokine production. Here we investigated the role of these neurotransmitters in the regulation of macrophage inflammatory protein (MIP)-1α expression.

2 Pretreatment of RAW 264.7 macrophages with NA or Ad decreased, in a concentration-dependent manner (1 nM – 100 μM), MIP-1α release induced by bacterial lipopolysaccharide (LPS 10 ng ml^{-1} LPS). The effect of NA was reversed by the selective β-adrenoceptor antagonist propranolol (10 μM), but not by the α-adrenoceptor antagonist phentolamine (10 μM).

3 In the concentration range of 10 nM – 10 μM, isoproterenol, a β-adrenoceptor agonist, but not phenylephrine (a selective α_1-adrenoceptor agonist) or UK-14304 (a selective α_2-adrenoceptor agonist) mimicked the inhibitory effects of catecholamines on MIP-1α production. Increases in intracellular cyclic adenosine monophosphate, elicited either by the selective type IV phosphodiesterase inhibitor rolipram (0.1 – 10 μM), or by prostaglandin E_2, (10 nM – 10 μM) decreased MIP-1α release, suggesting that increased cyclic AMP may contribute to the suppression of MIP-1α release by β-adrenoceptor stimulation.

4 Northern blot analysis demonstrated that NA (100 nM – 10 μM), Ad, isoproterenol, as well as rolipram (100 nM – 10 μM) decreased LPS-induced MIP-1α mRNA accumulation. NA and Ad (1 – 100 μM) also decreased MIP-1α production in thioglycollate-elicited murine peritoneal macrophages.

5 Pretreatment of mice with either isoproterenol (10 mg kg^{-1}, i.p.) or rolipram (25 mg kg^{-1}, i.p.) decreased LPS-induced plasma levels of MIP-1α, while propranolol (10 mg kg^{-1}, i.p.) augmented the production of this chemokine, confirming the role of a β-adrenoceptor mediated endogenous catecholamine action in the regulation of MIP-1α production *in vivo*.

6 Thus, based on our data we conclude that catecholamines are important endogenous regulators of MIP-1α expression in inflammation.

Keywords: Chemokines; inflammation; sympathetic nervous system; adrenergic; inflammation; macrophage

Introduction

The CC-chemokine macrophage inflammatory protein-1α (MIP-1α) was originally identified as a product of endotoxin-stimulated RAW 264.7 macrophages (Davatelis *et al*., 1988; Wolpe *et al*., 1988) and was subsequently shown to be produced by a number of cell types including neutrophils (Kasama *et al*., 1993), activated lymphocytes (Schall *et al*., 1992), and fibroblasts (Koch *et al*., 1994). MIP-1α is a member of the chemokine gene superfamily and is chemotactic for macrophages and T lymphocytes. In addition to its chemotactic activity, MIP-1α potently activates macrophages to secrete TNF-α, interleukin (IL)-1, and IL-6 (Fahey *et al*., 1992). MIP-1α has been shown to importantly contribute to the lung leukocyte accumulation, capillary leak, acute lung injury, and mortality in endotoxemia, influenza virus-infected mice, and by infection with group A Streptococci (Cook *et al*., 1995; Shanley *et al*., 1995, 1996; Standiford *et al*., 1995). We have recently shown that systemic appearance of MIP-1α can be also induced by the superantigen staphylococcal enterotoxin B (Haskó *et al*., 1998c). MIP-1α has also been implicated as a pathogenic factor in various other inflammatory states such as rheumatoid arthritis (Kasama *et al*., 1995), experimental autoimmune encephalomyelitis (Karpus *et al*., 1995), glomerulonephritis (Natori *et al*., 1997), and asthma (Strieter *et al*., 1996). Recently, it was shown that MIP-1α released by LPS-stimulated human macrophages inhibited HIV replication both in macrophages and T lymphocytes (Verani *et al*., 1997).

The catecholamines NA and Ad are among the most important stress hormones that can regulate macrophage function. Cells of the monocyte/macrophage line express catecholamine receptors and respond to occupation with selective ligands of these G-protein coupled cell surface structures by altering such diverse activities as cytokine production (Spengler *et al*., 1990, 1994; Severn *et al*., 1992; Pastores *et al*., 1996; van der Poll *et al*., 1996), free radical release (Feinstein *et al*., 1993; Szabó *et al*., 1997; Haskó *et al*., 1998a), migration (Pick, 1972), or tumoricidal activity (Koff & Dunegan, 1985). Inflammatory processes mediated by macrophage products induce the release of both NA and Ad (Hall & Hodge, 1971; Dunn, 1992; Besedovsky & Del Rey, 1996) from the sympathetic nervous system, providing the means for the feedback regulation of macrophage function.

In the present study, we examined, whether catecholamines regulate *in vitro* MIP-1α production in various cellular systems

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including RAW 264.7 macrophages, primary peritoneal macrophages, and *in vivo* production in endotoxemic mice. We report that catecholamines exert a substantial inhibitory activity on MIP-1α production both *in vitro* and *in vivo* demonstrating a novel mechanism whereby the sympathetic nervous system can modulate the immune response.

Methods

Mice

Male BALB/c mice (8 weeks) were purchased from Charles River Laboratories (Wilmington, MA, U.S.A.).

Materials

DMEM was purchased from Life Technologies (Grand Island, NY, U.S.A.). LPS from *Escherichia coli* 055:B5, prostaglandin $(PG)E_2$, (−) adrenaline, (−) arterenol (NA), and (±) isoproterenol was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). (±)-Propranolol hydrochloride, phentolamine mesylate, phenylephrine hydrochloride, U.K.-14,304, and rolipram were purchased from Research Biochemicals International (Natick, MA, U.S.A.). CH-38083 (7,8-(methylendioxi)-14-hydroxy-alloberbane HCl) was obtained from Chinoin (Budapest, Hungary).

In vitro *experiments*

Cell culture The mouse macrophage cell line RAW 264.7 was grown in DMEM supplemented with 10% FCS, 100 U ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin in a humidified atmosphere of 95% air and 5% CO_2. Cells were cultured in 96-well plates (200 μl medium per well) until 80% confluence. For MIP-1α measurement in RAW macrophages, cells were treated with various concentrations of NA, Ad, rolipram, PGE_2 or selective α- and β-adrenoceptor agonists 30 min before the addition of 10 ng ml^{-1} LPS and supernatants were taken at various time points after LPS. Selective antagonists of adrenoceptors were added 30 min before NA. MIP-1α was determined by ELISA as described below.

Preparation of peritoneal macrophages Mice were injected intraperitoneally (i.p.) with 1 ml of 1.5 % thioglycollate (TG) and peritoneal cells were harvested 3–4 days later. The cells were plated on 96-well plastic plates at 1 million cells ml^{-1} and incubated in DMEM 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 PBS. Cells were then stimulated with LPS (10 ng ml^{-1}) for 24 h.

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 (Haskó *et al*., 1996). Cells in 96-well plates were incubated with MTT (2×10^{-1} mg ml^{-1}) for 60 min at 37°C. Culture medium was removed by aspiration, and cells were solubilized in dimethylsulphoxide (100 μl). The extent of reduction of MTT to formazan within cells was quantitated by measurement of optical density at 550 nm (OD_{550}) using a Spectramax microplate reader.

Northern blot analysis Total cellular RNA was extracted from each well using a guanidinium isothiocyanate/chloroform based technique (TRIZOL; Gibco BRL, U.S.A.), followed by isopropanol precipitation. Cytoplasmic RNA (10–15 μg) was fractionated on a 1% formaldehyde gel and transferred to a nylon membrane. The cDNA for rat MIP-1α, which was cloned from IgG immune complex injured lungs (Shanley *et al*., 1995), has been shown to cross-hybridize with mouse MIP-1α was used for Northern blot analysis. This cDNA was radiolabelled with [^{32}P]-dCTP (specific activity, 3,000 Ci mM^{-1}; NAN DuPont) by random priming (Pharmacia, Piscataway, NJ, U.S.A.). Radioactivity of the probe was determined by scintillation counting and 1.5×10^7 c.p.m. were applied to the Northern blot and hybridization was performed at 42°C for 15 h. The hybridized membranes were serially washed at 52°C with 2 × sodium citrate, sodium chloride, 0.1% SDS (2 × SSC) solution. Autoradiography was done on either Kodak X-OMAT-AR film or phosphoimaging screens. After probing for MIP-1α, membranes were stripped with boiling 5 mM EDTA and rehybridized with a [^{3}P]-radiolabelled oligonucleotide probe for 18S ribosomal RNA for determination of equal loading.

In vivo *experiments*

Experimental design for plasma MIP-1α measurements Male BALB/c mice were injected i.p. with isoproterenol, propranolol, or CH-38083 in a volume of 0.1 ml 10^{-1} g body weight. Thirty minutes later, they were challenged with 5 mg kg^{-1} of LPS administered i.p. Animals were anaesthetized at various time-points after LPS treatment and blood was redrawn by the retroorbital route. The blood was then centrifuged for 10 min at 4°C. The plasma was stored at −70°C until assayed.

MIP-1α assay

MIP-1α levels in plasma and cell culture supernatants were determined by an ELISA kit that is specific against murine MIP-1α (R&D, Minneapolis, MN, U.S.A.). Plates were read at 450 nm by the Spectramax 250 microplate reader (Molecular Devices, Sunnyvale, CA, U.S.A.). The detection limit was 1.5 pg ml^{-1} for MIP-1α. The assay was performed according to the manufacturer's instructions.

Data analysis All values in the Figures and text are expressed as means ± s.e. mean of *n* observations, where *n* represents the number of wells studied (6–9 wells from 2–3 independent experiments). Data sets were examined by one- and two-way analysis of variance. After performing the ANOVA, a *post-hoc* analysis was performed, whereby individual group means were compared with Student's unpaired *t*-test. A *P*-value less than 0.05 was considered significant. Where Northern blots are presented, results shown are representative of at least three independent experiments performed on different experimental days.

Results

NA and Ad inhibit MIP-1 α production by RAW 264.7 macrophages

Low levels of MIP-1α were detectable without stimulation in RAW macrophages (not shown). LPS (10 ng ml^{-1}) induced a 10–20 fold elevation of the secretion of MIP-1α by 3 h after LPS exposure (Figure 1), which further increased by 24 h (not shown). The 3 h time point was chosen to examine the effect of adrenergic agonists and antagonists on the LPS-elicited increase in MIP-1α, because this time point represented

maximal MIP-1α plasma levels in the *in vivo* studies (see below), thus allowing for a good comparison of *in vitro* to *in vivo* results. Pretreatment of the cells with NA or Ad 30 min before the addition of LPS caused a concentration-dependent reduction of MIP-1α formation as assessed at the 3 h (Figure 1) and 24 h time-points (not shown). To investigate which adrenoceptors were responsible for the effects of catecholamines, cells were pretreated with NA in the presence of the nonselective α-adrenoceptor antagonist phentolamine (10 μM) or the nonselective β-adrenoceptor blocker propranolol (10 μM) and MIP-1α levels 3 h after LPS were determined. Figure 2 shows that propranolol, but not phentolamine prevented the effect of the non-selective adrenergic agonist NA, demonstrating that the inhibition of MIP-1α production is a β-receptor mediated process. Propranolol or phentolamine alone did not affect MIP-1α production by LPS-stimulated cells (Figure 2).

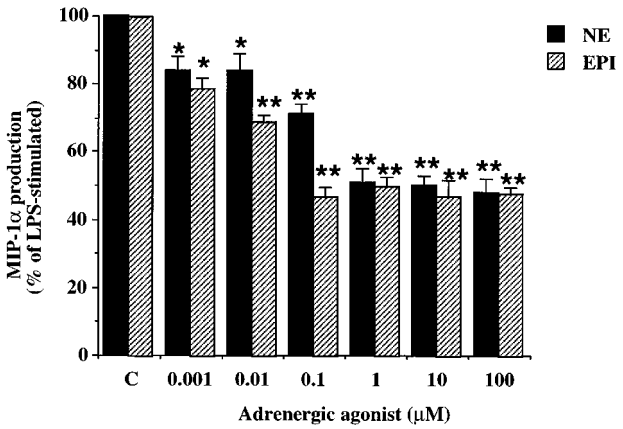

Figure 1 Effect of noradrenaline (NA) or adrenaline (Ad) on MIP-1α production by RAW 264.7 macrophages at 3 h after LPS (10 ng ml^{-1}). The production of MIP-1α increased from 1.5±0.1 to 28.1±2.2 ng ml^{-1} in response to LPS. NA or Ad decreased, in a concentration-dependent manner, MIP-1α release induced by LPS. *$P<0.05$ and **$P<0.01$ indicate significant suppression of MIP-1α in the presence of the catecholamines, when compared to LPS control (C), ($n=6-9$).

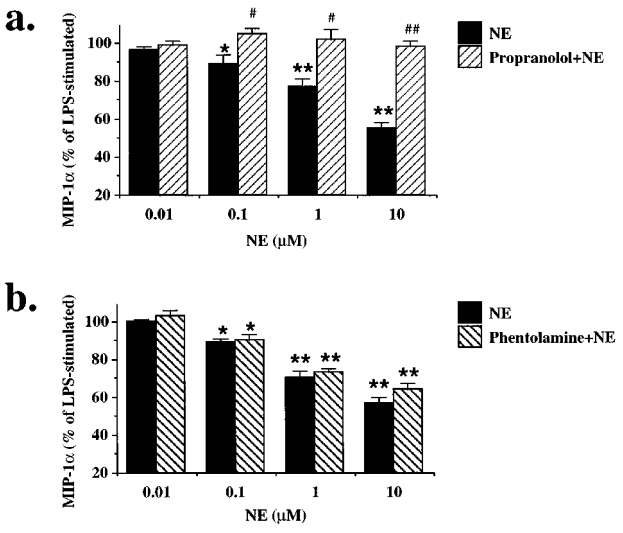

Figure 2 Effect of (a) the beta-blocker propranolol (10 μM) and (b) the alpha blocker phentolamine (10 μM) on the noradrenaline (NA) induced suppression on MIP-1α production by RAW 264.7 macrophages at 3 h after LPS (10 ng ml^{-1}). The production of MIP-1α increased from 1.4±0.2 to 26.0±2.4 ng ml^{-1} in response to LPS. *$P<0.05$ and **$P<0.01$ indicate significant suppression of MIP-1α in the presence of the catecholamines, when compared to LPS control; #$P<0.05$ and ##$P<0.01$ indicate protection by the beta-blocker against the NA-induced suppression of MIP-1α production, ($n=6-9$).

To confirm that β-adrenoceptors mediated the effect of catecholamines, the effects of the selective β-adrenoceptor agonist isoproterenol, the selective α_1-adrenoceptor agonist phenylephrine, or the selective α_2-adrenoceptor agonist UK-14304 were tested on the LPS-induced production of MIP-1α. Figure 3 shows that isoproterenol, but not phenylephrine or UK-14304 decreased MIP-1α production in a concentration-dependent manner, corroborating that β-adrenoceptors play a central role in the suppression of MIP-1 formation by catecholamines. The moderate decrease in MIP-1α production by phenylephrine and UK-14304 at their highest concentrations is likely to represent a non-specific action of these agonists on β-adrenoceptors at the highest dose used. None of the above agonists or antagonists of adrenoceptors used decreased cell viability as determined by the MTT test (not shown).

Role of elevated cyclic adenosine monophosphate (cyclic AMP) levels in the inhibition of MIP-1α production by β-adrenoceptor stimulation

Since the main pathway whereby activation of β-adrenoceptors produces its effects is related to the elevation of intracellular cyclic AMP levels and changes in intracellular cyclic AMP concentrations are known to alter cytokine production (Haskó *et al*., 1998b; Pastores *et al*., 1996), we next determined the effect other agents which elevate intracellular cyclic AMP levels on LPS-stimulated MIP-1α levels in RAW 264.7 cells. Both PGE_2 which augments cyclic AMP by binding to its own receptor, and rolipram, which causes cyclic AMP elevation by inhibiting the cyclic AMP degrading enzyme phosphodiesterase, inhibited, in a concentration-dependent manner, the release of MIP-1α (Figure 4). This observation suggests that the inhibition of MIP-1α production by β-adrenoceptor stimulation is most probably due to its ability to elevate intracellular cyclic AMP. Again, neither PGE_2 nor rolipram affected cell viability as determined by the MTT assay (not shown).

Catecholamines inhibit MIP-1α release by TG-elicited peritoneal adherent cells

LPS (10 μg ml^{-1}) induced a ~1.5–2 fold elevation of MIP-1α in thioglycollate (TG) elicited macrophages over baseline levels

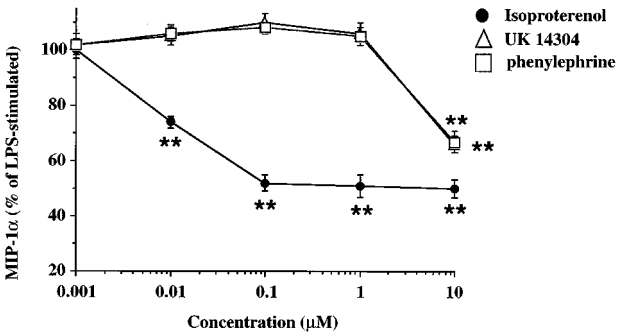

Figure 3 Effect of isoproterenol, UK-14304 and phenylephrine on MIP-1α production by RAW 264.7 macrophages at 3 h after LPS (10 ng ml^{-1}). The production of MIP-1α increased from 1.4±0.2 to 29.2±2.7 ng ml^{-1} in response to LPS. **$P<0.01$ indicates significant suppression of MIP-1α in the presence of the agonists, when compared to LPS control, ($n=6-9$).

measured 24 h after the addition of LPS, which were relatively high, probably owing to the activation caused by TG. Pretreatment of the cells with NA or Ad decreased the degree of LPS-induced MIP-1α production (Figure 5). Both catecholamines also suppressed basal MIP-1α release by these cells (Figure 5).

Effects of catecholamines on MIP-1α mRNA in RAW cells

In order to determine whether inhibition of MIP-1α observed with adrenergic stimulation was at the transcriptional level, steady state mRNA levels were determined under similar *in vitro* conditions as described above. In preliminary studies we established that peak transcript levels for MIP-1α in LPS-stimulated RAW 264.7 cells occurred between 1 and 3 h. Pretreatment of RAW cells with noradrenaline (100 nM – 10 μM, 30 min) resulted in a significant decrease in mRNA levels determined at 2 h post-LPS (Figure 6a). Similar results were found with adrenaline (not shown). Thus, the molecular level of MIP-1α inhibition by catecholamines appears to be at the transcriptional level.

The observation that pretreatment of RAW cells with both isoproterenol (100 nM – 10 μM, 30 min) (not shown) and rolipram (100 nM – 10 μM, 30 min pre-treatment) caused a dose-dependent decrease in steady state mRNA levels for MIP-1α (Figure 6b) is consistent with the proposal that inhibition by Ad and NA of MIP-1α production is mediated *via* β-adrenoceptor stimulation and as a result of increased cyclic AMP levels.

Overall, there was a fairly good agreement in the degree of the inhibition by MIP-1α protein and MIP-1α mRNA expression by the adrenergic agonists or rolipram. However, the degree of inhibition of MIP-1α mRNA levels and the protein levels did not always correspond to each other. For instance, using the ELISA method, we found that rolipram (at 1 μM) markedly inhibited MIP-1α protein production (Figure 4), while the inhibition of MIP-1α mRNA production was less pronounced at this concentration (Figure 6b). Differences in the time of the measurements (protein was measured at 3 h, mRNA at 2 h post-LPS), and/or differences in the sensitivity of the two assays used (ELISA vs Northern blotting) may be responsible for these differences.

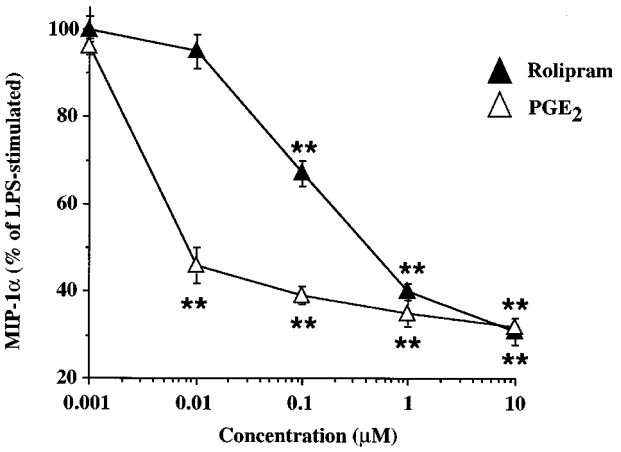

Figure 4 Effect of rolipram and prostaglandin E_2 on MIP-1α production by RAW 264.7 macrophages at 3 h after LPS (10 ng ml^{-1}). The production of MIP-1α increased from 1.4±0.2 to 29.2±2.7 ng ml^{-1} in response to LPS. $^{**}P<0.01$ indicates significant suppression of MIP-1α in the presence of the agonists, when compared to LPS control, ($n=6-9$).

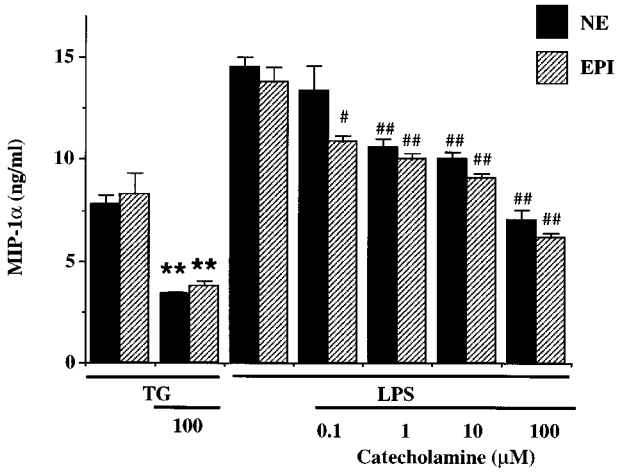

Figure 5 Effect of noradrenaline (NA) or adrenaline (Ad) on MIP-1α production by thioglycollate (TG) elicited macrophages at 24 h after LPS (10 ng ml^{-1}). NA or Ad decreased, in a concentration-dependent manner, MIP-1α release induced by LPS, and also suppressed basal MIP-1α production. $^{**}P<0.01$ indicates significant suppression of basal MIP-1α production by catecholamines, when compared to unstimulated control, $\#P<0.05$ and $\#\#P<0.01$ indicate significant suppression of MIP-1α production by catecholamines, when compared to LPS control ($n=6-9$).

Stimulation of β-adrenoceptors inhibits MIP-1α production in vivo

Intraperitoneal injection of 5 mg kg^{-1} LPS into BALB/c mice induced an elevation of the plasma MIP-1α level, which peaked at 2 h, and returned near baseline levels by 8 h (Figure 7a).

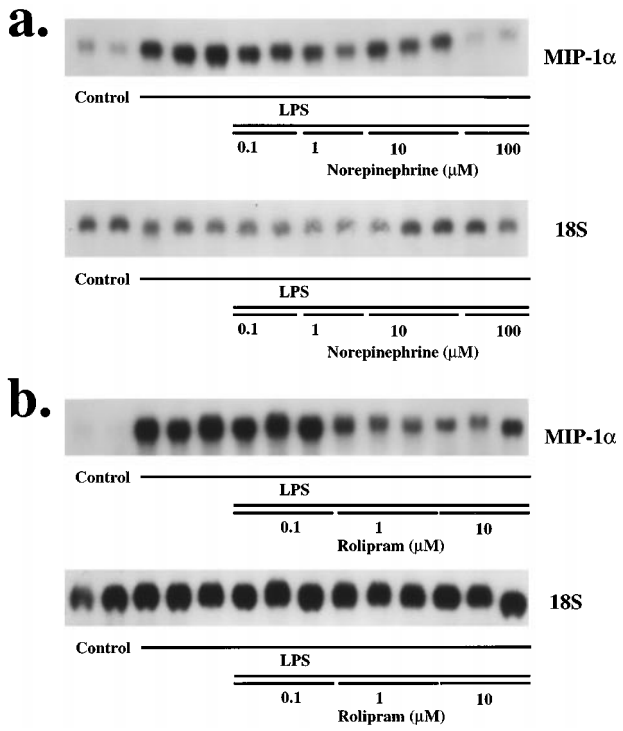

Figure 6 Effect of 100 nM – 100 μM noradrenaline (a), or 100 nM – 10 μM rolipram (b) on the LPS-induced MIP-1α and 18S mRNA production. Rolipram inhibited the expression of MIP-1α mRNA while there were no changes in the expression of 18S mRNA with noradrenaline or rolipram. Results shown are representative of $n=3$ experiments performed on different experimental days.

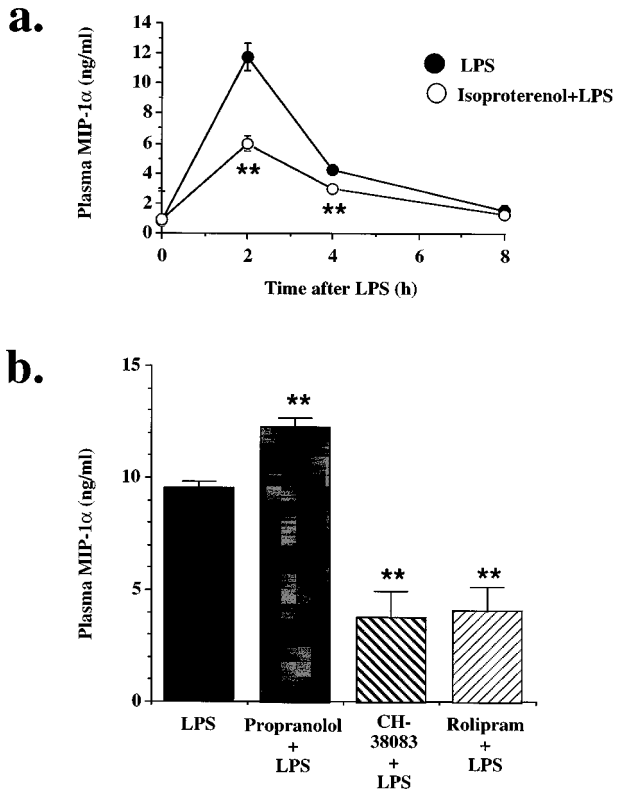

Figure 7 Effect of (a) isoproterenol (10 mg kg^{-1}), and (b) propranolol (10 mg kg^{-1}), CH-38083 (10 mg kg^{-1}), and rolipram (25 mg kg^{-1}) on the LPS-induced production of MIP-1α in mice. In (b), MIP-1α was measured at 3 h post-LPS. $*P<0.05$ and $**P<0.01$ indicate significant effect of the agents tested on plasma levels of MIP-1α; ($n=6-9$).

Pretreatment of the mice with isoproterenol 30 min before the injection of LPS resulted in a significant suppression of plasma MIP-1α levels at 2 and 4 h (Figure 7a). Conversely, pretreatment of animals with propranolol 30 min before LPS brought about a significant enhancement of plasma MIP-1α level (Figure 7b). Collectively, these data suggest that endogenous catecholamines decrease MIP-1α production *via* β-adrenoceptors.

Although, the results of the *in vitro* experiments suggest that α_2-adrenoceptors on macrophages are not involved in the regulation of MIP-1α release by catecholamines, the possible role of presynaptic α_2-adrenoceptors on catecholamine-releasing nerve terminals was tested by injecting the selective α_2-adrenoceptors antagonist CH-38083 (Haskó *et al*., 1995ab; Elenkov *et al*., 1995). Pretreatment of the animals with CH-38083 provoked a significant decrease of the plasma MIP-1α level (Figure 7b). This is consistent with the notion that α_2-adrenoceptor blockade enhances NA release from the sympathetic nerve terminals of lymphoid organs (Haskó *et al*., 1995a; Vizi *et al*., 1995) and the increased stimulation by NA released in excess of postsynaptic (macrophage) β-adrenoceptors suppresses the production of cytokines (Pastores *et al*., 1996; Haskó & Szabó, 1998). Finally, rolipram decreased plasma MIP-1α levels confirming that elevation of cyclic AMP suppresses MIP-1α production *in vivo* (Figure 7b).

Discussion

Over the past two decades a large body of evidence has accumulated demonstrating that immune cell function is under the control of the central nervous system (Madden *et al*., 1995; Vizi *et al*., 1995; Besedovsky & Del Rey, 1996; Haskó & Szabó, 1998). Signalling through the sympathetic nervous system represents one of the main pathways whereby this regulatory action is accomplished. The most important transmitters to relay information from the sympathetic nervous system to the cellular elements of the immune system are the catecholamine neurotransmitters NA and Ad. These molecules have been shown to be released in the vicinity of immune cells upon axonal action potential of sympathetic nerve fibres (Haskó *et al*., 1995a; Vizi *et al*., 1995). Catecholamine release generally occurs in response to stressful conditions caused by cognitive stimuli or non-cognitive stimuli, such as the immune response itself (Besedovsky & Del Rey, 1996). A highly sensitive immunological target for transmitters of the sympathetic nervous system is the mononuclear phagocyte. It is well established that the production of inflammatory mediators by the monocytes/macrophages is responsive to catecholamine action. Tumour necrosis factor (TNF)-α was the first cytokine whose production was shown to be regulated by occupation of α or β-adrenergic receptors by catecholamines (Spengler *et al*., 1990; Hu *et al*., 1991; Severn *et al*., 1992). Subsequently, a whole array of other cytokines have been demonstrated to be influenced by adrenergic receptor stimulation both *in vitro* and *in vivo* (Elenkov *et al*., 1996; van der Poll *et al*., 1996; Szabó *et al*., 1997; Haskó *et al*., 1998a). The present study demonstrates for the first time that classical transmitters of the sympathetic nervous system regulate the production of the CC chemokine MIP-1α. Our results with the RAW 264.7 macrophages and thioglycollate-elicited peritoneal adherent cells show that both catecholamines inhibit the production of this proinflammatory mediator, confirming the predominantly anti-inflammatory activity of catecholamines. The inhibitory action of NA was prevented by the selective β-blocker propranolol, while α-adrenergic blockade by phentolamine was without effect. Furthermore, stimulation of β-adrenoceptors by the selective β agonist isoproterenol, but not with the selective α_1 agonist phenylephrine or the α_2 agonist UK-14304, mimicked the inhibitory effect of NA and Ad. Together, these data implicate the β-adrenoceptor as the predominant substrate for mediating the inhibitory effect of catecholamines on MIP-1α production.

In various cell types, including macrophages, the main intracellular second messenger for β-adrenergic receptors is cyclic AMP (Severn *et al*., 1992; van der Poll *et al*., 1997; Haskó *et al*., 1998a). Since PGE_1 (Martin & Dorf, 1991), PGE_2 (VanOtteren *et al*., 1994, this study), a cell permeable analog of cyclic AMP (VanOtteren *et al*., 1994), and the phosphodiesterase inhibitor rolipram (this study) all suppress MIP-1α expression, it can be proposed that enhanced levels of intracellular cyclic AMP caused the decrease in MIP-1α secretion by β-adrenergic activation. In this respect, the regulation of MIP-1α production is similar to that of TNF-α, which is also suppressed by β-adrenergic stimulation *via* cyclic AMP (Severn *et al*., 1992; Haskó *et al*., 1998a). A further similarity is that both TNF-α (Spengler *et al*., 1989; Ignatowsky *et al*., 1996) and MIP-1α (Martin & Dorf, 1991, VanOtteren *et al*., 1994, this study) are regulated at the transcriptional level by β-adrenoceptor stimulation and/or elevated cyclic AMP levels.

In line with our *in vitro* observations, β-adrenergic stimulation with isoproterenol decreased MIP-1α production *in vivo*. Furthermore, β-adrenergic blockade with propranolol augmented MIP-1α plasma levels, providing evidence for an endogenous regulatory mechanism involving β-adrenoceptor stimulation by catecholamines released from the sympathetic nervous system. However, inhibition of α_2-adrenoceptors

suppressed MIP-1α plasma levels, while the *in vitro* studies showed no evidence for the presence of functional α_2-adrenoceptors on the macrophages. This difference can be explained by taking into account that presynaptic nerve terminals are equipped with inhibitory α_2-adrenoceptors and blockade of these receptors have been shown to enhance NA release (Haskó *et al*., 1995a, Vizi *et al*., 1995). Therefore, the augmented release of NA by blockade of presynaptic receptors can cause an increased stimulation of postsynaptic (macrophage) β-adrenoceptors, resulting in a decreased production of MIP-1α. Such a mechanism has been shown to account for the decrease in TNF-α production in mice treated with α_2-adrenoceptor antagonists (Haskó *et al*., 1995b; Pastores *et al*., 1996; Haskó & Szabó, 1998).

In summary, our results demonstrate for the first time that catecholamines inhibit MIP-1α production. The site of the regulation is transcriptional, and the mechanism is likely to involve increased intracellular cyclic AMP mediated by β-adrenergic stimulation. Our current observations may demonstrate an additional mode of the immunosuppressive action of β-adrenergic stimulation. Our current results may have relevance for a variety of pathophysiological states such as experimental autoimmune encephalomyelitis or asthma, where increased MIP-1α expression has been shown to contribute to disease development (Karpus *et al*., 1995; Strieter *et al*., 1996) and β-adrenergic stimulation is known to ameliorate the course of the disease (Twentyman *et al*., 1990; Wiegmann *et al*., 1995).

References

BESEDOVSKY, H.O. & DEL REY, A. (1996). Immune-neuro-endocrine interactions: facts and hypotheses. *Endocr. Rev.,* **17,** 64 – 102.

COOK, D.N., BECK, M.A., COFFMAN, T.M., KIRBY, S.L., SHERIDAN, J.F., PRAGNELL, I.B. & SMITHIES, O. (1995). Requirement of MIP-1α for an inflammatory response to viral infection. *Science,* **269,** 1583 – 1585.

DAVATELIS, G., TEKAMP-OLSON, T., WOLPE, S.D., HERMSEN, K., LUEDKE, C., GALLEGOS, C., COIT, D., MERRYWEATHER, J. & CERAMI, A. (1988). Cloning and characterization of a cDNA for murine macrophage inflammatory protein (MIP), a novel monokine with inflammatory and chemokinetic properties. *J. Exp. Med.,* **167,** 1939 – 1944.

DUNN, A.J. (1992). Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: Comparison with interleukin-1. *J. Pharmacol. Exp. Ther.,* **261,** 964 – 969.

ELENKOV, I.J., HASKÓ, G., KOVÁCS, K.J. & VIZI, E.S. (1995). Modulation of lipopolysaccharide-induced tumor necrosis factor-α production by selective α-and β-adrenergic drugs in mice. *J. Neuroimmunol.,* **61,** 123 – 131.

ELENKOV, I.J., PAPANICOLAOU, D.A., WILDER, R.L. & CHROUSOS, G.P. (1996). Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. *P. Assoc. Am. Physicians,* **108,** 374 – 381.

FAHEY, T.J. III, TRACEY, K.J., TEKAMP-OLSON, P., COUSENS, L.S., JONES, W.G., SHIRES, G.T., CERAMI, A. & SHERRY, B. (1992). Macrophage inflammatory protein 1 modulates macrophage function. *J. Immunol.,* **148,** 2764 – 2769.

FEINSTEIN, D.L., GALEA, E. & REIS, D.J. (1993). Norepinephrine suppresses inducible nitric oxide synthase activity in rat astroglial cultures. *J. Neurochem.,* **60,** 1945 – 1948.

HALL, R.C. & HODGE, R.L. (1971). Vasoactive hormones in endotoxin shock: a comparative study in cats and dogs. *J. Physiol.,* **213,** 69 – 84.

HASKÓ, G. & SZABÓ, C. (1998). Regulation of cytokine and chemokine production by transmitters of the sympathetic nervous system. *Biochem. Pharmacol.,* **56,** 1079 – 1087.

HASKÓ, G., ELENKOV, I.J. & VIZI, E.S. (1995a). Presynaptic receptors involved in the modulation of release of noradrenaline from the sympathetic nerve terminals of the rat thymus. *Immunol. Lett.,* **47,** 133 – 137.

HASKÓ, G., ELENKOV, I.J., KVETAN, V. & VIZI, E.S. (1995b). Differential effect of selective block of α_2-adrenoreceptors on plasma levels of tumour necrosis factor-α, interleukin-6 and corticosterone induced by bacterial lipopolysaccharide in mice. *J. Endocrinol.,* **144,** 457 – 462.

HASKÓ, G., NÉMETH, Z.H., SZABÓ, C., ZSILLA, G., SALZMAN, A.L. & VIZI, E.S. (1998a). Isoproterenol inhibits IL-10, TNF-α, and nitric oxide production in RAW 264.7 macrophages. *Brain Res. Bull.,* **45,** 183 – 187.

HASKÓ, G., SZABÓ, C., NÉMETH, Z.H., KVETAN, V., PASTORES, S.M. & VIZI, E.S. (1996). Adenosine receptor agonists differentially regulate IL-10, TNF-α, and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. *J. Immunol.,* **157,** 4634 – 4640.

HASKÓ, G., SZABÓ, C., NÉMETH, Z.H., SALZMAN, A.L. & VIZI, E.S. (1998b). Suppression of interleukin-12 production by phosphodiesterase inhibition in murine endotoxemia is interleukin-10 independent. *Eur. J. Immunol.,* **28,** 468 – 472.

HASKÓ, G., VIRÁG, L., EGNACZYK, G., SALZMAN A.L. & SZABÓ, C. (1998c). The crucial role of interleukin-10 in the suppression of the immune response in mice exposed to staphylococcal enterotoxin B. *Eur. J. Immunol.,* **28,** 1417 – 1425.

HU, X., GOLDMUNTZ, E.A. & BROSNAN, C.F. (1991). The effect of norepinephrine on endotoxin-mediated macrophage activation. *J. Neuroimmunol.,* **31,** 35 – 42.

IGNATOWSKY, T.A., GALLANT, S. & SPENGLER, R.N. (1996). Temporal regulation by adrenergic receptor stimulation of macrophage (Mφ)-derived tumor necrosis factor (TNF) production post-LPS challenge. *J. Neuroimmunol.,* **65,** 107 – 117.

KARPUS, W.J., LUKACS, N.W., McRAE, B.L., STRIETER, R.M., KUNKEL, S.L. & MILLER, S.D. (1995). An important role for the chemokine macrophage inflammatory protein-1α in the pathogenesis of the T cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis. *J. Immunol.,* **155,** 5003 – 5010.

KASAMA, T., STRIETER, R.M., LUKACS, N.W., LINCOLN, P.M., BURDICK, M.D. & KUNKEL, S.L. (1995). Interleukin-10 expression and chemokine regulation during the evolution of murine type II collagen-induced arthritis. *J. Clin. Invest.,* **95,** 2868 – 2876.

KASAMA, T., STRIETER, R.M., STANDIFORD, T.J., BURDICK, M.D. & KUNKEL S.L. (1993). Expression and regulation of human neutrophil-derived macrophage inflammatory protein 1α. *J. Exp. Med.,* **178,** 63 – 72.

KOCH, A.E., KUNKEL, S.L., HARLOW, L.A., MAZARAKIS, D.D., HAINES, G.K., BURDICK, M.D., POPE, R.M. & STRIETER, R.M. (1994). Macrophage inflammatory protein-1α: a novel chemotactic cytokine for macrophages in rheumatoid arthritis. *J Clin. Invest.,* **93,** 921 – 928.

KOFF, W.C. & DUNEGAN, M.A. (1985). Modulation of macrophage-mediated tumoricidal activity by neuropeptides and neurohormones. *J. Immunol.,* **135,** 350 – 354.

MADDEN, K.S., SANDERS, V.M. & FELTEN D.L. (1995). Catecholamine influences and sympathetic neural modulation of immune responsiveness. *Annu. Rev. Pharmacol. Toxicol.,* **35,** 417 – 448.

MARTIN, C.A. & DORF, M.E. (1991). Differential regulation of interleukin-6, macrophage inflammatory protein-1, and JE/MCP-1 cytokine expression in macrophage cell lines. *Cell. Immunol.,* **135,** 245 – 258.

NATORI, Y., SEKIGUCHI, M., OU, Z. & NATORI, Y. (1997). Gene expression of CC chemokines in experimental crescentic glomerulonephritis (CGN). *Clin. Exp. Immunol.,* **109,** 143 – 148.

PASTORES, S.M., HASKO, G., VIZI, E.S. & KVETAN, V. (1996). The role of vasoactive drugs in the regulation of cytokine production in sepsis. *New Horiz.,* **4,** 252 – 264.

PICK, E. (1972). Cyclic AMP affects macrophage migration. *Nature New Biol.,* **238,** 176 – 177.

SANDERS, V.M. (1995). The role of adrenoceptor-mediated signals in the modulation of lymphocyte function. *Adv. Neuroimmunol.,* **5,** 283 – 298.

SCHALL, T.J., O'HEHIR, R.E., GOEDDEL, D.V. & LAMB, J.R. (1992). Uncoupling of cytokine mRNA expression and protein secretion during the induction phase of T cell anergy. *J. Immunol.,* **148,** 381 – 387.

SEVERN, A., RAPSON, N.T., HUNTER, C.A. & LIEW, F.Y. (1992). Regulation of tumor necrosis factor production by adrenaline and β-adrenergic agonists. *J. Immunol.,* **148,** 3441 – 3445.

SHANLEY, T.P., SCHMAL, H., FRIEDL, H.P., JONES, M.L. & WARD, P.A. (1995). Role of macrophage inflammatory protein-1α (MIP-1α) in acute lung injury in rats. *J. Immunol.,* **154,** 4793–4802.

SHANLEY, T.P., SCHRIER, D., KAPUR, V., KEHOE, M., MUSSER, J.M. & WARD, P.A. (1996). Streptococcal cysteine protease augments lung injury induced by products of group A streptococci. *Infect. Immun.,* **64,** 870–877.

SPENGLER, R.N., ALLEN, R.M., REMICK, D.G., STRIETER, R.M. & KUNKEL, S.L. (1990). Stimulation of α-adrenergic receptor augments the production of macrophage-derived tumor necrosis factor. *J. Immunol.,* **145,** 1430–1434.

SPENGLER, R.N., CHENSUE, S.W., GIACHERIO, D.A., BLENK, N. & KUNKEL, S.L. (1994). Endogenous norepinephrine regulates tumor necrosis factor-α production from macrophages *in vitro*. *J. Immunol.,* **152,** 3024–3031.

SPENGLER, R.N., SPENGLER, M.L., LINCOLN, P., REMICK, D.G., STRIETER, R.M. & KUNKEL, S.L. (1989). Dynamics of dibutyryl cyclic AMP- and prostaglandin E_2-mediated suppression of lipopolysaccharide-induced tumor necrosis factor α gene expression. *Infect. Immun.,* **57,** 2837–2841.

STANDIFORD, T.J., KUNKEL, S.L., LUKACS, N.W., GREENBERGER, M.J., DANFORTH, J.M., KUNKEL, R.G. & STRIETER, R.M. (1995). Macrophage inflammatory protein-1α mediates lung leukocyte recruitment, lung capillary leak, and early mortality in murine endotoxemia. *J. Immunol.,* **155,** 1515–1524.

STRIETER, R.M., STANDIFORD, T.J., HUFFNAGLE, G.B., COLLETTI, L.M., LUKACS, N.W. & KUNKEL, S.L. (1996). ''The good, the bad, and the ugly'': the role of chemokines in models of human disease. *J. Immunol.,* **156,** 3583–3586.

SZABÓ, C., HASKÓ, G., ZINGARELLI, B., NÉMETH, Z.H., SALZMAN, A.L., KVETAN, V., PASTORES, S.M. & VIZI, E.S. (1997). Isoproterenol regulates TNF, IL-10, IL-6 and nitric oxide production and protects against the development of vascular hyporeactivity in endotoxemia. *Immunology,* **90,** 95–100.

TWENTYMAN, O.P., FINNERTY, J.P., HARRIS, A., PALMER, J. & HOLGATE, S.T. (1990). Protection against allergen-induced asthma by salmeterol. *Lancet,* **336,** 1338–1342.

VAN DER POLL, T., COYLE, S.M., BARBOSA, K., BRAXTON, C.C. & LOWRY, S.F. (1996). Epinephrine inhibits tumor necrosis factor-α and potentiates interleukin 10 production during human endotoxemia. *J. Clin. Invest.,* **97,** 713–719.

VAN DER POLL, T. & LOWRY, S.F. (1997). Lipopolysaccharide-induced interleukin 8 production by human whole blood is enhanced by epinephrine and inhibited by hydrocortisone. *Infect. Immun.,* **65,** 2378–2381.

VANOTTEREN, G.M., STANDIFORD, T.J., KUNKEL, S.L., DANFORTH, J.M., BURDICK, M.D., ABRUZZO, L.V. & STRIETER, R.M. (1994). Expression and regulation of macrophage inflammatory protein-1α by murine alveolar and peritoneal macrophages. *Am. J. Respir. Cell Mol. Biol.,* **10,** 8–15.

VERANI, A., SCARLATTI, G., COMAR, M., TRESOLDI, E., POLO, S., GIACCA, M., LUSSO, P. SICCARDI, A.G. & VERCELLI, D. (1997). C-C chemokines released by lipopolysaccharide (LPS)-stimulated human macrophages suppress HIV-1 infection in both macrophages and T cells. *J. Exp. Med.,* **185,** 805–816.

VIZI, E.S., ORSÓ, E., OSIPENKO, O.N., HASKÓ, G. & ELENKOV, I.J. (1995). Neurochemical, electrophysiological and immunocytochemical evidence for a noradrenergic link between the sympathetic nervous system and thymocytes. *Neuroscience,* **68,** 1263–1276.

WIEGMANN, K., MUTHYALA, S., KIM, D.H., ARNASON, B.G.W. & CHELMICKA-SCHORR, E. (1995). β-Adrenergic agonists suppress chronic/relapsing experimental allergic encephalomyelitis (CREAE) in Lewis rats. *J. Neuroimmunol.,* **56,** 201–206.

WOLPE, S.D., DAVATELIS, G., SHERRY, B., BEUTLER, B., HESSE, D., NGUYEN, H., MOLDAWER, L.L., NATHAN, C.F., LOWRY, S.F. & CERAMI, A. (1988). Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. *J. Exp. Med.,* **167,** 570–581.

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