British Journal of Pharmacology (2001) 133, 237–242

www.nature.com/bjp

Inhibitory effects of cyclic AMP elevating agents on lipopolysaccharide (LPS)-induced microvascular permeability change in mouse skin

*,[1]Kaoru Irie, [1,2]Emiko Fujii, [3]Hiroyasu Ishida, [1]Keiji Wada, [1]Taiyo Suganuma, [4]Tomohiro Nishikori, [1]Toshimasa Yoshioka & [1]Takamura Muraki

[1]Department of Pharmacology, Tokyo Women's Medical University, School of Medicine, Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan; [2]Department of Pharmacy, Tokyo Women's Medical University, School of Medicine, Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan; [3]Department of Anesthesiology, Nippon Medical School, Sendagi, Bunkyo-ku, Tokyo 113-9603, Japan and [4]Department of Anesthesiology, Tokyo Women's Medical University, School of Medicine, Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan

1 Anti-inflammatory effects of cyclic AMP elevating agents were examined in a mouse model of lipopolysaccharide (LPS)-induced microvascular permeability change.

2 Vascular permeability on the back skin was measured by the local accumulation of Pontamine sky blue (PSB) after subcutaneous injection of LPS (400 μg site^{-1}) from *Salmonella typhimurium*.

3 Dye leakage in the skin was significantly increased 2 h after injection of LPS. This LPS-induced dye leakage was suppressed by phosphodiesterase inhibitors, including pentoxifylline (160 mg kg^{-1}), milrinone (5–10 mg kg^{-1}), rolipram (0.5–10 mg kg^{-1}) and zaprinast (5–10 mg kg^{-1}). The dye leakage was also inhibited by β-adrenoceptor agonists, including isoproterenol (0.5–5 mg kg^{-1}) and salbutamol (0.05–5 mg kg^{-1}), an adenylate cyclase activator, forskolin (5 mg kg^{-1}), and a cell permeable cyclic AMP analogue, 8-bromo-cyclic AMP (8-Br-cAMP, 10 mg kg^{-1}).

4 LPS caused a transient increase in serum TNF-α level peaking at 1 h after the injection. This increase in serum TNF-α was completely blocked by a pretreatment with pentoxifylline (160 mg kg^{-1}), milrinone (5 mg kg^{-1}), rolipram (1 mg kg^{-1}), zaprinast (10 mg kg^{-1}), salbutamol (0.5 mg kg^{-1}), forskolin (1 mg kg^{-1}) and 8-Br-cAMP (10 mg kg^{-1}).

5 LPS caused an increase in serum IL-1α level peaking at 3 h after injection. This increase in serum IL-1α was not significantly suppressed by the cyclic AMP elevating agents.

6 Our study suggests that cyclic AMP elevating agents attenuate LPS-induced microvascular permeability change by suppressing TNF-α up regulation.

British Journal of Pharmacology (2001) **133,** 237–242

Keywords: Phosphodiesterase inhibitors; lipopolysaccharide; plasma leakage; cyclic AMP; tumour necrosis factor-α; β_2-adrenoceptor agonist; 8-bromo-cyclic AMP; forskolin; mouse skin

Abbreviations: 8-Br-cAMP, 8-bromo-cyclic AMP; IL-1α, interleukin-1α; LPS, lipopolysaccharide; PDE, phosphodiesterase; PSB, pontamine sky blue; TNF-α, tumour necrosis factor-α

Introduction

Endotoxin or lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria, causes an endotoxin syndrome, which is characterized by fever, hypotension, and multiple organ failure (Klosterhalfen & Bhardwaj, 1998; Karima *et al*., 1999). The endotoxin syndrome is associated with a release of inflammatory mediators including eicosanoids, cytokines, platelet-activating factor (PAF) and nitric oxide (Liao, 1996; Karima *et al*., 1999). Subcutaneous injection of LPS on the back of mice/rats induces a plasma leakage at the site of injection, which has been used as an *in vivo* model of inflammation (Fujii *et al*., 1996a, b; 2000; Iuvone *et al*., 1999). This LPS-induced increase in vascular permeability is mediated by TNF-α, IL-1α, histamine, nitric oxide, PAF and prostaglandins in the mouse (Fujii *et al*., 1996a, b; 2000; Wada *et al*., 2000) and by histamine, nitric oxide and TNF-α in the rat (Iuvone *et al*., 1999).

Cyclic AMP elevating agents, such as phosphodiesterase (PDE) inhibitors and β_2-adrenoceptor agonists, are reported to suppress LPS-induced TNF-α release to induce anti-inflammatory effects (Beavo *et al*., 1994; Sekut *et al*., 1995; Pettipher *et al*., 1996; Bergman & Holycross, 1996; Goncalves de Moraes *et al*., 1998; Teixeira *et al*., 1997). The present study was designed to examine the anti-inflammatory effects of cyclic AMP elevating agents, including PDE inhibitors, β_2-adrenoceptor agonists, an adenylate cyclase activator and a cyclic AMP analogue.

Methods

Animals

Male ddY mice (5–7 weeks old) were purchased from Sankyo Laboratory Service (Tokyo, Japan) and were housed in an air-conditioned room (22$\pm$1°C and 55$\pm$5% humidity)

*Author for correspondence; E-mail: kirie@research.twmu.ac.jp

with controlled light–dark cycle (0600–2000 h light on) and free access to standard chow and tap water. The experimental protocol was approved by the Institutional Animal Care Committee.

Materials

LPS (*Salmonella typhimurium*), pentoxifylline, zaprinast, salbutamol hemisulphate salt, 8-bromo adenosine 3′:5′-cyclic monophosphate (8-Br-cAMP) sodium salt, rolipram, (±)isoproterenol hemisulphate salt and forskolin were purchased from Sigma (St. Louis, MO, U.S.A.), Pontamine sky blue 6B (PSB) from Tokyo Kasei Kogyo (Tokyo, Japan), dimethyl sulphoxide (DMSO) from Wako Chemicals (Osaka, Japan), mouse TNF-α ELISA kit from BioSource International (Camarillo, CA, U.S.A.), and IL-1α ELISA kit from Endogen (Woburn, MA, U.S.A.). Milrinone was provided from Yamanouchi Pharmaceutical (Tokyo, Japan). Other reagents were purchased from Kanto Chemicals (Tokyo, Japan).

LPS (4 mg ml^{-1}) was dissolved in phosphate-buffered saline. PSB (5 mg ml^{-1}) was dissolved in 0.9% NaCl, filtered (0.22 μ) and stored in sterile tubes. Milrinone, rolipram, zaprinast and forskolin were dissolved in DMSO and then diluted with physiological saline into appropriate concentrations (final concentration of DMSO was less than 2%). Other drugs were dissolved in 0.9% NaCl.

Determination of plasma leakage in mouse skin

The microvascular permeability of the skin was assessed by an extravasation of PSB as previously described (Fujii *et al*., 1994). Briefly, PSB (50 mg kg^{-1}) was injected to the mouse *via* the tail vein, and 5 min later, LPS (400 μg $site^{-1}$) was subcutaneously (s.c.) administered at the back of the mouse. Two hours later, the mice were killed by cervical dislocation and the stained area of the skin at the site of injection was excised (about 1 g) and minced. The skin specimen was dispersed in 6 ml 0.5% Na_2SO_4 and the dye was extracted by an addition of 14 ml acetone. After 3.5 h of extraction period, the dye concentration was determined by a spectrophotometer UV-2100 (Shimadzu, Kyoto, Japan) at an absorbance of 590 nm. Pentoxifylline was intraperitoneally (i.p.) administered 1 h prior to PSB, and other cyclic AMP elevating agents 30 min prior to PSB.

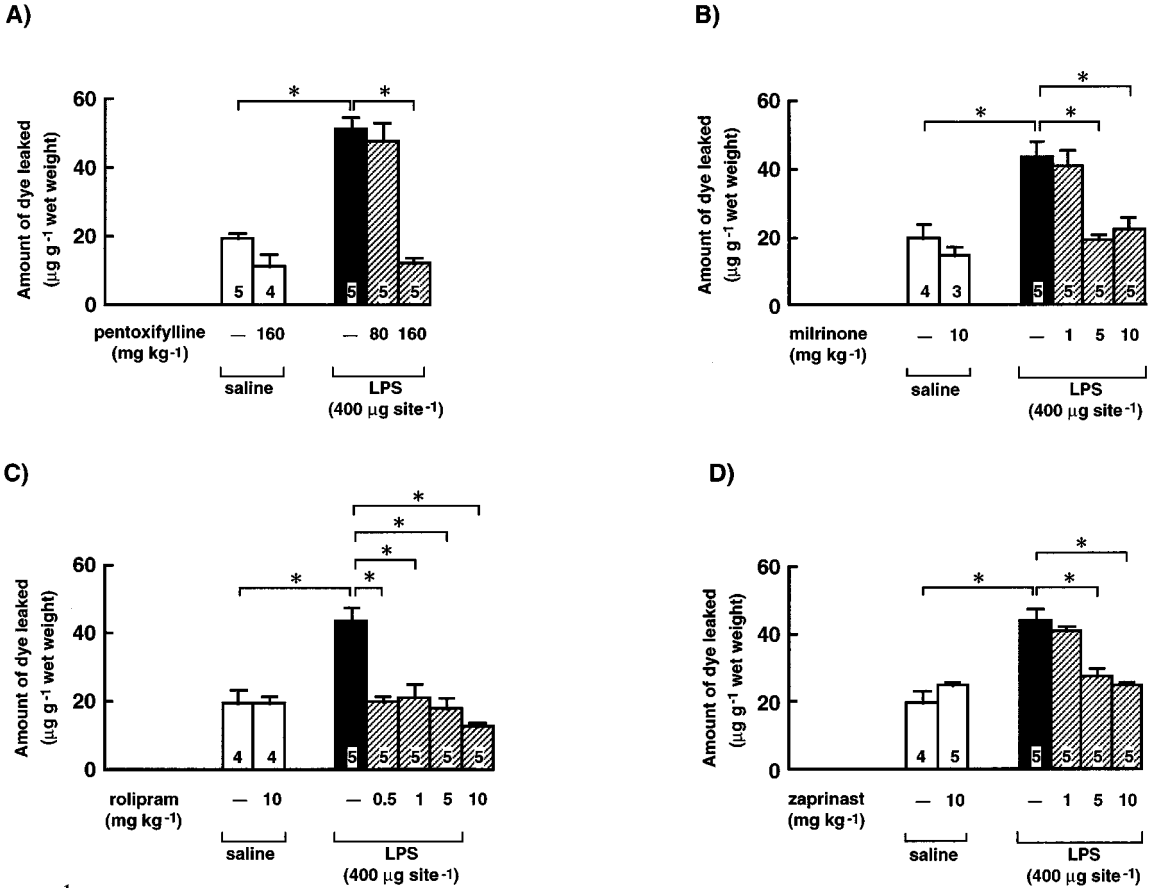

Figure 1 Inhibition of LPS-induced plasma leakage in mouse skin by PDE inhibitors, (A) pentoxifylline, (B) milrinone, (C) rolipram and (D) zaprinast. Pentoxifylline was administered (i.p.) 60 min prior to Pontamine sky blue (PSB, 50 mg kg^{-1}, i.v.) followed by LPS injection (400 μg $site^{-1}$, s.c., 5 min after PSB) on the back. Milrinone, rolipram and zaprinast were administered (i.p.) 30 min prior to PSB. Dye leakage in the skin was determined 2 h after LPS at the site of injection. Values are means±s.e.mean. Number of animals is given in columns. *$P<0.01$ by Bonferroni/Dunn's test.

Measurement of serum TNF-α and IL-1α

One to 3 h after an injection of LPS, blood samples were obtained. Serum was separated by centrifugation and stored at −20°C until assay. TNF-α and IL-1α were measured using mouse TNF-α and IL-1α ELISA kit as the manufacturer's specification.

Statistics

All the data were expressed as means ± s.e.mean. Results were analysed for statistical significance by one-way analysis of variance (ANOVA), followed by Bonferroni/Dunn's test.

Results

Effects of PDE inhibitors on LPS-induced dye leakage

The effects of drugs on LPS-induced dye leakage were evaluated at 2 h after topical application of LPS in this study, because we have previously shown that the dye leakage by LPS in the mouse skin reached maximum at 2 h (Fujii *et al*., 1996a). Pretreatment with pentoxifylline (160 mg kg^{-1}), a non-selective PDE inhibitor, blocked the LPS-induced dye leakage (Figure 1A). Then, the LPS-induced dye leakage was investigated with specific inhibitors of PDE subtypes, such as milrinone (PDE3), rolipram (PDE4) and zaprinast (PDE5). All of these PDE inhibitors significantly suppressed the LPS-induced plasma leakage (Figure 1B–D). Among these PDE inhibitors, rolipram demonstrated the strongest effect in inhibiting the LPS-induced dye leakage; rolipram was effective in smaller doses (0.5–1 mg kg^{-1}) compared to milrinone and zaprinast (Figure 1B–D).

Effects of β-agonists, forskolin and 8-Br-cAMP on the LPS-induced dye leakage

Because β_2-adrenoceptor agonists stimulate adenylate cyclase and increase in intracellular cylic AMP, effects of a non-selective β-agonist, isoproterenol, and a β_2-selective agonist, salbutamol, were studied on the LPS-induced dye leakage. Both isoproterenol (0.5–5 mg kg^{-1}) and salbutamol (0.05–5 mg kg^{-1}) dose-dependently inhibited the LPS-induced dye leakage (Figure 2A,B). Forskolin (5 mg kg^{-1}), an adenylate cyclase activator, also significantly suppressed the LPS-induced dye leakage (Figure 3).

The direct effect of cell permeable cyclic AMP, 8-Br-cAMP, was examined. Pretreatment with 8-Br-cAMP (10 mg kg^{-1}) significantly inhibited the LPS-induced dye leakage (Figure 4).

Serum TNF-α and IL-1α levels after LPS injection

The effect of cyclic AMP elevating agents on LPS-induced changes in serum TNF-α and IL-1α levels were determined. Doses of cyclic AMP elevating agents were chosen from the effective doses to inhibit the LPS-induced plasma leakage. Serum TNF-α increased transiently with a peak at 1 h after LPS injection (Figure 5A). Pretreatment with the cyclic AMP elevating agents markedly suppressed the LPS-stimulated elevation of serum TNF-α determined at 1 h after LPS administration (Figure 5B).

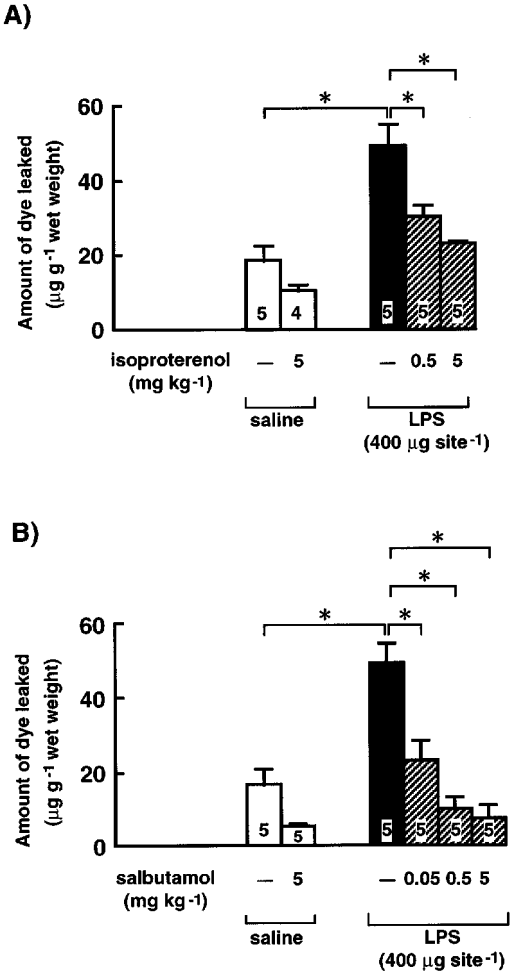

Figure 2 Inhibition of LPS-induced plasma leakage in mouse skin by β-agonists, (A) isoproterenol and (B) salbutamol. β-Agonists were administered (i.p.) 30 min prior to PSB followed by LPS injection. Two hours after LPS, dye leakage was determined. Values are means ± s.e.mean. Number of animals is given in columns. $*P<0.01$ by Bonferroni/Dunn's test.

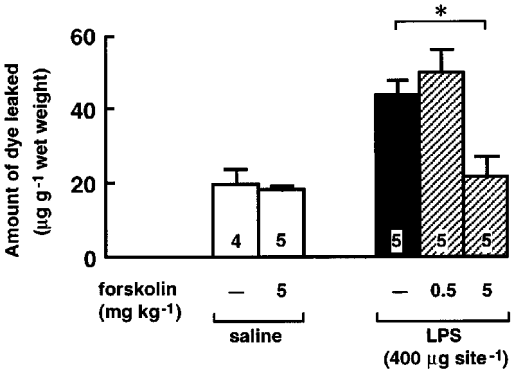

Figure 3 Effect of forskolin on LPS-induced plasma leakage. Forskolin was administered (i.p.) 30 min prior to PSB followed by LPS injection. Two hours after LPS, dye leakage was determined. Values are means ± s.e.mean. Number of animals is given in columns. $*P<0.01$ by Bonferroni/Dunn's test.

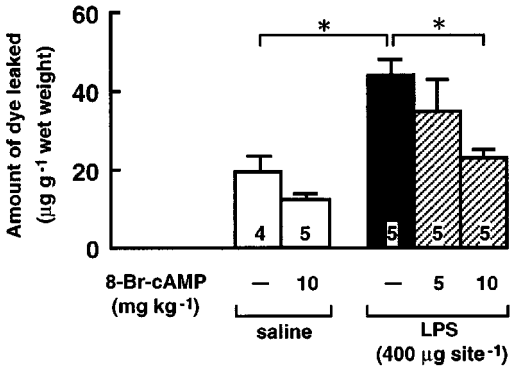

Figure 4 Effect of 8-Br-cAMP on LPS-induced plasma leakage. 8-Br-cAMP was administered (i.p.) 30 min prior to PSB followed by LPS injection. Two hours after LPS injection, dye leakage was determined. Values are means ± s.e.mean. Number of animals is given in columns. $*P<0.01$ by Bonferroni/Dunn's test.

In contrast, serum IL-1α increased gradually after LPS injection with a significant increase at 2–3 h (Figure 6A). The effect of cyclic AMP elevating agents was studied at 2 h after LPS injection. None of the cyclic AMP elevating agents examined significantly inhibited the LPS-induced increase in serum IL-1α level (Figure 6B).

Discussion

PDE inhibitors have been shown to suppress LPS-stimulated TNF-α release in both *in vivo* and *in vitro* studies (Pettipher *et al*., 1996; Noel *et al*., 1990; Sekut *et al*., 1995; Teixeira *et al*., 1997; Gantner *et al*., 1997; Goncalves de Moraes *et al*., 1998), and to have anti-inflammatory effects in airway inflammation models and carrageenan-induced paw oedema (Sekut *et al*., 1995; Ortiz *et al*., 1996; Teixeira *et al*., 1997). Our study demonstrated the anti-inflammatory effects of PDE inhibitors using a LPS-induced microvascular leakage in mice skin. We compared inhibitory potency of various PDE inhibitors, including pentoxifylline (non-selective PDE inhibitor), milrinone (PDE3 inhibitor; cyclic GMP inhibited PDE), rolipram (PDE4 inhibitor; cyclic AMP specific PDE) and zaprinast (PDE5 inhibitor; cyclic GMP specific PDE). All PDE inhibitors used significantly inhibited the LPS-induced plasma leakage. Among these PDE inhibitors, rolipram showed the strongest effect on the LPS-induced plasma leakage, with almost complete inhibition at 0.5 mg kg^{-1} to the saline control level. The PDE4 inhibitors have been proposed as anti-inflammatory drugs (Sekut *et al*., 1995; Ortiz *et al*., 1996; Teixeira *et al*., 1997; Cheng *et al*., 1997; Goncalves de Moraes *et al*., 1998). Our results of pentoxifylline accord with previous studies to demonstrate that this non-selective PDE inhibitor has an anti-inflammatory property by inhibiting TNF-α release (Noel *et al*., 1990; Neuner *et al*., 1994; Schratzberger *et al*., 1999). PDE3 inhibitors are clinically used for congestive heart failure. They are reported to inhibit TNF-α release from rat heart (Beavo *et al*., 1994; Bergman & Holycross, 1996), whereas no anti-inflammatory effect has been demonstrated in antigen-induced airway inflammation (Ortiz *et al*., 1996). Zaprinast,

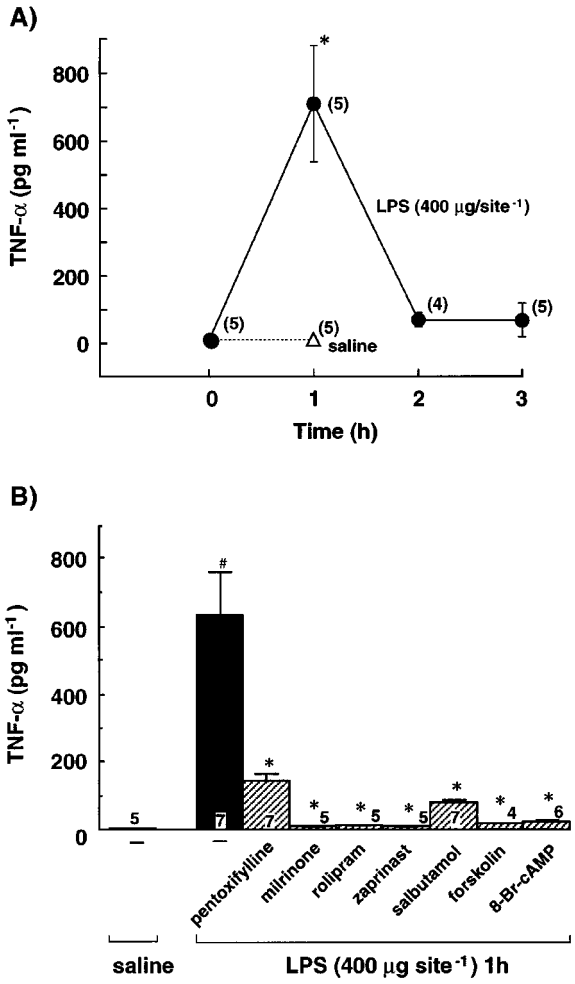

Figure 5 (A) Serum TNF-α level after subcutaneous injection of LPS (400 μg site^{-1}) on the back skin and (B) effects of cyclic AMP-elevating agents on the TNF-α release stimulated by s.c. LPS. (A) Blood was collected at 0, 1, 2 and 3 h after LPS. (B) Pentoxifylline (160 mg kg^{-1}), milrinone (5 mg kg^{-1}), rolipram (1 mg kg^{-1}), zaprinast (10 mg kg^{-1}), salbutamol (0.5 mg kg^{-1}), forskolin (1 mg kg^{-1}) and 8-Br-cAMP (10 mg kg^{-1}) were administered (i.p.) 35 min prior to LPS. Blood was collected for the assay at 1 h after LPS. Serum TNF-α concentration was measured by ELISA. Values are means ± s.e.mean. Number of determinations is given in parentheses/column. (A) $*P<0.01$ compared from 0 h/saline 1 h control by Bonferroni/Dunn's test. (B) $\#P<0.01$ compared from saline 1 h control and $*P<0.01$ compared from LPS only by Bonferroni/Dunn's test.

a PDE5 inhibitor, is reported to have little inhibitory effect on the LPS-stimulated TNF-α production in human monocytes (Gantner *et al*., 1997), while an anti-inflammatory effect on antigen-induced airway inflammation was shown in guinea-pigs (Ortiz *et al*., 1996). In our study, both milrinone and zaprinast showed a suppression of TNF-α release and an anti-inflammatory effect on microvascular permeability, although higher doses are needed compared to rolipram. It is not clear that the anti-inflammatory effects of zaprinase in this study are due to the inhibition on PDE5 or other non-specific inhibition on PDE3 and PDE4 isoenzymes. With this regard, dose-response relationship of these inhibitors is not clearly demonstrated in the present study.

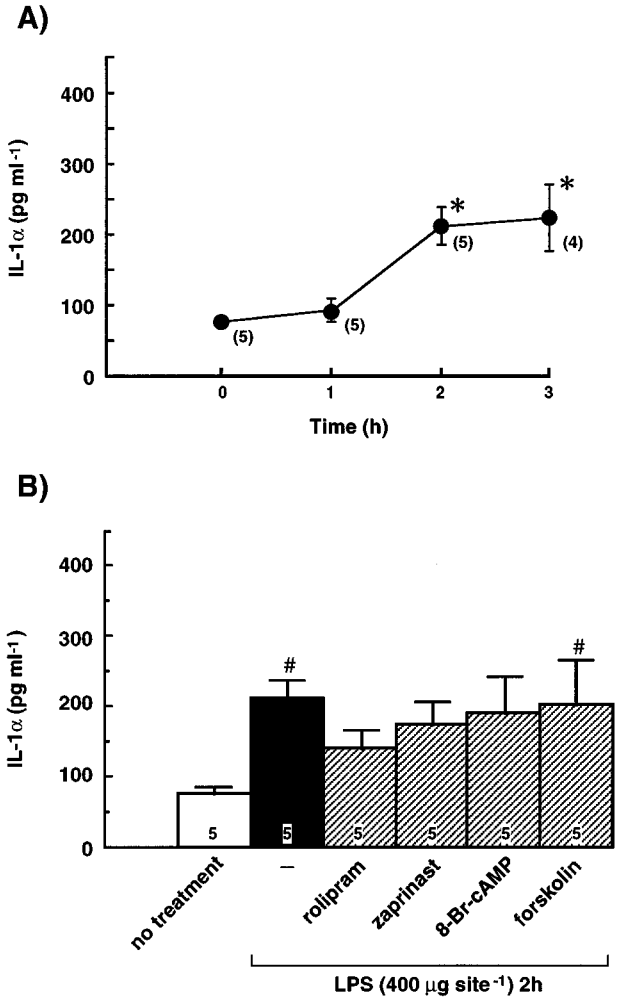

Figure 6 (A) Change in serum IL-1α after LPS (400 μg site^{-1}) injection on the back skin and (B) effects of cAMP-elevating agents on the IL-1α release stimulated by s.c. LPS. (A) Blood was collected at 0, 1, 2 and 3 h after LPS. (B) Rolipram (1 mg kg^{-1}), zaprinast (10 mg kg^{-1}), forskolin (1 mg kg^{-1}) and 8-Br-cAMP (10 mg kg^{-1}) were administered (i.p.) 35 min prior to LPS. Blood was collected for the assay at 2 h after LPS. Serum IL-1α was measured by ELISA. Values are means ± s.e.mean. Number of determinations is given in parentheses/column. (A) $*P<0.01$ compared from 0 h control by Bonferroni/Dunn's test. (B) $\#P<0.05$ compared from no treatment control by Bonferroni/Dunn's test.

β_2-Adrenoceptor agonists stimulate adenylate cyclase to cause an elevation in intracellular cyclic AMP. Although β_2-agonists are commonly used as a bronchodilator, they also possess anti-inflammatory activities by inhibiting the release of inflammatory mediators (Erjefalt & Persson, 1991; Bissonnette & Befus, 1997). The inhibitory effects of β_2-agonists on TNF-α production have been reported in human blood, leukocytes and mast cells (Severn *et al*., 1992; Bissonnette & Befus, 1997). Our studies showed that both a non-selective β-agonist and a β_2-selective agonist attenuate LPS-induced change in vascular permeability of mouse skin.

PDE inhibitors and β_2-agonists increase intracellular cyclic AMP; therefore, the direct effect of cyclic AMP on LPS-induced plasma leakage was examined. The cyclic AMP analogue, 8-Br-cAMP, and an adenylate cyclase activator, forskolin, demonstrated inhibitory effects on the LPS-induced plasma leakage and on the serum TNF-α level.

The cyclic AMP elevating agents studied thus far suppressed the serum TNF-α elevation and dye leakage induced by LPS. The anti-inflammatory effect of cyclic AMP elevating agents in mouse skin may be attributed to the suppression of TNF-α release from inflammatory cells in the skin. The elevation of cyclic AMP has been shown to inhibit the TNF-α production at the transcriptional level (Goncalves de Moraes *et al*., 1998; Farmer & Pugin, 2000). Using anti-TNF-α antibody, we have shown that TNF-α is one of the mediators in the LPS-stimulated plasma leakage (Fujii *et al*., 2000; Wada *et al*., 2000). IL-1α is another cytokine to mediate the LPS-induced plasma leakage of mouse skin (Fujii *et al*., 2000; Wada *et al*., 2000). The cyclic AMP elevating agents did not induce a significant inhibition in IL-1α level but elicit a great inhibition in plasma leakage in this study. Thus, an inhibition of IL-1α is not likely to be the mechanism of effect of PDE inhibitors on LPS-induced permeability change. An inhibition of TNF-α but not IL-1α production by cyclic AMP elevating agents has also been reported in LPS-stimulated human monocytes (Bailly *et al*., 1990). The origin of TNF-α and IL-1α detected in serum remained to be determined.

The present study provided evidence that the PDE inhibitors and β_2-adrenoceptor agonists have anti-inflammatory effects on the LPS-induced plasma leakage and a suppression of serum TNF-α elevation. Such properties of these cyclic AMP elevating agents may be applicable to clinical septic shock and cytokine-mediated inflammations.

This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan (Nos. 09672336, 10672158 and 11672279).

References

BAILLY, S., FERRUA, B., FAY, M. & GOUGEROT-POCIDALO, M.A. (1990). Differential regulation of IL 6, IL 1 A, IL 1β and TNFα production in LPS-stimulated human monocytes: role of cyclic AMP. *Cytokine,* **2,** 205–210.

BEAVO, J.A., CONTI, M. & HEASLIP, R.J. (1994). Multiple cyclic nucleotide phosphodiesterases. *Mol. Pharmacol.,* **46,** 399–405.

BERGMAN, M.R. & HOLYCROSS, B.J. (1996). Pharmacological modulation of myocardial tumor necrosis factor α production by phosphodiesterase inhibitors [published erratum appears in *J. Pharmacol. Exp. Ther.* (1997) **280,** 520]. *J. Pharmacol. Exp. Ther.,* **279,** 247–254.

BISSONNETTE, E.Y. & BEFUS, A.D. (1997). Anti-inflammatory effect of β_2-agonists: Inhibition of TNF-α release from human mast cells. *J. Allergy Clin. Immunol.,* **100,** 825–831.

CHENG, J.B., WATSON, J.W., PAZOLES, C.J., ESKRA, J.D., GRIFFITHS, R.J., COHAN, V.L., TURNER, C.R., SHOWELL, H.J. & PETTIPHER, E.R. (1997). 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.,* **280,** 621–626.

ERJEFALT, I. & PERSSON, C.G.A. (1991). Pharmacologic control of plasma exudation into tracheobronchial airways. *Am. Rev. Respir. Dis.* **143,** 1008 – 1014.

FARMER, P. & PUGIN, J. (2000). β-Adrenergic agonists exert their anti-inflammatory effects in monocytic cells through the IκB/NF-κB pathway. *Am. J. Physiol.,* **279,** L675 – L682.

FUJII, E., IRIE, K., OGAWA, A., OHBA, K. & MURAKI, T. (1996a). Role of nitric oxide and prostaglandins in lipopolysaccharide-induced increase in vascular permeability in mouse skin. *Eur. J. Pharmacol.,* **297,** 257 – 263.

FUJII, E., IRIE, K., UCHIDA, Y., TSUKAHARA, F. & MURAKI, T. (1994). Possible role of nitric oxide in 5-hydroxytryptamine-induced increase in vascular permeability in mouse skin. *Naunyn-Schmied. Arch. Pharmacol.,* **350,** 361 – 364.

FUJII, E., IRIE, K., UCHIDA, Y., TSUKAHARA, F., OHBA, K., OGAWA, A. & MURAKI, T. (1996b). Tolerance to lipopolysaccharide-induced increase in vascular permeability in mouse skin. *Eur. J. Pharmacol.,* **313,** 129 – 134.

FUJII, E., YOSHIOKA, T., ISHIDA, H., IRIE, K. & MURAKI, T. (2000). Evaluation of iNOS-dependent and independent mechanisms of the microvascular permeability change induced by lipopolysaccharide. *Br. J. Pharmacol.,* **130,** 90 – 94.

GANTNER, F., KUPFERSCHMIDT, R., SCHUDT, C., WENDEL, A. & HATZELMANN, A. (1997). In vitro differentiation of human monocytes to macrophages: change of PDE profile and its relationship to suppression of tumour necrosis factor-α release by PDE inhibitors. *Br. J. Pharmacol.,* **121,** 221 – 231.

GONCALVES DE MORAES, V.L., SINGER, M., VARGAFTIG, B.B. & CHIGNARD, M. (1998). Effects of rolipram on cyclic AMP levels in alveolar macrophages and lipopolysaccharide-induced inflammation in mouse lung. *Br. J. Pharmacol.,* **123,** 631 – 636.

KARIMA, R., MATSUMOTO, S., HIGASHI, H. & MATSUSHIMA, K. (1999). The molecular pathogenesis of endotoxic shock and organ failure. *Mol. Med. Today,* **5,** 123 – 132.

KLOSTERHALFEN, B. & BHARDWAJ, R.S. (1998). Septic shock. *General Pharmacol.,* **31,** 25 – 32.

IUVONE, T., DEN BOSSCHE, R.V., D'ACQUISTO, F., CARNUCCIO, R. & HERMAN, A.G. (1999). Evidence that mast cell degranulation, histamine and tumour necrosis factor α release occur in LPS-induced plasma leakage in rat skin. *Br. J. Pharmacol.,* **128,** 700 – 704.

LIAO, W. (1996). Endotoxin: Possible roles in initiation and development of atherosclerosis. *J. Lab. Clin. Med.,* **128,** 452 – 460.

NEUNER, P., KLOSNER, G., SCHAUER, E., POURMOJIB, M., MACHEINER, W., GRÜNWALD, C., KNOBLER, R., SCHWARZ, A., LUGER, T.A. & SCHWARZ, T. (1994). Pentoxifylline *in vivo* down-regulates the release of IL-1β, IL-6, IL-8 and tumour necrosis factor-α by human peripheral blood mononuclear cells. *Immunol.,* **83,** 262 – 267.

NOEL, P., NELSON, S., BOKULIC, R., BAGBY, G., LIPPTON, H., LIPSCOMB, G. & SUMMER, W. (1990). Pentoxifylline inhibits lipopolysaccharide-induced serum tumor necrosis factor and mortality. *Life Sci.,* **47,** 1023 – 1029.

ORTIZ, J.L., VALLÉS, J.M., MARTI-CABRERA, M., CORTIJO, J. & MORCILLO, E.J. (1996). Effects of selective phosphodiesterase inhibitors on platelet-activating factor- and antigen-induced airway hyperreactivity, eosinophil accumulation, and microvascular leakage in guinea pigs. *Naunyn-Schmied. Arch. Pharmacol.,* **353,** 200 – 206.

PETTIPHER, E.R., LABASI, J.M., SALTER, E.D., STAM, E.J., CHENG, J.B. & GRIFFITHS, R.J. (1996). Regulation of tumour necrosis factor production by adrenal hormones *in vivo*: insights into the antiinflammatory activity of rolipram. *Br. J. Pharmacol.,* **117,** 1530 – 1534.

SCHRATZBERGER, P., DUNZENDORFER, S., REINISCH, N., BURATTI, T., KÄHLER, C.M., TILG, H., HEROLD, M. & WIEDERMANN, C.J. (1999). Mediator-dependent effects of pentoxifylline on endothelium for transmigration of neutrophils: *Immunopharmacol.,* **41,** 65 – 75.

SEKUT, L., YARNALL, D., STIMPSON, S.A., NOEL, L.S., BATEMAN-FITE, R., CLARK, R.L., BRACKEEN, M.F., MENIUS, JR, J.A. & CONNOLLY, K.M. (1995). Anti-inflammatory activity of phosphodiesterase (PDE)-IV inhibitors in acute and chronic models of inflammation. *Clin. Exp. Immunol.,* **100,** 126 – 132.

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.

TEIXEIRA, M.M., GRISTWOOD, R.W., COOPER, N. & HELLEWELL, P.G. (1997). Phosphodiesterase (PDE)4 inhibitors: anti-inflammatory drugs of the future? *Trends Pharmacol. Sci.,* **18,** 164 – 171.

WADA, K., FUJII, E., ISHIDA, H., YOSHIOKA, T. & MURAKI, T. (2000). Effect of lipoteichoic acid on dermal vascular permeability in mice. *J. Pharmacol. Exp. Ther.,* **294,** 280 – 286.

(Received November 22, 2000
Revised February 20, 2001
Accepted March 9, 2001)